

Review Article

Styrylchromones: Biological Activities and Structure-Activity Relationship

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Styrylchromones (SC) are a group of oxygen-containing heterocyclic compounds, which are characterized by the attachment of a styryl group to the chromone core. SC can be found in nature or can be chemically synthesized in the laboratory. As their presence in nature is scarce, the synthetic origin is the most common. Two types of SC are known: 2-styrylchromones and 3-styrylchromones. However, 2-styrylchromones are the most common, being more commonly found in nature and which chemical synthesis is more commonly described. A wide variety of SC has been described in the literature, with different substituents in different positions, the majority of which are distributed on the A- and/or B-rings. Over the years, several biological activities have been attributed to SC. This work presents a comprehensive review of the biological activities attributed to SC and their structure-activity relationship, based on a published literature search, since 1989. The following biological activities are thoroughly revised and discussed in this review: antioxidant, antiallergic, antiviral, antibacterial, antifungal, anti-inflammatory, and antitumoral, affinity and selectivity for A₃ adenosine receptors, neuroprotective, and α -glucosidase inhibition. In general, SC are composed by a promising scaffold with great potential for the development of new drugs.

1. Introduction

Chromones are a group of oxygen-containing heterocyclic compounds with a benzoannulated γ -pyrone ring. The chromone ring system or chromone core (4*H*-chromen-4-ones or 4*H*-1-benzopyran-4-one, Figure 1) is found in several derivatives, and it is claimed as responsible for several biological activities, such as anti-inflammatory, antioxidant, anticancer, antiviral, and antimicrobial. Therefore, the chromone core is a privileged scaffold in drug discovery processes [1, 2].

Among the compounds derived from chromones, there is a group characterized by having a styryl group attached to the chromone core, known as styrylchromones (SC) [3]. Over the years, several biological activities have been attributed to SC, such as antioxidant, anti-inflammatory, anti-

microbial, antiviral, antitumor, neuroprotective, and antiallergic [3, 4]. This versatility has attracted the attention of several authors to the study of this group of chromones. Despite the various studies that already exist, there is still much more to discover and explore about the potential of SC. Thus, the present review gives a comprehensive insight into the biological activities of SC and their structure-activity relationship (SAR), based on the available information. This work provides an overview of the two types of SC that are known: 2-styrylchromones (2-SC) and 3-styrylchromones (3-SC), as well as their natural occurrence and synthetic approaches. Furthermore, their following biological activities are systematically described and discussed in terms of SAR: antioxidant, antiallergic, antiviral, antibacterial, antifungal, anti-inflammatory, and antitumoral. The neuroprotective activity, affinity and selectivity for A₃

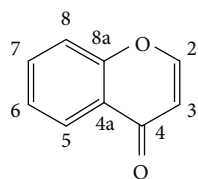


FIGURE 1: Chemical structure and numbering of the chromone core [1].

adenosine receptors, and α -glucosidase inhibition are also reported in this review.

2. Research Method

The bibliographic search for this review was carried out in the PubMed database using the keywords “styrylchromone” and “styryl-4*H*-chromen-4-one”. In the bibliographic search, 43 results were obtained, of which 4 were review articles (one of which was written in Japanese) and 39 were original articles. All the retrieved articles, written in English, from 1989 to the present (March 2021) have been analysed. From these articles, the cited references referring the biological activities of the SC were also included in this review. Furthermore, this review only includes studies with isolated compounds of synthetic and natural origin that are well characterized. Studies with SC's extracts or glycosylated derivatives were not included. Articles that do not present the methodologies used and with inconsistencies in the studied SC structures were also not included. The results presented throughout this review are presented according to the information available in the correspondent cited articles. Thus, the form of presentation of the results varies depending on the information available and may have the error associated (standard deviation or standard error of the mean) or not.

3. Styrylchromones

Styrylchromones belong to the group of compounds derived from chromones. These compounds have at least 17 carbons and are characterized by the attachment of a styryl group to the chromone core. Depending on the linking position of the styryl group, different SC are obtained. The position of this bond and the ring substituents determine the nomenclature of these compounds. The most common are 2-styrylchromones (2-SC) or 2-styryl-4*H*-chromen-4-one (1, Figure 2), where the bond occurs at C-2, and some 3-styrylchromones (3-SC) (2, Figure 2) are also known [3, 5].

SC are mostly of synthetic origin since their presence in nature is scarce. Gerwick and co-workers [6], in 1986, in the north coast of Puerto Rico, collected a material that was identified as the marine cryptophyte *Hormothamnion enteromorphaeoides* [6]. From this cryptophyte, was isolated the first natural SC-hormothamnione (3, Table 1). Subsequently, Gerwick [7] revised the taxonomy of the material previously collected, concluding that it was erroneously identified. The marine cryptophyte was instead a *Chryso-phaeum taylori*, from which they also isolated a new deriva-

tive – 6-desmethoxyhormothamnione (4, Table 1). Thus, hormothamnione (3) and its 6-desmethoxy analogue (4) are 2-SC that have been isolated from the marine cryptophyte *Chryso-phaeum taylori* [7]. Several years after the isolation of the first natural SC, Yoon and co-workers [8] isolated a new 2-SC (5, Table 1) from the methanolic extract of the rhizomes of *Imperata cylindrica*. Years later, Yang and co-workers [9] isolated 2-SC 6 (Table 1) from the ethanolic extract of Chinese eaglewood. Chinese eaglewood is a resinous wood from the tree of *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae) [9]. Also, from an ethanolic extract, but from of the bark of *Platanus × acerifolia* (Aiton) Willd, five new 2-SC were isolated: platachromones A–D (7–10, Table 1) and 2-SC 11 (Table 1) [10]. Jung and co-workers [11] and Chaniad and co-workers [12] also isolated 2-SC 11 from the methanolic extract of the heartwood of *Juniperus chinensis* and from the ethanolic extract of the bulbils of *Dioscorea bulbifera*, respectively [11, 12]. All compounds isolated from natural sources mentioned above were 2-SC. However, to the best of our knowledge, only one natural 3-SC was yet isolated: 3-SC 12 (Table 1) was isolated from the aerial parts of *Erucaria microcarpa* [13]. In some cases, SC derivatives were first chemically synthesized and only later were isolated from natural sources; examples of this are 2-SC 5 and 11 [14, 15].

Several approaches have been used and developed for the chemical synthesis of SC, leading to the obtention of a wide variety of compounds. The most common approach to 2-SC synthesis is the Baker-Venkataraman rearrangement, but there are others such as Allan-Robinson condensation, aldol condensation/oxidative cyclization, cyclization of an acetylenic ketone, condensation of 2-methylchromones with benzaldehydes, intramolecular Wittig reaction, and Knoevenagel condensation. In the synthesis of 3-SC, approaches such as oxidative rearrangement of 2'-hydroxychalcones to isoflavones using thallium (III) nitrate, Wittig reaction of 3-formylchromones with benzylic ylides, Knoevenagel condensation of chromone-3-carboxaldehyde with phenylacetic acids in the presence of potassium *tert*-butoxide under microwave irradiation, and Heck reaction of 3-bromochromone with styrene are used. These methodologies have been thoroughly reviewed and reported by several authors until now [3, 16–19].

4. Biological Activities

Several biological activities have been attributed to SC (Figure 3), namely, antioxidant, antiallergic, antiviral, antibacterial, antifungal, anti-inflammatory, antitumoral, neuroprotective, affinity and selectivity for A₃ adenosine receptors, and α -glucosidase inhibitory activity. Despite the several biological activities described, the *in vivo* absorption, distribution, metabolism, and excretion of SC, to the best of our knowledge, have not yet been reported in the literature. Below, the biological activities described for SC will be systematically described and discussed in terms of SAR.

4.1. Antioxidant Activity. Antioxidants are substances that prevent or delay oxidative degradation of the substrate,

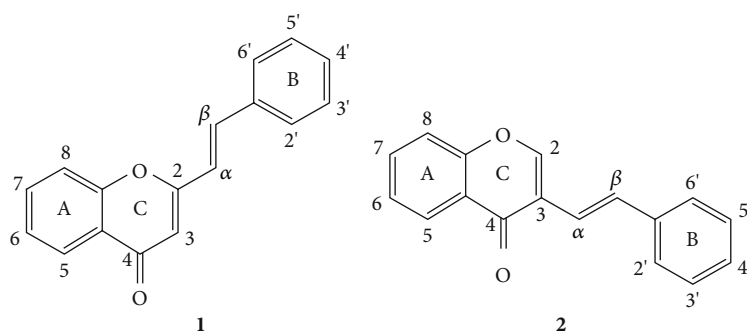


FIGURE 2: Chemical structures of 2-SC (1) and 3-SC (2) and numbering system to be adopted throughout this review [3].

protecting it against the action of reactive species. Reactive species are atoms or molecules, free and non-free radicals, which are extremely reactive [20, 21]. Several reactive species are generated during biological processes, such as reactive oxygen and nitrogen species (ROS and RNS, respectively), and this endogenous production has a physiological essential role in the organism [20, 21].

Oxidative stress can be understood as an imbalance between the production of reactive pro-oxidant species and the organism's capacity to counteract them by its antioxidant systems. Indeed, the human body has its own endogenous antioxidant system, able to fight against the oxidative attack and it is crucial for regular cellular functions [20]. The overproduction of these reactive pro-oxidant species produces severe damage in different biological functions and cell constituents, such as lipids, DNA, enzymes, or proteins, implicated in the development of various diseases as cancer, inflammatory diseases, or even ageing [20, 21]. Therefore, it became clear that, in some circumstances, the body's production of antioxidants is not enough to counteract this overproduction of reactive pro-oxidant species and their deleterious effects. Thus, the consumption of external antioxidants, as in the diet, is believed to be helpful, reinforcing the endogenous antioxidant defence system and bringing health benefits [20, 21]. Several 2-SC and 3-SC have shown potential as antioxidant agents and are below discussed, and all the observed antioxidant effects are summarized in Table 2, for 2-SC, and Table 3, for 3-SC.

4.1.1. 2-Styrylchromones. Fernandes and co-workers [22] evaluated the profile of some synthetic 2-SC derivatives (1, 5, 11, and 13–19, Figure 4) as inhibitors of xanthine oxidase (XO). XO is a highly versatile enzyme and exhibits a broad activity toward reducing substrates [22]. During these reactions with XO, ROS are generated, such as superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) [22, 23]. All the tested 2-SC derivatives were found to be inhibitors of XO in a concentration-dependent manner, considering the studied concentration range (0.625–100 μM), except for 1 (an unsubstituted 2-SC) that was practically inactive [concentration of the tested compound that produce 50% inhibition (IC_{50}) > 100 μM]. Some of the 2-SC were more potent than the tested positive control, allopurinol ($IC_{50} = 5.43 \pm 0.08 \mu M$). 2-SC 19 was the most active, with an IC_{50} value of $0.55 \pm 0.03 \mu M$, followed by 18, 14, and 17 (IC_{50} values

of $2.03 \pm 0.19 \mu M$, $2.52 \pm 0.08 \mu M$, and $4.36 \pm 0.57 \mu M$, respectively). The structure of the most active compounds indicates that the presence of the catechol group (C-3' and C-4') on the B-ring of the derivatives (17–19) potentiates XO inhibition. The presence of two –OH groups linked to the A-ring also contributes to the inhibition, especially at C-5 and C-7 (19) leading to an increase in the activity when compared with the presence of just one –OH on the A-ring (17 and 18). This effect is also observed in the result obtained for 2-SC 14, which, despite having only –OH groups at C-5 and C-7 on the A-ring, proved to be a potent XO inhibitor [22].

Some of the previous 2-SC derivatives (11 and 15–19, Figure 4) were also evaluated as having a possible protective activity against the *tert*-butylhydroperoxide (*t*-BHP)-induced pro-oxidant hepatotoxicity in freshly isolated rat hepatocytes [24]. All the tested 2-SC exhibited *in vitro* hepatoprotective activity, which was reflected in the preservation of the integrity of the plasma membrane of rat hepatocytes. In this study, the results and the associated error were presented only in graphs, activity values not being mentioned. The tested compounds can be divided into two groups: the group that has an –OH at C-4' on the B-ring (11, 15, and 16) and the group that has a catechol group (C-3' and C-4') on the B-ring (17–19). As in the previous study, the 2-SC derivatives that have the catechol group also proved to be much more active in this assay. For the group that has an –OH at C-4' on the B-ring (11, 15, and 16), the concentration range tested was 25–200 μM , while for the group that has a catechol group on the B-ring (17–19), it was 3.125–50 μM . These two groups showed differences in what concerns the qualitative and quantitative preservation of biochemical homeostasis: 2-SC 17–19 exerted their hepatoprotective effect through the prevention of lipid peroxidation and the inhibition of reduced glutathione depletion and oxidized glutathione formation, these effects being comparable to those demonstrated by the used positive control, quercetin; whereas 2-SC 11, 15, and 16 only partially prevented lipid peroxidation and had no effect on glutathione levels. Thus, 2-SC 17–19 can be considered the most promising antioxidant 2-SC in this study [24].

Filipe and co-workers [25] synthesized some already known 2-SC (17–19, Figure 5) and a new 2-SC (20, Figure 5). The authors studied their inhibitory effect on

TABLE 1: Chemical structures and natural occurring 2-SC and 3-SC.

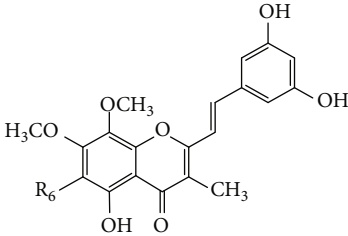
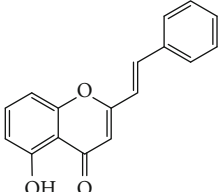
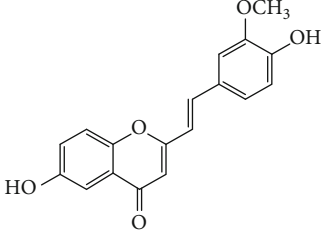
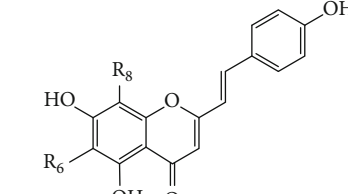
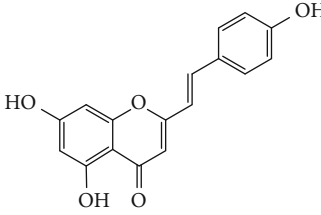
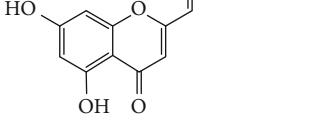
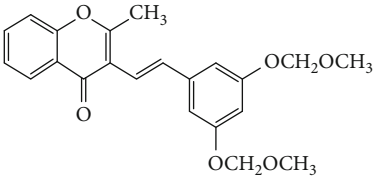
Chemical structure	2-SC	Natural occurrence*	Ref.
 <p data-bbox="240 591 352 655"> $3 R_6 = OCH_3$ $4 R_6 = H$ </p>	<i>Chrysophaeum taylori</i>	[6, 7]	
 <p data-bbox="236 900 248 917">5</p>	<i>Imperata cylindrica</i>	[8]	
 <p data-bbox="288 1208 301 1229">6</p>	<i>Aquilaria sinensis</i> (Lour.) Gilg (Thymelaeaceae)	[9]	
 <p data-bbox="150 1491 432 1515">7 $R_6 = CH_2CH=C(CH_3)_2$; $R_8 = H$</p> <p data-bbox="150 1534 432 1557">8 $R_6 = H$; $R_8 = CH_2CH=C(CH_3)_2$</p> <p data-bbox="150 1576 475 1600">9 $R_6 = CH_2CH(OH)CH=CH_2$; $R_8 = H$</p> <p data-bbox="134 1619 464 1640">10 $R_6 = H$; $R_8 = CH_2CH(OH)CH=CH$</p>	<i>Platanus × acerifolia</i> (Aiton) Willd	[10]	
 <p data-bbox="280 1889 304 1910">11</p>	<i>Platanus × acerifolia</i> (Aiton) Willd <i>Juniperus chinensis</i>	[10] [11]	
 <p data-bbox="280 1889 304 1910">11</p>	<i>Dioscorea bulbifera</i>	[12]	

TABLE 1: Continued.

Chemical structure	3-SC	Natural occurrence*	Ref.
 <p style="text-align: center;">12</p>		<i>Erucaria microcarpa</i>	[13]

*According to the information provided in the original article.

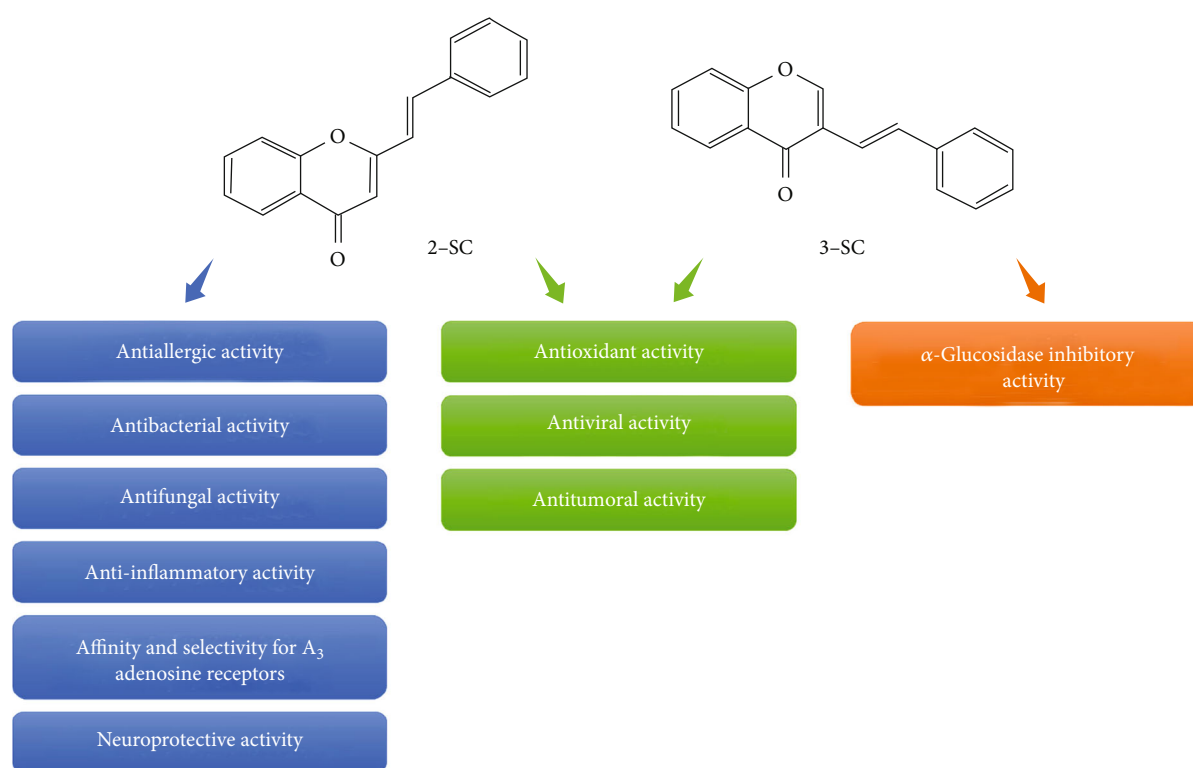


FIGURE 3: Biological activities described for 2-SC and 3-SC.

Cu²⁺-induced oxidation of isolated human serum low-density lipoproteins (LDL), an *in vitro* model of lipid peroxidation, via radical chain reaction. The results and the associated error were presented only in graphs, activity values not being mentioned. In this study, the most active 2-SC were the least hydroxylated one (**20**), with –OH groups only at C-3' and C-4' on the B-ring, and the 2-SC **17**, with an additional –OH at C-5 on the A-ring. The results observed in the assay of the inhibition of conjugated diene formation by 2-SC during Cu²⁺-induced LDL lipid peroxidation showed that 2-SC **17** and **20** (3 μM) and the used positive control (quercetin, 3 μM) totally inhibited the formation of conjugated dienes. 2-SC **18** and **19** (0.75 and 3 μM) were less active. These compounds have the catechol group on the B-ring, but **18** has only one –OH at C-7 on the A-ring and **19**

has –OH at C-5 and C-7 on the A-ring [25]. Despite this rather inactivity in this system, 2-SC **19** was above considered the most effective XO inhibitor [22, 25]. Thus, for the systems tested in this study, the antioxidant potential of 2-SC is not necessarily determined by the number of –OH substituents, contrary to what was concluded in the studies mentioned above [22, 24], since the increase of the number of –OH groups led to a decrease in the antioxidant effect of these 2-SC [25]. The number of –OH groups present may have influenced the partitioning of this hydrophobic 2-SC into LDL leading to the variations observed in its antioxidant effectiveness. In what concerns the study of the electron donating properties by pulse radiolysis in micellar solutions, Filipe and co-workers [25] found that all the tested 2-SC were equally capable to react with O₂^{•-} and

TABLE 2: 2-SC studied for the antioxidant activity and summary of the observed antioxidant effects. The table display the antioxidant activity shown for the studied 2-SC according to the criterion: activity greater or equal to 30%.

2-SC	Observed effect(s)	Ref.
3	Scavenging of DPPH*	[32]
5	Inhibition of XO and high oxidation potential	[22, 27]
11	Inhibition of XO; protective activity against <i>t</i> -BHP-induced hepatotoxicity in rat hepatocytes; scavenging effect against ROS and RNS ($O_2^{\bullet-}$; H_2O_2 ; HOCl; 1O_2 ; ROO* and ONOO ⁻); and moderate oxidation potential	[22, 24, 26, 27]
13	Inhibition of XO; scavenging effect against ROS ($O_2^{\bullet-}$ and HOCl); and high oxidation potential	
14	Inhibition of XO; scavenging effect against ROS and RNS (HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO); and high oxidation potential	[22, 26, 27]
15	Inhibition of XO; protective activity against <i>t</i> -BHP-induced hepatotoxicity in rat hepatocytes; scavenging effect against ROS and RNS (H_2O_2 and ONOO ⁻); and moderate oxidation potential	
16	Inhibition of XO; protective activity against <i>t</i> -BHP-induced hepatotoxicity in rat hepatocytes; scavenging effect against ROS and RNS ($O_2^{\bullet-}$; HOCl; 1O_2 ; ROO* and ONOO ⁻); and moderate oxidation potential	[22, 24, 26, 27]
17 - 19	Inhibition of XO; protective activity against <i>t</i> -BHP-induced hepatotoxicity in rat hepatocytes; inhibition of Cu^{2+} -induced oxidation of isolated human serum LDL; scavenging effect against ROS and RNS ($O_2^{\bullet-}$; H_2O_2 ; HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO); and low oxidation potential	[22, 24–27]
20	Inhibition of Cu^{2+} -induced oxidation of isolated human serum LDL; scavenging effect against ROS and RNS ($O_2^{\bullet-}$; H_2O_2 ; HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO); and low oxidation potential	[25–27]
21	Scavenging effect against ROS and RNS ($O_2^{\bullet-}$; HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO); and moderate oxidation potential	[26, 27]
22 - 24	Scavenging effect against ROS and RNS ($O_2^{\bullet-}$; H_2O_2 ; HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO); metal chelating capacity; and reducing activity	[28]
25	Scavenging effect against ROS and RNS ($O_2^{\bullet-}$; HOCl; 1O_2 ; ROO*; ONOO ⁻ , without $NaHCO_3$, and *NO)	[29]
26, 27	Scavenging effect against ROS and RNS ($O_2^{\bullet-}$; HOCl; 1O_2 ; ROO*; ONOO ⁻ and *NO)	
28 - 32	Scavenging of $O_2^{\bullet-}$	[30]
33	Inhibition of reactive species-induced oxidation of DCFH-DA	[32]

1O_2 : singlet oxygen; *t*-BHP: *tert*-butyl hydroperoxide; DCFH-DA: 2',7'-dichlorodihydrofluorescein diacetate; DPPH*: 2,2-Diphenyl-1-picrylhydrazyl radical; H_2O_2 : hydrogen peroxide; HOCl: hypochlorous acid; LDL: low-density lipoproteins; *NO: nitric oxide radical; ONOO⁻: peroxynitrite anion; RNS: reactive nitrogen species; ROS: reactive oxygen species; ROO*: peroxy radical; $O_2^{\bullet-}$: superoxide anion radical; XO: xanthine oxidase.

TABLE 3: 3-SC studied for the antioxidant activity and summary of the observed antioxidant effects.

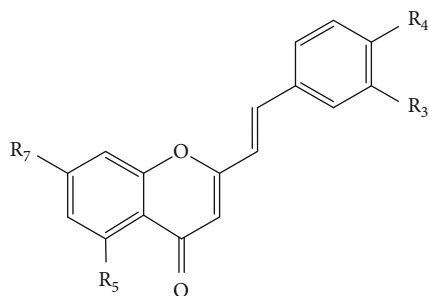
3-SC	Observed effect(s)	Ref.
12	Scavenging of 1O_2	[13]
44 - 47 49, 50	Scavenging of DPPH*	[33]

1O_2 : singlet oxygen; DPPH*: 2,2-diphenyl-1-picrylhydrazyl radical.

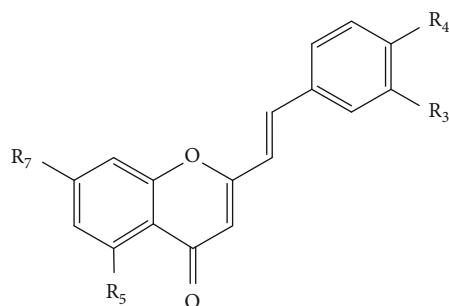
tryptophan radical in cationic micelles. When electron transfer reactions were carried out in neutral micelles, 2-SC 20 presented a higher effect when compared to the other tested compounds, since it was the one that exhibited a measurable reactivity [25].

The *in vitro* scavenging activities of ROS and RNS by several 2-SC derivatives (1, 5, 11, and 13–21, Figure 6) were also evaluated by Gomes and co-workers [26]. The scavenging activities of these 2-SC were tested against the following reactive species: $O_2^{\bullet-}$, H_2O_2 , hypochlorous acid (HOCl), singlet oxygen (1O_2), peroxy radical (ROO*), peroxynitrite anion (ONOO⁻), and nitric oxide radical (*NO), using various generating systems and probes, according to the reactive

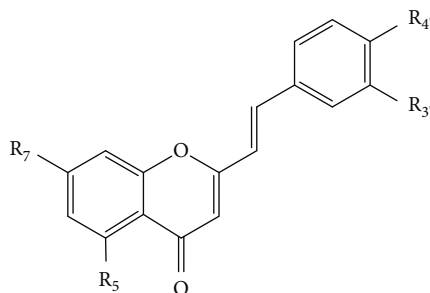
species to be detected [26]. Considering the –OH substituents present on the B-ring of the 2-SC, they can be divided into three groups: group 1, with a catechol group (C-3' and C-4') on the B-ring (17–20); group 2, with –OH at C-4' on the B-ring (11, 15, 16, and 21); and group 3, without any substitution on the B-ring (1, 5, 13, and 14). The studied concentration range and the used positive controls varied according to the reactive species under study. Under the tested experimental conditions, 2-SC 19 and 18 (IC₅₀ values of 48.9 ± 1.2 μM and 51.0 ± 1.4 μM, respectively) were the most active $O_2^{\bullet-}$ scavengers. The IC₅₀ found for the tested positive control (propyl gallate) was 24.6 ± 3.2 μM. 2-SC 11, 16, 17, and 20 were also able to scavenge $O_2^{\bullet-}$, while the 2-SC from group 3 presented very low (13 and 14) or no activity at all (1 and 5). The same did not apply to H_2O_2 scavenging activity, since only 2-SC from group 1 showed a significant scavenging effect. 2-SC 17 and 19 were the most active (IC₅₀ values of 48.9 ± 5.5 μM and 50.3 ± 7.8 μM, respectively). They were even more active than the used positive control, ascorbic acid (IC₅₀ = 625.5 ± 49.6 μM). The other compounds were less efficient (11 and 15) or completely ineffective (1, 5, 13, 14, 16, and 21) up to the highest tested concentration (250 μM). 2-SC 11, 14, and 19 were



- | | |
|---|---|
| 1 $R_5 = R_7 = R_{3'} = R_{4'} = H$ | 15 $R_5 = R_{4'} = OH; R_7 = R_{3'} = H$ |
| 5 $R_5 = OH; R_7 = R_{3'} = R_{4'} = H$ | 16 $R_5 = R_{3'} = H; R_7 = R_{4'} = OH$ |
| 11 $R_5 = R_7 = R_{4'} = OH; R_{3'} = H$ | 17 $R_5 = R_{3'} = R_{4'} = OH; R_7 = H$ |
| 13 $R_5 = R_{3'} = R_{4'} = H; R_7 = OH$ | 18 $R_5 = H; R_7 = R_{3'} = R_{4'} = OH$ |
| 14 $R_5 = R_7 = OH; R_{3'} = R_{4'} = H$ | 19 $R_5 = R_7 = R_{3'} = R_{4'} = OH$ |

FIGURE 4: Chemical structures of 2-SC **1**, **5**, **11**, and **13–19** [22, 24].

- | | |
|---|---|
| 17 $R_5 = R_{3'} = R_{4'} = OH; R_7 = H$ | 19 $R_5 = R_7 = R_{3'} = R_{4'} = OH$ |
| 18 $R_5 = H; R_7 = R_{3'} = R_{4'} = OH$ | 20 $R_5 = R_7 = H; R_{3'} = R_{4'} = OH$ |

FIGURE 5: Chemical structures of 2-SC **17–20** [25].

- | | | |
|---|---|---|
| 1 $R_5 = R_7 = R_{3'} = R_{4'} = H$ | 14 $R_5 = R_7 = OH; R_{3'} = R_{4'} = H$ | 18 $R_5 = H; R_7 = R_{3'} = R_{4'} = OH$ |
| 5 $R_5 = OH; R_7 = R_{3'} = R_{4'} = H$ | 15 $R_5 = R_{4'} = OH; R_7 = R_{3'} = H$ | 19 $R_5 = R_7 = R_{3'} = R_{4'} = OH$ |
| 11 $R_5 = R_7 = R_{4'} = OH; R_{3'} = H$ | 16 $R_5 = R_{3'} = H; R_7 = R_{4'} = OH$ | 20 $R_5 = R_7 = H; R_{3'} = R_{4'} = OH$ |
| 13 $R_5 = R_{3'} = R_{4'} = H; R_7 = OH$ | 17 $R_5 = R_{3'} = R_{4'} = OH; R_7 = H$ | 21 $R_5 = R_7 = R_{3'} = H; R_{4'} = OH$ |

FIGURE 6: Chemical structures of 2-SC **1**, **5**, **11**, and **13–21** [26, 27].

the most effective scavengers of HOCl, with IC_{50} values of $8.5 \pm 0.3 \mu M$, $5.7 \pm 0.7 \mu M$ and $3.9 \pm 0.2 \mu M$, respectively. This results' pattern was similar for 1O_2 scavenging activity; however, the most effective compound was **17** ($IC_{50} = 4.9 \pm 0.6 \mu M$). The other compounds of group 1 also showed activity but were less effective than the used positive control, penicillamine ($IC_{50} = 4.9 \pm 0.2 \mu M$). The results for ROO^\bullet scavenging activity showed that all the compounds from group 1 were able to delay the ROO^\bullet -dependent oxidation of

fluorescein, even more than some other 2-SC from the other groups. In group 2, only 2-SC **15** was not effective, whereas from group 3, only **14** was active. The $ONOO^-$ scavenging was evaluated in the presence and absence of $NaHCO_3$. The relative order of potencies of the 2-SC was the same for both conditions. From all the tested 2-SC, **20** was the most potent, with an IC_{50} value of $0.24 \pm 0.02 \mu M$, in the absence of $NaHCO_3$, and $0.44 \pm 0.02 \mu M$ in its presence. The 2-SC **20** was more potent than the positive control, ebselen (IC_{50} values

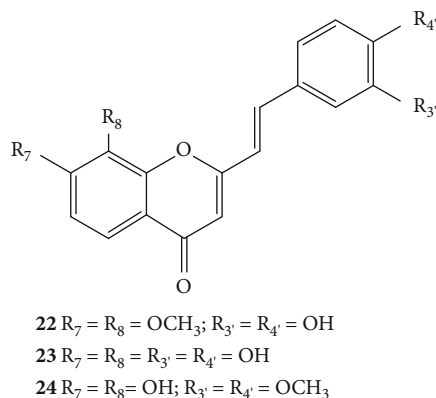


FIGURE 7: Chemical structures of 2-SC 22–24 [28].

of $0.91 \pm 0.06 \mu\text{M}$ in the absence of NaHCO_3 and $4.63 \pm 0.44 \mu\text{M}$ in its presence). Compounds from group 1 were the most effective, and all the compounds from group 2 also showed scavenging effect, while in group 3, only 2-SC 14 was active. In what concerns $\cdot\text{NO}$ scavenging activity, 2-SC from group 1 were the most active [IC_{50} varying from 0.24 ± 0.04 (17) to $0.51 \pm 0.13 \mu\text{M}$ (19)], being more potent than the used positive control, rutin ($\text{IC}_{50} = 3.66 \pm 0.43 \mu\text{M}$). The 2-SC from the other groups were less efficient (11, 14, 16, and 21) or completely ineffective (1, 5, 13, and 15). In summary, 2-SC from group 1 showed scavenging activity against all the detected ROS and RNS, while 2-SC from group 2 had a variable effect depending on the number and position of the $-\text{OH}$ substituents on the A-ring, and for some reactive species, they did not show any activity. The activity of 2-SC from group 3 was generally low or inexistent. Therefore, for most of the reactive species studied, the catechol on the B-ring appears to play an important role in the scavenging activity. However, for HOCl scavenging activity, the $-\text{OH}$ substituents on the A-ring assumed high importance, despite the existence of $-\text{OH}$ substituents on the B-ring. The authors also tested some flavonoids structurally related to the tested 2-SC, which allowed to conclude that the styryl moiety also contributes to their observed outstanding antioxidant activity. A possible explanation for this fact might be the contribution of the styryl pattern to the stabilization of the radicals that are formed during the scavenging reactions [26].

Considering these antioxidant properties, the electrochemical behaviour of these same 2-SC (1, 5, 11, and 13–21, Figure 6) was studied by cyclic voltammetry in order to understand the mechanism of ROS and RNS scavenging activity [27]. Cyclic voltammetry is an electroanalytic technique to determine the redox properties of molecules in solution. Higher scavenging effects corresponded to the lowest oxidation potentials, relating their electrochemical behaviour to the antioxidant capacity. The obtained results showed that 2-SC from group 1, with a catechol group on the B-ring, presented lower values of oxidation potentials corresponding to the oxidation of the $3',4'$ -OH of the catechol group, while for the compounds from group 2, the values of the oxidation potentials correspond to the oxidation of $-\text{OH}$ at C-4' on the B-ring. While for the com-

pounds 5, 13, and 14 from group 3, the values of oxidation potentials corresponded to the $-\text{OH}$ groups present on the A-ring. For 2-SC 1, without $-\text{OH}$ substituents, there were no detectable peaks. Correlations between the oxidation peak potentials and the scavenging activity against some ROS and RNS were also analysed. For H_2O_2 , $^1\text{O}_2$ and ONOO^- were found to have significant correlations, indicating that the scavenging mechanism against them is based on redox reactions; whereas for $\text{O}_2^{\cdot-}$ and HOCl , no significant correlations were found. The authors further suggested that the oxidation reaction involves only one electron leading to the formation of a semiquinone, which in turn undergoes disproportionation, and forms an orthoquinone and regenerates the initial 2-SC structure [27].

Gomes and co-workers [28] also published a work where a new group of 2-SC (22–24, Figure 7) was synthesized, with $-\text{OCH}_3$ or $-\text{OH}$ groups at C-7, C-8, C-3', and C-4' on the A- and B-rings, and studied their scavenging effects against the same ROS ($\text{O}_2^{\cdot-}$, H_2O_2 , HOCl , $^1\text{O}_2$, and $\text{ROO}\cdot$) and RNS (ONOO^- and $\cdot\text{NO}$). The metal chelating capacity and reducing activity of these compounds were also evaluated, as they are indicators of antioxidant activity [28]. The methodologies for the ROS and RNS scavenging assays used were similar to the previously mentioned studies of these authors [26, 28]. All the tested 2-SC showed $\text{O}_2^{\cdot-}$ scavenging activity, and the obtained IC_{50} values were much lower than the positive control, tiron ($\text{IC}_{50} = 287.7 \pm 32.0 \mu\text{M}$). 2-SC 23 was the most active ($\text{IC}_{50} = 17.8 \pm 3.8 \mu\text{M}$). This means that the $-\text{OH}$ groups on the A- and B-rings favour this activity and their methylation decreases the scavenging effect [28]. Comparing with the before tested 2-SC [26], this new group of 2-SC demonstrated an improved $\text{O}_2^{\cdot-}$ scavenging activity, as they presented lower IC_{50} values [28]. H_2O_2 was scavenged by 2-SC 22 ($\text{IC}_{50} = 701.9 \pm 58.2 \mu\text{M}$) that was the most active and by 2-SC 23 ($\text{IC}_{50} = 799.3 \pm 60.9 \mu\text{M}$). The positive control, ascorbic acid, presented an IC_{50} value of $567.4 \pm 33.0 \mu\text{M}$. All the tested 2-SC were able to scavenge HOCl but were less effective than the used positive control, lipoic acid ($\text{IC}_{50} = 1.95 \pm 0.06 \mu\text{M}$). 2-SC 22 was the most active ($\text{IC}_{50} = 4.67 \pm 0.64 \mu\text{M}$). For this reactive specie and once more, the methylation of $-\text{OH}$ on the B-ring decreased the activity. All the tested 2-SC were able to scavenge $^1\text{O}_2$, being more effective than the positive control, ascorbic acid ($\text{IC}_{50} = 10.2 \pm 1.5 \mu\text{M}$). The pattern of results of this assay was similar to the ones obtained for the $\text{O}_2^{\cdot-}$ scavenging assay. The results for the $\text{ROO}\cdot$ scavenging activity showed that all the 2-SC were able to delay the $\text{ROO}\cdot$ -dependent oxidation of fluorescein. Also, for the $\cdot\text{NO}$ scavenging activity, all the tested 2-SC were active and more potent than the positive control, rutin ($\text{IC}_{50} = 1.17 \pm 0.05 \mu\text{M}$). In terms of ONOO^- scavenging activity, in the absence of NaHCO_3 , 2-SC 24 was the most active ($\text{IC}_{50} = 0.59 \pm 0.08 \mu\text{M}$), being more potent than the positive control, ebselen ($\text{IC}_{50} = 1.00 \pm 0.11 \mu\text{M}$). In the presence of NaHCO_3 , 2-SC 22 was the most active ($\text{IC}_{50} = 0.93 \pm 0.21 \mu\text{M}$), being more potent than the positive control, ebselen ($\text{IC}_{50} = 5.27 \pm 0.32 \mu\text{M}$). Overall, the presented results demonstrate that the methylation of $-\text{OH}$ on the B-ring decreases the scavenging of ROS and RNS. Depending on the reactive species, $-\text{OH}$ at C-8

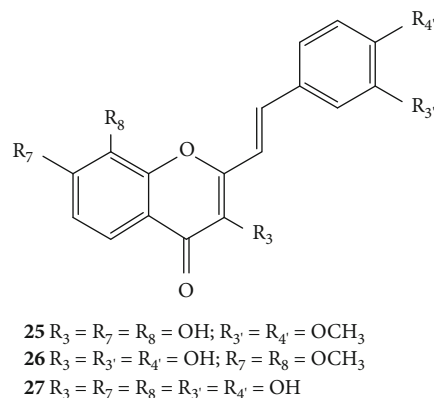


FIGURE 8: Chemical structures of 2-SC 25–27 [29].

on the A-ring sometimes favoured the scavenging effects, namely, for $\text{O}_2^{\bullet-}$ and $\bullet\text{NO}$. In what concerns the reducing activity, all the tested 2-SC were able to significantly reduce ferric ion. According to the figure presented by the authors, the order of potencies found was **22** > **24** > **23**, which indicates that methylation of the $-\text{OH}$ groups in the A- and B-rings improved the iron-reducing capacity. The determination of metal chelation capacity showed spectral changes of 2-SC with the addition of iron (II), indicating the formation of complexes [28].

Sousa and co-workers [29] synthesized 3-hydroxy-2-SC (25–27, Figure 8) and evaluated their scavenging activity against ROS and RNS ($\text{O}_2^{\bullet-}$, H_2O_2 , HOCl , $^1\text{O}_2$, ROO^\bullet , ONOO^- , and $\bullet\text{NO}$) [29]. The methodologies used were similar to the previously mentioned ones [26, 28]. The 3-hydroxy-2-SC synthesized in this study are derived from the 2-SC studied by Gomes and co-workers [28] (22–24) by introducing an $-\text{OH}$ at C-3 on the C-ring. $\text{O}_2^{\bullet-}$ was scavenged by 2-SC 25 and 27, for which the IC_{50} values were much lower than those found for the positive control used, quercetin (IC_{50} values of $35 \pm 2 \mu\text{M}$, $30 \pm 1 \mu\text{M}$, and $67 \pm 7 \mu\text{M}$, respectively), and compound 26 was less efficient than the previous ones. In this case, the methylation of $-\text{OH}$ at C-7 and C-8 on the A-ring decreased the scavenging activity [29]. Comparing these 3-hydroxy-2-SC with 2-SC 22–24, tested by Gomes and co-workers [28], the presence of an $-\text{OH}$ at C-3 on the C-ring does not seem to be relevant for the $\text{O}_2^{\bullet-}$ scavenging effect [29]. Also, none of the 3-hydroxy-2-SC showed any H_2O_2 scavenging activity, up to the highest tested concentration (1000 μM). These results indicate that an $-\text{OH}$ at C-3 on the C-ring is not favourable for the scavenging activity of H_2O_2 , when compared with the previously presented 2-SC (22–24), which showed activity [28]. All the tested 2-SC were able to scavenge HOCl . These compounds were even better scavengers than their structurally related previously presented 2-SC (22–24) [28], with much lower IC_{50} values. All the tested 2-SC were also able to scavenge $^1\text{O}_2$, and the IC_{50} values obtained were low when compared with the previously tested 2-SC (22–24) [28], leading to the conclusion that the presence of an $-\text{OH}$ at C-3 on the C-ring is also important for the $^1\text{O}_2$ scavenging activity. The results of the ROO^\bullet scavenging activity showed

that all the tested 2-SC were able to delay the ROO^\bullet -dependent oxidation of fluorescein. However, these compounds were less active than their analogous 2-SC (22–24) [28]. In the $\bullet\text{NO}$ scavenging assays, all the tested compounds were more potent than the positive control, quercetin ($\text{IC}_{50} = 1.3 \pm 0.1 \mu\text{M}$). These results, when compared to the previously presented 2-SC (22–24) [28], suggest that an $-\text{OH}$ at C-3 on the C-ring does not improve their $\bullet\text{NO}$ scavenging potential. The scavenging of ONOO^- was evaluated in the absence of NaHCO_3 , and the IC_{50} values of the tested 2-SC varied from 0.29 ± 0.02 (27) to $0.57 \pm 0.02 \mu\text{M}$ (25), being all more potent than the positive control, quercetin ($\text{IC}_{50} = 0.76 \pm 0.06 \mu\text{M}$). In the presence of NaHCO_3 , only 2-SC 26 and 27 were active (IC_{50} values of $1.3 \pm 0.3 \mu\text{M}$ and $0.98 \pm 0.09 \mu\text{M}$, respectively). Overall, the introduction of $-\text{OH}$ at C-3 on the C-ring considerably improved the ONOO^- scavenging potential, when compared to the previously tested 2-SC (22–24) [28, 29]. Generally, all the tested 2-SC showed to be potent scavengers of most of the evaluated reactive species, being more effective than the used positive control, quercetin. For the majority of the evaluated reactive species, the catechol on the B-ring appears to play an important role and the presence of $-\text{OH}$ at C-3 on the C-ring improves the scavenging activity. The authors, in addition to 2-SC, also tested some flavonoids structurally identical to the 2-SC tested and concluded that the styryl moiety also contributes to the observed outstanding antioxidant activity, for some reactive species (HOCl , $^1\text{O}_2$, and ONOO^-), since the 2-SC showed lower IC_{50} values than the flavonoids [29].

The $\text{O}_2^{\bullet-}$ scavenging activity of 2-SC 28–32 (Figure 9) was determined by the nitroblue tetrazolium chloride (NBT) photo reduction method [30]. All the tested compounds exhibited a good antioxidant activity, being more potent than the tested positive controls [butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), vitamin C, and vitamin E]. In this study, the results were presented as IC_{50} values but the associated error was not indicated, even though the assays were performed in triplicate. 2-SC 31 and 32 were the most potent (IC_{50} values of $234 \mu\text{M}$ and $243 \mu\text{M}$, respectively), followed by 28 and 30 (IC_{50} values of $268 \mu\text{M}$ and $275 \mu\text{M}$, respectively) and finally 29 ($\text{IC}_{50} = 287 \mu\text{M}$). The authors concluded that the same number of $-\text{OH}$ and $-\text{OCH}_3$ groups led to variations in activity due to their position in the 2-SC structure and the number and position of $-\text{OH}$ groups play a vital role in the antioxidant activity of 2-SC [30]. However, as the results were similar and were presented without indicating the associated error, it is difficult to draw solid conclusions about SAR as there is no guarantee about the magnitude of the difference between the results presented. In this study, the used methodology is significantly different from the one used in the studies mentioned above [26, 28, 29]. In the NBT photo reduction method, the $\text{O}_2^{\bullet-}$ generation was light induced by riboflavin, while in the other studies, $\text{O}_2^{\bullet-}$ was generated by the β -nicotinamide adenine dinucleotide (NADH)/phenazine methosulphate (PMS)/ O_2 system [26, 28–31]. Therefore, the results cannot be directly compared with those previously obtained [26, 28, 29].

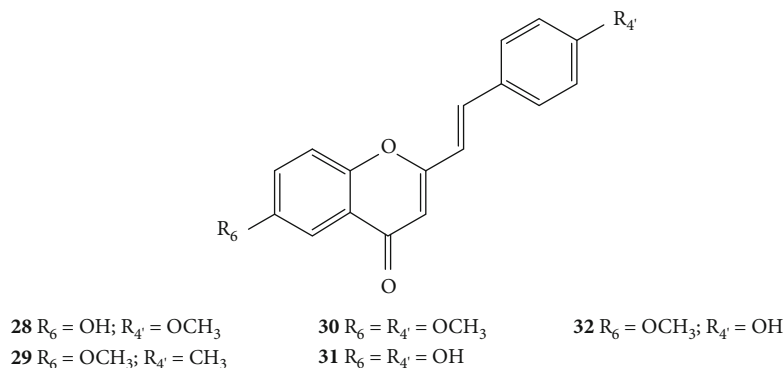


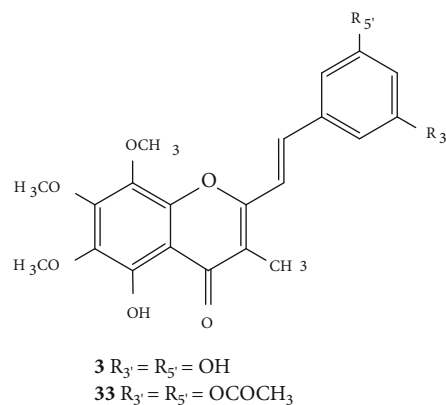
FIGURE 9: Chemical structures of 2-SC 28–32 [30].

The antioxidant activities of hormothamnione (**3**, Figure 10) and hormothamnione diacetate (**33**, Figure 10) were evaluated by Takamatsu and co-workers [32] by two antioxidant systems: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) solution-based chemical assay and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) cellular-based assay, in promyelocytic human leukaemia-60 (HL-60) cells. In this study, the results were presented as IC₅₀ values but the associated error was not indicated. The hormothamnione (**3**) only showed moderate antioxidant activity in the DPPH assay but did not show significant activity in the DCFH-DA assay. On the contrary, hormothamnione diacetate (**33**) showed no activity in DPPH assay but showed good activity in the DCFH-DA assay (IC₅₀ = 18.3 μM), which may indicate that it requires metabolic activation for antioxidant activity. The tested positive controls (vitamin E and vitamin C) showed strong activity in DPPH assay. In DCFH-DA assay, vitamin E and vitamin C exhibited IC₅₀ values of 255 μM and 9.7 μM, respectively. The differences in the antioxidant activity of 2-SC **3** and **33** may be due to the di-OCOCH₃ at C-3' and C-5' on the B-ring in 2-SC **33**, while 2-SC **3** presents –OH in the same positions [32].

The above-mentioned structural characteristics that demonstrated to favour the antioxidant activity of 2-SC are summarized in Figure 11.

4.1.2. 3-Styrylchromones. Hashem [13] showed the ability of the 3-SC **12** (Figure 12) to scavenge ¹O₂. This 3-SC was isolated from the aerial parts of *Erucaria microcarpa* and was well characterized in this article. Its antioxidant activity was determined by electron spin resonance. 3-SC **12** showed a positive reaction when mixed with ¹O₂, which was converted to oxygen, producing a free radical compound. This radical compound was rapidly combined with another radical compound to give a stable compound. This study suggests that the –OCH₃ groups and the conjugations of double bonds in the compound can possibly contribute to the ¹O₂ scavenging activity [13].

The antioxidant activity of a series of 3-SC derivatives (**34–50**, Figure 13) was evaluated by the DPPH free radical scavenging assay [33]. The synthesized compounds present several substituents such as halogen atoms (–F and –Cl), –OCH₃ and –OH groups in both A- and B-rings. In this

FIGURE 10: Chemical structures of 2-SC **3** and **33** [32].

study, the results were presented as the concentration of compounds that produce 50% of the maximum effect (EC₅₀) but the associated error and the number of assays done were not indicated. 3-SC **45**, **47**, and **50** were shown to be as or more potent than the tested positive control, ascorbic acid (EC₅₀ = 23 μM). In contrast, 3-SC **34–43** and **48** did not show DPPH[•] scavenging activity. These results indicate that the presence of an –OCH₃ group or a halo (–F or –Cl) substituent (**34–43** and **48**) on the B-ring did not favour the scavenging effect, while the existence of –OH groups is favourable. Indeed, 3-SC with –OH at C-3' and C-4' on the B-ring (**45** and **47**) were more potent than the 3-SC, with just an –OH at C-4' on the B-ring (**44** and **46**). This behaviour is similar to that previously observed for 2-SC: 2-SC with –OH at C-3' and C-4' on the B-ring (**17–20**) were more active than 2-SC with only one –OH at C-4' on the B-ring (**11**, **15**, **16**, and **21**) [26]. The introduction of an –OH at C-6 on the A-ring did not increase the scavenging activity (**34** versus **48**). In conclusion, this study corroborates the central role of –OH groups on the B-ring [33].

The above-mentioned structural characteristics that demonstrated to favour the antioxidant activity of 3-SC are summarized in Figure 14.

4.2. Antiallergic Activity. Allergy is one of the most common diseases and refers to abnormal adaptive immune response

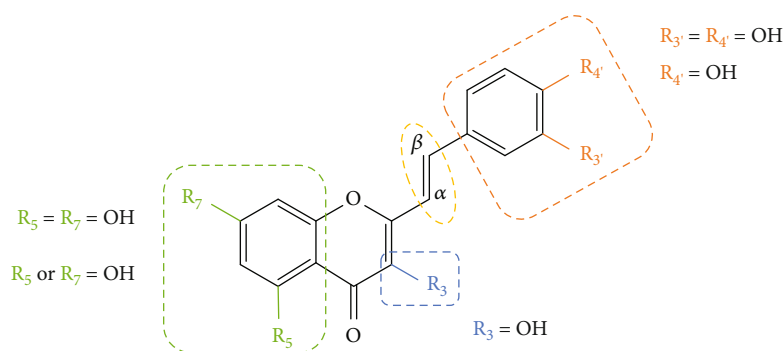
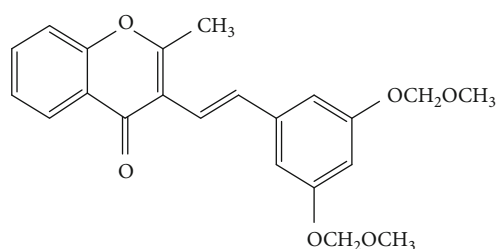


FIGURE 11: Structural characteristics that seem to favour the 2-SC antioxidant activity.



12

FIGURE 12: Chemical structure of 3-SC 12 [13].

against non-infectious environmental substances (allergens). This disease is characterized by the development of signs and symptoms of hypersensitivity reactions upon exposure to certain substances. Consequently, the production of allergen-specific immunoglobulin E and allergen-specific T-cell production occurs. In some cases, the allergic reaction can be extremely severe or even fatal, as in the case of anaphylactic reaction. The prevalence of allergy is increasing worldwide, which leads to the search for more effective therapeutic responses to this pathology [34]. Some 2-SC have shown potential as antiallergic agents and are below discussed, and all the observed antiallergic effects are summarized in Table 4. There are no studies on the antiallergic activity of 3-SC, to the best of our knowledge.

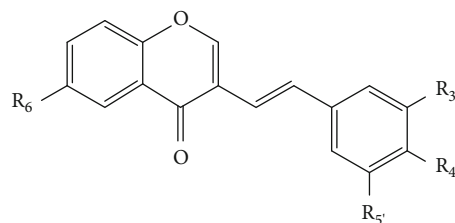
4.2.1. 2-Styrylchromones. The antiallergic activity of 2-SC has already been summarized in two previous reviews [4, 16]. In 1979, the antiallergic potential of some 2-SC, all substituted with $-\text{COOH}$ at C-6 on the A-ring, was evaluated (51–55, Table 4). The tested 2-SC displayed antiallergic activity, when orally administered to rats, in the passive cutaneous anaphylaxis test. This assay consisted in a sensitization test using rat serum rich in homocytotropic antibodies. These antibodies are responsible for triggering the release of pharmacological mediators of anaphylaxis. The antiallergic potential of 2-SC was also evaluated when the administration was parenteral, and also in this case, the studied 2-SC showed antiallergic effects in the passive cutaneous anaphylaxis test. These 2-SC were also able to inhibit histamine release from rat peritoneal cells passively sensitized with immunoglobulin E antibodies. The authors analysed the

SAR, and some conclusions were drawn: the smaller alkyl groups, such as ethyl (51) and *n*-propyl (52), at C-3 on the C-ring, increased the oral activity. The introduction of a single $-\text{CH}_3$ on the B-ring (53 and 55) increased the biological responses [4].

The above-mentioned structural characteristics that demonstrated to favour the antiallergic activity of 2-SC are summarized in Figure 15.

4.3. Antiviral Activity. Antivirals are a class of compounds that are used to treat viral infections and can be produced by living organism or obtained by chemical synthesis. An antiviral agent is capable to interfere in one or more steps of viral infection, arresting the viral replication cycle [35]. By attacking the virus's replication process, antivirals prevent the viral load from increasing to a point where it can cause pathogenesis, allowing the body's innate immune system to neutralize and eradicate the virus [35]. Viruses use the host's machinery to replicate and survive; thus, attacking the virus can also affect host cells causing deleterious effects, which can result in serious side effects to the host [35]. Since viral diseases are extremely widespread, the challenge is to find antivirals that interfere with viral replication without deleterious effects in the host cells. Other limitations of antivirals are related to the high mutation rate of virus, which favours the appearance of resistant viral strings. These questions make it essential to continually search and develop new antiviral compounds [35, 36]. Several 2-SC and 3-SC have shown potential as antiviral agents against human rhinovirus (HRV), murine norovirus (MNV), human immunodeficiency virus type 1 (HIV-1), and enterovirus (EV) and are below discussed. All the observed antiviral effects are summarized in Table 5, for 2-SC, and Table 6, for 3-SC.

4.3.1. 2-Styrylchromones. Desideri and co-workers [37] were the first to evaluate the antiviral potential of 2-SC, to the best of our knowledge. These authors tested the anti-rhinovirus activity of 2-SC 1, 21, and 56–61 (Figure 16) against two selected serotypes of HRV, 1B and 14. The HRV belongs to the picornavirus family, and is the most frequent cause of the common cold and has several serotypes, which makes it difficult to develop a vaccine, leading to continuous research in this field. In this work [37], serotypes 1B and 14 were selected as representative for viral groups B and A



34 $R_6 = R_{3'} = R_{5'} = H; R_{4'} = OCH_3$

35 $R_6 = R_{5'} = H; R_{3'} = R_{4'} = OCH_3$

36 $R_6 = H; R_{3'} = R_{4'} = R_{5'} = OCH_3$

37 $R_6 = R_{4'} = OCH_3; R_{3'} = R_{5'} = H$

38 $R_6 = R_{3'} = R_{4'} = OCH_3; R_{5'} = H$

39 $R_6 = R_{3'} = R_{4'} = R_{5'} = OCH_3$

40 $R_6 = R_{3'} = R_{5'} = H; R_{4'} = F$

41 $R_6 = R_{3'} = R_{5'} = H; R_{4'} = Cl$

42 $R_6 = OCH_3; R_{3'} = R_{5'} = H; R_{4'} = F$

43 $R_6 = OCH_3; R_{3'} = R_{5'} = H; R_{4'} = Cl$

44 $R_6 = R_{3'} = R_{5'} = H; R_{4'} = OH$

45 $R_6 = R_{5'} = H; R_{3'} = R_{4'} = OH$

46 $R_6 = OCH_3; R_{3'} = R_{5'} = H; R_{4'} = OH$

47 $R_6 = OCH_3; R_{3'} = R_{4'} = OH; R_{5'} = H$

48 $R_6 = OH; R_{3'} = R_{5'} = H; R_{4'} = OCH_3$

49 $R_6 = R_{4'} = OH; R_{3'} = R_{5'} = H$

50 $R_6 = R_{3'} = R_{4'} = OH; R_{5'} = H$

FIGURE 13: Chemical structures of 3-SC 34–50 [33].

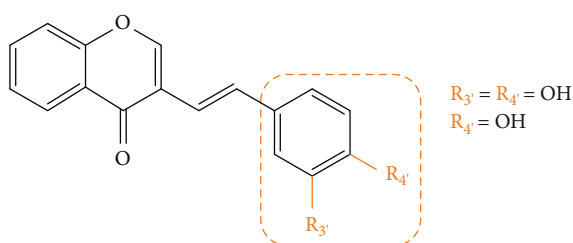


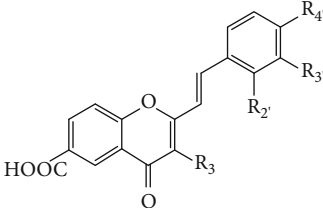
FIGURE 14: Structural characteristics that seem to favour the 3-SC antioxidant activity.

of HRVs, respectively. Antiviral activity was evaluated by a plaque reduction assay in cervical epithelioid carcinoma (HeLa) cell cultures infected with HRVs 1B and 14. First, the cytotoxicity of the compounds under study was evaluated, *in vitro*, by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) colorimetric assay, to determine the maximum non-toxic concentration (MNTC). In this study, the error associated with the results was not indicated, even though the assays were performed in triplicate. 2-SC 58 was the most toxic, since its MNTC was the lowest (3.12 μM). The evaluation of the antiviral activity was done from the maximum concentration that did not affect cell viability and growth and was expressed as IC_{50} (concentration of compound that reduces the plaque number by 50%). All the tested 2-SC showed antiviral effect against HRV 1B, but the same did not happen against HRV 14, since 2-SC 58 and 60 were inactive. Despite this difference, some 2-SC had better activity against HRV 14 than against serotype 1B (21, 56, 59, and 61). 2-SC 59 was the most potent against both serotypes (IC_{50} values of 3.89 μM and 1.33 μM for HRVs 1B and 14, respectively). The known *in vitro* potent inhibitor of group B HRV replication, 4',6-dichloroflavan, was used as positive control ($IC_{50} = 0.025 \mu\text{M}$). No known inhibitor was used as a control for HRV 14. For both serotypes, some compounds (21, 56, and 59–61), in addition to an effect on the plaque number, they also produced a reduction (about 50%) in viral plaque size. This fact suggested that these 2-SC caused a slowdown in the kinetics of viral replication. Analysing the structures of the tested compounds, the most active 2-SC (59) that dis-

played antiviral activity against both serotypes presents a single substituent, a $-NO_2$ at C-4' on the B-ring. Compared to 2-SC 59 (IC_{50} values of 3.89 μM and 1.33 μM against HRVs 1B and 14, respectively), the presence of an $-OH$ at C-4' on the B-ring (21, IC_{50} values of 9.29 μM and 6.41 μM against HRVs 1B and 14, respectively) or a $-NH_2$ at C-4' on the B-ring (61, IC_{50} values of 15.06 μM and 13.46 μM against HRVs 1B and 14, respectively) decreased the cytotoxicity, but also the antiviral effect against both serotypes; in the latter case, the decrease of the antiviral activity is more accentuated. The existence of $-Cl$ at C-4' on the B-ring (56) leads to antiviral activity against serotype 14 but not against 1B, while the addition of a second $-Cl$ at C-6 on the A-ring (60) increased the antiviral effect against HRV 1B but not against HRV 14, 2-SC 60 was inactive. The presence of a $-CH_3$ or an $-OCH_3$ at C-4' on the B-ring (57 and 58, respectively) resulted in a modest or no effect against HRVs 1B and 14 [37].

After this first approach, Desideri and co-workers [38] synthesized a new group of structurally related 2-SC (62–77, Figure 17) with $-OH$ or $-OCH_3$ at C-3 on the C-ring and studied their anti-rhinovirus activity against HRVs 1B and 14. The used methodology was the same with that of the previous study [37]. Also, in this study, the error associated with the results was not indicated, even though the assays were performed in triplicate. In this study [38], 2-SC 64, 69, 75, and 77 were the most toxic, since their MNTC was the lowest (3.12 μM). These new 2-SC were generally more toxic than those previously synthesized [37], since the majority presented MNTC values of 3.12 μM and 6.25 μM . All the tested 2-SC showed antiviral effect against HRV 1B, but the same did not happen against HRV 14, since two 2-SC were inactive (68 and 74). Despite this difference, some 2-SC had better activity against HRV 14 than against serotype 1B (63, 65, 69, 71, and 77). 2-SC 69 was the most potent against both serotypes (IC_{50} values of 0.94 μM and 0.73 μM against HRVs 1B and 14, respectively). 2-SC 70 also demonstrated high potency against both serotypes (IC_{50} values of 1.10 μM and 1.60 μM against HRVs 1B and 14, respectively). In this study, 4',6-dichloroflavan was used as a positive control ($IC_{50} = 0.025 \mu\text{M}$), and again, no known

TABLE 4: 2-SC studied for the antiallergic activity, their chemical structures, and summary of the observed antiallergic effects.

Chemical structure	2-SC	Observed effects	Ref.
	51-55	Antiallergic activity in the passive cutaneous anaphylaxis test in rats; and inhibition of histamine release from passively sensitized rat peritoneal cells	[4]
51 $R_3 = C_2H_5$; $R_2' = R_3' = R_4' = H$ 52 $R_3 = n-C_3H_7$; $R_2' = R_3' = R_4' = H$ 53 $R_3 = C_3H_7$; $R_2' = CH_3$; $R_3' = R_4' = H$ 54 $R_3 = C_3H_7$; $R_2' = R_4' = H$; $R_3' = CH_3$ 55 $R_3 = C_3H_7$; $R_2' = R_3' = H$; $R_4' = CH_3$			

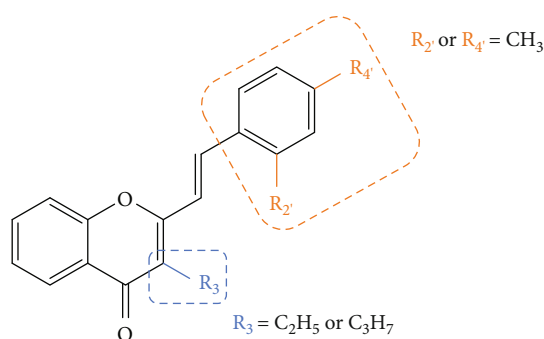


FIGURE 15: Structural characteristics that seem to favour the 2-SC antiallergic activity.

inhibitor was used as a control for HRV 14. For both serotypes, some compounds (**62**, **64**, **66**, **69**, **71**, and **74**, for HRV 1B, and **77** for HRV 14) in addition to an effect on plaque number also produced a reduction (about 50%) in viral plaque size. 2-SC **69** and **77** were the most potent 3-hydroxy- and 3-methoxy-2-SC, respectively (**69**, IC_{50} values of $0.94 \mu M$ and $0.73 \mu M$ for HRVs 1B and 14, respectively, and **77**, IC_{50} values of $3.00 \mu M$ and $0.96 \mu M$ for HRVs 1B and 14, respectively). Analysing the structure of the tested 2-SC for antiviral activity against HRV 1B, 2-SC **69** was the most active and presents an $-OH$ at C-3 on the C-ring, a $-Cl$ at C-6 on the A-ring, and a $-NO_2$ at C-4' on the B-ring. 2-SC **70**, which also showed high antiviral activity, has only one $-OCH_3$ at C-3 on the C-ring. For the antiviral activity against HRV 14, 2-SC **69** and **77** were the most active and both have $-Cl$ at C-6 on the A-ring and $-NO_2$ at C-4' on the B-ring, the difference being at C-3 on the C-ring, since **69** has an $-OH$ and **77** has an $-OCH_3$. For 2-SC **62** and **70**, the opposite happens, since **70**, with $-OCH_3$ at C-3 on the C-ring, was more active than **62**, with $-OH$ at C-3 on the C-ring. Thus, the influence of $-OH$ or $-OCH_3$ at C-3 on the C-ring in the anti-rhinovirus activity varies according to the substituents present in the structure and its location [38]. The comparison between these results and those previously obtained by the same authors [37] allows a more in-dept analysis of the SAR. 2-SC **59** was con-

sidered the most active 2-SC in the previous study [37], however the IC_{50} values found were higher than the ones found for 2-SC **69**, the most active in the present study. When comparing the structure of 2-SC **59** with the one of 2-SC **69**, the first lacks the $-Cl$ at C-6 on the A-ring and the $-OH$ at C-3 on the C-ring. This may mean that these substituents at these positions may be essential for a higher anti-rhinovirus activity. 2-SC with $-NO_2$ at C-4' on the B-ring, without substituents on the A-ring, but with $-OH$ or $-OCH_3$ at C-3 on the C-ring (**65** and **73**, respectively) were less potent than 2-SC **59**. Only these 2-SC showed a decrease in the antiviral effect with the introduction of $-OH$ or $-OCH_3$ at C-3 on the C-ring, since in general, the introduction of these groups enhanced the antiviral activity against both serotypes [37, 38].

These authors also evaluated the anti-rhinovirus activity against HRVs 1B and 14, resulting from the introduction of a $-F$ at C-6 on the A-ring in hydroxylated or methoxylated 2-SC (**78–80**, Figure 18) [39]. The methodology used to assess the antiviral activity was the same used in the previous studies by these authors [37, 38]. Also, in this study, only a positive control for HRV 1B (4'-dichloroflavan) was tested and the error associated with the results was not indicated [39]. 2-SC **78–80** were less toxic than those tested in the previous studies [37–39]. 2-SC **78** has only one $-F$ at C-6 on the A-ring as a substituent and showed a weak potency against both serotypes [47% and 13% of inhibition against HRV 1B and 14, respectively, up to the highest tested concentration ($12.5 \mu M$)] [39]. 2-SC **79** and **80** interfered with HRV 14 replication (IC_{50} values of $9.09 \mu M$ and $12.03 \mu M$, respectively), causing a reduction in viral plaque size (50 to 70%), and were inactive against serotype 1B. This effect suggests that these 2-SC may have a neutralizing action on the progeny virus and/or lead to a slowing down in the kinetics of viral replication. The results indicate that the introduction of $-OH$ (**79**) or $-OCH_3$ (**80**) at C-3 on the C-ring, when a $-F$ atom is present at C-6 on the A-ring, enhanced the activity against serotype 14 and led to the loss of efficacy against serotype 1B [39]. Analysing the previous study [37] and comparing the obtained results with the present ones, it may be concluded that the introduction of only a $-F$ at C-

TABLE 5: 2-SC studied for the antiviral activity and summary of the observed antiviral effects. The table displays the antiviral activity shown for the studied 2-SC according to the criterion: activity greater or equal to 30%.

2-SC	Observed effect(s)	Ref.
1	Anti-rhinovirus activity against HRV 1B in HeLa cells; and anti-norovirus activity against MNV in RAW cells	[37, 40]
5	Anti-norovirus activity against MNV in RAW cells	[40]
11	Anti-HIV-1 integrase activity	[12]
21	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[37]
56	Anti-rhinovirus activity against HRV 14 in HeLa cells	[37]
57, 58	Anti-rhinovirus activity against HRV 1B in HeLa cells; and anti-norovirus activity against MNV in RAW cells	[37, 40]
59	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[37]
60	Anti-rhinovirus activity against HRV 1B in HeLa cells	[37]
61	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[37]
62, 63	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[37]
64	Anti-rhinovirus activity against HRV 1B in HeLa cells	[37]
65, 66	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[38]
67, 68	Anti-rhinovirus activity against HRV 1B in HeLa cells	[38]
69	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[38]
70	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells; and anti-norovirus activity against MNV in RAW cells	[38, 40]
71	Anti-rhinovirus activity against HRV 14 in HeLa cells	[38]
72	Anti-rhinovirus activity against HRV 1B in HeLa cells	[38]
73	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[38]
74	Anti-rhinovirus activity against HRV 1B in HeLa cells	[38]
76	Anti-rhinovirus activity against HRV 1B in HeLa cells	[38]
77	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[38]
78	Anti-rhinovirus activity against HRV 1B in HeLa cells	[39]
79, 80	Anti-rhinovirus activity against HRV 14 in HeLa cells	[39]
81 - 83	Anti-norovirus activity against MNV in RAW cells	[40]

HeLa: cervical epithelioid carcinoma cells; HIV-1: human immunodeficiency virus type 1; HRV: human rhinovirus; MNV: murine norovirus; RAW cells: murine macrophage cell line RAW 264.7.

TABLE 6: 3-SC studied for the antiviral activity and summary of the observed antiviral effects. The table displays the antiviral activity shown for the studied 3-SC according to the criterion: activity greater or equal to 30%.

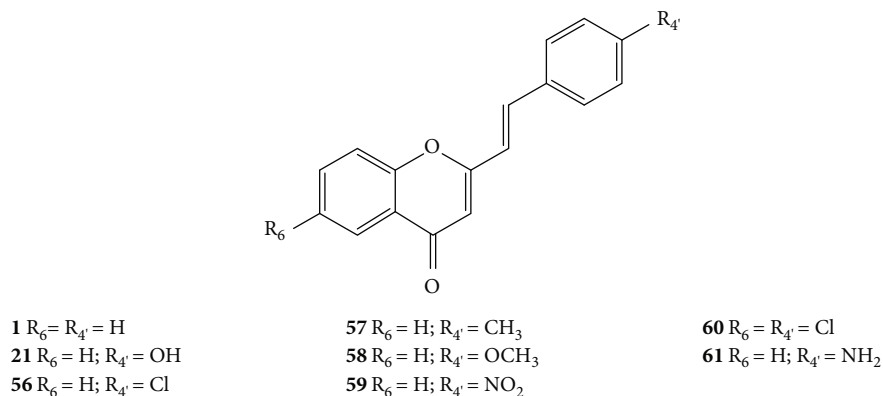
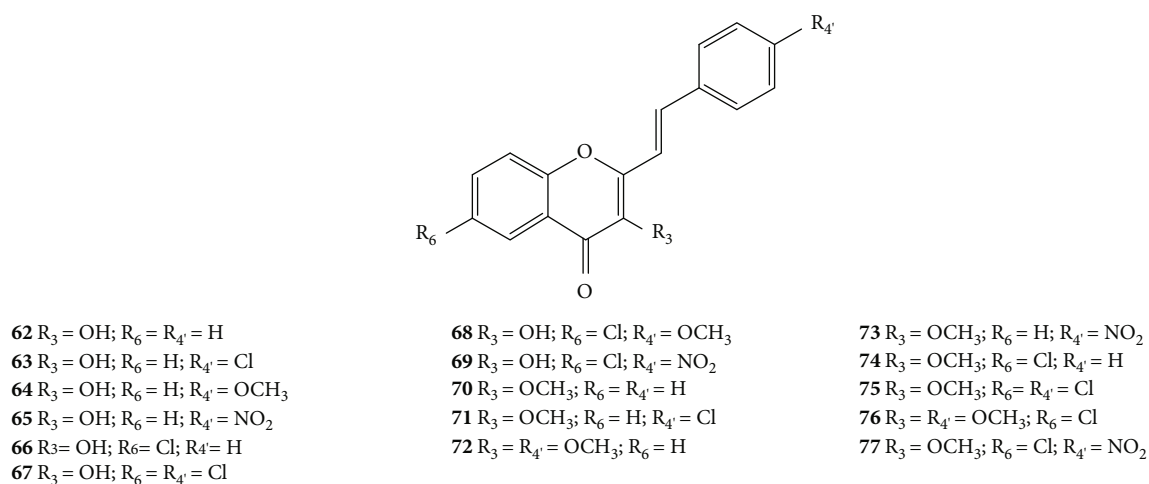
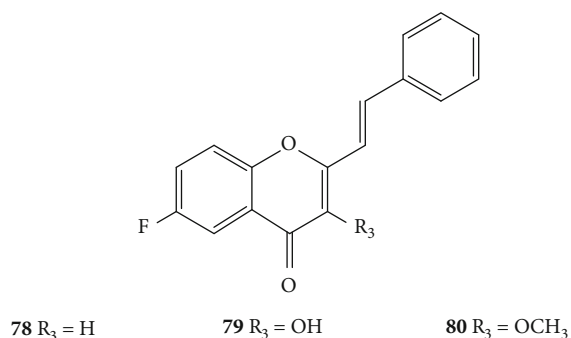
3-SC	Observed effect(s)	Ref.
2	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells; and anti-enterovirus activity against EV 71 in HEp-2 cells	[44]
41	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells	[44]
84	Anti-rhinovirus activity against HRV 1B in HeLa cells; and anti-enterovirus activity against EV 71 in HEp-2 cells	[44]
85	Anti-rhinovirus activity against HRVs 1B and 14 in HeLa cells; and anti-enterovirus activity against EV 71 in HEp-2 cells	[44]

EV: enterovirus; HeLa: cervical epithelioid carcinoma cells; HEp-2: human epithelial type 2 cells; HRV: human rhinovirus.

6 on the A-ring (**78**) decreased the activity against HRV 1B, when compared with the unsubstituted 2-SC **1** that was able to interfere with HRV 1B replication [39]. In conclusion, these new 2-SC (**78–80**) were not highly potent anti-rhinovirus agents and showed a preferential effect against HRV 14, contrary to the effects showed for 2-SC previously reported [37, 38].

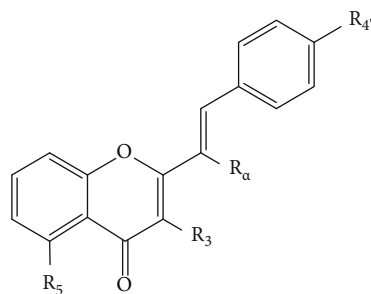
Rocha-Pereira and co-workers [40] evaluated the potential anti-norovirus activity of 2-SC (**1**, **5**, **21**, **56–58**, **62**, **70**, and **81–83**, Figure 19) using MNV in murine macrophage cell line RAW 264.7 (RAW cells), by a plaque reduction

assay. Human noroviruses (NoV) are the most frequent cause of outbreaks and sporadic cases of acute gastroenteritis and affect people of all ages [40, 41]. MNV was used in this study since it is genetically related to non-cultivable NoV and is able to replicate in RAW cells, being considered the best surrogate model for NoV [40]. First, the cytotoxicity of the compounds under study was evaluated, *in vitro*, by a colorimetric assay, to determine the concentration of compounds that cause 50% cytotoxicity (CC_{50}) and MNTC. Most of the tested 2-SC showed little toxicity, presenting a relatively large margin of security, at the highest

FIGURE 16: Chemical structures of 2-SC **1**, **21**, and **56–61** [37].FIGURE 17: Chemical structures of 2-SC **62–77** [38].FIGURE 18: Chemical structures of 2-SC **78–80** [39].

concentration tested ($150 \mu M$). However, 2-SC **62**, **70**, and **83** showed a different toxicity profile in RAW cells, with **62** and **83** being the most toxic (CC_{50} values of $52.1 \pm 10.6 \mu M$ and $46.7 \pm 8.7 \mu M$, respectively). Antiviral activity was evaluated by a plaque reduction assay in RAW cells. The results were presented as IC_{50} values and the selectivity index (SI) was also presented. 2-SC **5** and **58** were the most active (IC_{50} values of $7.0 \pm 0.7 \mu M$ and $7.4 \pm 1.3 \mu M$, respectively) and presented high SI values (>21.6 and >13.5 , respectively). 2-SC **1**, **57**, **70**, and **82** were less active but also

demonstrated antiviral effects, and **21**, **56**, and **62** showed a weak anti-norovirus activity. In this study, the possibility of 2-SC **5** and **58** interfering in an early or late stage of MNV replication cycle was also evaluated. The antiviral effects of compounds decreased when they were added during the first hour of viral infection and remained when they were added after viral infection and for a continuous period of 48 h. Therefore, it was concluded that 2-SC **5** and **58** interfere more in the late stages of the virus life cycle that follow the enhancement of virus in cells. The obtained results showed that the type of substituent present at C-4' on the B-ring influenced the potency of the anti-norovirus activity. As an example, this can be observed comparing the 4'-unsubstituted 2-SC **1** with 2-SC **21** (4'-OH substituted), **56** (4'-Cl substituted), **57** (4'-CH₃ substituted), and **58** (4'-OCH₃ substituted). The presence of -Cl or -OH at C-4' on the B-ring led to a loss of the antiviral effect of 2-SC **56** or **21**, respectively. 2-SC **57**, with -CH₃ at C-4' on the B-ring, showed some antiviral activity but was approximately three times less potent than 2-SC **58**, with -OCH₃ at C-4' on the B-ring. Thus, -OCH₃ at C-4' on the B-ring showed to be the best substituent, when there is no other substituent present in the 2-SC structure. In what concerns the C-5



1 $R_3 = R_5 = R_\alpha = R_4 = H$

5 $R_3 = R_\alpha = R_4 = H; R_5 = OH$

21 $R_3 = R_5 = R_\alpha = H; R_4 = OH$

56 $R_3 = R_5 = R_\alpha = H; R_4 = Cl$

57 $R_3 = R_5 = R_\alpha = H; R_4 = CH_3$

58 $R_3 = R_5 = R_\alpha = H; R_4 = OCH_3$

62 $R_3 = OH; R_5 = R_\alpha = R_4 = H$

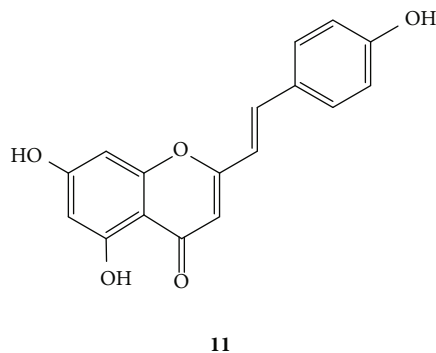
70 $R_3 = OCH_3; R_5 = R_\alpha = R_4 = H$

81 $R_3 = R_\alpha = R_4 = H; R_5 = OBn$

82 $R_3 = R_5 = R_4 = H; R_\alpha = CH_3$

83 $R_3 = OH; R_5 = R_4 = H; R_\alpha = CH_3$

FIGURE 19: Chemical structures of 2-SC **1**, **5**, **21**, **56–58**, **62**, **70**, and **81–83** [40].



11

FIGURE 20: Chemical structure of 2-SC **11** [12].

substitutions on the A-ring, the presence of $-OBn$ in 2-SC **81** led to a loss of antiviral effect, while the presence of $-OH$ in 2-SC **5** led to one of the most active compounds. The existence of the $-CH_3$ at C- α of the double bond of the styryl moiety led 2-SC **82** to show antiviral effect. The presence of the $-OCH_3$ at C-3 on the C-ring appears to confer some antinorovirus activity to 2-SC **70** [40]. Some of the tested 2-SC in this study (**1**, **21**, **56–58**, **62**, and **70**) were also previously evaluated for their anti-rhinovirus activity [37, 38, 40]. 2-SC **1**, **21**, **56–58**, **62**, and **70** showed anti-norovirus and anti-rhinovirus activities (against both serotypes, 1B and 14), while 2-SC **58** was only effective against HRV 1B [37, 38, 40]. 2-SC **70** presented only a modest anti-norovirus effect but was considered as one of the most potent against both serotypes of rhinovirus [38, 40]. 2-SC **58**, one the most active compounds for anti-norovirus activity, was inactive for HRV 14 and had little effect against HRV 1B [37, 40]. These results demonstrate a different interaction of the 2-SC with norovirus and rhinovirus and some selectivity, since the antiviral effects shown by the same 2-SC are very different for the two viruses, HRV and MNV [37, 38, 40].

Chaniad and co-workers [12] isolated the 2-SC **11** (Figure 20) from the ethanolic extract of the bulbils of *Dioscorea bulbifera* and evaluated its inhibitory activity against the HIV-1 integrase through the multiplate integration assay

[12]. HIV-1 is a human retrovirus of the lentivirus family that infects humans and results in the acquired immune deficiency syndrome (AIDS) [42]. HIV-1 integrase is one of the essential enzymes used for the replication of HIV, since it is responsible for the integration of viral DNA into the host cells [12, 42]. So, this enzyme is an attractive target for the development of novel anti-HIV drugs [12]. The anti-HIV-1 integrase activity of 2-SC **11** was evaluated and compared with the positive control, suramin ($IC_{50} = 2.3 \mu M$). 2-SC **11** was not much effective against the HIV-1 integrase, reaching only $47.89 \pm 1.54\%$ inhibition, at the highest tested concentration ($100 \mu M$) [12].

The above-mentioned structural characteristics that demonstrated to favour the antiviral activity of 2-SC against HRV and MNV are summarized in Figure 21.

4.3.2. 3-Styrylchromones. Shimada and co-workers [43] synthesized fifteen 3-SC (**34–39** and **42–50**, Figure 22) and studied their cytotoxicity and anti-HIV activity. For the anti-HIV assay, a human T-cell lymphotropic virus-I (HTLV-I) carrying human T-cell line MT4 that was infected with HIV-1_{IIIIB} was used [43]. HTLV-I is a human retrovirus of the oncornaviruses family [42]. First, the cytotoxicity of the 3-SC under study was evaluated by the MTT assay and the CC_{50} was determined. In this study, the error associated with the results was not indicated, even though the assays were performed in triplicate. According to their cytotoxicity, 3-SC can be divided into two groups: those with high to moderate toxicity (**37**, **39**, **44–47**, **49**, and **50**) and those with low toxicity (**34–36**, **38**, **42**, **43**, and **48**). For the anti-HIV assay, the results were expressed as SI. The SI was calculated by dividing the CC_{50} by the 50% cytoprotective concentration from HIV infection (EC_{50}). All the tested 3-SC showed SI values of <1 , meaning that none of the tested 2-SC protected the cells from the HIV infection. Four positive controls were used: dextran sulphate, curdlan sulphate, azidothymidine, and 2',3'-dideoxycytidine, with SI values between 2445 and 20421 [43].

Conti and Desideri [44] also evaluated the potential antiviral activity of four 3-SC (**2**, **41**, **84**, and **85**, Figure 23)

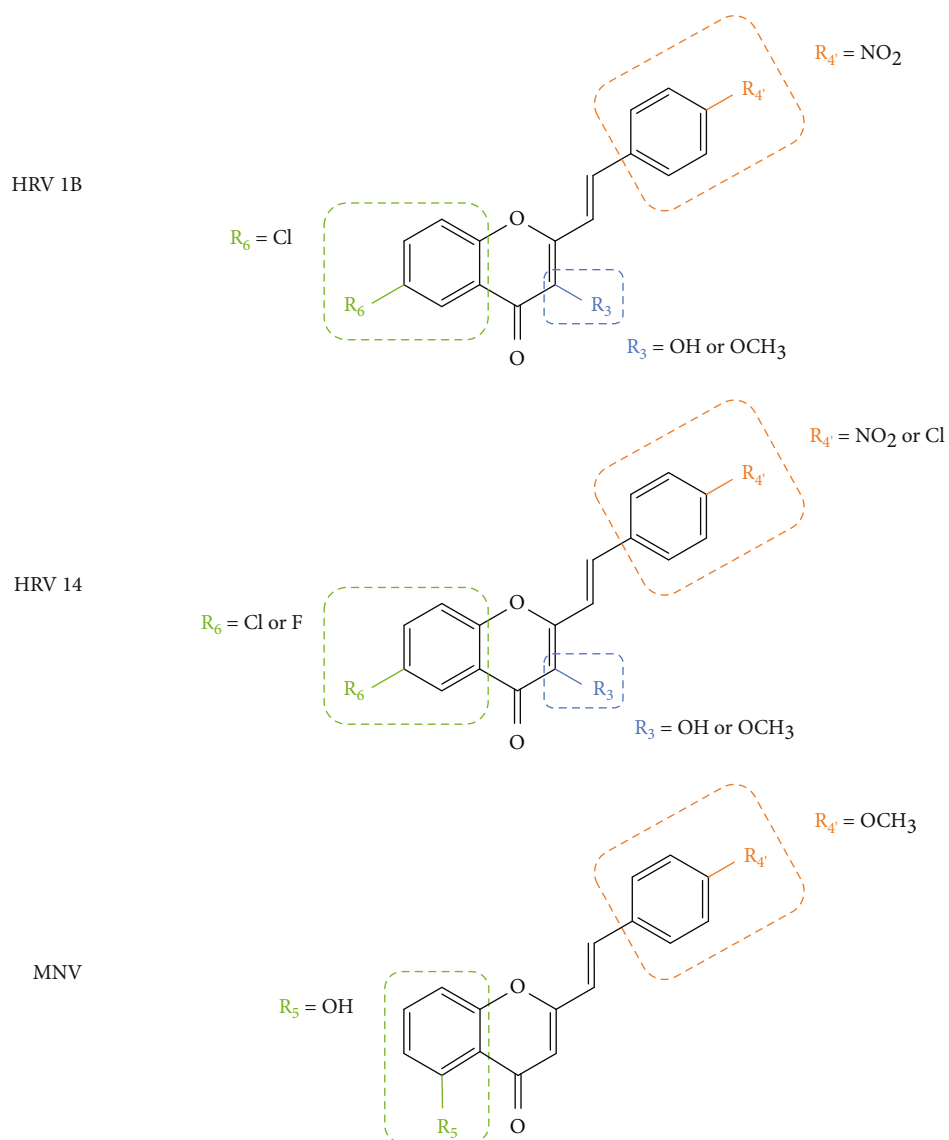
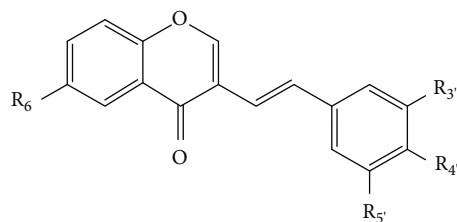


FIGURE 21: Structural characteristics that seem to favour the 2-SC antiviral activity against serotypes 1B and 14 of human rhinovirus (HRV) and murine norovirus (MNV).



34 $R_6 = R_3' = R_5' = \text{H}; R_4' = \text{OCH}_3$
 35 $R_6 = R_5' = \text{H}; R_3' = R_4' = \text{OCH}_3$
 36 $R_6 = \text{H}; R_3' = R_4' = R_5' = \text{OCH}_3$
 37 $R_6 = R_4' = \text{OCH}_3; R_3' = R_5' = \text{H}$
 38 $R_6 = R_3' = R_4' = \text{OCH}_3; R_5' = \text{H}$

39 $R_6 = R_3' = R_4' = R_5' = \text{OCH}_3$
 42 $R_6 = \text{OCH}_3; R_3' = R_5' = \text{H}; R_4' = \text{F}$
 43 $R_6 = \text{OCH}_3; R_3' = R_5' = \text{H}; R_4' = \text{Cl}$
 44 $R_6 = R_3' = R_5' = \text{H}; R_4' = \text{OH}$
 45 $R_6 = R_5' = \text{H}; R_3' = R_4' = \text{OH}$

46 $R_6 = \text{OCH}_3; R_3' = R_5' = \text{H}; R_4' = \text{OH}$
 47 $R_6 = \text{OCH}_3; R_3' = R_4' = \text{OH}; R_5' = \text{H}$
 48 $R_6 = \text{OH}; R_3' = R_5' = \text{H}; R_4' = \text{OCH}_3$
 49 $R_6 = R_4' = \text{OH}; R_3' = R_5' = \text{H}$
 50 $R_6 = R_3' = R_4' = \text{OH}; R_5' = \text{H}$

FIGURE 22: Chemical structures of 3-SC 34–39 and 42–50 [43].

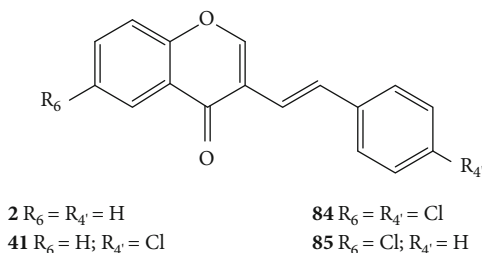


FIGURE 23: Chemical structures of 3-SC **2**, **41**, **84**, and **85** [44].

against HRVs 1B and 14 and EV 71. The EV belongs to the family of picornavirus and its infection can constitute a serious health threat in children under young children [35]. Antiviral activity was evaluated by a plaque reduction assay in HeLa cell culture infected with HRV types 1B and 14 and in human epithelial type 2 (HEp-2) cell cultures infected with EV 71. First, the cytotoxicity of the 3-SC under study was evaluated by XTT assay, to determine the MNTC. The error associated with the results was not indicated, even though the assays were performed in triplicate. 4',6-Dichloroflavan was included as a positive control for serotype 1B ($IC_{50} = 0.026 \mu M$), but no positive control was included for HRV 14 and EV 71. The tested compounds presented low cytotoxicity against HeLa and HEp-2 cells, and all the tested 3-SC were able to interfere with the replication of both HRV serotypes and EV 71, but the sensibility of each virus was considerably different. For HRV, the tested 3-SC exhibited a higher potency against the serotype 1B than against 14. 3-SC **84** was the most potent against HRV 1B ($IC_{50} = 2.19 \mu M$), while 3-SC **2** and **41** were the most potent against HRV 14 (IC_{50} values of $4.36 \mu M$ and $5.52 \mu M$, respectively). The results seem to indicate that the presence of a -Cl at C-6 on the A-ring (**85**) or -Cl at C-4' on the B-ring (**41**) slightly decreases the antiviral activity of these 3-SC against both HRV serotypes, when compared with unsubstituted 3-SC (**2**). When comparing the activity of 3-SC **84**, with -Cl at C-6 on the A-ring and C-4' on the B-ring, with 3-SC **2**, it was observed that the presence of these substituents enhanced the antiviral activity of 3-SC against HRV 1B but decreased the antiviral effect against serotype 14. For the antiviral activity against EV 71, the tested 3-SC showed low activities and only reached 21.6–37.0% of inhibition, at the highest tested concentration ($25 \mu M$ for **41**, **84**, and **85**, and $50 \mu M$ for **2**) [44].

The above-mentioned structural characteristics that demonstrated to favour the antiviral activity of 3-SC against HRV 1B are summarized in Figure 24.

4.4. Antibacterial Activity. Antibacteria are a class of compounds that are used to treat bacterial infections. The antibacterial activity is associated with the compounds that kill bacteria or slow down their rate of growth, without being extensively toxic to nearby tissues. The wide use as well as the abuse of antibacterial agents has led to the development of multidrug-resistant bacteria. The emergence of drug-resistant bacteria has become the major cause of failure in

the treatment of infectious diseases, since most bacteria are resistant to a minimum of one of the antibiotics that are generally used to eliminate the infection. The inefficacy of currently available antibiotics urges the searching for new types of antibacterial agents against the drug-resistant bacteria [45]. Several 2-SC have shown potential as antibacterial agents against *Enterococcus faecium*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. sciuri*, *S. xylosus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Xanthomonas campestris*, and *Agrobacterium tumefaciens* and are below discussed. All the observed antibacterial effects are summarized in Table 7. There are no studies on the antibacterial activity of 3-SC, to the best of our knowledge.

4.4.1. 2-Styrylchromones. Momin and co-workers [46] synthesized ten 2-SC (**58**, **78**, and **86–93**, Figure 25) and evaluated their antibacterial activity by the disc diffusion method against the Gram-positive bacteria *E. faecium* (ATCC 51299), *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 29212 and ATCC 43300), *S. sciuri* (ATCC 29062), and *S. xylosus* (ATCC 35033) and the Gram-negative bacteria *E. coli* (ATCC 25922 and ATCC 35218), *P. aeruginosa* (ATCC 35032), and *K. pneumoniae* (ATCC 700603). In this study, we used two standard antibiotics as positive controls: tetracycline and ampicillin. In general, the 2-SC were more effective against the Gram-positive bacteria and less potent than the standard antibiotics used. The exception was 2-SC **93** which showed no antibacterial activity against any of the studied bacterial organisms. The other 2-SC showed to be effective against *B. subtilis*. Only 2-SC **78** showed antibacterial effect against *E. faecium* [diameter of inhibition zone (DIZ) = 10 ± 0.06 mm]. In what concerns *S. aureus*, two strains were studied: ATCC 29212 and ATCC 43300. 2-SC **78** and **86–89** were effective against both strains for ATCC 43300 strain; however, 2-SC **92** was only effective against the ATCC 29212 strain (DIZ = 9 ± 0.12 mm). 2-SC **78** were effective against *S. sciuri* and *S. xylosus* (DIZ values of 8 ± 0.06 mm and 10 ± 0.06 mm, respectively), while 2-SC **90** was only effective against *S. sciuri* (DIZ = 12 ± 0.06 mm). In what concerns the Gram-negative bacteria, the studied 2-SC did not show any antibacterial activity against *P. aeruginosa* and *K. pneumoniae*. 2-SC **86–90** showed activity against *E. coli* strain ATCC 25922, and additionally, 2-SC **89** and **90** also showed activity against *E. coli* strain ATCC 35218. Analysing the structures of the tested 2-SC, in general, the 2-SC with -F on the A- and/or B-rings (**78** and **86–91**) were effective against a large number of studied bacterial organisms, which seems to indicate that -F plays an important role for the antibacterial activity. 2-SC **78**, with only one -F at C-6 on the A-ring, showed an inhibitory effect against all the studied Gram-positive bacteria but did not show any activity against any of the Gram-negative bacteria. 2-SC **90**, with -F at C-7 on the A-ring and C-4' on the B-ring, showed antibacterial activity only against two Gram-positive bacteria (*B. subtilis* and *S. sciuri*) but was more effective than 2-SC **78** against these specific strains. Additionally, **90** also showed an inhibitory effect against both strains of *E. coli* (Gram-negative bacteria). 2-SC **89**, with -F at C-3' and

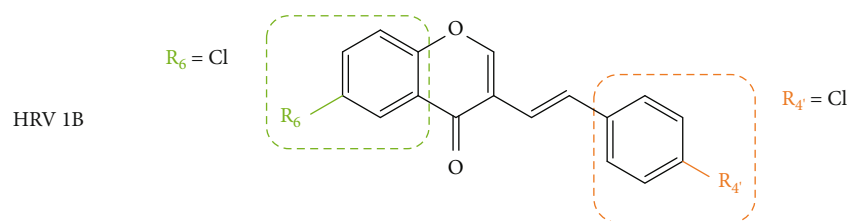
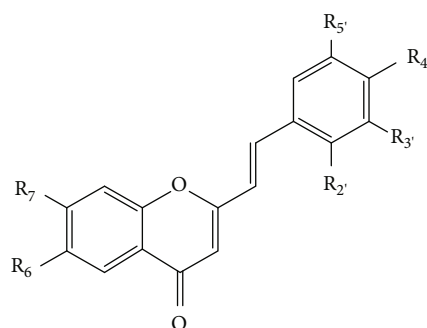


FIGURE 24: Structural characteristics that seem to favour the 3-SC antiviral activity against serotype 1B of human rhinovirus (HRV).

TABLE 7: 2-SC studied for the antibacterial activity and summary of the observed antibacterial effects.

2-SC	Observed effect(s)	Ref.
1		[48]
13		[48]
28–32	Antibacterial activity against <i>X. campestris</i> and <i>A. tumefaciens</i>	[30]
56		[48]
58	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633), <i>X. campestris</i> and <i>A. tumefaciens</i>	[46, 48]
78	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633), <i>E. faecium</i> (ATCC 51299), <i>S. aureus</i> (ATCC 29212 and ATCC 43300), <i>S. sciuri</i> (ATCC 29062) and <i>S. xylosus</i> (ATCC 35033)	
86–88	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633), <i>S. aureus</i> (ATCC 29212 and ATCC 43300) and <i>E. coli</i> (ATCC 25922)	
89	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633), <i>S. aureus</i> (ATCC 29212 and ATCC 43300) and <i>E. coli</i> (ATCC 25922 and ATCC 35218)	[46]
90	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633), <i>S. sciuri</i> (ATCC 29062) and <i>E. coli</i> (ATCC 25922 and ATCC 35218)	
91	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633)	
92	Antibacterial activity against <i>B. subtilis</i> (ATCC 6633) and <i>S. aureus</i> (ATCC 29212)	
94–101	Antibacterial activity against <i>B. subtilis</i> (NCIM-2063), <i>S. aureus</i> (NCIM-2901), <i>E. coli</i> (NCIM-2256) and <i>P. aeruginosa</i> (NCIM-2036)	[47]
102	Antibacterial activity against <i>X. campestris</i> and <i>A. tumefaciens</i>	[48]



58 R₆ = R₇ = R_{2'} = R_{3'} = R_{5'} = H; R_{4'} = OCH₃

78 R₆ = F; R₇ = R_{2'} = R_{3'} = R_{4'} = R_{5'} = H

86 R₆ = R₇ = R_{3'} = R_{4'} = R_{5'} = H; R_{2'} = F

87 R₆ = R₇ = R_{2'} = R_{4'} = R_{5'} = H; R_{3'} = F

88 R₆ = R₇ = R_{2'} = R_{3'} = R_{5'} = H; R_{4'} = F

89 R₆ = R₇ = R_{2'} = R_{4'} = H; R_{3'} = R_{5'} = F

90 R₆ = R_{2'} = R_{3'} = R_{5'} = H; R₇ = R_{4'} = F

91 R₆ = R_{2'} = R_{3'} = R_{4'} = R_{5'} = H; R₇ = F

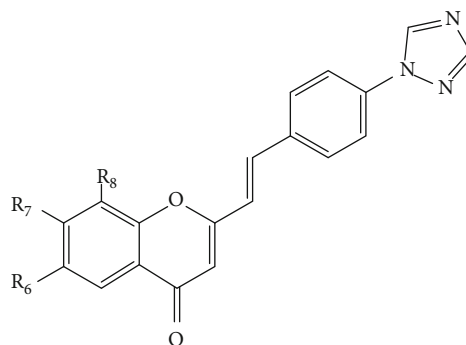
92 R₆ = R₇ = R_{2'} = R_{5'} = H; R_{3'} = R_{4'} = OCH₃

93 R₆ = R₇ = R_{2'} = R_{5'} = H; R_{3'} = R_{4'} = -OCH₂O-

FIGURE 25: Chemical structures of 2-SC 58, 78, and 86–93 [46].

C-5' on the B-ring, also exhibited inhibitory effect against both strains of *E. Coli* and against *B. subtilis* and both strains of *S. aureus*. The presence of only one -F at C-2' (86) or at

C-3' (87) or at C-4' (88) on the B-ring led these 2-SC to be effective against *B. subtilis* and both strains of *S. aureus* and only one strain of *E. coli* (ATCC 25922). The obtained



94 $R_6 = R_7 = R_8 = H$

95 $R_6 = CH_3; R_7 = R_8 = H$

96 $R_6 = R_8 = CH_3; R_7 = H$

97 $R_6 = Cl; R_7 = CH_3; R_8 = H$

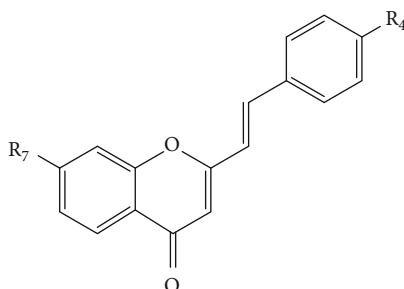
98 $R_6 = F; R_7 = R_8 = H$

99 $R_6 = Cl; R_7 = R_8 = H$

100 $R_6 = R_8 = Cl; R_7 = H$

101 $R_6 = Br; R_7 = R_8 = H$

FIGURE 26: Chemical structures of 2-SC **94–101** [47].



1 $R_7 = R_4' = H$

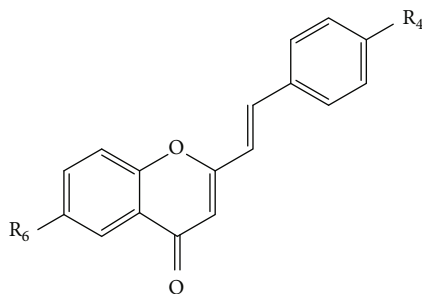
13 $R_7 = OH; R_4' = H$

56 $R_7 = H; R_4' = Cl$

58 $R_7 = H; R_4' = OCH_3$

102 $R_7 = OH; R_4' = OCH_3$

FIGURE 27: Chemical structures of 2-SC **1, 13, 56, 58, and 102** [48].



28 $R_6 = OH; R_4' = OCH_3$

29 $R_6 = OCH_3; R_4' = CH_3$

30 $R_6 = R_4' = OCH_3$

31 $R_6 = R_4' = OH$

32 $R_6 = OCH_3; R_4' = OH$

FIGURE 28: Chemical structures of 2-SC **28–32** [30].

results in this study seem to indicate that the position and number of $-F$ substituents in the A- or B-rings influence the antibacterial activity of 2-SC. 2-SC **58**, **92**, and **93** have no $-F$ substituents in their structure. 2-SC **58**, with $-OCH_3$ at C-4' on the B-ring, was only active against *B. subtilis*. The presence of $-OCH_3$ at C-3' and C-4' on the B-ring (**92**) led to an inhibitory effect against *B. subtilis* and one strain of *S. aureus* (ATCC 29212). The 2-SC **93** is a methylenedioxy derivative and displayed no antibacterial activity,

which apparently indicates that this group does not favour this activity [46].

Nikam and co-workers [47] synthesized a novel series of 2-SC with 1,2,4-triazole ring at C-4' on the B-ring (**94–101**, Figure 26) and evaluated their antibacterial activity by the agar dilution method against the Gram-positive bacteria *B. subtilis* (NCIM-2063) and *S. aureus* (NCIM-2901) and the Gram-negative bacteria *E. coli* (NCIM-2256) and *P. aeruginosa* (NCIM-2036). In this study, the results were presented

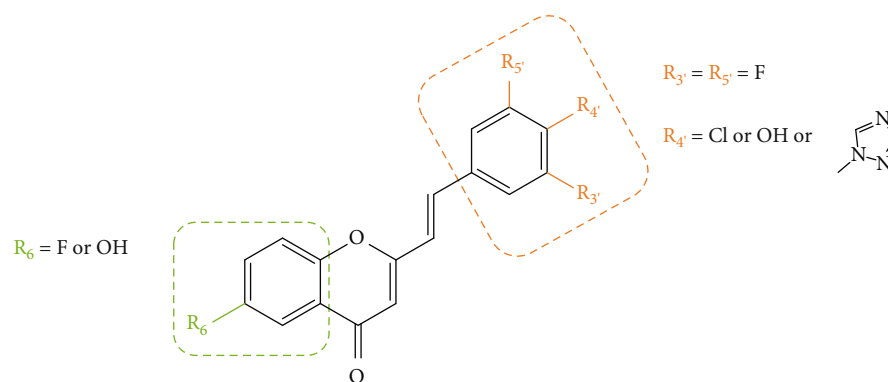


FIGURE 29: Structural characteristics that seem to favour the 2-SC antibacterial activity.

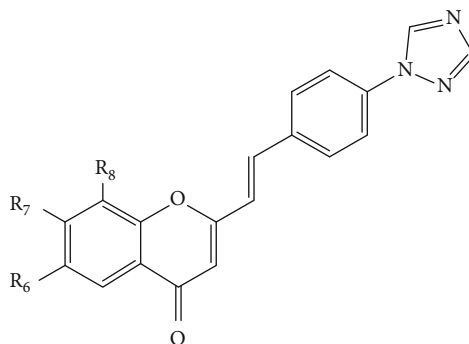
as the minimum inhibitory concentration (MIC) but the associated error was not indicated, even though the assays were performed in triplicate. Taking this in consideration, 2-SC **96** was the most potent (MIC = 25 $\mu\text{g}/\text{mL}$) against *S. aureus*, being more potent than the positive control, ciprofloxacin (MIC = 50 $\mu\text{g}/\text{mL}$). 2-SC **94**, **96**, and **99** were the most potent against *B. subtilis* (MIC = 50 $\mu\text{g}/\text{mL}$), being equipotent with ciprofloxacin. In what concerns Gram-negative bacteria, 2-SC **94** was the most potent against *E. coli* (MIC = 25 $\mu\text{g}/\text{mL}$), being as powerful as ciprofloxacin, and against *P. aeruginosa* (MIC = 50 $\mu\text{g}/\text{mL}$). The positive control, ciprofloxacin, showed a MIC value of 25 $\mu\text{g}/\text{mL}$. Analysing the substitution pattern of the studied 2-SC, **94**, only with 1,2,4-triazole ring at C-4' on the B-ring as a substituent, showed an excellent antibacterial activity against all the bacterial panel tested, being the most potent for the majority of them, with exception of *S. aureus*. The introduction of a $-\text{CH}_3$ at C-6 on the A-ring of 2-SC **94** resulted in **95** and led to a significant loss of antibacterial activity, which was recovered with the addition of a second $-\text{CH}_3$ at C-8 on the A-ring (**96**). The presence of a single $-\text{Cl}$ at C-6 on the A-ring in 2-SC **99** made it equipotent with a ciprofloxacin against *B. subtilis* but decreased its antibacterial potential against the other tested bacterial strains, when compared to 2-SC **94**. The addition of a second $-\text{Cl}$ at C-8 on the A-ring to 2-SC **99**, giving **100**, improved the antibacterial effect against *S. aureus* and *E. coli* but decreased the potential against *B. subtilis* and *P. aeruginosa*. The introduction of a $-\text{F}$ or a $-\text{Br}$ at C-6 on the A-ring (**98** and **101**, respectively) led to a weaker antibacterial activity, when compared to 2-SC **94**. The tested 2-SC were also evaluated for their absorption, distribution, metabolism, excretion, and toxicity (ADMET). All synthesized 2-SC showed to obey the Lipinski's rule of five and Veber rule, which indicates good drug-like properties. It has also been suggested that 2-SC have a good oral bioavailability, due the values obtained for the total polar surface area (TPSA) (<100), logarithm of partition coefficient of compound between *n*-octanol and water ($\log P < 5$), and molecular weight (<500). The number of rotatable (<25) and rigid (<10) bonds, the number of hydrogen bond acceptors (<10) and donors (<5) indicated that these 2-SC have good intestinal bioavailability [47].

Ujwala and co-workers [48] evaluated the antibacterial activity of five 2-SC (**1**, **13**, **56**, **58**, and **102**, Figure 27) by the agar cup method against *Xanthomonas campestris* and *Agrobacterium tumefaciens*. In this study, the results were expressed as DIZ values but the authors did not indicate the associated error. The results of DIZ were presented for each of the volumes of the compounds used, 50 μL and 100 μL , with no concentration of solutions being indicated. Thus, two values of DIZ will always be indicated, which correspond in the same order to 50 μL and 100 μL . Streptomycin was used as a positive control, and only a volume of the compound (10 μL) was tested, thus only one DIZ value was reported. All the tested 2-SC exhibited some antibacterial effect against the tested bacteria. The order of potencies that can be established, based on the reported values, against *X. campestris* is **56** > **58** > **102** > **13** > **1** (DIZ values of 4.2 and 11.2 mm; 4.0 and 10.8 mm; 4.1 and 9.8 mm; 3.6 and 9.5 mm; and 1.2 and 4.2 mm, respectively). Streptomycin showed a DIZ value of 15 mm. The order of potencies that may be suggested against *A. tumefaciens* is the same: **56** > **58** > **102** > **13** > **1**, with DIZ values of 3.9 and 9.6 mm; 3.8 and 8.9 mm; 3.7 and 8.6 mm; 3.4 and 7.8 mm; and 2.2 and 5.3 mm, respectively. For streptomycin, the DIZ value was 12 mm against *A. tumefaciens*. In what concerns the structure of the tested 2-SC, the presence of a $-\text{Cl}$ at C-4' on the B-ring in 2-SC **56** appears to increase the antibacterial potential against both tested bacteria, compared with unsubstituted 2-SC **1**. The 2-SC **58**, with $-\text{OCH}_3$ at C-4' on the B-ring, and **102**, with $-\text{OCH}_3$ at C-4' and $-\text{OH}$ at C-7 on the B- and A-rings, also had a greater antibacterial activity than the unsubstituted 2-SC **1** and the 2-SC **13**, with only one $-\text{OH}$ at C-7 on the A-ring. In conclusion, the 2-SC with $-\text{Cl}$ or $-\text{OCH}_3$ at C-4' on the B-ring resulted in a better antibacterial activity against *X. campestris* and *A. tumefaciens* [48].

The same authors also evaluated the antibacterial activity of a series of 2-SC derivatives with $-\text{OH}$, $-\text{CH}_3$, and $-\text{OCH}_3$ at C-6 on the A-ring and/or C-4' on the B-ring (**28–32**, Figure 28) by the same previous method against *X. campestris* and *A. tumefaciens* [30]. In this study, the results were expressed as DIZ values but the authors did not indicate the associated error. The results of DIZ were presented for each of the volumes of the compounds used, 50 μL and

TABLE 8: 2-SC studied for the antifungal activity and summary of the observed antifungal effects.

2-SC	Observed effect(s)	Ref.
1		[48]
13		[48]
28 - 32	Antifungal activity against <i>A. niger</i> and <i>P. chrysogenum</i>	[30]
56		[48]
58		[48]
94 - 101	Antifungal activity against <i>C. albicans</i> , <i>A. niger</i> and <i>A. flavus</i>	[47]
102	Antifungal activity against <i>A. niger</i> and <i>P. chrysogenum</i>	[48]



94 $R_6 = R_7 = R_8 = H$

95 $R_6 = CH_3$; $R_7 = R_8 = H$

96 $R_6 = R_8 = CH_3$; $R_7 = H$

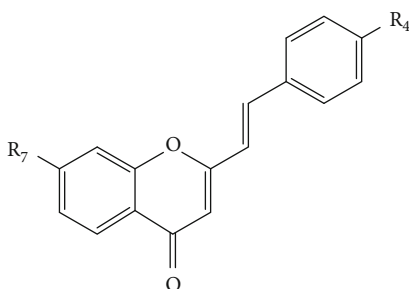
97 $R_6 = Cl$; $R_7 = CH_3$; $R_8 = H$

98 $R_6 = F$; $R_7 = R_8 = H$

99 $R_6 = Cl$; $R_7 = R_8 = H$

100 $R_6 = R_8 = Cl$; $R_7 = H$

101 $R_6 = Br$; $R_7 = R_8 = H$

FIGURE 30: Chemical structures of 2-SC **94–101** [47].

1 $R_7 = R_4' = H$

13 $R_7 = OH$; $R_4' = H$

56 $R_7 = H$; $R_4' = Cl$

58 $R_7 = H$; $R_4' = OCH_3$

102 $R_7 = OH$; $R_4' = OCH_3$

FIGURE 31: Chemical structures of 2-SC **1, 13, 56, 58, and 102** [48].

100 μ L, with no concentration of solutions being indicated. Thus, two values of DIZ will always be indicated, which correspond in the same order to 50 μ L and 100 μ L. Streptomycin was used as a positive control and only a volume of the compound (10 μ L) was tested, thus only one DIZ value was reported. All the tested 2-SC exhibited some antibacterial effect against the tested bacteria, and 2-SC **31** was the most active one (DIZ values of 5.7 and 11.3 mm against *X. campestris* and 5.2 and 11.0 mm against *A. tumefaciens*). Streptomycin showed a DIZ value of 15 and 12 mm against *X. campestris* and *A. tumefaciens*, respectively. In what concerns the structure of the tested 2-SC, the presence of $-OH$ at C-6 on the A-ring and C-4' on the B-ring appears

to favour the antibacterial potential against both tested bacteria, while the presence of $-OCH_3$ at C-6 on the A-ring and C-4' on the B-ring appears to decrease the activity (**30**) [30].

The existing works on the antibacterial activity of 2-SC include studies with different strains of Gram-positive and Gram-negative bacteria. The antibacterial activity demonstrated by 2-SC showed some variability, and it is not possible to define a clear SAR for each strain, resulting in the establishment of a generic SAR for the antibacterial activity. Thus, the above-mentioned structural characteristics that demonstrated to favour the antibacterial activity of 2-SC are summarized in Figure 29.

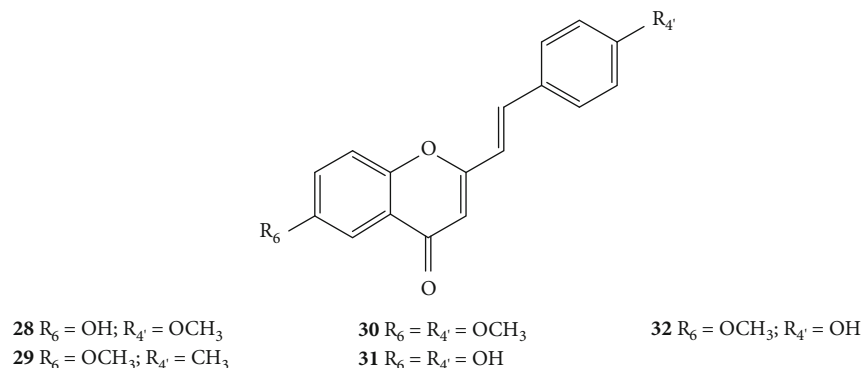


FIGURE 32: Chemical structures of 2-SC 28–32 [30].

4.5. Antifungal Activity. Antifungal agents are used to treat fungal infections, in which the most common types are cutaneous, and ringworm infections of the skin and nails [49, 50]. Another type of common fungal infections includes mucosal infections of the oral and genital tracts. Fungal infections can be superficial or invasive, the latter having a much lower incidence but are of great concern [49]. Invasive fungal infections have a higher incidence in immunocompromised individuals, as their immune system is weakened. The body's immune system plays an essential role and has effective mechanisms for the prevention fungal infections [49, 50]. Fungi are omnipresent and a generally benign part of our environment, for healthy individuals. Antifungal agents work by arresting or killing pathogenic fungal cells in preference to normal cells [50]. The development of successful antifungal agents becomes urgent, especially with the emergence of new fungal pathogens and knowing that fungal infections substantially contribute to human mortality [49, 50]. Several 2-SC have shown potential as antifungal agents against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* and are below discussed. All the observed antifungal effects are summarized in Table 8. There are no studies on the antifungal activity of 3-SC, to the best of our knowledge.

4.5.1. 2-Styrylchromones. Nikam and co-workers [47] evaluated the antifungal potential of 2-SC 94–101 (Figure 30) against *C. albicans* (NCIM-3417), *A. niger* (NCIM-1196), and *A. flavus* (NCIM-539) by the agar dilution method. In this study, the results were presented as MIC values but the associated error was not indicated, even though the assays were performed in triplicate. 2-SC 94 and 96 were the most potent (MIC = 25 $\mu\text{g}/\text{mL}$) against *C. albicans*, being two times more potent than positive control, fluconazole (MIC = 50 $\mu\text{g}/\text{mL}$). For the *A. niger*, 2-SC 94 and 96–98 were the most potent (MIC = 25 $\mu\text{g}/\text{mL}$) and were equipotent with fluconazole. Against *A. flavus*, 2-SC 94, 96, and 98 were the most potent (MIC = 25 $\mu\text{g}/\text{mL}$), being equipotent with fluconazole. Analysing the substitution pattern, all the tested 2-SC have a 1,2,4-triazole ring at C-4' on the B-ring. Interestingly, 2-SC 94, with only a 1,2,4-triazole ring at C-4' on the B-ring as a substituent, showed an excellent antifungal activity against all the tested fungal strains. The

presence of only one $-\text{CH}_3$ at C-6 on the A-ring (95) led to a decrease in antifungal activity, when compared to 2-SC 94 and 96. The presence of a $-\text{Cl}$ or $-\text{Br}$ at C-6 on the A-ring (99 and 101, respectively) also led to a decrease in antifungal potential compared to 2-SC 94. The existence of a $-\text{Cl}$ at C-6 and C-8 on the A-ring (100) also did not favour antifungal activity. The introduction of $-\text{F}$ at C-6 on the A-ring (98) maintained the antifungal activity against *A. niger* and *A. flavus* but decreased the potential against *C. albicans*, when compared to 2-SC 94. The studied 2-SC were also evaluated for their ADMET, as already mentioned in Section 4.4.1. In order to understand the possible mechanism of antifungal activity, docking studies were performed to rationalize the 2-SC binding interaction at the active site of fungal enzyme cytochrome P450 lanosterol 14 α -demethylase. The 1,2,4-triazole ring at C-4' on the B-ring was mainly responsible for the interaction in the active site. Overall, a good binding interaction was shown between 2-SC 94–101 and the active site of fungal enzyme cytochrome P450 lanosterol 14 α -demethylase [47].

Ujwala and co-workers [48] evaluated the antifungal activity of 2-SC 1, 13, 56, 58, and 102 (Figure 31) by the disc diffusion method against *A. niger* and *P. chrysogenum*. The results were expressed as DIZ values, but the authors did not indicate the associated error. The results of DIZ were presented for each of the volumes of the compounds used, 50 μL and 100 μL , with no concentration of solutions being indicated. Thus, two values of DIZ will always be indicated, which correspond in the same order to 50 μL and 100 μL . Nystatin was used as a positive control and only a volume of the compound (10 μL) was tested, thus only one DIZ value was reported. All tested 2-SC exhibited antifungal activity. 2-SC 56 was the most active against both tested fungal strains (DIZ values of 7.5 and 13.4 mm against *A. niger* and 6.9 and 12.2 mm against *P. chrysogenum*); nystatin presented DIZ values of 19 and 13 mm against *A. niger* and *P. chrysogenum*, respectively. 2-SC 56 has a $-\text{Cl}$ at C-4' on the B-ring, suggesting that this feature contributes to the increase of antifungal activity, when compared to the unsubstituted 2-SC 1. The 2-SC 58, with $-\text{OCH}_3$ at C-4' on the B-ring, and 2-SC 102, with $-\text{OCH}_3$ at C-4' on the B-ring and $-\text{OH}$ at C-7 on the A-ring, also had a greater antifungal activity. 2-SC 13, with only one $-\text{OH}$ at C-7 on the A-ring,

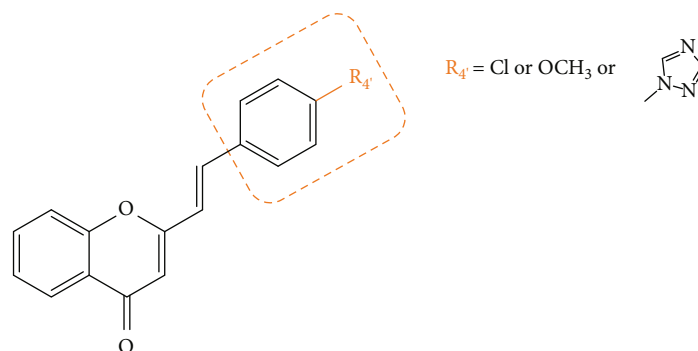


FIGURE 33: Structural characteristics that seem to favour the 2-SC antifungal activity.

TABLE 9: 2-SC studied for the anti-inflammatory activity and summary of the observed anti-inflammatory effects.

2-SC	Observed effect(s)	Ref.
1	Inhibition of LTB ₄ production in human neutrophils	
5		[54]
11	Inhibition of LTB ₄ production in human neutrophils and COX-1 activity	
15	Inhibition of LTB ₄ production in human neutrophils, NF-κB activation and production of TNF-α and IL-6; and induction of the production of IL-1β and IL-8	[54, 59]
16	Inhibition of LTB ₄ production in human neutrophils; and reducing activity	[54]
17	Inhibition of LTB ₄ production in human neutrophils; metal chelating capacity; reducing activity; inhibition of NF-κB activation and production of TNF-α, IL-1β, IL-6 and IL-8	[54, 59]
18, 19	Inhibition of LTB ₄ production in human neutrophils and COX-1 activity; metal chelating capacity; and reducing activity	[54]
20	Inhibition of LTB ₄ production in human neutrophils; metal chelating capacity; and reducing activity	
21	Inhibition of LTB ₄ production in human neutrophils, NF-κB activation and production of TNF-α, IL-1β and IL-6	[54, 59]
33	Inhibition of cell-cell adhesion between HL-60 cells and CHO-ICAM-1 cells	[52]

CHO: Chinese hamster ovary; COX-1: cyclooxygenase-1; HL-60: promyelocytic human leukaemia-60; ICAM-1: intercellular cell adhesion molecule-1; IL: interleukin; LT: leukotriene; NF-κB: nuclear factor kappa B; TNF-α: tumor necrosis factor α.

showed antifungal activity lower than those mentioned above (**56**, **58**, and **102**) but higher than 2-SC **1**. Thus, 2-SC with -Cl or -OCH₃ at C-4' on the B-ring had better antifungal activity, suggesting that these groups may be responsible for the greater effects [48].

The same authors evaluated the antifungal activity of 2-SC **28–32** (Figure 32), with -OH, -CH₃, and -OCH₃ at C-6 and/or C-4' on the A- and B-rings, against *A. niger* and *P. chrysogenum*, by the disc diffusion method [30]. The results were expressed as DIZ values, but the authors did not indicate the associated error. The results of DIZ were presented for each of the volumes of the compounds used, 50 μL and 100 μL, with no concentration of solutions being indicated. Thus, two values of DIZ will always be indicated, which correspond in the same order to 50 μL and 100 μL. Nystatin was used as a positive control and only a volume of the compound (10 μL) was tested, thus only one DIZ value was reported. 2-SC **30** was the most active against both tested fungal strains (DIZ values of 6.7 and 11.9 mm against *A. niger* and 6.3 and 11.7 mm against *P. chrysogenum*); nystatin presented DIZ values of 19 and 13 mm against *A. niger* and *P. chrysogenum*, respectively. These results indicate that the

presence of -OCH₃ simultaneously at C-6 on the A-ring and C-4' on the B-ring appears to favour the antifungal potential. 2-SC **28**, with -OH at C-6 on the A-ring and an -OCH₃ at C-4' on the B-ring, also showed antifungal potential, as well as 2-SC **32**, with -OCH₃ at C-6 on the A-ring and an -OH at C-4' on the B-ring. 2-SC **31** has -OH simultaneously at C-6 on the A-ring and C-4' on the B-ring and showed a decrease in the antifungal effects when compared to 2-SC **30** and **28**. 2-SC **29** has an -OCH₃ at C-6 on the A-ring, while in C-4' on the B-ring, it has an -CH₃ group. This structure showed a greater decrease in antifungal effects compared to 2-SC **30**, which may indicate that the -CH₃ group may not favour the antifungal activity. Thus, the -OCH₃ group can be identified as contributing for the antifungal activity of the 2-SC but its effects vary according to their position and the additional substituents present in the 2-SC scaffold [30].

The studies on the antifungal activity of 2-SC included different substitution patterns of 2-SC and diverse methodologies used in each study, which difficulties the establishment of a specific SAR for each fungus. Thus, it was only possible to establish a generic SAR for antifungal activity. The above-

mentioned structural characteristics that demonstrated to favour the antifungal activity of 2-SC are summarized in Figure 33.

4.6. Anti-Inflammatory Activity. Inflammation is the body's first response to processes such as infection, tissue injury, cell death, and degeneration. The inflammatory process involves the innate and the adaptive immune responses. The adaptive immune system involves the activity of more specialized cells such as B and T cells, while the innate immune system involves the activity of various cells. During inflammatory responses, numerous inflammatory mediators are synthesized and secreted and they are usually divided into pro- and anti-inflammatory mediators. The search for compounds that are able to regulate these mediators and interfere with the inflammation mechanisms is still ongoing [51]. Several 2-SC have shown potential as anti-inflammatory agents through several mechanisms that are discussed below, such as the inhibition of lymphocyte function-associated molecule-1/intercellular cell adhesion molecule-1 (LFA-1/ICAM-1)-mediated cell adhesion, interference with the arachidonic acid metabolic pathways, inhibition of nuclear factor kappa B (NF- κ B) activation, and modulation of cytokines/chemokine production. All the observed anti-inflammatory effects are summarized in Table 9. The anti-inflammatory activity of SC has already been reviewed [3, 4]; the most recent review article was published in 2017. To the best of our knowledge, since this year, no article has been published describing the anti-inflammatory activity of SC. Thus, in this work, this activity will be presented and discussed in more detail than in the two previously referred reviews [3, 4]. There are no studies on the anti-inflammatory activity of 3-SC, to the best of our knowledge.

4.6.1. 2-Styrylchromones. Takamatsu and co-workers [52] evaluated the profile of hormothamnione (**3**, Figure 34) and hormothamnione diacetate (**33**, Figure 34) as inhibitors of the adhesion of HL-60 cells to Chinese hamster ovary (CHO)-ICAM-1 cells through LFA-1/ICAM-1 interaction. LFA-1 is a leukocyte integrin adhesion molecule which promotes intercellular adhesion of most leukocytes to each other and to other cells, playing a crucial role in immunological and inflammatory reactions [52, 53]. ICAM-1 is a glycosylated integral membrane protein, that is expressed by most tissues at low levels. Its expression readily increases by the action of inflammatory cytokines. ICAM-1 plays an important role in inflammation and in T-cell-mediated host defence system. LFA-1 typically binds to ICAM-1, and this interaction mediates a cell adhesion pathway that is important in the progression of inflammatory response [52, 53]. In this study, the expression of LFA-1 occurred in HL-60 cells, while CHO-ICAM-1 cells express high levels of ICAM-1. The authors only indicated the associated error for some results. Thus, the results presented here are in accordance with the information provided in the original article. First, a primary screening was carried out to the selection of candidates of cell adhesion inhibitors, among the compounds selected for the study, using cell aggregation

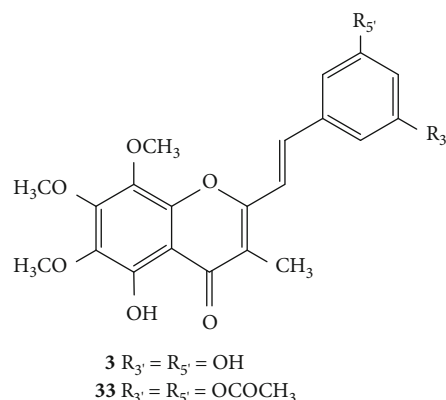
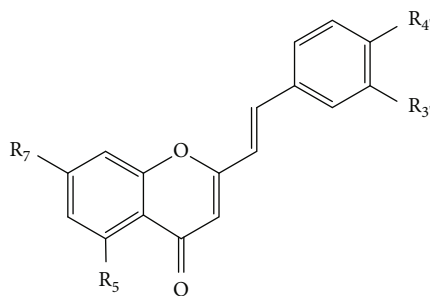


FIGURE 34: Chemical structures of 2-SC **3** and **33** [52].

assay and XTT method. After this screening, the cytotoxicity of the selected compounds to HL-60 cells was evaluated by XTT assay, whereas to CHO-ICAM-1 cells, the MTT assay was used. 2-SC **3** and **33** did not affect the cell proliferation of HL-60 cells but did affect the proliferation of CHO-ICAM-1 cells. 2-SC **3** and **33** inhibited cell aggregation of HL-60 cells (MIC values of 3.1 and 2.6 μM , respectively). 2-SC **3** did not significantly inhibit the cell adhesion of HL-60 to CHO-ICAM-1, while 2-SC **33** potently inhibited this adhesion ($\text{IC}_{50} = 1.5 \pm 0.2 \mu\text{M}$). Cytochalasin B was used as positive control and showed to inhibit cell adhesion of HL-60 to CHO-ICAM-1 cells with an IC_{50} value of $1.2 \pm 0.2 \mu\text{M}$. The difference between the results obtained for 2-SC **3** and **33**, regarding the inhibition of cell-cell adhesion, may be attributed to the $-\text{OCOCH}_3$ present at C-3' and C-5' on the B-ring of 2-SC **33**, whereas 2-SC **3** has $-\text{OH}$ at the same positions. Thus, as 2-SC **33** inhibited cell-cell adhesion between HL-60 cells and CHO-ICAM-1 cell, it may prove to be beneficial in the treatment of inflammation [52].

Gomes and co-workers [54] evaluated the anti-inflammatory potential of 2-SC **1**, **5**, **11**, and **13–21** (Figure 35) by studying their interference with the arachidonic acid metabolic pathways. The authors assessed the ability of these 2-SC to inhibit cyclooxygenase (COX) -1 and -2 (ovine and human recombinant origin, respectively) and leukotriene (LT) B_4 production (in human neutrophils). The metal chelating activity and reducing power of these compounds were also evaluated [54]. Arachidonic acid is a polyunsaturated fatty acid and an integral constituent of biological cell membranes, conferring their fluidity and flexibility [55]. Arachidonic acid is metabolized by COXs and 5-lipoxygenase, resulting in the synthesis of prostanoids and leukotrienes, respectively. COX-1 and COX-2 are isoforms of the COXs. COX-1 is a constitutive enzyme expressed in all tissues that induces an acute inflammation in response to an inflammatory stimuli, while COX-2 is an inducible isoform, usually absent in most tissues and that is upregulated by inflammatory stimuli as cytokines, hormones, and growth factors. COX-1 is involved in several homeostatic processes, such as the modulation of platelet aggregation and the preservation of gastrointestinal mucosal integrity, so its inhibition affects not only the inflammatory response



1 R ₅ = R ₇ = R _{3'} = R _{4'} = H	14 R ₅ = R ₇ = OH; R _{3'} = R _{4'} = H	18 R ₅ = H; R ₇ = R _{3'} = R _{4'} = OH
5 R ₅ = OH; R ₇ = R _{3'} = R _{4'} = H	15 R ₅ = R _{4'} = OH; R ₇ = R _{3'} = H	19 R ₅ = R ₇ = R _{3'} = R _{4'} = OH
11 R ₅ = R ₇ = R _{4'} = OH; R _{3'} = H	16 R ₅ = R _{3'} = H; R ₇ = R _{4'} = OH	20 R ₅ = R ₇ = H; R _{3'} = R _{4'} = OH
13 R ₅ = R _{3'} = R _{4'} = H; R ₇ = OH	17 R ₅ = R _{3'} = R _{4'} = OH; R ₇ = H	21 R ₅ = R ₇ = R _{3'} = H; R _{4'} = OH

FIGURE 35: Chemical structures of 2-SC **1**, **5**, **11**, and **13–21** [54, 59].

but also the normal function of the body. COX-2 is expressed primarily in cells involved in inflammation, e.g., macrophages and fibroblasts, so its selective inhibition is preferable for an anti-inflammatory activity [55–57]. As mentioned above, COXs are involved in the metabolism of arachidonic acid to prostanoids, which include prostaglandins, thromboxanes, and prostacyclins. The prostanoids are lipid mediators and are involved in physiologic and pathologic processes, namely, in inflammation. Prostaglandins are pro-inflammatory mediators and their biosynthesis is increased during the inflammatory response. These mediators increase vascular permeability and are involved in common inflammation signalling processes, such as pain, oedema, and redness [55, 56, 58]. LTB₄ is a LT that induces inflammation via its chemotactic and degranulating actions on polymorphonuclear lymphocytes [55]. In the study of Gomes and co-workers, the results were expressed as percentages of inhibition and the values are indicated by the authors in the “Results” Section. Thus, the results presented here are in accordance with the information provided in the original article, where only a few activity values were mentioned. In what concerns the inhibition of COX-1 and COX-2 by 2-SC, this was determined in a cell-free system by quantifying the levels of prostaglandin F_{2α}, produced by catalysis of arachidonic acid. The results were expressed as the percent inhibition of COX-1 or COX-2 activity. 2-SC **17–20** were able to inhibit COX-1. The 2-SC **19** was the most potent, significantly inhibiting the enzyme’s activity at the concentrations of 100 and 250 μM (46.0 ± 5.6% and 74.4 ± 12.8%, respectively). From 2-SC with –OH at C-4’ on the B-ring (**11**, **15**, **16**, and **21**), only 2-SC **11** and **16** inhibited COX-1 at a concentration of 250 μM, with 2-SC **11** being significantly more potent (66.5 ± 11.4% inhibitory effect). None of the other 2-SC (**1**, **5**, **13**, **14**, **15**, and **21**) was able to inhibit COX-1 and none of the studied 2-SC inhibited COX-2 at the tested concentrations (100 and 250 μM). Indomethacin (1 μM), a non-selective COX inhibitor, and a selective COX-2 inhibitor, celecoxib (10 μM), were tested as positive controls. Indomethacin showed 26.6 ± 6.6% inhibitory effect of COX-1 and 92.6 ± 2.1% inhibitory effect of COX-2. Celecoxib inhibited 68.2 ± 3.0% of

COX-2 activity. In general, 2-SC with –OH groups at C-3’ and C-4’ on the B-ring appear to be better COX-1 inhibitors than 2-SC with –OH at C-4’ on the B-ring or unsubstituted B-ring. All the tested 2-SC demonstrated inhibitory effects of LTB₄ production by human neutrophils. A lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA), at a concentration of 1 μM, and two concentrations of 2-SC (10 and 25 μM) were tested, but only the results for the concentration 25 μM of SC were shown. In general, 2-SC **17–20**, with –OH at C-3’ and C-4’ on the B-ring, were more effective inhibitors of LTB₄ production than the other 2-SC (**1**, **5**, **11**, **13–16**, and **21**). 2-SC **17**, **19**, and **20** were the most effective compounds, reaching ≈90% inhibitory effect. Among the 2-SC that have in common an –OH at C-4’ on the B-ring (**11**, **15**, **16**, and **21**), 2-SC **15** was the most effective with 78.0 ± 9.0% inhibitory effect. In general, 2-SC without any substitution in the B-ring (**1**, **5**, **13**, and **14**) were less effective. 2-SC **1** was the most effective of these group of compounds (48.9 ± 10.3% inhibitory effect). NDGA (1 μM) reached 76.1 ± 11.0% inhibitory effect. In what concerns the reducing power, 2-SC **16–20** were able to reduce ferric ion, at the final concentration of 25 μM, being 2-SC **17–20** more efficient reducers than the positive control, ascorbic acid. 2-SC **17–20** also showed metal chelating capacity. In this study, the authors established correlations with the results obtained in a previous work [26] with these 2-SC. Thus, a significant correlation was established between inhibition of LTB₄ production and the reducing activity and between inhibition of LTB₄ production and the scavenging activity against ¹O₂ and ONOO[–] (with and without NaHCO₃) of the tested 2-SC [54]. Overall, 2-SC with substitutions on the B-ring (–OH at C-3’ and C-4’ or –OH at C-4’) appear to have a more significant influence on the inhibition of LTB₄ production in human neutrophils than 2-SC with substitutions on the A-ring (–OH at C-5 and/or C-7 or unsubstituted). In this study, it was also proposed that inhibition of LTB₄ production by 2-SC probably involves the inhibition of 5-lipoxygenase. The majority of 5-lipoxygenase inhibitors act in the catalytic domain of the enzyme, reducing or chelating iron from the active site or by eliminating radical intermediates in the iron redox cycle.

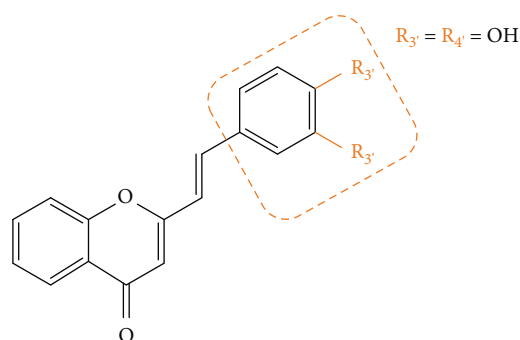


FIGURE 36: Structural characteristics that seem to favour the 2-SC anti-inflammatory activity.

Thus, the 2-SC that were most effective in inhibiting the production of LTB_4 (17–20) were also strong reducers, supporting the idea that the inhibition of 5-lipoxygenase is related to the mechanism that leads to the inhibition of LTB_4 production by 2-SC. Significant correlations were found between the inhibition of LTB_4 production and the scavenging activity against 1O_2 and $ONOO^-$ also indicating that the elimination of radical intermediates may contribute to the LTB_4 production inhibitory effect. For the inhibition of COX-1 activity, it has been proposed that the mechanism by which 2-SC inhibit COX-1 activity probably consists in the scavenging of the radical intermediates involved in COX enzyme catalysis. This can be corroborated by the fact that only 2-SC that previously showed high ROS and RNS scavenging activity [26] have been effective [54]. The fact that 2-SC only inhibits COX-1 activity seems to indicate some selectivity for this isoform.

Gomes and co-workers [59] also evaluated the effects of several 2-SC derivatives (1, 5, 11, and 13–21, Figure 35) on lipopolysaccharide (LPS)-induced NF- κ B activation and consequent production of pro-inflammatory cytokines/chemokine, using a human monocytic leukaemia cell line (THP-1). NF- κ B is one of the most important transcription factors and a regulator of immune and inflammatory responses. When not stimulated, the NF- κ B is located in the cytoplasm, but when activated, it translocates to the nucleus and induces the expression of many mediators of the inflammation, including cytokines, enzymes, and chemokines. The activation of NF- κ B can occur due to the action of several pro-inflammatory stimuli, such as cytokines, e.g., tumor necrosis factor (TNF)- α or interleukin (IL)-1, and infectious agents. The cytokines and chemokine evaluated in this study, e.g., TNF- α , IL-1 β , IL-6, and IL-8, are inflammatory mediators, involved in the regulation of the inflammatory response. Cytokines act as signalling molecules, participating in the inflammatory response, mainly regulating their intensity, propagation, and duration. There are several types of cytokines and their specific function depends on the cell type that produce them and their location. Chemokines are generally produced by leukocytes and endothelial cells and their main function is to induce cell migration. Thus, chemokines chemoattract leukocytes to inflammation sites and induce integrin expression [60, 61]. The cytotoxicity of the tested 2-SC to THP-1 cells was evaluated for the concentration of 50 μ M and none showed cyto-

toxicity. For NF- κ B activation, 2-SC 17 was the most active ($65.7 \pm 11.5\%$ inhibitory effect, 50 μ M) and exhibited a concentration-dependent effect. The other 2-SC (1, 5, 11, 13, 14, and 16–20) displayed a lower inhibition of NF- κ B activation. The ability of 2-SC 15, 17, and 21 to reduce pro-inflammatory cytokines/chemokine production was tested for the concentration of 50 μ M. In the original article, the obtained results and the associated errors were presented only in graphs and the exact activity values were not mentioned. The three tested 2-SC were able to almost completely inhibit the production of IL-6. 2-SC 17 was capable to significantly inhibit the production of TNF- α , IL-1 β , IL-6, and IL-8, being the most potent. 2-SC 15 also inhibited production of TNF- α and IL-6 but appears to induce the production of IL-1 β and IL-8. 2-SC 21 inhibited the production of TNF- α , IL-1 β , and IL-6, while it appears to induce the production of IL-8, in a lower extent. Thus, 2-SC 15, 17, and 21 showed to be capable to significantly inhibit the NF- κ B activation and to reduce the production of the pro-inflammatory cytokines/chemokine. However, 2-SC 17 was the most active for the two assays. The three 2-SC with better activity (15, 17, and 21) have in common the presence of an -OH at C-4' on the B-ring, which seems to indicate that this substitution favours the inhibition of NF- κ B activation and the reduction of cytokines/chemokine production. In addition to the -OH at C-4' on the B-ring, the simultaneous presence of an -OH at C-3' on the B-ring (catechol group) in 2-SC 17 seemed to potentiate the anti-inflammatory activity [59].

The above-mentioned structural characteristics that demonstrated to favour the anti-inflammatory activity of 2-SC are summarized in Figure 36.

4.7. Antitumoral Activity. A tumor can be defined as an abnormal mass of tissue, resulting from the abnormal growth of cells in the body. Tumors, also known as neoplasms, may be benign or malignant. Benign tumors are slow-growing masses; they remain localized, neither spreading nor invading the surrounding tissues. These types of tumors are less harmful, are easily removed by surgery, and generally do not grow back after removal. On the other hand, malignant tumors, commonly known as cancer, are invasive and tend to spread through metastases and to reappear after their removal. The growth of these tumors is characterized by a disordered and uncontrolled multiplication of cells, causing cancer cells to gain a degree of self-sufficiency [62, 63]. Cancer can be considered as a genetic disease and include different types of cancer diseases. This is one of the main causes of death in the world, strongly affecting the world population, since its incidence is increasing [62]. Thus, these cancer diseases have become a worldwide health problem, which leads to the search for effective responses to these pathologies. Several 2-SC and 3-SC have shown potential as antitumoral agents and are below discussed. The observed antitumor effects, in the published works that studied tumor and normal cell lines, are summarized in Table 10, for 2-SC, and Table 11, for 3-SC.

4.7.1. 2-Styrylchromones. Gerwick and co-workers [6] were the first to describe the antitumor potential of 2-SC, to the

TABLE 10: Summary of the 2-SC in which the antitumoral activity was studied in works using tumor and normal cell lines and description of the observed effects in each type of cells used. The table displays the antitumoral activity shown for the studied 2-SC according to the criterion: selectivity index greater or equal to 2.

2-SC	Studied cell lines		Observed effect(s)	Ref.
	Tumoral	Non-tumoral		
1	Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells		Decreased cell viability of Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells	[64, 66]
30	Ca9-22, HSC-2, HSC-3 and HSC-4 cells	HGF, HPC and HPLF cells	Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells; and apoptosis induction of HSC-2 cells	[66]
56			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	
57	HSC-2, HSC-3, HSG and HL-60 cells		Decreased cell viability of HSC-2, HSC-3, HSG and HL-60 cells	[64]
58	MCF-7, NCI-H460, Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells	MRC-5 cells, human lymphocytes, HGF, HPLF and HPC cells	Growth inhibition of MCF-7 and NCI-H460 cells; cell cycle arrest at the G ₂ /M phase of MCF-7 and NCI-H460 cells; microtubule-stabilizing antimitotic effect in MCF-7 and NCI-H460 cells; weak inhibition of peripheral human lymphocytes proliferation; decreased cell viability of Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells; apoptosis induction of HSC-2 cells; DNA fragmentation induction in HL-60 and HSC-2 cells; and activation of caspase 3, 8 and 9 in HL-60 cells	[64–66]
88	Ca9-22, HSC-2, HSC-3 and HSC-4 cells		Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	[66]
92	Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells		Decreased cell viability of Ca9-22, HSC-2, HSC-3, HSC-4, HSG and HL-60 cells	[64, 66]
103	HSC-2, HSC-3, HSG and HL-60 cells	HGF, HPLF and HPC cells	Decreased cell viability of HSC-2, HSC-3, HSG and HL-60 cells; DNA fragmentation induction in HL-60 and HSC-2 cells; and activation of caspase 3, 8 and 9 in HL-60 cells	[64]
104 - 108				
110, 111	Ca9-22, HSC-2, HSC-3 and HSC-4 cells		Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	[66]
114, 115				
152	AREc32 and K562 cells	Peripheral blood mononuclear cells	Nrf2-inducing activity in AREc32 cells; decreased cell viability and proliferation of K562 cells	[70]

AREc32: human mammary epithelial adenocarcinoma cell line; Ca9-22: human oral squamous cell carcinoma Ca9-22; HGF: human gingival fibroblast; HL-60: promyelocytic human leukaemia-60 cell line; HPC: human pulp cell; HPLF: human periodontal ligament fibroblast; HSC-2: human squamous cell carcinoma-2; HSC-3: human squamous cell carcinoma-3; HSC-4: human squamous cell carcinoma-4; HSG: human submandibular gland carcinoma; K562: human chronic myeloid leukaemia; MCF-7: breast adenocarcinoma cell line; MRC-5: diploid embryonic lung fibroblast cell line; NCI-H460: non-small cell lung cancer; Nrf2: nuclear factor erythroid 2-related factor 2.

best of our knowledge. These authors evaluated the cytotoxic potential of the natural derivative hormothamnione (**3**, Figure 37). In this study, the associated error, the number of assays done, and the positive control used were not mentioned. The hormothamnione (**3**) was defined as a potent cytotoxic agent, by the inhibition of the cell growth of P388 lymphocytic leukaemia and HL-60 cell lines, with IC₅₀ values of 4.6 ng/mL and 0.1 ng/mL, respectively. The possible mechanism of action of hormothamnione (**3**) was studied by the evaluation of macromolecule biosynthesis (DNA, RNA, and protein), through radioactive precursor incorporation studies in HL-60 and KB cells (human epidermoid carcinoma). The 2-SC **3** demonstrated to be effective in the inhibition of RNA synthesis, apparently being a selective inhibitor (IC₅₀ values of 1.0 ng/mL and 140 ng/mL for HL-60 and KB cells, respectively), but was not effective in the inhibition of the synthesis of DNA and proteins [6].

Gerwick [7] also evaluated the antitumor potential of 6-desmethoxyhormothamnione (**4**, Figure 37). Again, the associated error, the number of assays done, and positive control were not indicated. 2-SC **4** showed good cytotoxicity in 9 KB cells (median lethal dose ~ 1 μg/mL) [7].

Momoi and co-workers [64] evaluated the cytotoxic activity of 2-SC **1**, **57**, **58**, **92**, **93**, and **103** (Figure 38) against three normal oral human cells [human gingival fibroblast (HGF), human pulp cell (HPC), and human periodontal ligament fibroblast (HPLF)] and four human tumor cell lines [squamous cell carcinoma (HSC-2 and HSC-3), human submandibular gland carcinoma (HSG), and HL-60]. Cytotoxic activity was assessed by the MTT assay, except for HL-60 cells for which the viable cell number was determined by the trypan blue exclusion assay. The CC₅₀ was calculated and tumor specificity of the studied 2-SC was evaluated by SI, presented by the authors as an interval of values. The associated error and the number of assays done were not

TABLE 11: Summary of the 3-SC which antitumoral activity was studied in works using tumor and normal cell lines and description of the observed effects in each type of cells used. The table displays the antitumoral activity shown for the studied 3-SC according to the criterion: selectivity index greater or equal to 2.

3-SC	Studied cell lines		Observed effect(s)	Ref.
	Tumoral	Non-tumoral		
2				[78]
34			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	[43]
36 - 39				
41			High cytotoxicity against HGF, HPLF and HPC cells	[78]
42 - 47				
49, 50			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	[43]
165	Ca9-22, HSC-2, HSC-3 and HSC-4 cells	HGF, HPLF and HPC cells	High cytotoxicity against HGF, HPLF and HPC cells	
167				
168			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells; induction of apoptosis and mitotic arrest in HSC-2 cells	
169			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells; and high cytotoxicity against HGF, HPLF and HPC cells	[78]
170 - 174			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells	
175			Decreased cell viability of Ca9-22, HSC-2, HSC-3 and HSC-4 cells; induction of apoptosis and mitotic arrest in HSC-2 cells	

Ca9-22: human oral squamous cell carcinoma Ca9-22; HGEP: primary gingival epithelial cell; HOK: human oral keratinocyte; HPC: human pulp cell; HGF: human gingival fibroblast; HPLF: human periodontal ligament fibroblast; HSC-2: human squamous cell carcinoma-2; HSC-3: human squamous cell carcinoma-3; HSC-4: human squamous cell carcinoma-4.

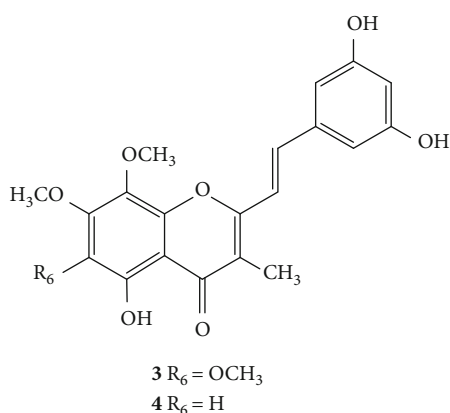


FIGURE 37: Chemical structures of 2-SC 3 and 4 [6, 7].

indicated and a positive control was also not mentioned. All the tested 2-SC showed higher cytotoxicity against tumor cell lines than against normal cells. 2-SC **58** and **103** (SI values of 10.0–13.8 and 7.5–27.3, respectively) were the most cytotoxic against tumor cell lines, followed by 2-SC **92** (SI values of 2.1–8.2). These results indicate that 2-SC with $-OCH_3$ on the B-ring (**58**, **92**, and **103**) showed greater tumor specificity. The log P was also theoretically calculated, and a relationship was established between SI and log P , in which 2-SC **58**, **92**, and **103** showed low log P values (2–2.8), despite the greater SI. Low log P indicates greater hydrophilicity, which makes it more difficult to compounds to cross the cell phospholipid bilayer and produce the desired effects. In this case, despite the low values of log P , the 2-SC were able to affect the cell viability, as demonstrated by the SI values. The effect of NADH on the cyto-

toxic activities of 2-SC **58** and **103** in HSC-2 cells was also evaluated, which allowed to assess whether cell viability was affected by enzymatic reactions of 2-SC with NADH. Lower concentrations of NADH (0.05–0.5 mM) slightly reduced the cytotoxic activity of the two 2-SC, whereas a higher concentration of NADH (5 mM) improved the cytotoxic activity of the 2-SC **58**. In this study, it was also evaluated whether the tested 2-SC could induce apoptosis-associated characteristics, such as internucleosomal DNA fragmentation and caspase activation, in the tumor cell lines, and whether radical-mediated reactions were involved in the cytotoxicity induction. In HL-60 cells, 2-SC **58** and **103** induced internucleosomal DNA fragmentation, with this fragmentation being more accentuated at 24 h. The authors also indicated that apoptotic bodies were produced. In HSC-2 cells, 2-SC **58** and **103** induced the production of large DNA fragments without induction of internucleosomal DNA fragmentation. 2-SC **58** and **103** were able to activate caspases 3, 8, and 9 in HL-60 cells, but the activation of caspases by 2-SC **58** was superior to the positive control, UV irradiation. The authors suggested that 2-SC **58** and **103** activated the non-mitochondrial extrinsic and the mitochondrial intrinsic apoptotic pathways, involved with caspases 8 and 9, respectively, and that they induce caspase 3 activation by both these pathways, leading to DNase activation inducing internucleosomal DNA fragmentation, which may depend on the type of cell line. It was also assessed whether radical-mediated reactions were involved in the induction of cytotoxicity. In these assays, all the tested 2-SC did not produce a detectable amount of radicals, under alkaline condition (pH 10 and 12.5), and did not scavenge the $O_2^{\bullet-}$ generated by the hypoxanthine-xanthine oxidase reaction. As mentioned, the results indicate that 2-SC **58**,

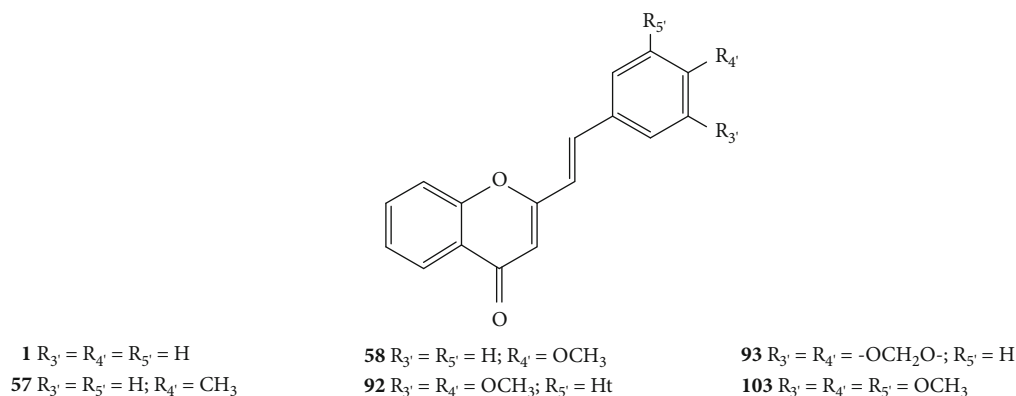


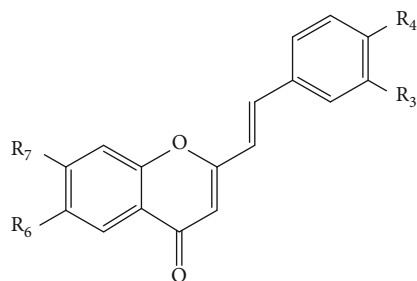
FIGURE 38: Chemical structures of 2-SC **1**, **57**, **58**, **92**, **93**, and **103** [64].

92, and **103** showed greater tumor specificity. These 2-SC have in common the presence of $-OCH_3$ on the B-ring: **58**, at C-4'; **92**, at C-3' and C-4'; **103**, at C-3', C-4', and C-5'. The other 2-SC (**1**, **57**, and **93**) have less tumor specificity than those above mentioned. 2-SC **1** is unsubstituted; 2-SC **57** has only one $-CH_3$ at C-4' on the B-ring, instead of an $-OCH_3$ (**58**). The decrease in the tumor specificity was even more accentuated with the presence of an $-OCH_2O-$, forming a ring with C-3' and C-4' on the B-ring (**93**), when compared to **92**, with $-OCH_3$ at C-3' and C-4' on the B-ring. Based on the results of the assay with different concentrations of NADH, it was proposed that the addition of NADH stimulated the formation of orthoquinone in 2-SC **92** and **103** and quinone in 2-SC **58** by enzymatic demethylation of the $-OCH_3$ groups. This hypothesis is supported by the fact that 2-SC **58** and **103** showed tumor specificity and apoptosis-inducing activity, even though these 2-SC have not been oxidized under alkaline conditions, since they did not produce a detectable amount of radicals. On the other hand, 2-SC **93** with $-OCH_2O-$ at C-3' and C-4' on the B-ring did not show any tumor specificity, which may indicate that this group is not dehydrolyzed due to ether bonding, which means that the cytotoxic orthoquinone does not form. In conclusion, 2-SC **58** and **103** appear to be very promising compounds, relating $-OCH_3$ groups to the cytotoxic activity against human tumor cell lines [64].

Marinho and co-workers [65] also studied 2-SC **58** (Figure 38). The authors evaluated whether 2-SC **58**, identified as a potent growth inhibitor of human tumor cell lines, may be equally potent for non-tumor cell lines. In this study, the following human tumor cell lines were used: breast adenocarcinoma cells (MCF-7) and non-small cell lung cancer (NCI-H460) and human non-tumor cell line: diploid embryonic lung fibroblast cell (MRC-5). 2-SC **58** (2.2–180 μM) was able to inhibit the growth of MCF-7 and NCI-H460 cells in a concentration-dependent manner [concentration of compound that produce 50% of cell growth inhibition (GI_{50}) of $4.4 \pm 0.8 \mu M$ and $5.2 \pm 0.3 \mu M$, respectively]. Interestingly, 2-SC **58** was five to six times less potent at inhibiting the growth of MRC-5 cells ($GI_{50} = 26.9 \pm 3.2 \mu M$). Regarding proliferation of peripheral human lymphocytes, 2-SC **58** (9.4–600 μM) showed to be a weak inhibitor ($IC_{50} = 74.7$

$\pm 4.8 \mu M$). The cell cycle of non-tumor MRC-5 cells does not appear to be affected by 2-SC **58**, since the cells presented a profile of DNA content similar to the untreated control. On the other hand, 2-SC **58** led to the hold in the G_2/M phase of the cell cycle of MCF-7 cells in a concentration- and time-dependent manner. Also, for NCI-H460 cells, the effects of 2-SC **58** on the cell cycle were concentration and time dependent and led to an arrest at the G_2/M phase of the cell cycle. These results led the authors to analyse the effect of 2-SC **58** (2.2–180 μM) on confluent (non-proliferative) and non-confluent (exponential growth) cells with the assumption that 2-SC **58** specifically affects mitosis. The results of both cell lines showed a high cellular protein content for the confluent cells, indicating that they were less sensitive to the 2-SC **58** treatment than non-confluent cells. The evaluation of cell growth by DNA synthesis for the NCI-H460 cell line indicates an inhibition of cell growth in non-confluent cells, in a concentration-dependent manner. The evaluation of the effects of 2-SC **58** (100 μM) on cytoskeleton components (actin filaments and microtubules) of MCF-7 and NCI-H460 cells during the interphase showed that the microtubule cytoskeleton appeared rich and intact in both cell lines, similar to the untreated cells of the control. Mitotic cells have undergone serious alterations, such as monopolar microtubule arrays, and rarely, normal mitotic spindles were found. For actin filaments, no significant changes were detected in both cell lines after 2-SC **58** treatment. These results suggest that 2-SC **58** specifically affects microtubules during mitosis. In the *in vitro* tubulin polymerization assay, 2-SC **58** (100–500 μM) led to a slight increase in tubulin polymerization, in a concentration-dependent manner, and the highest concentration tested (500 μM) resulted in the maximum polymerization plateau. In conclusion, 2-SC **58** appears to be a potential microtubule-stabilizing agent and a selective proliferation inhibitor of human tumor cell lines over normal cells, acting preferentially on proliferating cells [65].

Uesawa and co-workers [66] investigated the cytotoxic activity of 2-SC **1**, **30**, **56**, **58**, **88**, **92**, and **104–115** (Figure 39) against four human oral squamous cell carcinoma (Ca9-22, derived from gingival tissue; HSC-2, HSC-3, and HSC-4, derived from tongue) and three human oral mesenchymal cells (HGF, HPLF, and HPC). Cytotoxic



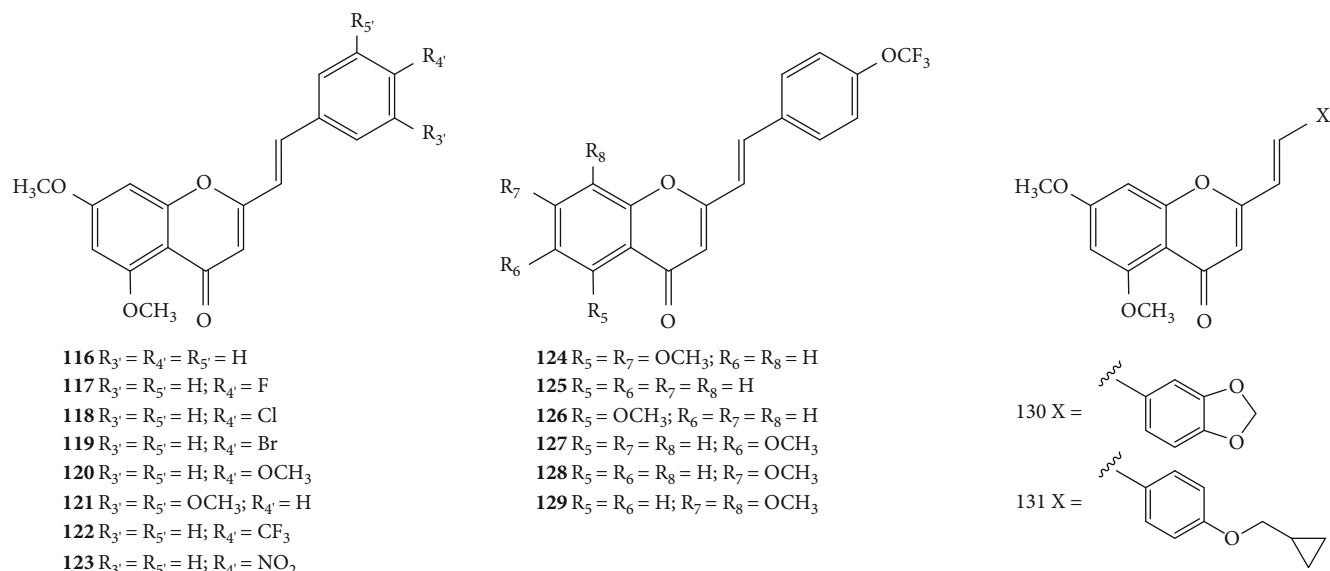
- | | | |
|--|---|---|
| 1 R ₆ = R ₇ = R _{3'} = R _{4'} = H | 104 R ₆ = R ₇ = R _{3'} = H; R _{4'} = Br | 110 R ₆ = R _{3'} = R _{4'} = H; R ₇ = OCH ₃ |
| 30 R ₆ = R _{4'} = OCH ₃ ; R ₇ = R _{3'} = H | 105 R ₆ = OCH ₃ ; R ₇ = R _{3'} = R _{4'} = H | 111 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = F |
| 56 R ₆ = R ₇ = R _{3'} = H; R _{4'} = Cl | 106 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = F | 112 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = Cl |
| 58 R ₆ = R ₇ = R _{3'} = H; R _{4'} = OCH ₃ | 107 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = Cl | 113 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = Br |
| 88 R ₆ = R ₇ = R _{3'} = H; R _{4'} = F | 108 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = Br | 114 R ₆ = R _{3'} = H; R ₇ = R _{4'} = OCH ₃ |
| 92 R ₆ = R ₇ = H; R _{3'} = R _{4'} = OCH ₃ | 109 R ₆ = R _{3'} = R _{4'} = OCH ₃ ; R ₇ = H | 115 R ₆ = H; R ₇ = R _{3'} = R _{4'} = OCH ₃ |

FIGURE 39: Chemical structures of 2-SC **1**, **30**, **56**, **58**, **88**, **92**, and **104–115** [66].

activity was assessed by the MTT assay, and the results were presented as CC₅₀, for each cell line (without the associated error) and the mean values of the four tumor cell lines and the three normal cells (with the associated error), SI, and potency-selectivity expression (PSE). Doxorubicin is an anthracycline anticancer drug and was used as a positive control. 2-SC **58** was the most cytotoxic against tumor cell lines (CC₅₀ values between 1.2 and 2.6 μM and mean CC₅₀ = 1.9 ± 0.6 μM), followed by 2-SC **30** (CC₅₀ values between 2.8 and 4.9 μM and mean CC₅₀ = 3.8 ± 1.1 μM). Doxorubicin showed a mean CC₅₀ value of 0.101 ± 0.046 μM against tumor cell lines and 6.8 ± 5.5 μM against normal oral cells. For normal oral cell lines, 2-SC **113** was the most cytotoxic (mean CC₅₀ = 27.3 ± 16.8 μM). The SI calculation showed that the 2-SC **30** has a high tumor specificity (SI = 89.1), followed by 2-SC **58** (SI = 84.1). The PSE values showed that 2-SC **58** has a high potency-selectivity expression (PSE = 4443.4), followed by 2-SC **30** (PSE = 2364.8). Therefore, the tested tumor oral cell lines were more sensitive to 2-SC **30** and **58** than normal oral cell lines. Thus, the effect of 2-SC **30** (8–32 μM) and **58** (4–16 μM) on the cell cycle of HSC-2 cells was also analysed for 24 h. 2-SC **30** and **58** led to an accumulation of the cell population in the subG₁ and G₂/M phase and induced mitotic arrest. The subG₁ population is a marker of apoptosis. Thus, these two 2-SC induced apoptosis of HSC-2 cell more potently than the positive control, actinomycin D (1 μM) [66]. The results obtained for 2-SC **58** were in line with those obtained in the previous study [65], where this 2-SC demonstrated an antiproliferative and microtubule-stabilizing effect [65]. Quantitative structure-activity relationship (QSAR) analysis of all the tested 2-SC, in this study, indicated that tumor specificity was correlated with the following descriptors: topological and 3D shape, size, electric state, and ionization potential [66]. The study of these authors also reinforces the idea, previously mentioned [64], that the –OCH₃ on the B-ring are related to the cytotoxic activity, more specifically at C-4' on the B-ring [66]. Looking at the structure of the most cytotoxic derivatives, 2-SC **58** has an –OCH₃ at C-4' on the B-ring,

while 2-SC **30** has an –OCH₃ at C-6 on the A-ring and C-4' on the B-ring [66].

Shaw and co-workers [67] synthesized a novel series of 2-SC with –OCH₃ at various positions on the A-ring and diverse substituents and modifications on the B-ring (**116–131**, Figure 40) and evaluated the antiproliferative activity against five human carcinoma cell lines [prostate carcinoma cell (PC-3), non-small cell lung adenocarcinoma cell (A549), mammary gland adenocarcinoma cell (BT483), HeLa, and hepatocellular carcinoma cell (SKHep)]. Cell proliferation studies were performed by the MTT assay and no standard compound was mentioned. Only 2-SC **125** exhibited toxicity against PC-3 cells (IC₅₀ = 28.9 ± 0.7 μM). 2-SC **116**, **117**, **121**, **123–125**, and **131** exhibited cytotoxicity against the A549 cell line, with IC₅₀ values from 15.6 ± 2.6 (**121**) to 24.2 ± 1.5 μM (**124**). Results of cytotoxicity against BT483 cells showed that the majority of 2-SC had moderate antiproliferative activity [IC₅₀ values from 18.2 ± 2.3 (**126**) to 30.9 ± 1.1 μM (**129**)], except for 2-SC **116**, **119**, **122**, and **127** that were not active. Finally, 2-SC **121**, **125**, and **131** demonstrated good antiproliferative activity against BT483 cells, with IC₅₀ values of 16.6 ± 2.4 μM, 15.5 ± 1.9 μM, and 16.6 ± 1.3 μM, respectively. The HeLa cell line was the most sensitive to the tested 2-SC, since most the tested 2-SC had better antiproliferative effects against this cell line when compared to the other tested cell lines, only 2-SC **119** and **124** were not active. 2-SC **129** was the most cytotoxic against the HeLa cell line (IC₅₀ = 4.9 ± 1.0 μM). The results obtained for the SKHep cell line showed that 2-SC **119**, **120**, **125**, **127**, and **128** were not active and 2-SC **124** and **129** were the most potent (IC₅₀ values of 12.5 ± 2.9 μM and 12.4 ± 1.5 μM, respectively). The results of 2-SC **129** obtained for the HeLa cell line led the authors to make a mechanistic study of its antiproliferative effects. Thus, HeLa cells were treated with several concentrations of 2-SC **129** (1–10 μM), and the results showed that suppression of cell proliferation was concentration dependent. The effects of 2-SC **129** (5–20 μM) on HeLa cell morphology were also studied, and significant morphological changes have

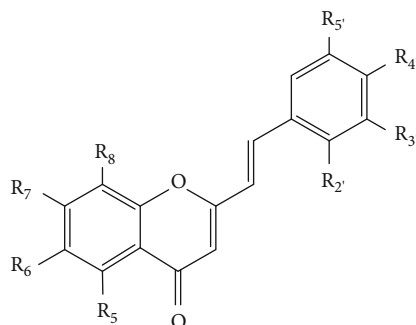
FIGURE 40: Chemical structures of 2-SC **116–131** [67].

occurred, such as cell rounding and detachment from the substratum. The effects of 2-SC **116**, **122**, and **129** on apoptosis and cell cycle profile of HeLa cells were also evaluated to establish a relationship between the antiproliferative effect and cell cycle arrest. 2-SC **116** and **122** ($20 \mu M$) led to some cell cycle arrest in the sub G_1 phase, while 2-SC **129** ($5–20 \mu M$) induced cell cycle arrest in the sub G_1 phase in a concentration-dependent manner, which may indicate that the antiproliferative effect of 2-SC **129** observed in HeLa cells may be due to DNA fragmentation, resulting in cell death. Therefore, the effect of 2-SC **129** (5 and $10 \mu M$) on DNA fragmentation in HeLa cells was also studied, by 4',6-diamidino-2-phenylindole (DAPI) staining, and this fragmentation clearly occurred after treatment with $10 \mu M$ of 2-SC **129**. The possibility of 2-SC **129** ($10 \mu M$) to have a microtubule-stabilizing effect was also evaluated by *in vitro* microtubule polymerization assay, and no effect was observed, suggesting that the antiproliferative effect on HeLa cells occurs by the other mechanisms mentioned above. In this study, the 3D-QSAR model was established by comparative molecular field analysis (CoMFA) on the BT483 cell line, to investigate how the 2-SC substituents influence the activities of the BT483 cell line. For this analysis, it was assumed that each 2-SC would adopt a conformation in its lowest energy, since the binding site is unknown. The results of the predictive model were in line with those obtained in cellular model. In what concerns the SAR, in this study, it is difficult to establish a relationship between the antiproliferative effects against the studied cell lines and the structures of the studied 2-SC, since the observed cytotoxic effects varied considerably among cell lines and regardless of the common structural characteristics among the studied 2-SC [67].

Lin and co-workers [68] synthesized 2-SC **1**, **56**, **58**, **59**, **92**, **93**, **103**, **104**, **125**, and **132–147** (Figure 41), with several substituents on the A- and/or B-rings, and evaluated their growth inhibition activity against a panel of human carci-

noma cell lines, by MTT assay, with concentrations varying between 0.1 and 1 mM, and the results were presented as GI_{50} .

For 2-SC **1**, **56**, **58**, **59**, **92**, **93**, **104**, **125**, and **132–137**, growth inhibition against gastric carcinoma (AGS), HeLa, ovarian carcinoma (OVCA), SKHep, and large lung carcinoma (H460) was assessed. In addition, for 2-SC **103**, growth inhibition against BT483 and colorectal adenocarcinoma (SW620) cell lines was also studied. For 2-SC **138–147**, growth inhibition against AGS, PC-3, H460, and SW620 cell lines was studied. 2-SC **138** was the most active growth inhibitor ($GI_{50} = 1.3 \pm 0.07 \mu M$) of AGS cells, followed by 2-SC **103** ($GI_{50} = 2 \pm 0.2 \mu M$). 2-SC **1**, **56**, **92**, **93**, **104**, **125**, **132–134**, and **143–147** did not inhibit AGS cell growth. Results of growth inhibition of 2-SC against HeLa cells showed that 2-SC **58**, **59**, **103**, and **136** were the most potent, with GI_{50} varying from 6 ± 1 (**103**) to $9.9 \pm 1 \mu M$ (**136**). 2-SC **56**, **92**, **132**, and **134** did not inhibit HeLa cell growth. Against OVCA cells, 2-SC **56**, **58**, **103**, and **135** were the most active, with GI_{50} values between 6 ± 1 (**58**) and $11 \pm 1 \mu M$ (**56**), and **59**, **93**, **125**, **132**, **134**, and **137** did not inhibit OVCA cell growth. 2-SC **58** was the most potent growth inhibitor ($GI_{50} = 5 \pm 1 \mu M$) against the SKHep cells, and **1**, **92**, **93**, **125**, **132**, and **135** did not inhibit SKHep cell growth. Results of growth inhibition of 2-SC against H460 cells showed that 2-SC **58**, **103**, **135**, **139**, and **141** presented the lowest GI_{50} values, varying from 6 ± 1 (**103**) to $10 \pm 2 \mu M$ (**139** and **141**), and **1**, **92**, **93**, **132**, **138**, **140**, and **142–147** did not inhibit their growth. Only 2-SC **138** and **139** were able to inhibit the growth of PC-3 cells (GI_{50} values of $20 \pm 4 \mu M$ and $29 \pm 5 \mu M$, respectively). For SW620 cells, 2-SC **138**, **139**, and **141** were able to inhibit their growth, while 2-SC **140** and **142–147** did not. Only 2-SC **103** was tested against BT483 cells and a GI_{50} value of $10 \pm 2 \mu M$ was found. Doxorubicin was tested as a positive control against all carcinoma cell lines and showed GI_{50} values between 0.02 ± 0.01 and $1.76 \pm 0.71 \mu M$. The results



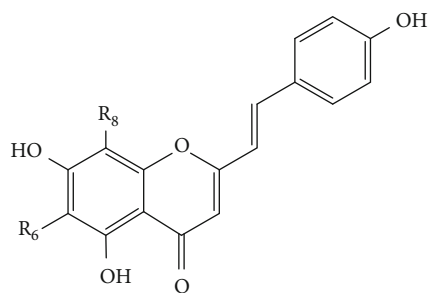
- 1** $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_4' = R_5' = H$
56 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = Cl$
58 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = OCH_3$
59 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = NO_2$
92 $R_5 = R_6 = R_7 = R_8 = R_2' = R_5' = H; R_3' = R_4' = OCH_3$
93 $R_5 = R_6 = R_7 = R_8 = R_2' = R_5' = H; R_3' = R_4' = -OCH_2O-$
103 $R_5 = R_6 = R_7 = R_8 = R_2' = H; R_3' = R_4' = R_5' = OCH_3$
104 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = Br$
125 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = OCF_3$
132 $R_5 = R_6 = R_7 = R_8 = R_3' = R_4' = R_5' = H; R_2' = OCH_3$
133 $R_5 = R_6 = R_7 = R_8 = R_2' = R_4' = R_5' = H; R_3' = OCH_3$
134 $R_5 = R_6 = R_7 = R_8 = R_2' = R_4' = H; R_3' = R_5' = OCH_3$
135 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = N(CH_3)_2$
136 $R_5 = R_6 = R_7 = R_8 = R_2' = R_5' = H; R_3' = R_4' = F$
137 $R_5 = R_6 = R_7 = R_8 = R_2' = R_3' = R_5' = H; R_4' = CF_3$
138 $R_5 = R_3' = R_4' = R_5' = OCH_3; R_6 = R_7 = R_8 = R_2' = H$
139 $R_5 = R_7 = R_8 = R_2' = H; R_6 = R_3' = R_4' = R_5' = OCH_3$
140 $R_5 = R_6 = R_8 = R_2' = H; R_7 = R_3' = R_4' = R_5' = OCH_3$
141 $R_5 = R_7 = R_3' = R_4' = R_5' = OCH_3; R_6 = R_8 = R_2' = H$
142 $R_5 = R_6 = R_2' = H; R_7 = R_8 = R_3' = R_4' = R_5' = OCH_3$
143 $R_5 = R_6 = R_7 = R_8 = R_2' = H; R_3' = R_4' = R_5' = OH$
144 $R_5 = R_7 = R_8 = R_2' = H; R_6 = R_3' = R_4' = R_5' = OH$
145 $R_5 = R_6 = R_8 = R_2' = H; R_7 = R_3' = R_4' = R_5' = OH$
146 $R_5 = R_7 = R_3' = R_4' = R_5' = OH; R_6 = R_8 = R_2' = H$
147 $R_5 = R_6 = R_2' = H; R_7 = R_8 = R_3' = R_4' = R_5' = OH$

FIGURE 41: Chemical structures of 2-SC **1**, **56**, **58**, **59**, **92**, **93**, **103**, **104**, **125**, and **132–147** [68].

obtained in this study reinforces the idea, previously mentioned [64, 66], that the presence of $-OCH_3$ on the B-ring is related to the antitumoral activity. The results obtained for 2-SC **58** show that an $-OCH_3$ at C-4' on the B-ring is favourable for the inhibition of growth against the studied carcinoma cell lines, with GI_{50} values being relatively low. 2-SC **58** was one of the 2-SC with the best activity. 2-SC **103** that has $-OCH_3$ at C-3', C-4' and C-5' on the B-ring also presented good activity. Thus, the position that seems to most favour the activity is $-OCH_3$ at C-4' on the B-ring (**58**) or simultaneously at C-3', C-4', and C-5' on the B-ring (**103**). In addition to $-OCH_3$, the tested 2-SC also displayed other groups as substituents on the B-ring. Interestingly, different halogen substituents resulted in different activities: 2-SC **104**, with $-Br$ at C-4' on the B-ring, showed growth inhibition activity against HeLa, OVCA, SKHep, and H460 cells; 2-SC **136**, with $-F$ at C-3' and C-4' on the B-ring, showed activity against all the studied carcinoma cells; 2-SC **56**, with $-Cl$ at C-4' on the B-ring, demonstrated activity against OVCA, SKHep, and H460 cells. The introduction of $-CF_3$ into C-4' on the B-ring led 2-SC **137** to display activity against AGS, HeLa, SKHep, and H460 cells. In turn, the introduction of an $-OCF_3$ into C-4' on the B-ring led 2-SC **125** to only display activity against HeLa and H460 cells. 2-SC **59** has a $-NO_2$ at C-4' on the B-ring and was only inactive against OVCA cells and showed better activity than 2-SC **125** and **137**. Since the 2-SC **103** demonstrated good activity, analogues were synthesized with $-OCH_3$ at C-3', C-4' and C-5' on the B-ring but with modifications on the A-ring (**138–142**). In general, the analogues (**138–142**) were less active than 2-SC **103**, except for 2-SC **138** against AGS cells, which showed great potency. Another series of 2-SC

(**143–147**) was also synthesized, but now with $-OH$ at C-3', C-4', and C-5' on the B-ring and modifications in several positions on the A-ring. However, none of these 2-SC showed growth inhibitory activity against the studied carcinoma cell lines, which seems to indicate that the simultaneous presence of $-OH$ groups on the A- and B-rings does not favour this activity. The importance of double bond of the styryl moiety and the rigidity of the 2-SC structure for growth inhibitory activity was also evaluated. For this, a structure analogous to 2-SC **103** was tested, in which the double bond of the styryl moiety was hydrogenated to a single bond. This analogue and 2-SC **103** were tested against seven carcinoma cell lines (AGS, BT483, HeLa, OVCA, SKHep, H460, and SW620 cells). The new analogue showed a decreased activity when compared to 2-SC **103**. Thus, the double bond of the styryl moiety seems to favour the growth inhibitory activity [68].

Yang and co-workers [10] isolated 2-SC **7–11** (Figure 42) and reported their cytotoxic activity against human hepatocyte carcinoma (HepG2), human breast adenocarcinoma (MDA-MB-231), human hepatocarcinoma (SMMC-7721), and KB cell lines. 2-SC **7–10** are also known as platachromones A–D. The cytotoxicity of 2-SC (0.5–100 $\mu g/mL$) was assayed by the MTT assay, and taxol was used as positive control. In general, 2-SC **7–11** showed to be more effective against HepG2 and KB cells, being more cytotoxic for these two cell lines than for MDA-MB-231 and SMMC-7721. 2-SC **7** was the most cytotoxic against HepG2 cells ($IC_{50} = 6.8 \pm 0.3 \mu M$), followed by 2-SC **8** ($IC_{50} = 7.4 \pm 0.1 \mu M$). 2-SC **8** showed to be the most potent against MDA-MB-231 cells ($IC_{50} = 8.7 \pm 0.3 \mu M$), followed by 2-SC **7** ($IC_{50} = 11.9 \pm 0.6 \mu M$). 2-SC **9** was the most



7 $R_6 = \text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$; $R_8 = \text{H}$

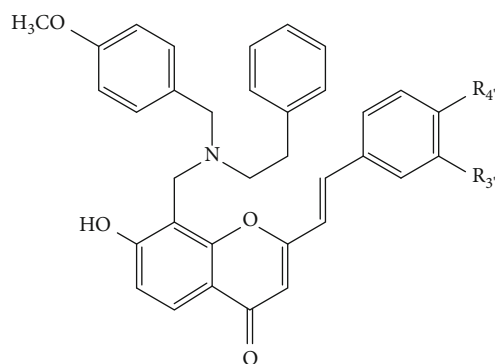
8 $R_6 = \text{H}$; $R_8 = \text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$

9 $R_6 = \text{CH}_2\text{CH}(\text{OH})\text{CH}=\text{CH}_2$; $R_8 = \text{H}$

10 $R_6 = \text{H}$; $R_8 = \text{CH}_2\text{CH}(\text{OH})\text{CH}=\text{CH}_2$

11 $R_6 = R_8 = \text{H}$

FIGURE 42: Chemical structures of 2-SC 7–11 [10].



148 $R_3 = R_4 = \text{H}$

149 $R_3 = \text{H}$; $R_4 = \text{OCH}_3$

150 $R_3 = R_4 = \text{OCH}_3$

151 $R_3 = \text{Br}$; $R_4 = \text{H}$

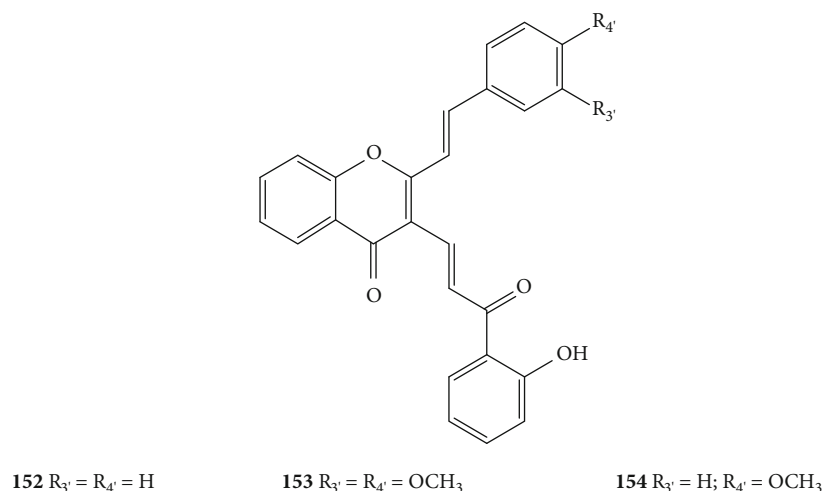
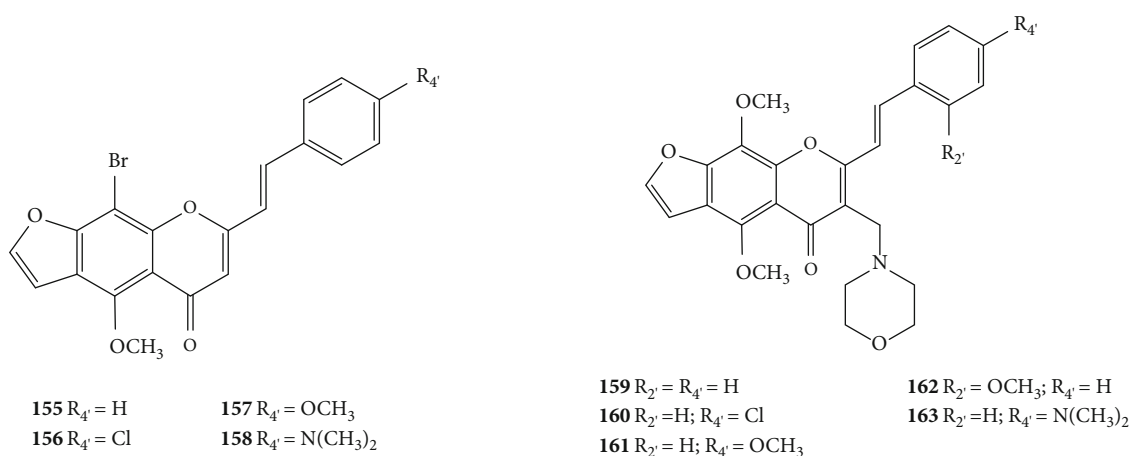
FIGURE 43: Chemical structures of 2-SC 148–151 [69].

cytotoxic against KB cells ($\text{IC}_{50} = 3.0 \pm 0.2 \mu\text{M}$) and SMMC-7721 ($\text{IC}_{50} = 14.6 \pm 0.3$), and **11** was considered inactive in the last ones. Taxol was tested against the four cancer cell lines and showed IC_{50} values that ranged from 0.04 ± 0.002 to $1.1 \pm 0.2 \mu\text{M}$. These 2-SC have in common an $-\text{OH}$ at C-5 and C-7 on the A-ring and at C-4' on the B-ring, and their structure differ in the substituents present at C-6 and C-8 on the A-ring. The $-\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$ group seems to contribute to the greater cytotoxic effect observed against HepG2 and MDA-MB-231 cells (**7** and **8**), while the $-\text{CH}_2\text{CH}(\text{OH})\text{CH}=\text{CH}_2$ group seems to favour cytotoxicity against SMMC-7721 and KB cells (**9** and **10**). In conclusion, the presence of substituents at C-6 and C-8 on the A-ring (**7–10**) seems to favour the cytotoxic activity over the unsubstituted structure (**11**) at these positions [10].

Lee and co-workers [69] synthesized a series of 2-SC based on the lavendustin A structure (**148–151**, Figure 43) and reported their cytotoxic activity against A549, human colon cancer (HCT-15), KB, and malignant melanoma (SK-MEL-2) cell lines. The cytotoxicity of 2-SC was assayed by the sulforhodamine B (SRB) assay, and cisplatin was used as positive control. 2-SC **148** was the most cytotoxic against A549 cells ($\text{IC}_{50} = 17.9 \pm 2.9 \mu\text{g}/\text{mL}$). 2-SC **151** showed potent cytotoxicity against HCT-15 ($\text{IC}_{50} = 9.22 \pm 1.8 \mu\text{g}/\text{mL}$). Only 2-SC **148** showed cytotoxicity against KB cells ($\text{IC}_{50} = 34.98 \pm 6.6 \mu\text{g}/\text{mL}$). None of the 2-SC **148–151** showed cytotoxic

activity against SK-MEL-2 cells, up to a concentration of $100 \mu\text{g}/\text{mL}$. The positive control, cisplatin, was tested against four cancer cell lines and showed IC_{50} values that ranged from 0.56 ± 0.12 to $1.65 \pm 0.12 \mu\text{g}/\text{mL}$. 2-SC **148–151** have in common an $-\text{OH}$ at C-7 on the A-ring and a substituent structurally derived from lavendustin A at C-8 on the A-ring, and differ in the substituents on the B-ring. 2-SC **148** that has no substituent on the B-ring exhibited cytotoxicity against A549, HCT-15, and KB cells. In turn, 2-SC **149** that has an $-\text{OCH}_3$ at C-4' on the B-ring showed a decrease in the cytotoxic effect when compared to 2-SC **148**, as it only showed effect against A549 and HCT-15 cells and with higher IC_{50} values. 2-SC **150**, with $-\text{OCH}_3$ at C-3' and C-4' on the B-ring, also revealed a decrease in the cytotoxic effect when compared with the activity of 2-SC **148**. The presence of $-\text{Br}$ at C-3' on the B-ring (**151**) seems to favour the cytotoxic effect against HCT-15 cells, but to decrease the activity against A549 cells, when compared to 2-SC **148**. In general, in the presence of a substituent structurally derived from lavendustin A at C-8 on the A-ring, the modifications at C-3' and/or C-4' on the B-ring did not favour the cytotoxic activity [69].

Talhi and co-workers [70] evaluated the anticarcinogenic potential of 2-SC **152–154** (Figure 44). These authors measured the induction of the cytoprotective Kelch ECH-associating protein 1-nuclear factor erythroid 2-related factor 2 (Keap1-Nrf2) signalling pathway through the luciferase reporter assay and also evaluated the cytotoxic activity through the SRB assay, in human mammary epithelial adenocarcinoma MCF-7 (AREc32) cells. The antileukemic activity of 2-SC **152** and **153** was also evaluated against human chronic myeloid leukaemia cells (K562) [70]. The Nrf2 is a transcription factor that regulates cytoprotective genes and mediates the cellular response to inducers (e.g., electrophiles and oxidants) in the regulatory regions of target cytoprotective genes. Keap1 is a cysteine-rich repressor protein that provides Nrf2 degradation when bound to it. Thus, the Keap1-Nrf2 pathway is the major regulator of cytoprotective responses and plays a role in cancer prevention, being considered a chemopreventive pathway [71, 72]. The ability of 2-SC **152–154** ($1.56–100 \mu\text{M}$) to activate the Keap1-Nrf2 pathway was evaluated and compared to xanthohumol and sulforaphane, which were tested as positive controls, in AREc32 cells. The results were presented as the concentration required to increase luciferase activity

FIGURE 44: Chemical structures of 2-SC **152–154** [70].FIGURE 45: Chemical structures of 2-SC **155–163** [73].

by fivefold in relation to solvent control (designated as C5 value), but the authors did not mention the associated error. 2-SC **152–154** presented a concentration-dependent activity and showed C5 values of $2.9 \mu M$, $18 \mu M$, and $10.2 \mu M$, respectively. The xanthohumol and sulforaphane presented C5 values of $7.8 \mu M$ and $4.8 \mu M$, respectively. Thus, 2-SC **152** had better Nrf2-inducing potential than the two positive controls. 2-SC **152–154** did not show cytotoxic effects against AREc32 cells. 2-SC **152** was able to reduce the viability and proliferation ($IC_{50} = 4.5 \pm 1.9 \mu M$, after 72 h of treatment), while **153** did not affect cell proliferation or induce the death of K562 cells. When the effect of 2-SC **152** on the viability of peripheral blood mononuclear cells from healthy donors was evaluated, this compound did not affect the viability of these cells, which may indicate selective toxicity for cancer cells. The obtained results revealed that the introduction of $-OCH_3$ at C-3' and C-4' (**153**) or only at C-4' (**154**) on the B-ring reduced the Nrf2-inducing activity, when compared with 2-SC **152**. The same conclusion can be drawn for the antiproliferative activity against the K562 cell line, since 2-SC **153** did not have an antiproliferative effect and 2-SC **152** did. In this study, the antioxidant activity of

2-SC **152–154** was also evaluated, using the ferric-reducing antioxidant power and DPPH methods. However, these 2-SC demonstrated very low or non-existent activity, therefore this study was not mentioned in the Section 4.1 [70].

Ragab and co-workers [73] synthesized 2-SC **155–163** (Figure 45), with a furan ring attached at C-6 and C-7 on the A-ring. The compounds were screened for a first single dose of $10 \mu M$ against a panel of sixty cell lines, where cells were continuously exposed to the compounds for 48 h and cell viability or growth was determined by the SRB assay. The panel of sixty cancer lines represented nine types of cancer: leukaemia, lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, and breast. In this study, the error associated with the results was not indicated. In the first screening, 2-SC **160** showed growth inhibitory activity against four leukaemia cell lines (% GI values between 60 and 93%), two non-small cell lung cancer cell lines (% GI values of 95% and 88%), one CNS cancer cell line (% GI = 67%), two ovarian cancer cell lines (% GI values of 60% and 68%), three renal cancer cell lines (% GI values between 61 and 66%), one prostate cancer cell line (% GI = 72%), and one breast cancer cell line (% GI = 86%). 2-SC **163** inhibited

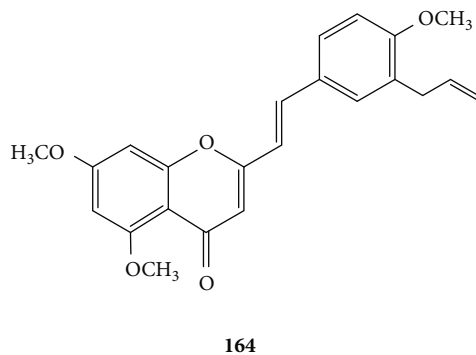


FIGURE 46: Chemical structure of 2-SC **164** [76].

the growth of five leukaemia cell lines (% GI values between 64 and 95%), two non-small cell lung cancer cell lines (% GI values of 80% and 84%), four colon cancer cell lines (% GI values between 67 and 82%), two CNS cancer cell lines (% GI values of 69% and 99%), three melanoma cell lines (% GI values between 63 and 76%), four ovarian cancer cell lines (% GI values between 78 and 88%), three renal cancer cell lines (% GI values between 63 and 95%), two prostate cancer cell lines (% GI values of 87% and 93%), and one breast cancer cell line (% GI = 70%). After this primary screening, 2-SC **160** and **163** (0.01–100 μM) were selected by the authors for an assay against cell lines of the nine types of cancer mentioned above. In this assay, GI_{50} , the compound concentration resulting in total growth inhibition (TGI), and LC_{50} (compound concentration affecting 50% net loss in initial cells, at the end of the 48 h incubation period) were calculated for each cell line. Summarizing and in general, 2-SC **163** (median $\text{GI}_{50} = 1.9 \mu\text{M}$) was more active than **160** (median $\text{GI}_{50} = 2.45 \mu\text{M}$) against the tested cell lines. The structures of these two 2-SC differ in the substituent present at C-4' on the B-ring: **160** has a $-\text{Cl}$, while **163** has $-\text{N}(\text{CH}_3)_2$. These results indicate that the $-\text{N}(\text{CH}_3)_2$ at C-4' on the B-ring favours the growth inhibitory activity in relation to the $-\text{Cl}$ in the same position. After the results are obtained in the screening, the authors submitted the 2-SC **160** and **163** to the kinase inhibition assay to determine whether the activity of these 2-SC was related to their enzyme inhibition ability of protein kinase targets. Thus, the profile of 2-SC **160** and **163** against cyclin-dependent kinase (CDK) 2/cyclin E1, CDK4/cyclin D1, and glycogen synthase kinase 3-beta (GSK-3 β) was performed [73]. CDKs play an important role in the regulation of the cell cycle, controlling gene transcription and other processes, while GSK-3 β is an enzyme that regulates numerous cellular signalling pathways and is involved in a wide range of cellular processes, such as cell cycle regulation and proliferation [74, 75]. Profiling results for the CDK2/cyclin E1 protein kinase showed that 2-SC **160** and **163** did not inhibit the enzyme activity. 2-SC **160** and **163** showed weak inhibition against the CDK4/cyclin D protein kinase target but caused stimulation of GSK-3 β activity (5% and 13%, respectively) instead of inhibiting it. Given that 2-SC **163** was very potent in cell growth inhibition assays, it was not expected to stimulate of GSK-3 β activity, but to inhibit it.

Thus, this result led the authors to progress to *in vivo* studies with 2-SC **163** against breast cancer induced in female BALB/mice (Ehrlich solid tumor), in an attempt to correlate the high *in vitro* antiproliferative activity with the stimulation of GSK-3 β activity. The tumor was induced and grew for two weeks, after which the mice received intraperitoneal injections of 2-SC **163** (3 mg/kg and 6 mg/kg) for five days, daily. The treatment with 2-SC **163** inhibited the growth of the tumor in a dose-dependent manner. The treatment with 2-SC **163** at low and high dose (3 mg/kg and 6 mg/kg, respectively) significantly decreased the level of cyclin D1 in the tumor, whereas it increased the level of GSK-3 β , when compared to the untreated group. Thus, the 2-SC **163** anti-tumor effect was accompanied by a significant decrease in cyclin D1 levels and a significant increase in GSK-3 β levels. These results seem to indicate that tumor suppression is partially mediated by an increase in GSK-3 β and a decrease in cyclin D1 levels, which suggests that cyclin D1 levels can be reduced by the activation of GSK-3 β that phosphorylates cyclin D1, leading to its proteolysis [73].

Peixoto and co-workers [76] studied the effects of 2-SC **164** (Figure 46) on rat liver mitochondrial bioenergetics related with a potential cancer chemopreventive activity. Mitochondrial assays can be used as models to study the cellular toxicity, since mitochondria is involved in the regulation of apoptosis [76, 77]. The influence of 2-SC **164** on the membrane potential was evaluated by the detection of changes induced in mitochondrial membrane energization and respiration. 2-SC **164** (200 and 500 nmol/mg mitochondrial protein) decreased the mitochondrial membrane potential, which was more pronounced at the highest concentration. 2-SC **164** showed an inhibitory effect, in a concentration-dependent manner, on respiratory rates. Thus, the decrease in transmembrane potential induced by **164** can result from the inhibition in any respiratory complex (II, III, or IV), since it must interact with the phosphorylation system and the mitochondrial redox chain. The authors, in an attempt to elucidate the exact action of 2-SC **164** in the respiratory redox chain and in the phosphorylation system (directly on the respiratory complexes or by an uncoupler effect), evaluated the effect of 2-SC **164** on the respiratory complexes (II, III, and IV) and in the mitochondria swelling. 2-SC **164** decreased the ATP synthase and ATPase activities (80% and 50%, respectively) and inhibited succinate dehydrogenase activity (17%). 2-SC **164** partially inhibited cytochrome *c* oxidase (27%), however it inhibited succinate cytochrome *c* reductase ($\approx 62\%$). These results may indicate that 2-SC **164** essentially interacts with electron transfer at the level of complex III. Experiments in the osmotic potassium acetate medium showed that in the presence of 2-SC **164**, only a small mitochondrial swelling was observed, in a concentration-dependent manner, when compared to carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone, used as a positive control. In conclusion, the results of this study indicate that the possible toxicological effect of 2-SC **164** may be related to the inhibition of the phosphorylation system and the electron transfer via the respiratory chain of rat liver mitochondria, especially in complex III (succinate cytochrome *c* reductase). The authors also

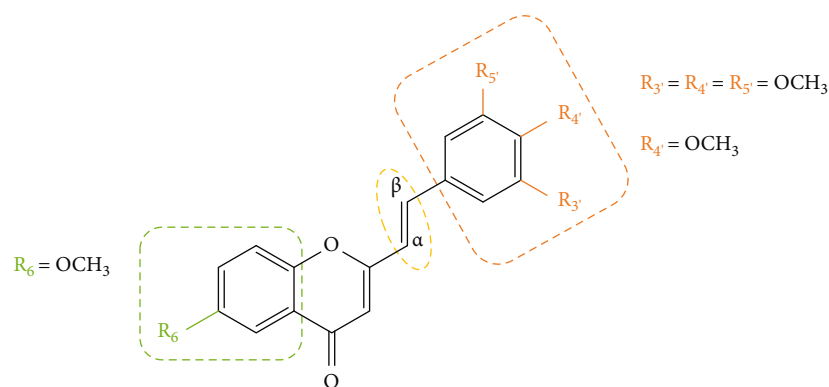
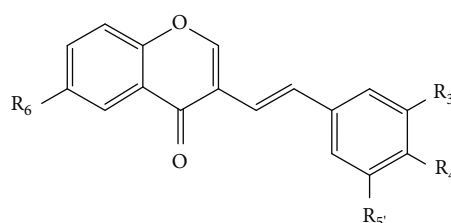


FIGURE 47: Structural characteristics that seem to favour the 2-SC antitumoral activity.



34 $R_6 = R_3 = R_5 = \text{H}; R_4 = \text{OCH}_3$

35 $R_6 = R_5 = \text{H}; R_3 = R_4 = \text{OCH}_3$

36 $R_6 = \text{H}; R_3 = R_4 = R_5 = \text{OCH}_3$

37 $R_6 = R_4 = \text{OCH}_3; R_3 = R_5 = \text{H}$

38 $R_6 = R_3 = R_4 = \text{OCH}_3; R_5 = \text{H}$

39 $R_6 = R_3 = R_4 = R_5 = \text{OCH}_3$

42 $R_6 = \text{OCH}_3; R_3 = R_5 = \text{H}; R_4 = \text{F}$

43 $R_6 = \text{OCH}_3; R_3 = R_5 = \text{H}; R_4 = \text{Cl}$

44 $R_6 = R_3 = R_5 = \text{H}; R_4 = \text{OH}$

45 $R_6 = R_5 = \text{H}; R_3 = R_4 = \text{OH}$

46 $R_6 = \text{OCH}_3; R_3 = R_5 = \text{H}; R_4 = \text{OH}$

47 $R_6 = \text{OCH}_3; R_3 = R_4 = \text{OH}; R_5 = \text{H}$

48 $R_6 = \text{OH}; R_3 = R_5 = \text{H}; R_4 = \text{OCH}_3$

49 $R_6 = R_4 = \text{OH}; R_3 = R_5 = \text{H}$

50 $R_6 = R_3 = R_4 = \text{OH}; R_5 = \text{H}$

FIGURE 48: Chemical structures of 3-SC 34–39 and 42–50 [5, 43].

suggested that 2-SC **164** may induce apoptosis through a decrease in the cellular energetic charge and promotion of the release of cytochrome *c* in the cytosol. Thus, the induction of mitochondrial cytochrome *c* release, shown in these biochemical studies, can subsequently trigger an apoptotic mechanism, an indicator of the potential cancer chemopreventive activity of 2-SC **164** [76].

The great number of studies found in the literature about 2-SC antitumoral activity covers a wide variety of tumor cell lines, some also include normal cell lines, but not all the studies. Moreover, they comprise a wide range of 2-SC with numerous substituents in different locations, making it difficult to indicate which type of cancer is most studied and to establish a SAR. However, the above-mentioned structural characteristics that demonstrated to favour the antitumoral activity of 2-SC are summarized in Figure 47.

4.7.2. 3-Styrylchromones. Shimada and co-workers [43] synthesized fifteen 3-SC (**34–39**, and **42–50**, Figure 48) and investigated their cytotoxic activity against four human oral squamous cell carcinoma (Ca9-22, HSC-2, HSC-3, and HSC-4) and three human normal oral cells (HGF, HPLF, and HPC) and performed the QSAR analysis. Cytotoxic activity was assessed by the MTT assay, and CC_{50} and SI were calculated and presented. The authors mentioned the obtained CC_{50} values for each cell line, without the associated error, and the mean of the CC_{50} values obtained for the four tumor cell lines and the three normal cells, with

the associated error. 3-SC **46** was the most cytotoxic against the four tumor cell lines (mean $CC_{50} = 2.0 \pm 1.2 \mu\text{M}$), followed by **37** (mean $CC_{50} = 6.4 \pm 2.4 \mu\text{M}$). Against normal oral cells, 3-SC **46** and **37** showed lower cytotoxicity (mean CC_{50} values of $138 \pm 116 \mu\text{M}$ and $258 \pm 126 \mu\text{M}$, respectively). 3-SC **39** was also quite cytotoxic against tumor cell lines (mean $CC_{50} = 13 \pm 6.1 \mu\text{M}$) and showed lower cytotoxicity against normal cell lines (mean $CC_{50} = 339 \pm 183 \mu\text{M}$). Thus, these 3-SC showed high tumor specificity (SI values of 69, 40.3, and 26.1) and their results can be compared to those obtained for the positive controls, doxorubicin and 5-fluorouracil (SI values of >26.0 and >55.6 , respectively). Only 3-SC **48** did not show cytotoxicity against tumor cell lines nor against normal oral cell lines. In what concerns QSAR analysis, several chemical descriptors were used. The effects of functional groups on cytotoxicity of the studied 3-SC against tumor cell lines were evaluated, and the substituents $-\text{OCH}_3$ at C-6 on the A-ring and $-\text{OH}$ at C-4' on the B-ring were defined to be crucial to increase the cytotoxicity against tumor cell lines. Accordingly, 3-SC **37** that has $-\text{OCH}_3$ at C-6 on the A-ring and at C-4' on the B-ring showed a greater cytotoxicity and tumor specificity than 3-SC **39**, with $-\text{OCH}_3$ at C-6 on the A-ring and at C-3', C-4' and C-5' on the B-ring. 3-SC **38** and **39** have $-\text{OCH}_3$ at C-6 on the A-ring and at C-3' and C-4' on the B-ring, but **39** also have an $-\text{OCH}_3$ at C-5' on the B-ring and showed better tumor specificity than **38**, which indicates that this

additional $-\text{OCH}_3$ may contribute to the cytotoxic activity when in the presence of multiple $-\text{OCH}_3$ groups on the B-ring. 3-SC **34** (with $-\text{OCH}_3$ at C-4' on the B-ring), **35** (with $-\text{OCH}_3$ at C-3' and C-4' on the B-ring), and **36** (with $-\text{OCH}_3$ at C-3', C-4' and C-5' on the B-ring) showed low tumor specificity, which seems to indicate that the presence of $-\text{OCH}_3$ groups only on the B-ring (without substituents at C-6 on the A-ring) decreases the cytotoxic activity against tumor cell lines. When an $-\text{OCH}_3$ at C-6 on the A-ring is present, $-\text{Cl}$ at C-4' on the B-ring (**43**) seems to favour the cytotoxic activity against tumor cell lines over the presence of $-\text{F}$ in the same position (**42**). 3-SC **44** (with $-\text{OH}$ at C-4' on the B-ring) and **45** (with $-\text{OH}$ at C-3' and C-4' on the B-ring) have no substituents in the A-ring and showed better tumor specificity than 3-SC also without substituents in the A-ring but with $-\text{OCH}_3$ on the B-ring, in the same positions (**34–36**), which may indicate that $-\text{OH}$ on the B-ring is important for the cytotoxic activity against tumor cell lines. In 3-SC **46**, $-\text{OCH}_3$ is at C-6 on the A-ring and $-\text{OH}$ is at C-4' on the B-ring; while in 3-SC **48**, $-\text{OCH}_3$ is at C-4' on the B-ring and $-\text{OH}$ is at C-6 on the A-ring; this inversion of the substituents' positions led to the loss of 3-SC **48** cytotoxic activity. As mentioned, 3-SC **46** has an $-\text{OCH}_3$ at C-6 on the A-ring and $-\text{OH}$ at C-4' on the B-ring and it showed better tumor specificity than **47**, which has an additional $-\text{OH}$ at C-3' on the B-ring. Moreover, 3-SC **49** and **50** both have $-\text{OH}$ at C-6 on the A-ring and C-4' on the B-ring but **50** has an additional $-\text{OH}$ at C-3' on the B-ring that led to a better tumor specificity than **49**. This result indicates that when C-6 on the A-ring is $-\text{OH}$ instead of $-\text{OCH}_3$, the addition of $-\text{OH}$ to the B-ring can improve tumor specificity. In conclusion, 3-SC **46**, with $-\text{OCH}_3$ at C-6 on the A-ring and $-\text{OH}$ at C-4' on the B-ring, was the most cytotoxic and showed high tumor specificity, which indicates that these two groups, at these positions, favour the cytotoxic activity against the studied tumor cell lines [43].

As described, 3-SC **37**, **39**, and **46** (Figure 48) showed to have much higher cytotoxicity against four epithelial human oral squamous cell carcinoma lines (Ca9-22, HSC-2, HSC-3, and HSC-4) than against human normal mesenchymal cells (HGF, HPLF, and HPC) [43]. These results led Sakagami and co-workers [5] to continue the study of the tumor specificity of 3-SC **37**, **39**, and **46** against both epithelial malignant and non-malignant cells through the MTT assay. As in the previous study [43], the cytotoxicity activity against epithelial malignant cell lines had already been tested; in the present study [5], the authors only tested the cytotoxicity activity against epithelial non-malignant cell lines [human oral keratinocyte (HOK) and primary gingival epithelial cell (HGEP)]. The obtained results show that 3-SC **37**, **39**, and **46** were less cytotoxic against epithelial non-malignant cell lines (CC_{50} values between 19 and 800 μM) than against epithelial malignant cell lines (mean CC_{50} values between 2.0 ± 1.2 and $13 \pm 6.1 \mu\text{M}$) [5, 43]. Doxorubicin and 5-fluorouracil were tested as positive controls and were highly cytotoxic against epithelial non-malignant cell lines, showing weak tumor specificity (SI values between 0.2 and 8.7).

So, 3-SC **37**, **39**, and **46** demonstrated greater tumor specificity than the positive controls [5]. The PSE was also calculated to identify 3-SC that have both good potency and selective toxicity, and 3-SC **46** showed high potency-selectivity to malignant tumors (PSE values between 475 and 20000). To determine the minimum treatment time required for cytotoxicity induction, pulse-chase experiments were performed, where HSC-2 cells were incubated until 47 h with increasing concentrations of **37**, **39**, and **46** (0–1000 μM), and the relative viable cell number was determined by the MTT assay. 3-SC **46** was the most cytotoxic (CC_{50} values varied between 18 and <0.39 , for 1 h and 47 h of exposure, respectively), followed by **37** (CC_{50} values varied between 61 and 2.4 μM , for 1 h and 47 h of exposure, respectively) and **39** (CC_{50} values varied between 65 and 2.2 μM , for 1 h and 47 h of exposure, respectively). In this assay, it was also observed that high concentrations (100–1000 μM) of 3-SC **37** and **46** led to a cell death greater than 90%, while 3-SC **39**, for the same range of concentrations, was less cytotoxic, showing a 30% cell viability. The obtained results led the authors to conclude that 3-SC **46** had the highest cytotoxicity and tumor specificity among the three 3-SC (**37**, **39**, and **46**), with cytotoxicity being triggered at early 3 h after treatment. Therefore, the effects of 3-SC **46** on fine cell structures and the type of cell death were evaluated, as well as the metabolic profile induced by this 3-SC in HSC-2 cells. 3-SC **46** (3 and 10 μM) induced concentration-dependent mitochondrial vacuolization, which suggests induction of mitochondrial injury. 3-SC **46** (10 μM) suppressed autophagy and induced apoptotic cell death. In what concerns metabolomic analysis, 3-SC **46** (1, 3, and 10 μM) did not significantly affect major cellular metabolic pathways, however the authors reported changes in metabolites involved in amino acid and glycerophospholipid metabolism [5].

Takao and co-workers [78] synthesized fourteen 3-SC (**2**, **40**, **41**, and **165–175**, Figure 49) and investigated their tumor specificity. The authors evaluated the cytotoxic activity of these 3-SC against four human oral squamous cell carcinoma cell lines (Ca9-22, HSC-2, HSC-3, and HSC-4) and three human normal oral mesenchymal cells (HGF, HPLF, and HPC). In addition, QSAR analyses were performed. First, the cytotoxic activity of 3-SC (0.3–400 μM) was assessed by the MTT assay, and CC_{50} , SI, and PSE were calculated and presented. In this study, the associated error to CC_{50} values was not indicated, even though the determination of the relative viable cell number was performed in triplicate. The CC_{50} values were presented for each cell line and the mean of the CC_{50} values of the four tumor cell lines, and the three normal cells were also presented, with the associated error. Doxorubicin (0.078–10 μM) and 5-fluorouracil (7.8–1000 μM) were used as positive controls. 3-SC **168** and **175** were the most cytotoxic against the tumor cell lines (mean CC_{50} values of $0.6 \pm 0.27 \mu\text{M}$ and $0.65 \pm 0.34 \mu\text{M}$, respectively), followed by **169** (mean $\text{CC}_{50} = 1.1 \pm 0.31 \mu\text{M}$). Against normal oral cells, 3-SC **168** and **175** showed low cytotoxicity (mean CC_{50} values of $181.9 \pm 195.0 \mu\text{M}$ and $118.8 \pm 142.6 \mu\text{M}$, respectively), while 3-SC **169** showed high toxicity (mean CC_{50} values of $4.1 \pm 1.6 \mu\text{M}$). Thus, 3-SC **168** and

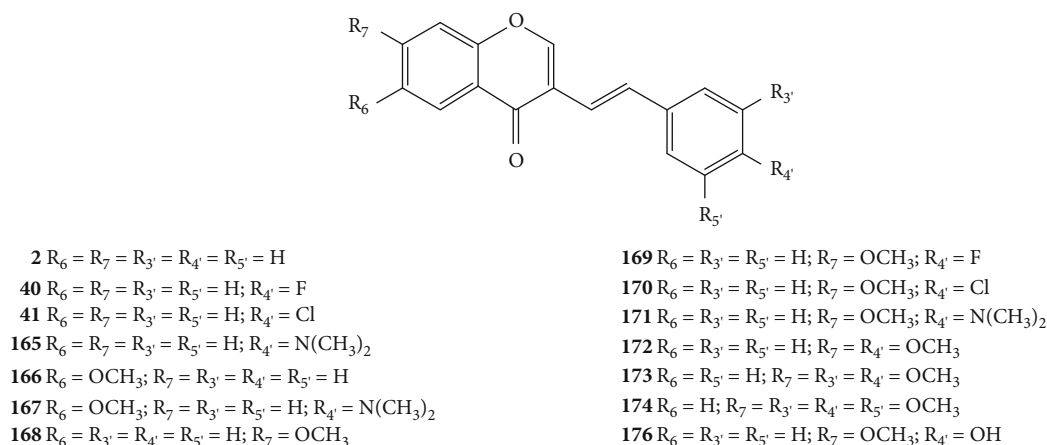
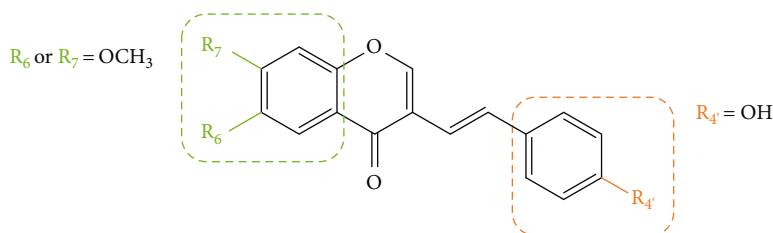
FIGURE 49: Chemical structures of 3-SC **2**, **40**, **41**, and **165–175** [78].

FIGURE 50: Structural characteristics that seem to favour the 3-SC antitumoral activity.

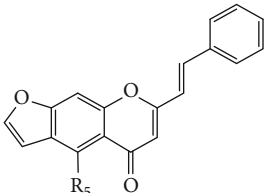
175 showed high tumor specificity (SI values of 301.1 and 182.0, respectively), being better than doxorubicin and 5-fluorouracil (SI values of 54.9 and 16.2, respectively); and **169** showed low tumor specificity (SI = 3.7). 3-SC **41** and **167** were also quite cytotoxic against tumor cell lines (mean CC_{50} values of $5.3 \pm 0.8 \mu M$ and $6.5 \pm 2.6 \mu M$, respectively), but nevertheless also showed high cytotoxicity against normal oral cell lines (mean CC_{50} values of $3.7 \pm 1.1 \mu M$ and $6.1 \pm 0.8 \mu M$, respectively). 3-SC **168** showed a high potency-selectivity expression (PSE = 49842.4), followed by **175** (PSE = 27898.1), being these values higher than those obtained for the positive controls (PSE = 24953.8 for doxorubicin and PSE = 26.2 for 5-fluorouracil). These results indicate that all tumor oral cell lines tested were more sensitive to 3-SC **168** and **175** than normal oral cells. Thus, the effects of 3-SC **168** (1–4 μM) and **175** (2–8 μM) on the apoptosis induction and growth arrest were further monitored by cell cycle analysis of HSC-2 cells. 3-SC **168** and **175** and the positive control, actinomycin D (1 μM), led to an accumulation of cell population in the sub G_1 ; however, only 3-SC **168** and **175** induced the accumulation at G_2/M phase cells and induced mitotic arrest. Thus, the results demonstrated that these two 3-SC induced the apoptosis and mitotic arrest in HSC-2 cell more potently than actinomycin D. The QSAR analysis of all the tested 3-SC indicated that tumor specificity was correlated with the following descriptors: topological and 3D shape, size, electric state, aromaticity index, energy of the lowest unoccupied molecular orbital (LUMO), and partial charges. The authors also indicated that the presence of $-OCH_3$ at C-6 on the A-ring reduced the tumor specificity and the presence of $-OCH_3$ at C-7 on the A-ring led to an increase in tumor spec-

ificity. In fact, the 3-SC with the highest tumor specificity (**168**) has only $-OCH_3$ at C-7 on the A-ring. Furthermore, the fact that 3-SC **166**, with only an $-OCH_3$ at C-6 on the A-ring, has a very low tumor specificity is an example that demonstrates that in addition to the type of substituents, their position also influences the tumor specificity. In general, the introduction of halogen atoms ($-F$ and $-Cl$) at C-4' on the B-ring (3-SC **40** and **41**) reduced the tumor specificity, even in the presence of additional $-OCH_3$ at C-7 on the A-ring (**169** and **170**). Unsubstituted 3-SC **2** also showed low tumor specificity. For 3-SC **165**, **167**, and **171**, a low tumor specificity was found. These three 3-SC have in common the $-N(CH_3)_2$ group at C-4' on the B-ring, which does not seem to favour tumor specificity. In conclusion, this study suggests that the $-OCH_3$ group at C-7 on the A-ring favours the cytotoxic activity against the studied tumor cell lines and improves the tumor specificity of 3-SC [78].

As for 2-SC, studies found in the literature on the antitumor activity of 3-SC include different tumor cell lines and a wide range of 3-SC with various substituents in different locations, making it difficult to establish a clear SAR. However, the above-mentioned structural characteristics that demonstrated to favour the antitumoral activity of 3-SC are summarized in Figure 50.

4.8. Other Activities. In addition to the biological activities already described in the previous topics of this review, several SC have shown other less explored biological properties, such as affinity and selectivity for A_3 adenosine receptors, neuroprotective activity, and α -glucosidase inhibitory activity and are below discussed.

TABLE 12: 2-SC studied for A₃ adenosine antagonists, their chemical structures, and summary of the observed effects.

Chemical structure	2-SC	Observed effect(s)	Ref.
	176	Binding affinity for A ₁ , A _{2A} and A ₃ adenosine receptors	[79]
	177	Binding affinity for A ₁ , A _{2A} and A ₃ adenosine receptors; and selectivity for A ₃ adenosine receptor	
	178	Binding affinity for A ₁ , A _{2A} and A ₃ adenosine receptors	
176 R ₅ = OCH ₃ 177 R ₅ = OC ₂ H ₅ 178 R ₅ = O(CH ₂) ₂ CH ₃			

Affinity and selectivity for A₃ adenosine receptors have only been reported in one study, to the best of our knowledge, in which three 2-SC were studied. Adenosine receptors are important targets for drug development as they have been associated with several biological functions, such as immune regulation and vascular function, but also with pathological processes, such as inflammatory and neurodegenerative diseases. Karton and co-workers [79] evaluated the potential of 2-SC **176–178** (Table 12) as A₃ adenosine antagonists, which the authors described to serve as anti-inflammatory, cerebroprotective, or antiasthmatic molecules. For this purpose, the affinity and selectivity of these 2-SC for A₁, A_{2A}, and A₃ adenosine receptors were evaluated and the observed effects are described in Table 12 [79].

The potential of 2-SC as neuroprotective agents has only been studied in four studies, to the best of our knowledge. Yoon and co-workers [8] evaluated the neuroprotective activity of the natural 2-SC **5** (Table 13) against glutamate-induced neurotoxicity in primary cultures of Sprague-Dawley rat cortical cells. Yang and co-workers [9] isolated the 2-SC **6** (Table 13) from the ethanolic extract of Chinese eaglewood and studied its neuroprotective activities against glutamate-induced and corticosterone-induced neurotoxicity in P12 pheochromocytoma and human U251 glioma cells, respectively. In another study, Jung and co-workers [11] isolated a 2-SC (**11**, Table 13) and evaluated its inhibitory potential against β -site amyloid precursor protein-cleaving enzyme 1 (BACE1), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). Years later, Takao and co-workers [80] synthesized a series of 2-SC (**1**, **30**, **56**, **58**, **88**, **92**, and **104–115**, Table 13) and evaluated their inhibitory activity against recombinant human monoamine oxidase (MAO)-A and MAO-B. The observed effects are described in Table 13.

Remarkably, a new series of 2-SC has been synthesized and evaluated for their possible application in the diagnostic as imaging probes for cerebral amyloid- β plaques and for abnormal prion protein aggregates. Even though it is not a biological activity, it demonstrates the great potential of 2-SC in the medical diagnostic field. For more detailed information, the works by Ono and co-workers [81], Fuchigami and co-workers [82], and Fuchigami and co-workers [83] elegantly describe these properties of 2-SC.

The potential of 3-SC as inhibitors of α -glucosidase has only been studied once, to the best of our knowledge. α -Glucosidase is a carbohydrase enzyme that catalyses the final step in the digestive process of carbohydrates. The inhibition of this enzyme activity is one of the therapeutic approaches for the treatment of diabetes *mellitus* [84]. Takao and co-workers [33] evaluated the α -glucosidase inhibitory activity of a series of 3-SC (**34–50**, Table 14), and the observed effects are described in Table 14.

5. Discussion

Several biological activities have been attributed to SC. Currently, two types of SC have been studied: 2-SC and 3-SC. The presence of SC in nature is scarce, however 2-SC are more common and are also the most chemically synthesized. Thus, they are most often reported in the literature for their biological properties. 2-SC have been reported to display antioxidant, antiallergic, antiviral, antibacterial, antifungal, anti-inflammatory, antitumor, neuroprotective properties and affinity and selectivity for A₃ adenosine receptors. 3-SC have only been attributed to have the following activities: antioxidant, antiviral, antitumor properties, and α -glucosidase inhibitory activities. This work presents a comprehensive review of the biological activities attributed to SC and their SAR. However, the number of existing reports as well as the variety of structural changes between the studied SC and the experimental differences between studies make it difficult to establish a generic SAR. Nevertheless, for each biological activity, some important structural aspects were identified.

The antioxidant activity of SC is one of the most studied biological activities, with many associated reports in the literature. These studies refer to different antioxidant effects, which leads to significant differences regarding the type of important substituents and their location in the structure. Nevertheless, in general, the presence of –OH groups on the B-ring seems to play an important role for this activity, particularly the presence of a catechol group. The presence of only one –OH at C-4' on the B-ring also revealed some importance, however it was overcome by the presence of the catechol group in the same ring. In addition, the presence of –OH groups on the A-ring also seems to contribute

TABLE 13: 2-SC studied for the neuroprotective activity, their chemical structures, and summary of the observed neuroprotective effects.

Chemical structure	2-SC	Observed effect(s)	Ref.	
	1	Inhibition of MAO-A and MAO-B activities, displaying MAO-B selectivity	[80]	
	5	NA	[8]	
	6	Neuroprotective activity against glutamate-induced neurotoxicity in P12 pheochromocytoma cells	[9]	
5 R ₅ = OH; R ₆ = R ₇ = R _{3'} = R _{4'} = H 6 R ₅ = R ₇ = H; R ₆ = R _{4'} = OH; R _{3'} = OCH ₃ 11 R ₅ = R ₇ = R _{4'} = OH; R ₆ = R _{3'} = H	11	Inhibition of AChE, BChE and BACE1	[11]	
	30	Inhibition of MAO-A and MAO-B activities	[80]	
	56			
	58			
	88	Inhibition of MAO-A and MAO-B activities, displaying MAO-B selectivity		
	92			
	104 - 111			
	30 R ₆ = R _{4'} = OCH ₃ ; R ₇ = R _{3'} = H 56 R ₆ = R ₇ = R _{3'} = H; R _{4'} = Cl 58 R ₆ = R ₇ = R _{3'} = H; R _{4'} = OCH ₃ 88 R ₆ = R ₇ = R _{3'} = H; R _{4'} = F 92 R ₆ = R ₇ = H; R _{3'} = R _{4'} = OCH ₃ 104 R ₆ = R ₇ = R _{3'} = H; R _{4'} = Br 105 R ₆ = OCH ₃ ; R ₇ = R _{3'} = R _{4'} = H 106 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = F 107 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = Cl 108 R ₆ = OCH ₃ ; R ₇ = R _{3'} = H; R _{4'} = Br 109 R ₆ = R _{3'} = R _{4'} = OCH ₃ ; R ₇ = H 110 R ₆ = R _{3'} = R _{4'} = H; R ₇ = OCH ₃ 111 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = F 112 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = Cl 113 R ₆ = R _{3'} = H; R ₇ = OCH ₃ ; R _{4'} = Br 114 R ₆ = R _{3'} = H; R ₇ = R _{4'} = OCH ₃ 115 R ₆ = H; R ₇ = R _{3'} = R _{4'} = OCH ₃	112, 113		Inhibition of MAO-B activity, displaying MAO-B selectivity
	114	Inhibition of MAO-A and MAO-B activities, displaying MAO-B selectivity		
	115	Inhibition of MAO-B activity, displaying MAO-B selectivity		

NA: no activity was found under tested experimental conditions. AChE: acetylcholinesterase; BACE1: β -site amyloid precursor protein cleaving enzyme 1; BChE: butyrylcholinesterase; MAO: monoamine oxidase.

to the antioxidant effects, especially if they are at C-5 and C-7 on the A-ring. Studies for the scavenging effects of ROS and RNS have shown that, depending on the reactive species, the styryl moiety also influences the antioxidant activity of 2-SC and can improve it. The importance of the role of -OH groups (C-3' and/or C-4') on the B-ring was also confirmed, because when they were methylated, the scavenging activity of 2-SC decreased. In contrast, the presence of

-OCH₃ at C-3' and C-4' on the B-ring improved the iron reducing activity of 2-SC. Finally, the presence of -OH at C-3 on the C-ring also seems to favour the scavenging activity against ROS and RNS. The studies of the antioxidant activity of 3-SC also corroborate the importance of the -OH groups on the B-ring for their antioxidant effects.

For the antiallergic activity, the presence of only one -CH₃ on the B-ring (C-2' or C-4') seems to increase it.

TABLE 14: 3-SC studied for α -glucosidase inhibitory activity, their chemical structures, and summary of the observed effects.

Chemical structure	3-SC	Observed effect(s)	Ref.
	34 - 39	NA	
	40, 41	Inhibition of α -glucosidase	
	42	NA	
	43 - 45	Inhibition of α -glucosidase	
	46	NA	
	34 $R_6 = R_3 = R_5 = H; R_4' = OCH_3$		
	35 $R_6 = R_5 = H; R_3' = R_4' = OCH_3$		
	36 $R_6 = H; R_3' = R_4' = R_5' = OCH_3$		
	37 $R_6 = R_4' = OCH_3; R_3' = R_5' = H$		
	38 $R_6 = R_3' = R_4' = OCH_3; R_5' = H$		
39 $R_6 = R_3' = R_4' = R_5' = OCH_3$			
40 $R_6 = R_3' = R_5' = H; R_4' = F$			
41 $R_6 = R_3' = R_5' = H; R_4' = Cl$			
42 $R_6 = OCH_3; R_3' = R_5' = H; R_4' = F$	47 - 50	Inhibition of α -glucosidase	
43 $R_6 = OCH_3; R_3' = R_5' = H; R_4' = Cl$			
44 $R_6 = R_3' = R_5' = H; R_4' = OH$			
45 $R_6 = R_5' = H; R_3' = R_4' = OH$			
46 $R_6 = OCH_3; R_3' = R_5' = H; R_4' = OH$			
47 $R_6 = OCH_3; R_3' = R_4' = OH; R_5' = H$			
48 $R_6 = OH; R_3' = R_5' = H; R_4' = OCH_3$			
49 $R_6 = R_4' = OH; R_3' = R_5' = H$			
50 $R_6 = R_3' = R_4' = OH; R_5' = H$			

[33]

NA: no activity was found under tested experimental conditions.

The presence of small alkyl groups at C-3 on the C-ring also seems to contribute to the antiallergic activity of the studied 2-SC.

The antiviral potential of SC has been studied for different viruses, and the same SC have demonstrated different interactions with the different studied viruses and viruses' strains. These differences between the antiviral effects shown by SC indicate some selectivity, being an advantage for their use as antiviral agents. The analysis of the antiviral activity of 2-SC showed that it is significantly influenced by the substituents present in the structure and its location, however it is difficult to indicate in a generic way which substituents play the most important role. The presence of $-Cl$ at C-6 on the A-ring, $-OH$ or $-OCH_3$ at C-3 on the C-ring, and $-NO_2$ at C-4' on the B-ring seems to favour 2-SC antiviral activity against serotype 1B of HRV. These substituents also seem to be important for the antiviral activity of 2-SC against serotype 14 of HRV; however, the presence of $-F$ at C-6 on the A-ring and $-Cl$ at C-4' on the B-ring also seems to contribute to this activity. 2-SC antiviral activity against MNV was also studied and demonstrated the importance of the presence of the $-OH$ group at C-5 on the A-ring and the $-OCH_3$ group at C-4' on the B-ring for the antiviral activity against MNV. The inhibitory activity of 2-SC against the HIV-1 integrase was another of the studied antiviral effects, but only one 2-SC was evaluated, which was not very effective against the HIV-1 integrase. In what concerns 3-SC antiviral activity, there are few studies related to their antiviral properties, however in the reports found in the literature, 3-SC have shown few antiviral effects. 3-SC were studied

against HIV, EV, and the serotypes 1B and 14 of HRV. The studied 3-SC did not show anti-HIV activity. In addition, it was only possible to conclude that the presence of $-Cl$ at C-6 on the A-ring and C-4' on the B-ring seems to play an important role for the antiviral effect against HRV 1B. The studied 3-SC showed low efficacy against EV and HRV 14, so it was not possible to establish a SAR.

As previously mentioned, for the antibacterial and antifungal activities of SC, only 2-SC were studied and there are no reports of 3-SC with antibacterial and antifungal potential, to the best of our knowledge. The antibacterial activity of 2-SC was studied for different strains of Gram-positive and Gram-negative bacteria, with the tested 2-SC showing some variability in what concerns the results observed for each strain and between Gram-positive and negative bacteria, which makes it difficult to establish a clear SAR. Additionally, the studied 2-SC included several substituents which did not present coherent activities patterns, which also makes it difficult to establish of a specific SAR. Thus, only a generic SAR has been established for the antibacterial activity. The antibacterial activity of 2-SC seems to be influenced by the position and the number of $-F$ substituents present on the A- or B-rings, as well as by the existence of $-Cl$ or $-OH$ at C-4' on the B-ring. The C-6 on the A-ring and C-4' on the B-ring also seem to be important substituents' positions for the antibacterial effects of 2-SC. For most 2-SC, the authors who reported their antibacterial properties, also assessed their antifungal potential. As reported for the antibacterial activity, the 2-SC studied included different substitution patterns. This fact, combined

with differences in the used methodology between studies, only allowed the establishment of a generic SAR for antifungal activity. Similar to what was reported for the bacterial activity, the presence of the 1,2,4-triazole ring at C-4' on the B-ring appears to contribute to the antifungal activity, as well as the existence of -Cl at C-4' on the B-ring. The -OCH₃ group at C-4' on the B-ring also seems important for this activity. Summarizing, in part, the substituents that favour antibacterial activity also favour antifungal activity, which seems to indicate a weak selectivity by 2-SC.

Several anti-inflammatory mechanisms have been described for 2-SC, namely, inhibition of LFA-1/ICAM-1-mediated cell adhesion, interference with the arachidonic acid metabolic pathways, inhibition of NF- κ B activation, and modulation of cytokines/chemokine production. Despite the reported studies being quite different and evaluating several mediators, the presence of -OH at C-3' and C-4' on the B-ring seems to generically favour the anti-inflammatory activity, similarly to what has been demonstrated for the antioxidant activity.

The antitumor potential is one of the most explored biological activities in the literature for SC. The antitumor properties of 2-SC are widely described, however there are few studies describing the antitumoral effects of 3-SC. Some of the existing studies, in addition to the evaluation of the effects of SC on tumor cell lines, also evaluate their effects on normal cells, which allows to understand whether the effects of SC are tumor selective. In addition to selectivity, it is important to know whether SC are also cytotoxic to normal cells. This fact is determinant for their use as possible antitumor agents, since the major objective is to demonstrate cytotoxicity against tumor cells, affecting as little as possible normal cells. Unfortunately, many of the studies reported did not include normal cell lines in their evaluation, making it difficult to understand if and in what extension SC are selective for tumor cell lines. In the cases where both cell types were studied, in general, SC were cytotoxic to tumor cells and did not show cytotoxicity to normal cells. These studies cover a wide variety of tumor cell lines, so it is difficult to indicate which type of cancer is most studied and to establish SAR. Nevertheless, in general, the presence of -OCH₃ groups on the B-ring seems to play an important role in the antitumor activity performed by 2-SC, especially when the -OCH₃ is at C-4' on the B-ring. Furthermore, the simultaneous presence of -OCH₃ groups at C-3', C-4', and C-5' on the B-ring also seems to potentiate the antitumor effects of 2-SC. The styryl moiety seems to favour the growth inhibitory activity. The existence of -OCH₃ at C-6 on the A-ring also appear to favour cytotoxic activity against tumor cell lines. All in all, the studies of the antitumoral activity of 3-SC seem to indicate that the presence of -OCH₃ at C-6 or C-7 on the A-ring and a-OH at C-4' on the B-ring favour cytotoxic activity against tumor cell lines.

The neuroprotective activity of SC is one of the biological activities less explored in the literature, since only the possible neuroprotective effects of three 2-SC were reported. Among these three 2-SC studied, only two showed neuroprotective properties: neuroprotective activity against

glutamate-induced neurotoxicity in P12 pheochromocytoma cells and inhibition of AChE, BChE, and BACE1. Since the neuroprotective activity of 2-SC has been less studied and the effects evaluated are very different, it is difficult to identify the substituents that favour their effect, because in each study, a different 2-SC was evaluated.

As mentioned in the beginning of this section, in addition to the biological activities mentioned above, other activities have also been reported for SC, namely, affinity and selectivity for the A₃ adenosine receptor, inhibitory activity of MAO-A and MAO-B, and inhibitory activity of α -glucosidase. In what concerns the affinity and selectivity of 2-SC for adenosine receptors, the presence of -OC₂H₅ at C-5 on the A-ring appears to favour the affinity and selectivity for the A₃ adenosine receptor. For the MAO inhibitory activity, the presence of -OCH₃ at C-6 on the A-ring and halogenated groups (-F, -Cl, or -Br) at C-4' on the B-ring favoured the MAO-A inhibitory activity; while the presence of -OCH₃ at C-6 on the A-ring and -Cl or -Br at C-4' on the B-ring favoured the MAO-B inhibitory activity and selectivity. The α -glucosidase inhibitory activity of 3-SC was influenced by the presence of the -OH group at C-6 on the A-ring and -OH at C-3' and C-4' or at C-4' on the B-ring; however, the 3-SC with -OH at C-3' and C-4' on the B-ring was more potent than the 3-SC with just an -OH at C-4' on the B-ring.

6. Conclusions

This work demonstrates that SC exhibit a large variety of biological properties. Throughout this work, it can be observed that a wide variety of SC structures has already been studied, most of which with substituent variations on the A- and/or B-rings. The type of substituents in the SC structure and their location assume an essential role in each one of the activities shown. This variety, added to the experimental differences between studies, makes it difficult to establish a generic SAR for each activity. The antioxidant and antitumor activities of SC are undoubtedly the most explored ones in the literature. In conclusion, SC present a molecular scaffold that demonstrates an enormous potential for the development of new drugs envisioning the treatment of several oxidative process-related diseases, namely, inflammatory, carcinogenic, and neurodegenerative.

Abbreviations

2-SC:	2-Styrylchromones
3-SC:	3-Styrylchromones
CC ₅₀ :	Concentration that causes 50% of cytotoxicity
CDK:	Cyclin-dependent kinase
CHO:	Chinese hamster ovary
CNS:	Central nervous system
COX:	Cyclooxygenase
DCFH-DA:	2',7'-Dichlorodihydrofluorescein diacetate
DIZ:	Diameter of inhibition zone
DPPH [•] :	2,2-Diphenyl-1-picrylhydrazyl radical
EC ₅₀ :	Concentration that produces 50% of the maximum effect

EV:	Enterovirus
GI:	Growth inhibition
GI ₅₀ :	Concentration that produces 50% of cell growth inhibition
GSK-3 β :	Glycogen synthase kinase 3-beta
HIV-1:	Human immunodeficiency virus type 1
HRV:	Human rhinovirus
IC ₅₀ :	Concentration that produces 50% of inhibition
ICAM-1:	Intercellular cell adhesion molecule-1
IL:	Interleukin
Keap1:	Kelch ECH-associating protein 1
LDL:	Low-density lipoproteins
LFA-1:	Lymphocyte function-associated molecule-1
log <i>P</i> :	Logarithm of partition coefficient of compound between <i>n</i> -octanol and water
LT:	Leukotriene
MAO:	Monoamine oxidase
MIC:	Minimum inhibitory concentration
MNTC:	Maximum non-toxic concentration
MNV:	Murine norovirus
MTT:	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA:	No activity
NADH:	β -Nicotinamide adenine dinucleotide
NF- κ B:	Nuclear factor kappa B
NoV:	Human noroviruses
Nrf2:	Nuclear factor erythroid 2-related factor 2
PSE:	Potency-selectivity expression
QSAR:	Quantitative structure-activity relationship
Ref.:	Reference
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
SAR:	Structure-activity relationship
SC:	Styrylchromones
SI:	Selectivity index
SRB:	Sulforhodamine B
<i>t</i> -BHP:	<i>tert</i> -Butyl hydroperoxide
TNF- α :	Tumor necrosis factor- α
XO:	Xanthine oxidase
XTT:	2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 <i>H</i> -tetrazolium-5-carboxanilide.

Conflicts of Interest

The authors declare no conflict of interest.

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References

- [1] A. Gaspar, M. J. Matos, J. Garrido, E. Uriarte, and F. Borges, "Chromone: a valid scaffold in medicinal chemistry," *Chemical Reviews*, vol. 114, no. 9, pp. 4960–4992, 2014.
- [2] R. S. Keri, S. Budagumpi, R. K. Pai, and R. G. Balakrishna, "Chromones as a privileged scaffold in drug discovery: a review," *European Journal of Medicinal Chemistry*, vol. 78, pp. 340–374, 2014.
- [3] C. M. M. Santos and A. M. S. Silva, "An overview of 2-styrylchromones: natural occurrence, synthesis, reactivity and biological properties," *European Journal of Organic Chemistry*, vol. 2017, no. 22, pp. 3115–3133, 2017.
- [4] A. Gomes, M. Freitas, E. Fernandes, and J. L. F. C. Lima, "Biological activities of 2-styrylchromones," *Mini-Reviews in Medicinal Chemistry*, vol. 10, no. 1, pp. 1–7, 2010.
- [5] H. Sakagami, C. Shimada, Y. Kanda et al., "Effects of 3-styrylchromones on metabolic profiles and cell death in oral squamous cell carcinoma cells," *Toxicology Reports*, vol. 2, pp. 1281–1290, 2015.
- [6] W. H. Gerwick, A. Lopez, G. D. van Duyne, J. Clardy, W. Ortiz, and A. Baez, "Hormothamnione, a novel cytotoxic styrylchromone from the marine cyanophyte *Hormothamnion entermorphoides* grunow," *Tetrahedron Letters*, vol. 27, no. 18, pp. 1979–1982, 1986.
- [7] W. H. Gerwick, "6-Desmethoxyhormothamnione, a new cytotoxic styrylchromone from the marine cryptophyte *Chrysophaeum taylori*," *Journal of Natural Products*, vol. 52, no. 2, pp. 252–256, 1989.
- [8] J. S. Yoon, M. K. Lee, S. H. Sung, and Y. C. Kim, "Neuroprotective 2-(2-phenylethyl)chromones of *Imperata cylindrica*," *Journal of Natural Products*, vol. 69, no. 2, pp. 290–291, 2006.
- [9] L. Yang, L. Qiao, D. Xie et al., "2-(2-Phenylethyl)chromones from Chinese eaglewood," *Phytochemistry*, vol. 76, pp. 92–97, 2012.
- [10] C.-H. Yang, Y. Yang, and J. H. Liu, "Platachromone A-D: Cytotoxic 2-styrylchromones from the bark of *Platanus \times acerifolia* (Aiton) Willd.," *Phytochemistry Letters*, vol. 6, no. 3, pp. 387–391, 2013.
- [11] H. J. Jung, H. A. Jung, B.-S. Min, and J. S. Choi, "Anticholinesterase and β -site amyloid precursor protein cleaving enzyme 1 inhibitory compounds from the heartwood of *Juniperus chinensis*," *Chemical and Pharmaceutical Bulletin*, vol. 63, no. 11, pp. 955–960, 2015.
- [12] P. Chaniad, C. Wattanapiromsakul, S. Pianwanit, and S. Tewtrakul, "Anti-HIV-1 integrase compounds from *Dioscorea bulbifera* and molecular docking study," *Pharmaceutical Biology*, vol. 54, no. 6, pp. 1077–1085, 2016.
- [13] F. A. Hashem, "Phenolic compounds of *Erucaria microcarpa*-Boiss. And their effect as scavengers for singlet oxygen," *Journal of Herbs, Spices & Medicinal Plants*, vol. 12, no. 3, pp. 27–41, 2007.
- [14] D. C. G. A. Pinto, A. M. S. Silva, and J. A. S. Cavaleiro, "A convenient synthesis of new (*E*)-5-hydroxy-2-styrylchromones by modifications of the Baker–Venkataraman method," *New Journal of Chemistry*, vol. 24, no. 2, pp. 85–92, 2000.

- [15] C. M. M. Santos, A. M. S. Silva, and J. A. S. Cavaleiro, "Synthesis of new hydroxy-2-styrylchromones," *European Journal of Organic Chemistry*, vol. 2003, no. 23, pp. 4575–4585, 2003.
- [16] J. A. S. Cavaleiro, W. A. Price, and A. M. S. Silva, "2-Styrylchromones: biological action, synthesis and reactivity," *Heterocycles*, vol. 36, no. 11, pp. 2601–2612, 1993.
- [17] A. M. S. Silva, D. C. G. A. Pinto, J. A. S. Cavaleiro, A. Levai, and T. Patonay, "Synthesis and reactivity of styrylchromones," *ARKIVOC*, vol. 2004, no. 7, pp. 106–123, 2004.
- [18] V. L. M. Silva, A. M. S. Silva, D. C. G. A. Pinto, J. A. S. Cavaleiro, A. Vasas, and T. Patonay, "Syntheses of (*E*)- and (*Z*)-3-styrylchromones," *Monatshefte für Chemie - Chemical Monthly*, vol. 139, no. 11, pp. 1307–1315, 2008.
- [19] D. H. A. Rocha, D. Pinto, and A. Silva, "One-pot synthesis of 3-Methylflavones and their transformation into (*E*)-3-Styrylflavones via Wittig reactions," *Synlett*, vol. 24, no. 20, pp. 2683–2686, 2013.
- [20] A. M. Pisoschi and A. Pop, "The role of antioxidants in the chemistry of oxidative stress: a review," *European Journal of Medicinal Chemistry*, vol. 97, pp. 55–74, 2015.
- [21] A. Gomes, E. Fernandes, and J. L. F. C. Lima, "Fluorescence probes used for detection of reactive oxygen species," *Journal of Biochemical and Biophysical Methods*, vol. 65, no. 2-3, pp. 45–80, 2005.
- [22] E. Fernandes, F. Carvalho, A. M. S. Silva et al., "2-Styrylchromones as novel inhibitors of xanthine oxidase. A structure-activity study," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 17, no. 1, pp. 45–48, 2002.
- [23] M. G. Battelli, L. Polito, M. Bortolotti, and A. Bolognesi, "Xanthine oxidoreductase-derived reactive species: physiological and pathological effects," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 3527579, 8 pages, 2016.
- [24] E. Fernandes, M. Carvalho, F. Carvalho et al., "Hepatoprotective activity of polyhydroxylated 2-styrylchromones against *tert*-butylhydroperoxide induced toxicity in freshly isolated rat hepatocytes," *Archives of Toxicology*, vol. 77, no. 9, pp. 500–505, 2003.
- [25] P. Filipe, A. M. S. Silva, P. Morlière et al., "Polyhydroxylated 2-styrylchromones as potent antioxidants," *Biochemical Pharmacology*, vol. 67, no. 12, pp. 2207–2218, 2004.
- [26] A. Gomes, E. Fernandes, A. M. S. Silva et al., "2-Styrylchromones: novel strong scavengers of reactive oxygen and nitrogen species," *Bioorganic & Medicinal Chemistry*, vol. 15, no. 18, pp. 6027–6036, 2007.
- [27] A. Gomes, E. Fernandes, M. B. Q. Garcia et al., "Cyclic voltammetric analysis of 2-styrylchromones: relationship with the antioxidant activity," *Bioorganic & Medicinal Chemistry*, vol. 16, no. 17, pp. 7939–7943, 2008.
- [28] A. Gomes, O. Neuwirth, M. Freitas et al., "Synthesis and antioxidant properties of new chromone derivatives," *Bioorganic & Medicinal Chemistry*, vol. 17, no. 20, pp. 7218–7226, 2009.
- [29] J. L. C. Sousa, C. Proença, M. Freitas, E. Fernandes, and A. M. S. Silva, "New polyhydroxylated flavon-3-ols and 3-hydroxy-2-styrylchromones: synthesis and ROS/RNS scavenging activities," *European Journal of Medicinal Chemistry*, vol. 119, pp. 250–259, 2016.
- [30] V. M. Rao, B. Ujwala, P. Priyadarsini, and P. Krishna Murthy, "Synthesis, antioxidant and antimicrobial activity of three new 2-styrylchromones and their analogues," *Der Pharma Chemica*, vol. 8, no. 7, pp. 1–6, 2016.
- [31] R. J. Anto, G. Kuttan, R. Kuttan, K. Sathyanarayana, and M. N. A. Rao, "Tumor-reducing and antioxidant activities of sydnone-substituted chalcones," *Journal of Clinical Biochemistry and Nutrition*, vol. 17, no. 2, pp. 73–80, 1994.
- [32] S. Takamatsu, T. W. Hodges, I. Rajbhandari, W. H. Gerwick, M. T. Hamann, and D. G. Nagle, "Marine natural products as novel antioxidant prototypes," *Journal of Natural Products*, vol. 66, no. 5, pp. 605–608, 2003.
- [33] K. Takao, R. Ishikawa, and Y. Sugita, "Synthesis and biological evaluation of 3-styrylchromone derivatives as free radical scavengers and α -glucosidase inhibitors," *Chemical and Pharmaceutical Bulletin*, vol. 62, no. 8, pp. 810–815, 2014.
- [34] S. J. Galli, M. Tsai, and A. M. Piliponsky, "The development of allergic inflammation," *Nature*, vol. 454, no. 7203, pp. 445–454, 2008.
- [35] T. G. Villa, L. Feijoo-Siota, J. L. R. Rama, and J. M. Ageitos, "Antivirals against animal viruses," *Biochemical Pharmacology*, vol. 133, pp. 97–116, 2017.
- [36] M.-Z. Zhang, Q. Chen, and G.-F. Yang, "A review on recent developments of indole-containing antiviral agents," *European Journal of Medicinal Chemistry*, vol. 89, pp. 421–441, 2015.
- [37] N. Desideri, C. Conti, P. Mastromarino, and F. Mastropaolo, "Synthesis and anti-rhinovirus activity of 2-styrylchromones," *Antiviral Chemistry and Chemotherapy*, vol. 11, no. 6, pp. 373–381, 2000.
- [38] N. Desideri, P. Mastromarino, and C. Conti, "Synthesis and evaluation of antirhinovirus activity of 3-hydroxy and 3-methoxy 2-styrylchromones," *Antiviral Chemistry and Chemotherapy*, vol. 14, no. 4, pp. 195–203, 2003.
- [39] C. Conti, P. Mastromarino, P. Goldoni, G. Portalone, and N. Desideri, "Synthesis and anti-rhinovirus properties of fluoro-substituted flavonoids," *Antiviral Chemistry and Chemotherapy*, vol. 16, no. 4, pp. 267–276, 2005.
- [40] J. Rocha-Pereira, R. Cunha, D. C. G. A. Pinto, A. M. S. Silva, and M. S. J. Nascimento, "(*E*)-2-Styrylchromones as potential anti-norovirus agents," *Bioorganic & Medicinal Chemistry*, vol. 18, no. 12, pp. 4195–4201, 2010.
- [41] M. M. Patel, A. J. Hall, J. Vinjé, and U. D. Parashar, "Noroviruses: a comprehensive review," *Journal of Clinical Virology*, vol. 44, no. 1, pp. 1–8, 2009.
- [42] J. W. Sleasman and M. M. Goodenow, *Principles of Molecular Medicine*, J. L. Jameson, Ed., Humana Press, Totowa, NJ, 1998.
- [43] C. Shimada, Y. Uesawa, R. Ishii-Nozawa et al., "Quantitative structure-cytotoxicity relationship of 3-styrylchromones," *Anticancer Research*, vol. 34, no. 10, pp. 5405–5411, 2014.
- [44] C. Conti and N. Desideri, "New 4*H*-chromen-4-one and 2*H*-chromene derivatives as anti-picornavirus capsid-binders," *Bioorganic & Medicinal Chemistry*, vol. 18, no. 17, pp. 6480–6488, 2010.
- [45] K. Singh, A. Mishra, and D. Sharma, Eds. K. Singh, *Applications of Targeted Nano Drugs and Delivery Systems*, K. Singh, A. Mishra, D. Sharma, R. K. Mishra, S. Thomas, , , and , Eds., Elsevier, 2019.
- [46] M. Momin, D. Ramjugernath, H. Chenia, and N. A. Koorbanally, "Synthesis and evaluation of novel fluorinated 2-styrylchromones as antibacterial agents," *Journal of Chemistry*, vol. 2013, Article ID 436758, 13 pages, 2013.
- [47] M. D. Nikam, P. Mahajan, M. Damale et al., "Novel amalgamation of 2-styrylchromones and 1,2,4-triazole: synthesis, antimicrobial evaluation and docking study," *Letters in Drug Design & Discovery*, vol. 12, no. 8, pp. 650–660, 2015.

- [48] B. Ujwala, P. Priyadarsini, and V. M. Rao, "Synthesis and bioactivity evaluation of 2-styrylchromones," *International Journal of Pharma and Bio Sciences*, vol. 4, no. 1, pp. 199–206, 2013.
- [49] G. D. Brown, D. W. Denning, N. A. R. Gow, S. M. Levitz, M. G. Netea, and T. C. White, "Hidden killers: human fungal infections," *Science Translational Medicine*, vol. 4, no. 165, p. 165rv13, 2012.
- [50] K. C. Howard, E. K. Dennis, D. S. Watt, and S. Garneau-Tsodikova, "A comprehensive overview of the medicinal chemistry of antifungal drugs: perspectives and promise," *Chemical Society Reviews*, vol. 49, no. 8, pp. 2426–2480, 2020.
- [51] A. Azab, A. Nassar, and A. Azab, "Anti-inflammatory activity of natural products," *Molecules*, vol. 21, no. 10, p. 1321, 2016.
- [52] S. Takamatsu, D. G. Nagle, and W. H. Gerwick, "Secondary metabolites from marine cyanobacteria and algae inhibit LFA-1/ICAM-1 mediated cell adhesion," *Planta Medica*, vol. 70, no. 2, pp. 127–131, 2004.
- [53] E. Martz, *Encyclopedia of Immunology*, P. J. Delves, Ed., Elsevier, Oxford, Second Edition edition, 1998.
- [54] A. Gomes, E. Fernandes, A. M. S. Silva et al., "Anti-inflammatory potential of 2-styrylchromones regarding their interference with arachidonic acid metabolic pathways," *Biochemical Pharmacology*, vol. 78, no. 2, pp. 171–177, 2009.
- [55] V. S. Hanna and E. A. A. Hafez, "Synopsis of arachidonic acid metabolism: a review," *Journal of Advanced Research*, vol. 11, pp. 23–32, 2018.
- [56] F. Cipollone, G. Cicolini, and M. Bucci, "Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives," *Pharmacology & Therapeutics*, vol. 118, no. 2, pp. 161–180, 2008.
- [57] D. Ribeiro, M. Freitas, J. L. F. C. Lima, and E. Fernandes, "Pro-inflammatory pathways: the modulation by flavonoids," *Medicinal Research Reviews*, vol. 35, no. 5, pp. 877–936, 2015.
- [58] E. Ricciotti and G. A. FitzGerald, "Prostaglandins and inflammation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 5, pp. 986–1000, 2011.
- [59] A. Gomes, J. Capela, D. Ribeiro et al., "Inhibition of NF- κ B activation and cytokines production in THP-1 monocytes by 2-styrylchromones," *Medicinal Chemistry*, vol. 11, no. 6, pp. 560–566, 2015.
- [60] G. Ramesh, A. G. MacLean, and M. T. Philipp, "Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain," *Mediators of Inflammation*, vol. 2013, Article ID 480739, 20 pages, 2013.
- [61] M. D. Turner, B. Nedjai, T. Hurst, and D. J. Pennington, "Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease," *Biochimica et Biophysica Acta*, vol. 1843, no. 11, pp. 2563–2582, 2014.
- [62] N. Korgaonkar and K. S. Yadav, "Understanding the biology and advent of physics of cancer with perspicacity in current treatment therapy," *Life Sciences*, vol. 239, article 117060, 2019.
- [63] "U. S. National Institutes of Health: National Cancer Institute SEER Training Modules: Cancer Terms," 2020, <https://training.seer.cancer.gov/disease/cancer/terms.html>.
- [64] K. Momoi, Y. Sugita, M. Ishihara et al., "Cytotoxic activity of styrylchromones against human tumor cell lines," *In Vivo*, vol. 19, no. 1, pp. 157–163, 2005.
- [65] J. Marinho, M. Pedro, D. C. G. A. Pinto et al., "4'-Methoxy-2-styrylchromone a novel microtubule-stabilizing antimetabolic agent," *Biochemical Pharmacology*, vol. 75, no. 4, pp. 826–835, 2008.
- [66] Y. Uesawa, J. Nagai, H. Shi et al., "Quantitative structure–cytotoxicity relationship of 2-styrylchromones," *Anticancer Research*, vol. 39, no. 12, pp. 6489–6498, 2019.
- [67] A. Y. Shaw, C.-Y. Chang, H.-H. Liao et al., "Synthesis of 2-styrylchromones as a novel class of antiproliferative agents targeting carcinoma cells," *European Journal of Medicinal Chemistry*, vol. 44, no. 6, pp. 2552–2562, 2009.
- [68] C. Lin, P.-J. Lu, C.-N. Yang, C. Hulme, and A. Y. Shaw, "Structure-activity relationship study of growth inhibitory 2-styrylchromones against carcinoma cells," *Medicinal Chemistry Research*, vol. 22, no. 5, pp. 2385–2394, 2013.
- [69] K. Y. Lee, D. H. Nam, C. S. Moon, S. H. Seo, J. Y. Lee, and Y. S. Lee, "Synthesis and anticancer activity of lavendustin a derivatives containing aryloxychromone substituents," *European Journal of Medicinal Chemistry*, vol. 41, no. 8, pp. 991–996, 2006.
- [70] O. Talhi, L. Brodziak-Jarosz, J. Panning et al., "One-pot synthesis of benzopyran-4-ones with cancer preventive and therapeutic potential," *European Journal of Organic Chemistry*, vol. 2016, no. 5, pp. 965–975, 2016.
- [71] L. Baird and A. T. Dinkova-Kostova, "The cytoprotective role of the Keap1–Nrf2 pathway," *Archives of Toxicology*, vol. 85, no. 4, pp. 241–272, 2011.
- [72] E. Kansanen, S. M. Kuosmanen, H. Leinonen, and A.-L. Levonen, "The Keap1–Nrf2 pathway: mechanisms of activation and dysregulation in cancer," *Redox Biology*, vol. 1, no. 1, pp. 45–49, 2013.
- [73] F. A. Ragab, T. A. A. Yahya, M. M. El-Naa, and R. K. Arafa, "Design, synthesis and structure-activity relationship of novel semi-synthetic flavonoids as antiproliferative agents," *European Journal of Medicinal Chemistry*, vol. 82, pp. 506–520, 2014.
- [74] D. O. Morgan, "Cyclin-dependent kinases: engines, clocks, and microprocessors," *Annual Review of Cell and Developmental Biology*, vol. 13, no. 1, pp. 261–291, 1997.
- [75] C. A. Grimes and R. S. Jope, "The multifaceted roles of glycogen synthase kinase β in cellular signaling," *Progress in Neurobiology*, vol. 65, no. 4, pp. 391–426, 2001.
- [76] F. Peixoto, A. I. R. N. A. Barros, and A. M. S. Silva, "Interactions of a new 2-styrylchromone with mitochondrial oxidative phosphorylation," *Journal of Biochemical and Molecular Toxicology*, vol. 16, no. 5, pp. 220–226, 2002.
- [77] L. M. Knobeloch, G. A. Blondin, H. W. Read, and J. M. Harkin, "Assessment of chemical toxicity using mammalian mitochondrial electron transport particles," *Archives of Environmental Contamination and Toxicology*, vol. 19, no. 6, pp. 828–835, 1990.
- [78] K. Takao, K. Hoshi, H. Sakagami et al., "Further quantitative structure-cytotoxicity relationship analysis of 3-styrylchromones," *Anticancer Research*, vol. 40, no. 1, pp. 87–95, 2020.
- [79] Y. Karton, J. L. Jiang, X. D. Ji et al., "Synthesis and biological activities of flavonoid derivatives as A_3 adenosine receptor antagonists," *Journal of Medicinal Chemistry*, vol. 39, no. 12, pp. 2293–2301, 1996.
- [80] K. Takao, S. Endo, J. Nagai et al., "2-Styrylchromone derivatives as potent and selective monoamine oxidase B inhibitors," *Bioorganic Chemistry*, vol. 92, article 103285, 2019.
- [81] M. Ono, Y. Maya, M. Haratake, and M. Nakayama, "Synthesis and characterization of styrylchromone derivatives as β -

- amyloid imaging agents," *Bioorganic & Medicinal Chemistry*, vol. 15, no. 1, pp. 444–450, 2007.
- [82] T. Fuchigami, A. Ogawa, Y. Yamashita et al., "Development of alkoxy styrylchromone derivatives for imaging of cerebral amyloid- β plaques with SPECT," *Bioorganic & Medicinal Chemistry Letters*, vol. 25, no. 16, pp. 3363–3367, 2015.
- [83] T. Fuchigami, Y. Yamashita, M. Kawasaki et al., "Characterisation of radioiodinated flavonoid derivatives for SPECT imaging of cerebral prion deposits," *Scientific Reports*, vol. 5, no. 1, p. 18440, 2015.
- [84] S. Kumar, O. Prakash, S. Kumar, and S. Narwal, " α -Glucosidase inhibitors from plants: a natural approach to treat diabetes," *Pharmacognosy Reviews*, vol. 5, no. 9, pp. 19–29, 2011.