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MINI REVIEW

A mini-review on Biginelli adducts with notable pharmacological properties

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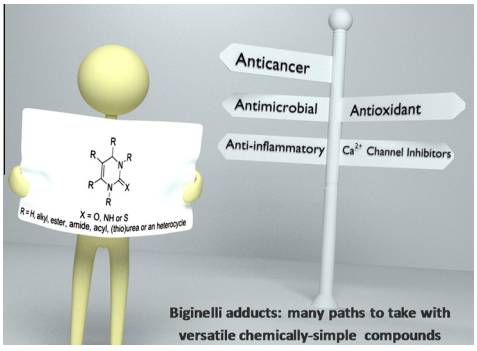
GRAPHICAL ABSTRACT



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ABSTRACT

Since the disclosure of Biginelli reaction by the chemist Pietro Biginelli, functionalized 3,4-dihydropyrimidin-2(1*H*)-ones/thiones (DHPMs) have emerged as prototypes for the design of compounds with a broad variety of biological activities. This mini-review describes over 100 Biginelli adducts demonstrated to be promising anticancer, inhibitors of calcium channel,

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anti-inflammatory, antimicrobial and antioxidant agents. Thus, this compilation presents the most notable *in vitro* and *in vivo* results for such fascinating class of organic compounds.

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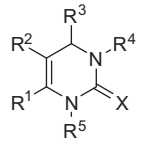
Introduction

The year 1891 was a milestone for the discovery of a new class of heterocycle molecules named Biginelli adducts after the chemist Pietro Biginelli who first report the simple one-pot process that furnish organic compounds of this kind [1]. The multicomponent reaction that provides Biginelli adducts, also



X = O, NH or S
R^{1-5} = H, alkyl, aryl, ester, amide, acyl, (thio)urea or an heterocycle

Fig. 1 Basic structure of Biginelli adducts.

known as 3,4-dihydropyrimidin-2(1*H*)-ones/thiones (DHPMs; Fig. 1), involves the reaction of 1,3-dicarbonyl compounds with aldehydes and (thio)urea [2]. Three main mechanisms have been proposed for the Biginelli reaction, but this subject is still under debate in the literature. Detailed information on these three mechanisms is addressed elsewhere [3]. Variation of all three building blocks has broadened the molecular diversity of DHPMs with wide variety biological activities. Indeed, a series of pharmacological properties of DHPMs have been reported, which include antiviral, antitumor, anti-inflammatory, antibacterial, antifungal, anti-epileptic, antimalarial, antileishmanial, among others. The next topics will cover for some of the most notable Biginelli adducts reported as anticancer, calcium channel inhibitors, anti-inflammatory, antimicrobial and antioxidant agents since the listed pharmacological properties are some of the most investigated for DHPMs.

Anticancer activity

Biginelli adducts are promising compounds for the treatment of cancers in which monastrol (**1**) is the most studied with this regard (Fig. 2). The first work that explored the effect of monastrol on cancer cells was reported in 1999 [4]. Monastrol was found to interrupt mitosis by inhibiting the motor activity of the kinesin Eg5, a protein involved in spindle bipolarity formation [5].

Since then, monastrol has been used as an inspiration for the design of new anticancer agents. Out of eleven monastrol analogues synthesized, the Biginelli adduct **2** was identified as a potent anticancer agent based on the concentration of this adduct necessary to inhibit cell growth by 50% (EC_{50}, IC_{50} or GI_{50}) as it follows: MCF-7 breast (1.9 μg mL^{-1}), 786-0 kidney (2.0 μg mL^{-1}), HT-29 colon (2.5 μg mL^{-1}), UACC.62 melanoma (6.0 μg mL^{-1}) and OVCAR03 ovarian (6.6 μg mL^{-1}) cancer cells [5].

Compounds **3**–**10** (Fig. 2) were described as some of the most effective pyrimidinone-peptoid hybrids against SK-BR-3 breast cancer cells, exhibiting GI_{50} values in the range of 6.0–8.8 μM [6].

Biginelli adducts bearing cinnamoyl (**11** and **12**), pyridin-4-yl (**13**) or furan-2-yl (**14** and **15**) groups (Fig. 2) showed significant cytotoxic effects against the MCF-7 breast cancer cell line, in which at concentration of 50 μg mL^{-1} prevented cell growth by at least 70% [7].

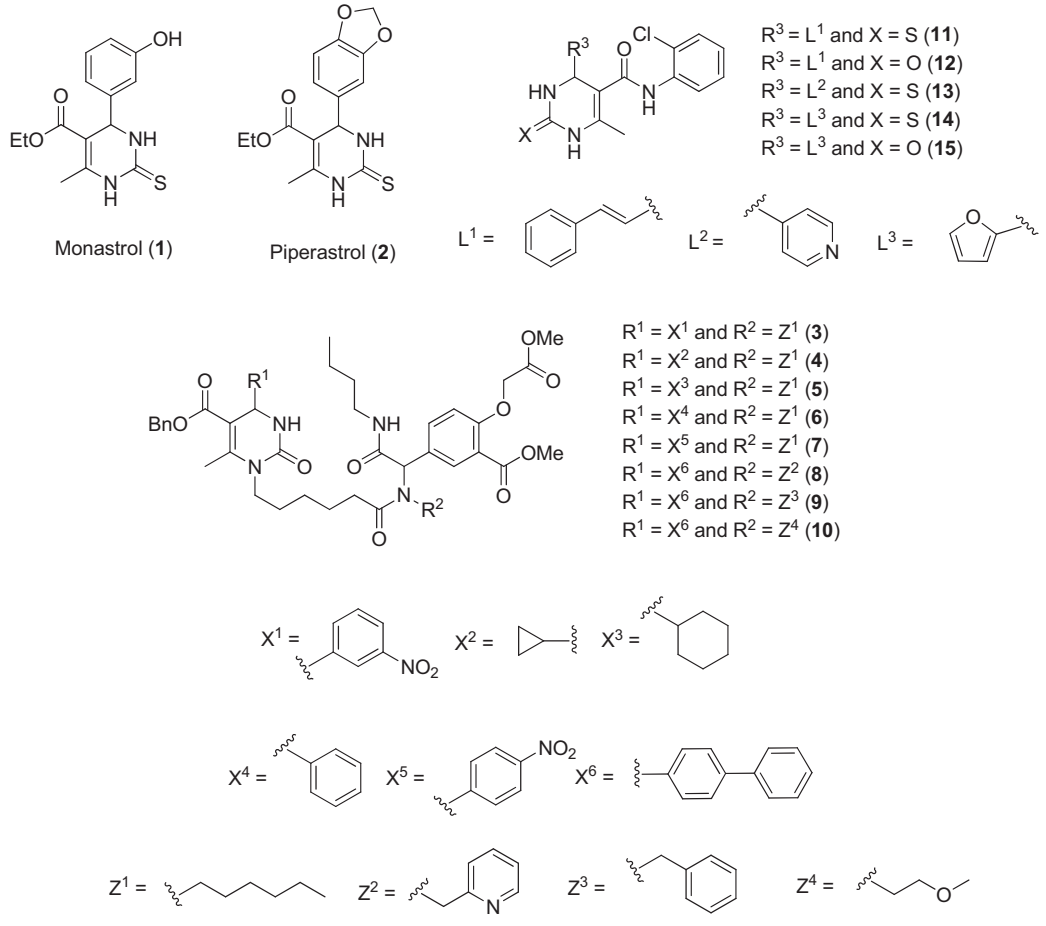


Fig. 2 Example of Biginelli adducts that possess antiproliferative activity against cancer cells.

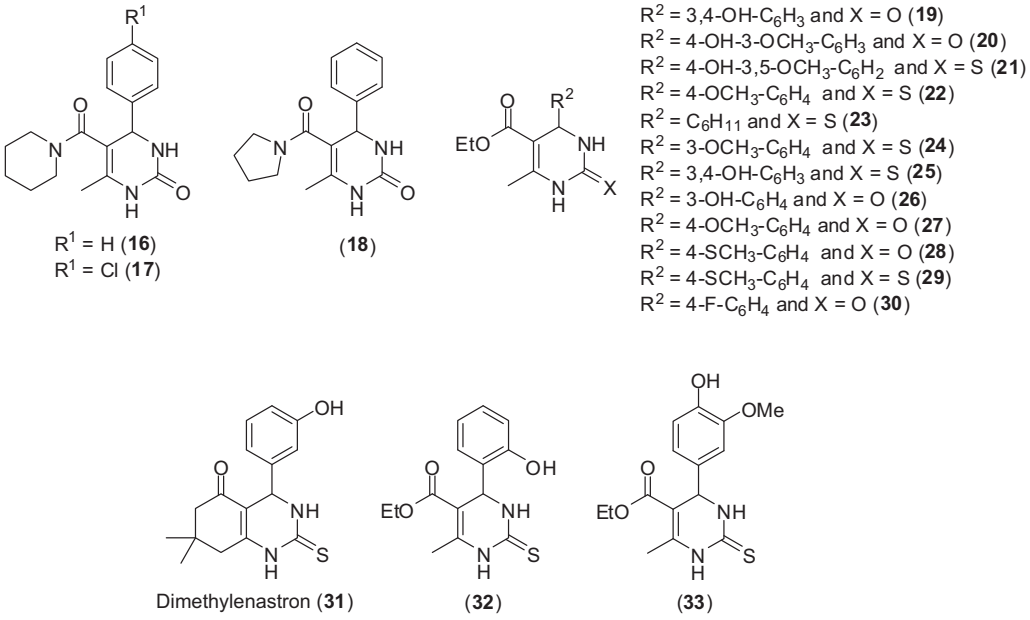


Fig. 3 Other examples of Biginelli adducts well-known for their ability to inhibit cancer cells growth.

Table 1 Potency (in folds) of Biginelli adducts relative to monastrol (**1**) with respect to the antiproliferative activity against cancer cells of different histological origins. Adapted from da Silva and coworkers [9].

Biginelli adduct	U251	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-03	HT-29
19	96.0	10.6	(–)	(–)	(–)	9.0	(–)
20	5.0	88.8	(–)	(–)	(–)	8.4	1.0
21	31.0	1.0	(–)	(–)	(–)	7.5	1.0
22	1.0	7.0	(–)	(–)	1.0	14.3	1.0
23	1.0	1.0	1.0	3.0	11.0	6.5	1.0
24	5.7	7.0	(–)	(–)	(–)	(–)	1.0
25	1.0	1.0	(–)	(–)	(–)	11.0	1.0
26	4.7	2.0	(–)	(–)	(–)	2.4	1.0
27	(–)	4.0	(–)	(–)	(–)	(–)	1.0
28	3.0	8.0	1.5	3.0	(–)	1.4	3.0
29	4.0	(–)	(–)	(–)	(–)	1.7	1.7
30	1.0	6.0	(–)	(–)	3.0	(–)	1.0

GI_{50} values for monastrol were in range of 4.0–29.6 μg mL^{-1} [9]. (–) Indicates that the Biginelli adduct was less potent than monastrol (**1**). U251, glioma cells; NCI-ADR/RES, multiple drug-resistant ovarian cancer cells; 786, renal cancer cells; NCI-H460, non-small lung cancer cells; PC-3, prostate cancer cells; OVCAR-03, ovarian cancer cells and HT-29, colon cancer cells.

Biginelli adducts-amide derivatives such as **16** and **17** (Fig. 3) exhibited moderate antiproliferative activity against HepG2 epithelial carcinoma in which the IC_{50} value for both compounds was *ca.* 120 μg mL^{-1} [8]. On the other hand, the derivatives **17** and **18** showed IC_{50} values of around 190 μg mL^{-1} against HeLa hepatocellular carcinoma cells [8].

Other monastrol (**1**) analogues were synthesized and tested against cancer cell lines of different histological origins [9]. Twelve Biginelli adducts (**19**–**30**; Fig. 3) were more potent than monastrol (GI_{50} in the range of 4.0–29.6 μg mL^{-1}) against one or more of the seven cancer cell lines studied (Table 1) [9]. Notably, compound **19** was determined to be over 90- and 10-fold more potent than monastrol (**1**) against U251 glioma cells and NCI-ADR/RES multiple drug-resistant ovarian cancer cells, respectively (Table 1). Compound **20** was found to be almost 90-fold more potent than monastrol against NCI-ADR/RES multiple drug-resistant ovarian cancer cells while the GI_{50} value for **21** is about 30-fold lower than that of monastrol toward U251 cells (Table 1). The results also indicate that six Biginelli adducts present GI_{50} values at least 5-fold lower than those of monastrol against some of the following cancer cells: U251 glioma, NCI-ADR/RES multiple drug-resistant ovarian, 786 renal, NCI-H460 non-small lung, PC-3 prostate, OVCAR-03 ovarian and HT-29 colon cancer (Table 1).

Morphological alterations in MCF-7 breast cancer cells that culminated in the death of over 80% cells were observed after 72 h of treatment with the Biginelli adducts **31** (dimethylenastron) to **33** (Fig. 3) at concentrations in the range of 400 μM to 1 mM. Such compounds showed minute toxic effects against fibroblast healthy cells [10].

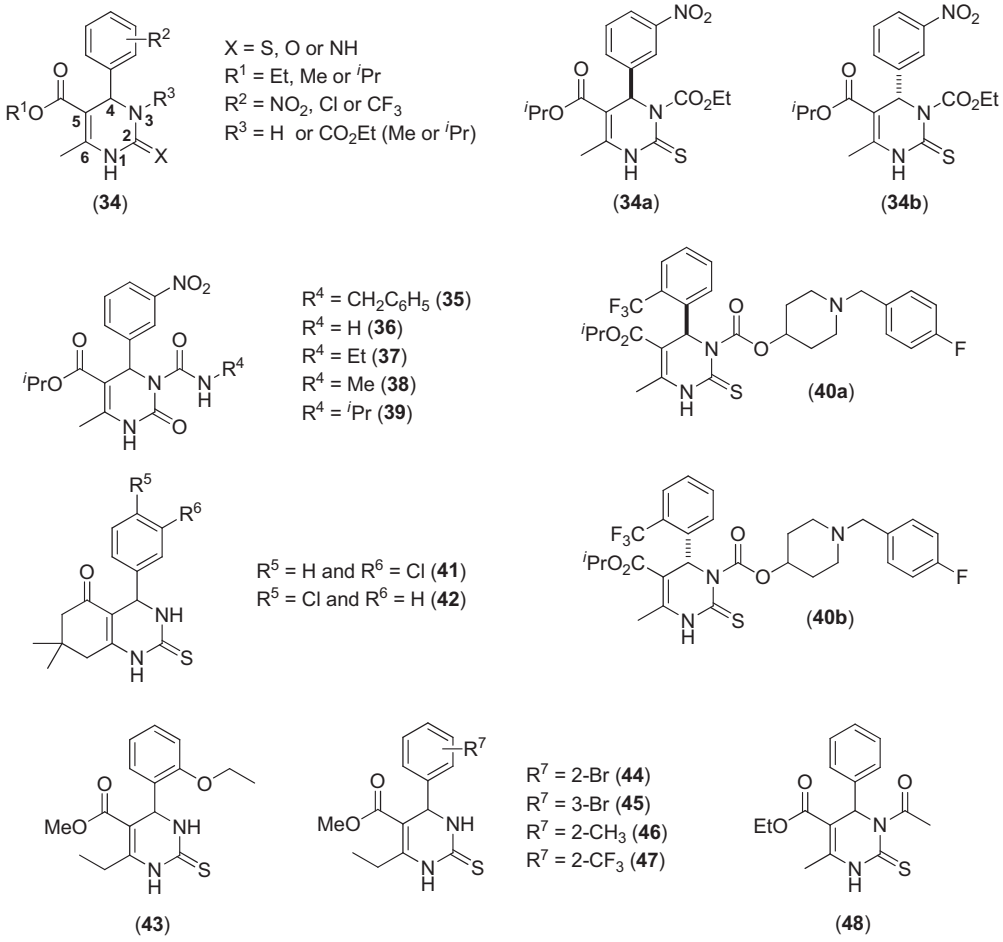


Fig. 4 Example of Biginelli adducts that exhibit inhibitory effect on calcium channels.

Calcium channel inhibition

Dihydropyridines such as nifedipine were introduced to the market in 1975 for the treatment of cardiovascular diseases (hypertension, cardiac arrhythmias and angina) due to the ability to inhibit calcium channels [11]. After the discovery of this drug several analogues, including Biginelli adducts, were synthesized to verify the potential to block calcium channels.

A structure–activity relationship study with Biginelli adducts was reported in 1990 with respect to the ability to target calcium channels [12,13]. It was determined that *thio*-adducts were the most potent Biginelli compounds in comparison with *oxo*- and *aza*-analogues [12,13]. *In vitro* assays revealed that the adduct bearing a nitro group at *ortho*-position of aromatic ring was more effective antihypertensive compound than that containing CF_3 or Cl as substituent (Fig. 4) [13]. Interestingly, the presence of an isopropyl ester group at C5 improved the Biginelli adduct potency by 10- and 60-fold in comparison with the effect of the ones bearing an ethyl ester or methyl ester group at the same carbon, respectively [13]. Although compounds bearing substituents at N3 are potent calcium channel blockers *in vitro*, their antihypertensive properties are lost in *in vivo* experiments as a result of metabolization by rats [13]. Additionally, *oxo*-analogues were found to be more stable as homogenates from rat liver did not present metabolites derived from such these compounds [13]. Finally, the stereocenter at C4 also plays a key role in the activity of such Biginelli adducts toward calcium channel; the (*R*)-enantiomer (**34a**; Fig. 4) is 750-fold more potent vasorelaxant agent than the corresponding (*S*)-enantiomer (**34b**; Fig. 4) [13]. Atwal and coworkers then substituted the acyl at N3 for a carbamoyl group to check whether such structural changes would affect the inhibition of calcium channel by Biginelli adducts related to **34** [14]. The best compounds (**35**–**39**; Fig. 4) tested *in vitro* exhibited IC_{50} values of 3, 12, 13, 16 and 60 nM, respectively. Thus, it was concluded that the presence of substituents at carbamoyl group influenced compounds potency as it follows: benzyl group > hydrogen, methyl or ethyl group > isopropyl group [14]. Compounds bearing 1-(phenylmethyl)-4-piperidinyl carbamate at N3 were described as the most promising calcium channel blockers in *in vivo* experiments, in which the presence of CF_3 at *ortho*-position of aromatic ring enhanced compounds effect when compared to the ones bearing nitro group [15]. Additionally, fluorine at *para*-position of benzyl moiety prevented the Biginelli adduct from metabolization by rat cells and conferred much higher potency than that of the reference drug amlodipine. Again, *in vitro* experiments demonstrated that the (*R*)-enantiomer (**40a**; Fig. 4) is much more potent than the corresponding (*S*)-enantiomer (**40b**; Fig. 4), since the

former exhibits an IC_{50} value of 15 nM while the IC_{50} value for the latter is determined to be higher than 1000 nM [15].

The *thio*-Biginelli adducts **41** and **42** were determined to be relaxant agents as effective as the reference drug nicardipine (inhibition of stimulus by 35.5 ± 4.2%) on KCl-stimulated lamb carotid strips when used at 100 μM [16]. Compounds **41** and **42** present a Cl atom as substituent at *meta*- and *para*-position, respectively (Fig. 4). The relaxant effect of the *thio*-Biginelli adduct **43** (Fig. 4) on KCl-stimulated contractions in rat thoracic aorta was comparable to that of nicardipine (inhibition of stimulus by 20.5 ± 2.9%) [17]. Other *oxo*-Biginelli adducts were investigated for the calcium channel blockage-dependent relaxant effect on KCl-stimulated lamb carotid strips. Compounds containing Br, CH_3 or CF_3 at *ortho*-position or Br at *meta*-position in aromatic ring (**44**–**47**; Fig. 4) at 1 μM were either as potent or as more potent than nicardipine that at the same concentration was able to inhibit the stimulus by 2.5 ± 1.8% [18].

The acetylated *thio*-Biginelli adduct derivative **48** (Fig. 4) effectively caused the relaxation of KCl-stimulated guinea pig ileum as attested by its value of negative log molar concentration of antagonist required to reduce the response of agonist by 50% (PA_2 = 6.06) in relation to the reference drug verapamil [19].

Anti-inflammatory activity

Inflammation process can be characterized by five phases that may or may not occur simultaneously, named pain, heat, redness, swelling and ultimately loss of function. They comprise a defensive body response to invasion of a foreign material. Acute inflammation can cause several damages in tissues or organs. The anti-inflammatory potential of a certain molecule can be investigated by various means, such as the analgesic effect using paw edema as model, the inhibition of proinflammatory cytokines (e.g. tumor necrosis factor (TNF-α) and interleukin 6 (IL-6)) [20], the effect on prostaglandin E_2 and/or hialuronidase, nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and transient receptor A1 (TRPA1), among others [20–24].

Biginelli adducts have received great attention with respect to their potential as anti-inflammatory agents. Based on the duration of action and percentage of inflammation inhibition on Albino rats paw edema, the propanoic acid derivatives *thio*-adducts (**49**–**53**; Fig. 5) were found to be the most promising anti-inflammatory compounds when compared to diclofenac, a reference drug [25]. The Biginelli derivative **54**, which bears a 1,3,4-oxadiazol-2-yl moiety (Fig. 5), controls inflammation process by inhibiting the carrageenan-induced rat paw edema by 75% after 3 h of treatment, an effect comparable to that exhibited by diclofenac [26].

The potential of the *thio*-analogue Biginelli adduct **55** (Fig. 5) to inhibit the production of proinflammatory cytokines in LPS-induced human monocytic leukemia cells (THP-1) was addressed [20]. The production of TNF-α and IL-6 in THP-1 cells in the presence of compound **55** at 10 μM was 78% and 96% lower than that of cells incubated in the absence of this Biginelli adduct, respectively. Under the same experimental conditions, dexamethasone (reference drug at 1 μM) inhibit TNF-α and IL-6 production by 71% and 84%, respectively [20].

Chronic inflammation is known to be associated with increased activity of hyaluronidases, enzymes that catalyzes the degradation of hyaluronic acid [27,28]. Based on this,

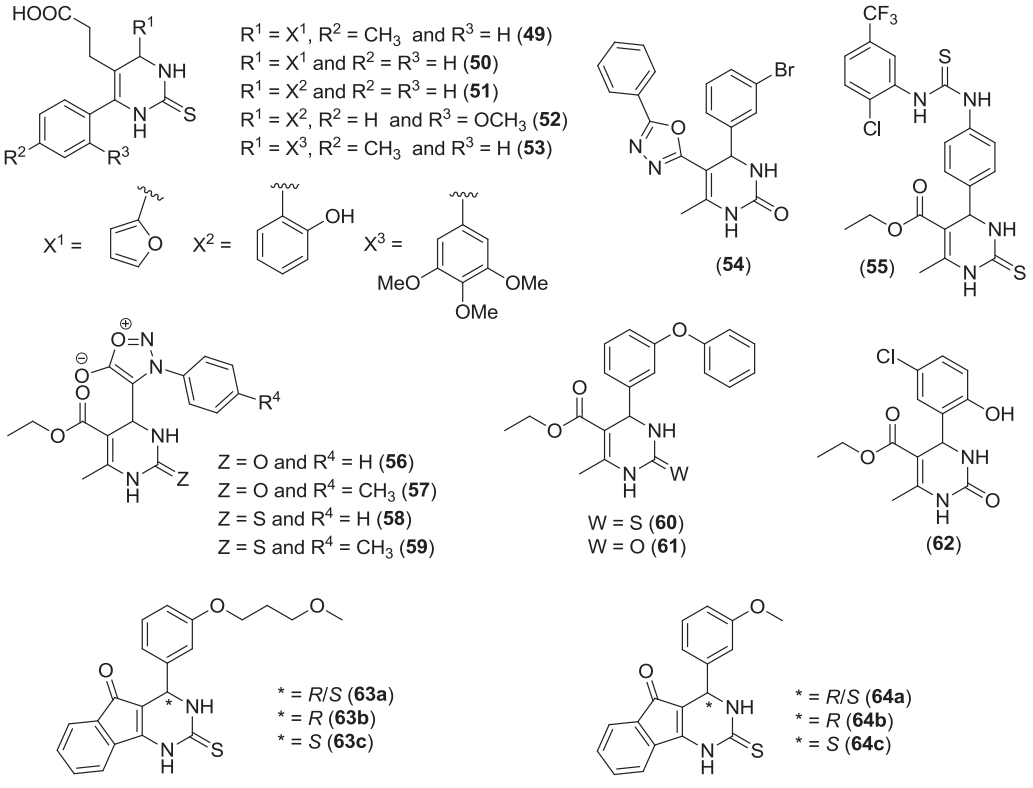


Fig. 5 Example of Biginelli adducts that exhibit anti-inflammatory effect.

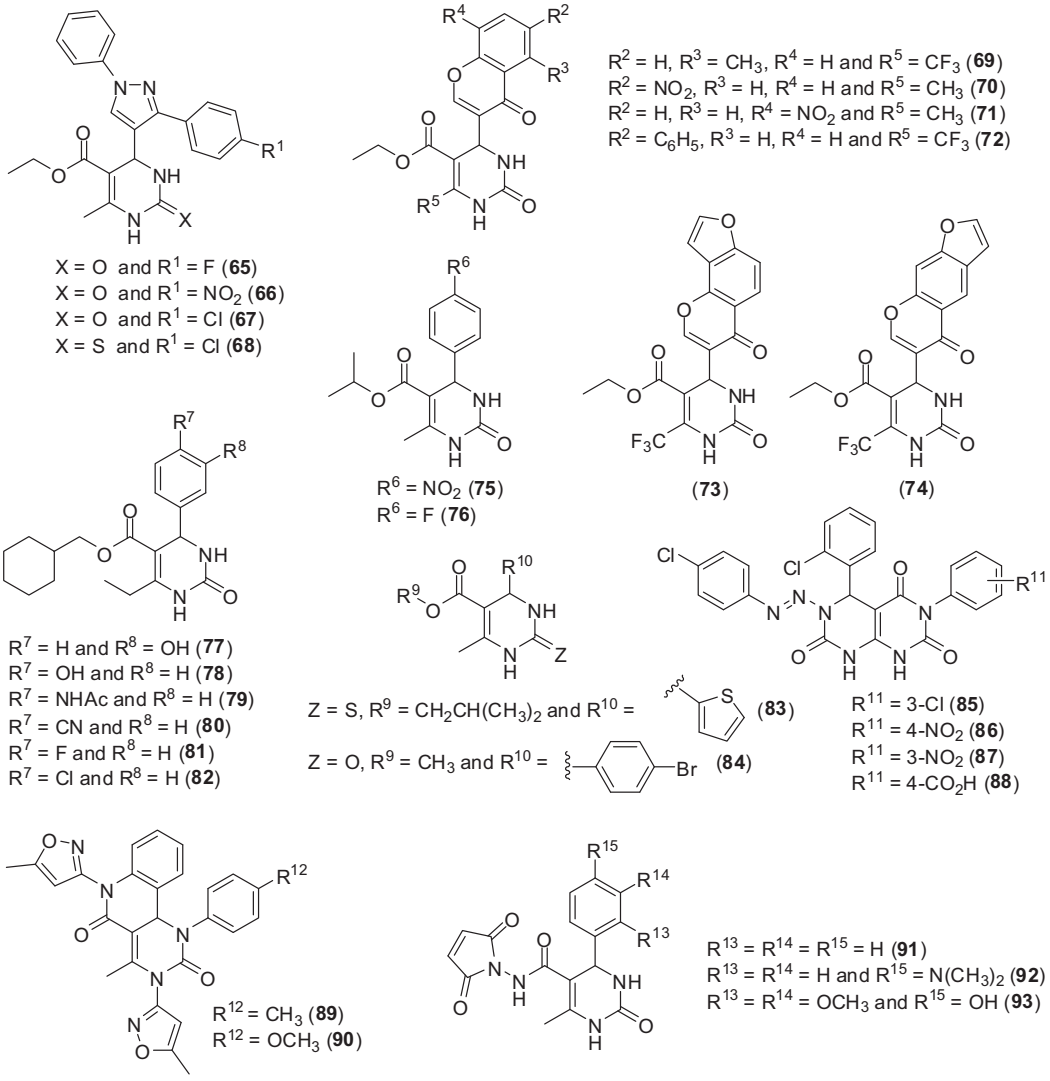


Fig. 6 Example of Biginelli adducts that exhibit antimicrobial activity.

Gireesh and coworkers performed molecular docking studies using some Biginelli adducts and related derivatives to identify compounds with potential to inhibit hyaluronidase [24]. Indeed, *in vitro* assays confirmed that 100 μg of compounds **56**–**59** (Fig. 5) was able to inhibit the activity of hyaluronidase (3–5 units) in the range from 89% to 100%. Similar results were achieved when compounds **56**–**59** were substituted for indomethacin, a reference drug [24].

The anti-inflammatory properties of Biginelli adducts **60**–**62** (Fig. 5) were attested by their capacity to inhibit NO production in LPS-activated microglia at IC_{50} values ranging from 41.3 to 67.3 μM [29]. Compound **60** was also the most potent among these Biginelli adducts in the inhibition of prostaglandin E_2 (PGE_2) production and iNOS and COX-2 genes expression. Additionally, **60** negatively affected the production of TNFα and interleukin-1 β (IL-1β) [23].

Biginelli adducts bearing *meta*-substituents have been described as very promising anti-inflammatory agents in studies carried out with human embryonic kidney 293 cell lines (HEK293) overexpressing the transient receptor potential A1 (TRPA1) either from human or rat [22]. Thus, compounds **63a-b** and **64a-b** (Fig. 5) were able to inhibit both human and rat TRPA1 at concentrations ranging from 4 to 75 nM. The *R* isomers (**63b** and **64b**), however, were identified as the most potent inhibitors acting on rat TRPA1 at IC_{50} values as low as 4 and 12 nM, respectively, while the IC_{50} for the corresponding *S* isomers (**63c** and **64c**; Fig. 5) were found to be higher than 10,000 nM [22].

Antibacterial activity

Biginelli compounds bearing a 1,3-diarylpyrazole moiety (**65**–**68**; Fig. 6) exhibited minimal inhibition concentration (MIC) of 20 ng mL^{-1}, 20 ng mL^{-1}, 250 ng mL^{-1} and 125 ng mL^{-1} against the *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB $H_{37}Rv$), respectively [30,31]. The effect of **65** and **66** on normal kidney-derived African green monkey cells (VERO line) was assessed, revealing that both Biginelli adducts are highly selective to MTB $H_{37}Rv$ (selectivity index > 500) [30]. Other 16 Biginelli adducts (**69**–**74**; Fig. 6) were found to be as potent as or more potent than the reference drugs ethambutol (MIC = 7.6 μM) and ciprofloxacin (MIC = 9.4 μM) against MTB $H_{37}Rv$. The MIC values for compounds **69**–**74** ranged from 3.4 to 76.2 μM [32].

Compounds **75** and **76**, containing a nitro group and fluorine at *para*-position, respectively, exhibited MIC values of 12.5 μg mL^{-1} (for the former) and 12.5–25.0 μg mL^{-1} (for the latter) against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*, which make these compounds more potent than ciprofloxacin [33]. Biginelli adducts bearing a 1,3-dihydro-2*H*-indol-2-one core showed moderate antibacterial activities (62.5–250.0 μg mL^{-1}) against *Bacillus subtilis* (MTCC-441), *E. coli* (MTCC-443), *K. pneumonia* (MTCC-109), *P. aeruginosa* (MTCC-1688), *S. typhi* (MTCC-98), *S. aureus* (MTCC-96) and *Staphylococcus pyogenus* (MTCC-442) [34].

Antiviral activity

Kim and coworkers showed the potential of some Biginelli adducts as agents for preventing human immunodeficiency virus HIV-1 replication [35,36]. Notably, compounds **77**–**82** (Fig. 6) compromised the HIV-1 replication in CEMx174-LTR-GFP cells (clone CG8) by 50% when employed at concentrations lower than 90 nM. At the same experimental conditions, the reference drug nevirapine exhibited an EC_{50} value of 150 nM [35,36]. The (*S*)-enantiomer was determined to be more potent than the corresponding (*R*)-enantiomer with respect to the antiviral activity. Indeed, it was shown that (*S*)-**77** is at least 26-fold more potent than (*R*)-**77** [35,36].

The potential of the Biginelli-type pyrimidines **83** (IC_{50} = 1.8 μM) and **84** (IC_{50} = 0.9 μM) against herpes simplex virus (HSV-KOS strain) was shown elsewhere (Fig. 6) [37]. Notably, the analogue **84** exhibited negligible toxicity toward the mammalian cells tested indicating its selectivity to the studied virus. A time-of-addition study was then performed with **84** revealing that the administration of such compound to cells 2 and 4 h post inoculation was sufficient to negatively affect virus replication. The lack of inhibition of virus adhesion and/or entry to the cells suggests that compound **84** inhibits virus replication in late stages [37].

Antifungal activity

Fungi have emerged worldwide as some of the most frequent causes of healthcare-associated infections. Invasive fungal infections can be life-threatening and the number of antifungal agents currently available in the market is very limited [38].

Although Biginelli adducts have been poorly explored with respect to the antifungal activity, some examples of promising compounds are described in the literature. Eleven Biginelli-type pyrimido[4,5-*d*]pyrimidine-2,5-diones were described as potential anti-*Aspergillus niger* and anti-*Candida albicans* agents, exhibiting MIC values raging from 11 to 57 μg mL^{-1} [39]. The most active compounds (**85**–**88**; Fig. 6) showed MIC values near to or lower than 20 μg mL^{-1} in comparison with the reference antifungal clotrimazole, whose MIC values against *A. niger* and *C. albicans* were 20 and 25 μg mL^{-1}, respectively. Thus, analogues bearing withdrawing groups, with exception of 4-Cl substituent, were the most active against *A. niger* and *C. albicans* [39].

The Biginelli adducts **75** and **76** (Fig. 6) efficiently inhibited the growth of *C. albicans*, *Aspergillus flavus*, *Rhizopus* sp. and *Mucor* sp. as attested by the MIC values in the range of 12.5–25 μg mL^{-1}, being most of the time more potent than amphotericin B (MIC = 25–50 μg mL^{-1}) [33].

According to Rajanarendar and coworkers [40], isaxole Biginelli adducts are promising antifungal agents against *A. niger*, *Chrysosporium tropicum*, *Rhizopusoryzae*, *Fusarium moniliformae* and *Curvularia lunata*. When tested at 100 μg mL^{-1}, compounds **89** and **90** (Fig. 6) were able to induce the formation of a zone of fungal growth inhibition from 60 mm to 65 mm against the strains tested, which confers to these compounds higher potency in comparison with clotrimazole (inhibition zone of up to 35 mm) [40]. Studies of formation of zones of fungal growth inhibition were also carried out with *C. albicans* and *Aspergillus parasiticus* and the adducts **91**–**93** (Fig. 6) [41]. An average zone of inhibition of 16.5 mm was verified in cultures of *C. albicans* in the presence of Biginelli

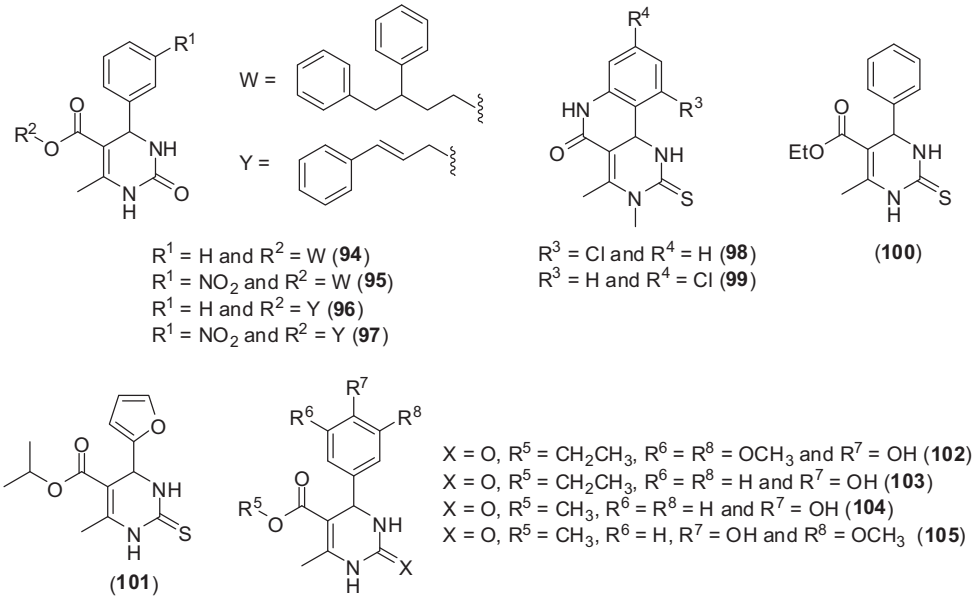


Fig. 7 Example of Biginelli adducts with ability to scavenge oxygen and/or nitrogen reactive species.

adducts at 10 μg mL^{-1}, while clotrimazole triggered the formation of a 21 mm-inhibition zone. As for *A. parasiticus*, the inhibition zone in the presence of compounds **91**–**93** and clotrimazole (all at 10 μg mL^{-1}) were, respectively, 13 mm, 17 mm, 18 mm and 22 mm [41].

Antioxidant activity

Oxygen and nitrogen reactive species (ROS and RNS, respectively) are ubiquitous in nature being a result of electron escape from electron transport chain (present in mitochondria and chloroplast). The overproduction of ROS and/or RNS can be deleterious to cells if the cellular antioxidant system is not able to efficiently restore the normal levels, which can ultimately cause pathologies [9,42].

The first report on the antioxidant properties of Biginelli adducts was published in 2006 in a study that investigated the potential of such molecules to prevent ROS formation and lipid peroxidation in male adult albino Wistar rats [42]. The Biginelli adducts **94** and **95** (Fig. 7) restored the lipid hydroperoxide to normal levels in liver cells when administered at 200 μM. These results indicate that the presence of a nitro group on aromatic ring is not mandatory for adduct **95** preventing lipid peroxidation. Compounds **94** and **96** (Fig. 7) were found to be more efficient than the corresponding nitro-analogues **95** and **97** (Fig. 7) to prevent the overproduction of ROS [42].

The potency of the *thio*-adducts **98** and **99** (87.5%; Fig. 7) to scavenge hydroxyl radicals was comparable to that of the reference antioxidant quercetol (92.3%) when all compounds were used at 100 μM [43]. The *thio*-adducts **22** (Fig. 3) and **100** (Fig. 7) exhibit IC_{50} values of 10 μM and 76 μM, respectively, regarding the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [44]. Also, compounds **22** and **100** at 300 μM diminished, at similar extents, the lipid hydroperoxide levels in homogenates of cerebral cortex from rats [44].

The adduct **101** (Fig. 7) effectively scavenged DPPH radicals exhibiting an IC_{50} value of 0.6 mg mL^{-1}, while the IC_{50} value for gallic acid (a known radical scavenger) was 0.8 μg mL^{-1} [45].

A series of Biginelli adducts were tested by da Silva and coworkers to compare the ability of *thio*- and *oxo*-derivatives to scavenge RNS and ROS [9]. Compounds **19**, **21**, **25** (Fig. 3) and **102** (Fig. 7) were determined to be the most promising RNS scavengers among the tested adducts, as they showed IC_{50} values of 20.3, 29.7, 23.3 and 24.2 μM, respectively, while resveratrol exhibited an IC_{50} of 34.4 μM in reactions containing DPPH 100 μM. As for ROS scavenging, the IC_{50} values for **19**, **21**, **25** and **102** and resveratrol toward O_2^- were 33.0, 25.7, 122.3, 78.0, 121.4 μM, respectively [9].

Compounds **20** (Fig. 3) and **103**–**105** (Fig. 7) were demonstrated to be as efficient as gallic acid in the scavenging of DPPH at 40 μg mL^{-1} as the IC_{50} values for these adducts ranged from 2.1 to 5.0 μg mL^{-1} [46].

Concluding remarks

The diverse biological profile of Biginelli adducts brought perspectives for the development of novel drugs to improve human and animal health. Here, we compiled the effect of over 100 Biginelli adducts on cancer cells, calcium channels, inflammation, microorganisms (bacteria, viruses and fungi) and ROS and RNS scavenging. Some progress has been made with respect to the mechanism of action by which monastrol (**1**) and related molecules trigger the inhibition of cancer cells growth. However, the mechanisms of action of Biginelli adducts that lead to the attenuation and/or prevention of other pathologies are still incipient. Therefore, advances in this matter will certainly contribute to the rational design of more efficient and selective calcium channel inhibitor, anti-inflammatory, antimicrobial and antioxidant agents based on Biginelli adducts core.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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