RESEARCH ARTICLE
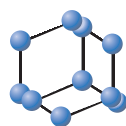
Synthesis of Novel Fluorine Compounds Substituted-4-thiazolidinones Derived from Rhodanine Drug as Highly Bioactive Probes

Mohammad S.T. Makki, Reda M. Abdel-Rahman and Nawaa A.H. Alshammari[*]

Department of Chemistry, Faculty of Science, King Abdul Aziz University, P.O. Box. 42805, Jeddah 21551, Saudi Arabia

Abstract: ***Aim and Objective***: It is known that rhodanine drug has various biocidal activities. The aim of this work was to improve the structure of rhodanine drug *via* alkylation at N, S, and O- centers in addition to the introduction of fluorine atoms. The new fluorinated modified rhodanines **2-16** were evaluated as enzymatic probes for cellobiase activity produced by fungi and as CDK2 inhibitors of tumor cells.

Materials and Methods: Novel fluorine substituted N-alkyl, S-alkyl and amino-rhodanines were obtained *via* Hydroxy methylation, Mannich reactions, chlorination and amination of 5-(4'-fluorophenylene)-2-thioxo-thiazolidin-4-one, and the enzymatic effects of cellobiase produced by fungi and /or CDK2 inhibition of tumor cells were evaluated.

Results: Most of the targets were obtained in high yield and in the form of very pure crystals with characteristic colors. Only compounds **5**, **8**, **10**, **13**, and **14** exhibited a higher activity as cellobiase while compounds **2** and **5** showed a highly enzymatic effect on tumor cells. In addition, compounds **2** and **10** can be used as Olomoucine (standard referees).

Conclusion: Various N, S and O-alkyl derivatives of fluorine-substituted rhodanines were prepared *via* a simple method and used as enzymatic probes for cellobiase activity produced by fungi and CDK2 inhibitors for tumor cells. The more bioactive compounds had rich fluorine atoms as p-fluorophenyl and p-fluorobenzoyl bearing N, S, O-alkyl rhodanine. The highly active compounds may be used as enzymatic materials for various biological transformations in the future.

1. INTRODUCTION

Most of the substituted-4-thiazolidinones exhibit a wide range of pharmacological, medicinal and biological properties as antibacterial [1, 2], antifungal [3], antitubercular [4], anticancer [5], antiinflammatory [6], antianalgesic [7], anticonvulsant [8], antidepressant [9], antiviral /anti HIV [10], antidiaberic [11], a muscarinic receptor1-agonist [12], an FSH receptor agonist [13], trypanocidal (anti-epimastigote) [14] and antiarrhythmic activity [15, 16].

Introduction of fluorine atoms to heterocyclic nitrogen systems often improves their physical with chemical advantages and enhances their biological activity [17-20]. Based on these observations, the present work describes the development of new fluorine compound substituted rhodanine derivatives which may enhance the biological effects, as cellobiase produced by fungi as well as CDK2 inhibitors for tumor cell (DNA damage).

The structure-activity relationship has been discussed and their binding conformation is further clarified by molecular modification studies.

2. MATERIALS AND METHODS

All the melting points were determined on Stuart scientific SMP30 (Bibby, UK) melting point apparatus and reported as uncorrected. The FT-IR spectra were recorded on a Perkin-Elmer spectrum 100 spectrometer in ATR. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Brucker advance DPX NMR spectrometer (400 and 100 MHz, respectively), by using DMSO-d_6 as a solvent (chemical shift in δ, ppm), and TMS as an internal standard. Elemental analysis was conducted by a 2400 Perkin Elmer series 2 analyzer. Mass spectra were determined by using quadruple MS (Electronic ionization mod E1 mode with source temperature: 200ºC) at 70 eV. All the reactions were monitored by TLC, using silica gel coated Al plates with a fluorescent indicator F254 (Merck).

^{19}F NMR spectra of all the compounds 2-16 showed resonated signals as d,d at -119.75 and -123.16 ppm.

3. RESULTS AND DISCUSSION

3.1. Chemistry

A literature survey reported a new fluorine compound substituted for rhodanine at positions SH, NH$_3$, and active CH$_2$ positions [21] and is used for detecting Tau pathology in Alzheimer's Brains [22] as well as inhibitors of JSP-1 [23].

In view of these results, the aim of this work was to search for new fluorine compounds derived from rhodanine drug with a hope to enhance their biological activity.

Thus, hydroxyl methylation and/ or Mannich bases of 5-(4-fluorobenzylidene)-2-thioxothiazolidin-4-one (**2**) [24] *via* refluxing with HCHO-MeOH and /or HCHO-MeOH with secondary amines afforded N^3-substituted rhodanine **3-5** (Scheme **1**).

*Address correspondence to this author at the Department of Chemistry, Faculty of Science, King Abdul Aziz University, P.O. Box. 42805, Jeddah 21551, Saudi Arabia; Tel: +966531097330; E-mail: ro-0s@hotmail.com

A simple nucleophilic attack of primary aliphatic/aromatic amines to SH group at position-2 of rhodanine by refluxing with ethanolamine, 4-fluoroaniline and sulfanilamide (1:1 by mol) in EtOH, furnished 2-substituted amino-5-(4'-fluorobenzylidene)-4-thiazolidinones (**6-8**) (Scheme **2**).

Also, the treatment of compound **2** with POCl₃ under fusion condition yielded 4-chloro-5-(4-fluorobenzylidene)thiazol-2(5*H*)-thione (**9**). Chlorine atom at 4-position of rhodanine **9** was removed

by treatment with primary amines. Thus, compound **9** upon attack by primary amines such as 4-fluoroaniline, hydrazine hydrate and /or 4-chlorophenyl-hydrazine under refluxing with DMF produced 5-(4-fluorobenzylidene)-4-[(4-fluorophenyl) amino]thiazol-2(5*H*)-thione (**10**); 3-(4-fluorophenyl)-1,3-dihydro-pyrazolo[3,4-*d*]thiazol-5-thione (**11**), and / or 2-(4-chlorophenyl)-3-(4-fluorophenyl)-1,2-dihydropyrazolo[3,4-*d*]thiazol-5-thione (**12**) (Scheme **3**).

Formation of **4** and **5** from **2** is shown in Fig. (**1**).
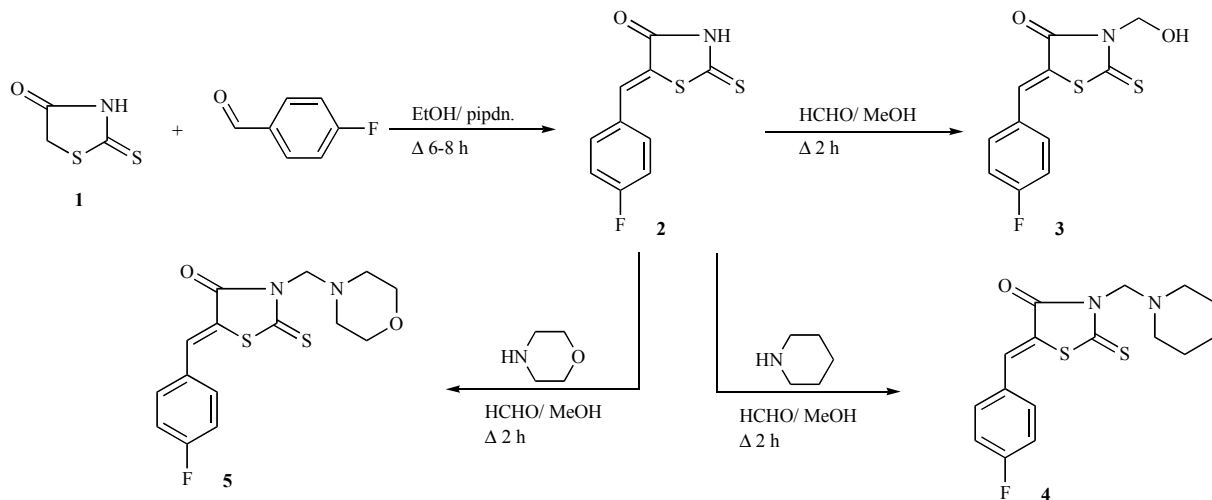
Scheme 1. Formation of compounds **3-5** from **2**.
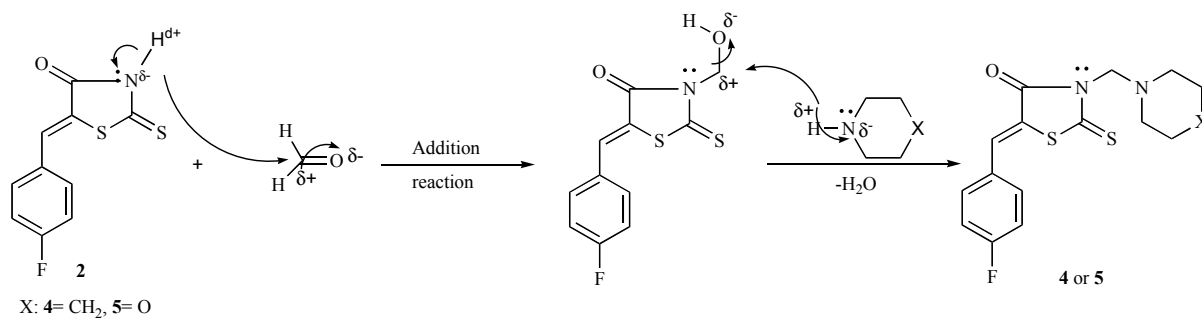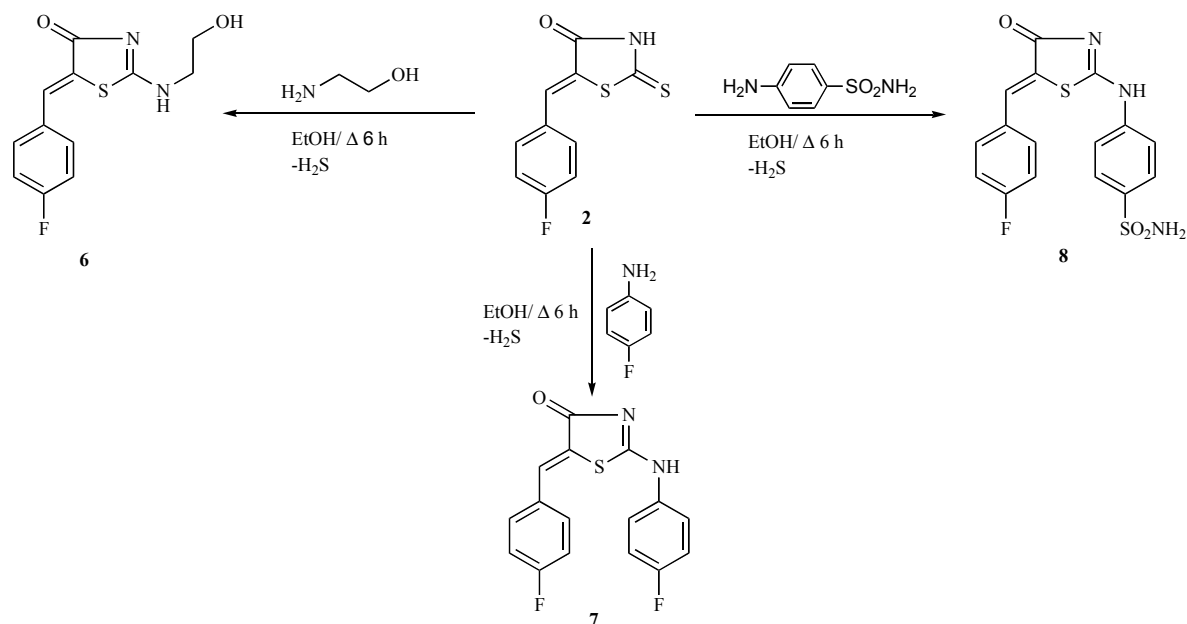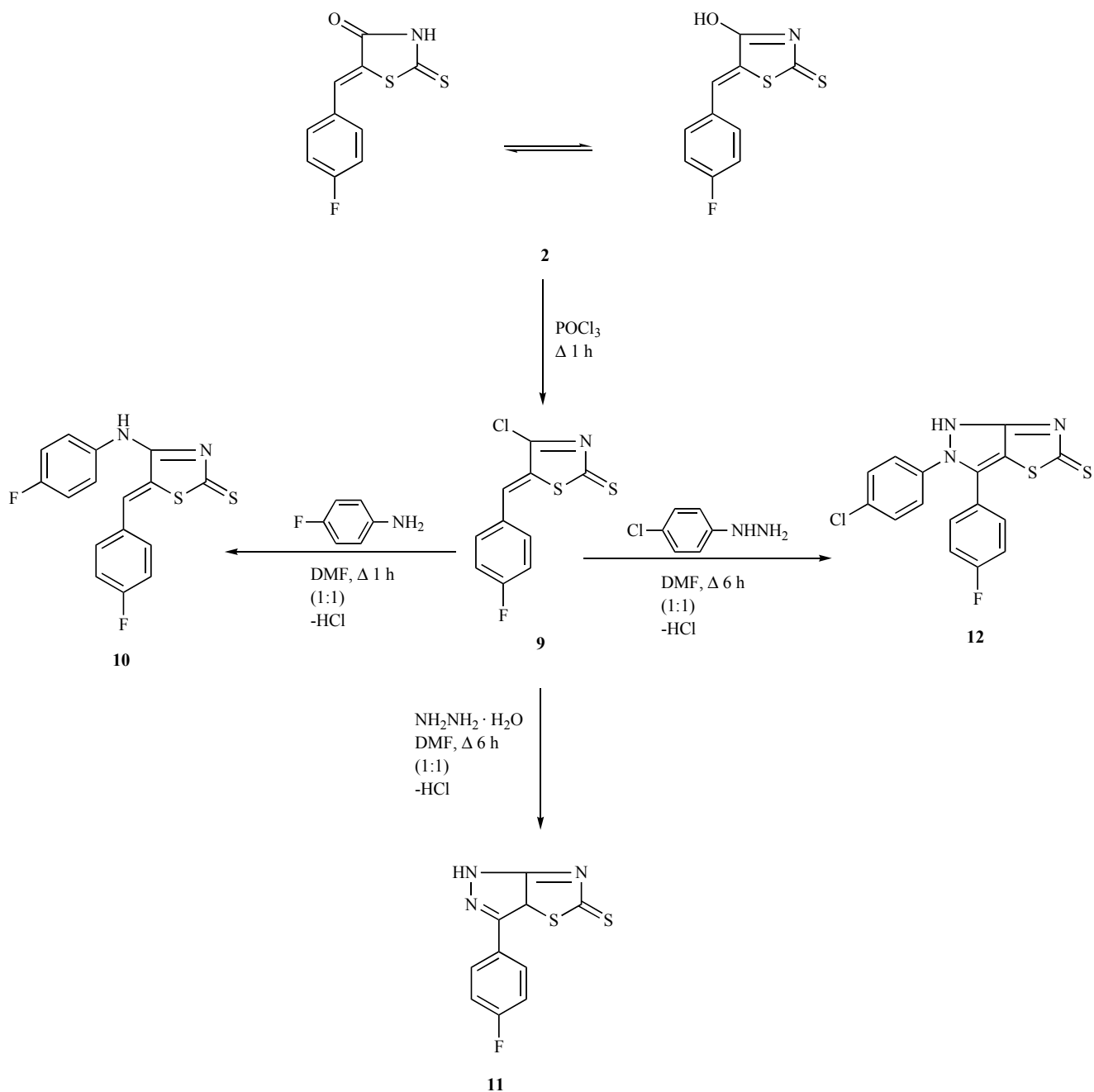

X: **4**= CH₂, **5**= O

Fig. (1). Formation of **4** and **5** from **2**.

Scheme 2. Formation of compounds **6** and **7** from **2**.

Scheme 3. Formation of compounds **9-12** from **2**.

The formation of compound **11** is shown in Fig. (**2**).

Alkylation of compound **2** by refluxing with monochloroacetic acid in DMF yielded 2-[(5-(4-fluorobenzylidene)-4-oxo-4, 5-dihydrothiazol-2-yl)thio] acetic acid (13), which on decarboxylation *via* warming with aqueous K_2CO_3 gave 5-(4-fluorobenzylidene)-2-(methylthio) thiazol-4(5H)-one (**14**) (Scheme **4**).

The structural formula of new systems was obtained from the correct elemental analysis and spectral data.

FT-IR absorption spectra of compounds **3-5** showed the absorption bands at $\bar{\upsilon}$ 2980 & 2888 cm^{-1}, attributed to endo CH= and exo CH$_2$ with lack of NH group, and with the presence of $\bar{\upsilon}$ at 1700, 1610, 1240 and 1180 cm^{-1} due to C=O, C=C, C-F, and C=S respectively. Also, FT-IR spectra of compounds **6-8** recorded $\bar{\upsilon}$ at 3180-

3150 cm^{-1} for bonded NH which lacks the SH groups. Moreover, FT-IR spectrum of compound **9** indicates a lack of C=O with the presence of $\bar{\upsilon}$ at 700 cm^{-1} for C-Cl. Also, FT-IR spectra of both the compounds **10** and **12** recorded $\bar{\upsilon}$ at 3180, 1230 and 1188 cm^{-1} which were attributed to the presence of NH, C-F, and C=S with lack of C=O, while **11** showed $\bar{\upsilon}$ at 3200, 3180, 1590 and 1240 cm^{-1} for NH, NH$_2$, C=N, C-F functional groups.

It is interesting to note that 1,4-disubstitutedthiazolo[5,4-*d*]pyrazole-piperazine (**16**) isolated from the interaction of compound **2** with piperazine (2:1 by mole) / in refluxing EtOH gave N^1, N^4-disubstituted piperazine **15** followed by hydrazinolysis in EtOH (Scheme **5**).

FT-IR spectra of compounds **13** and **14** showed $\bar{\upsilon}$ at 2980 and 2880 cm^{-1} of alkyl groups, with the presence of two $\bar{\upsilon}$ at 1720, and
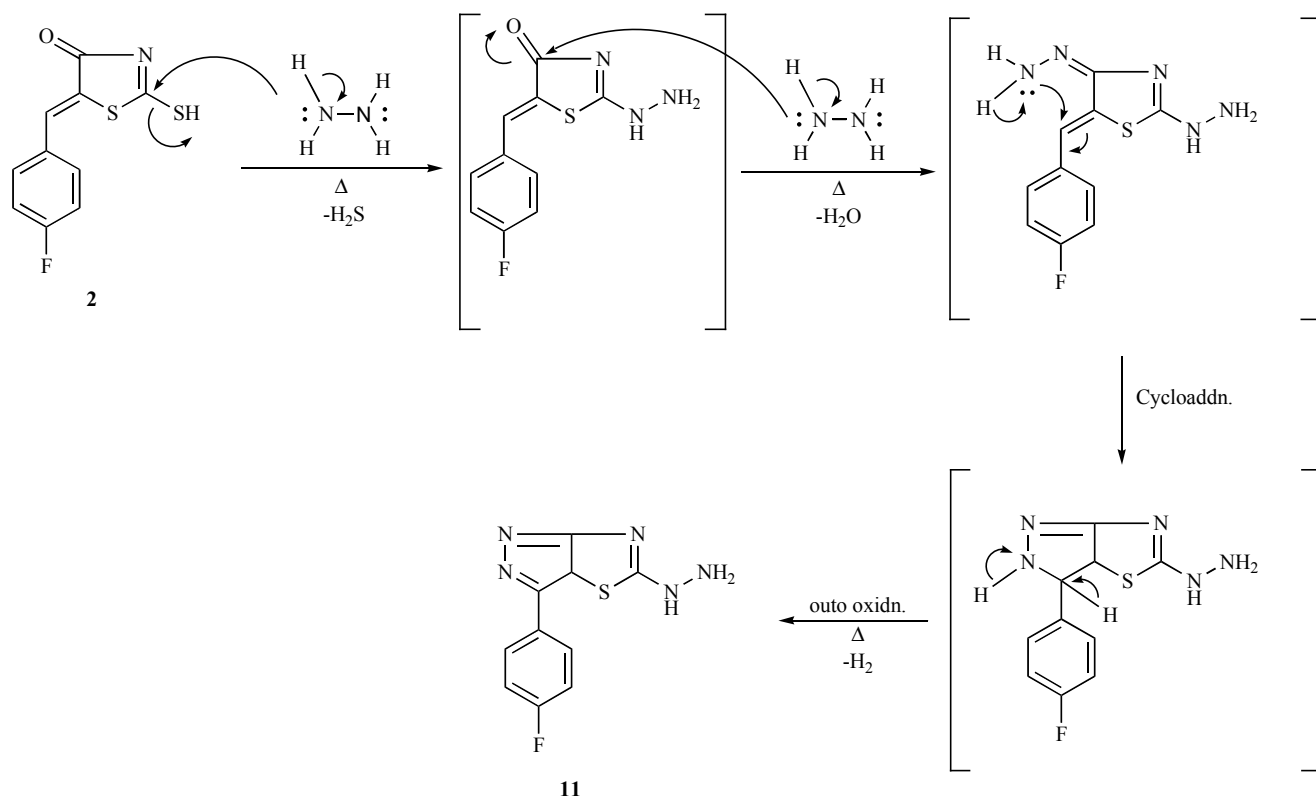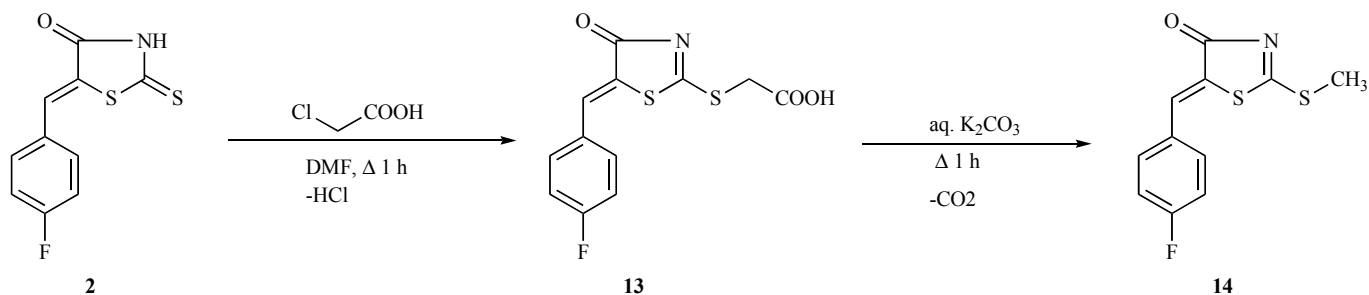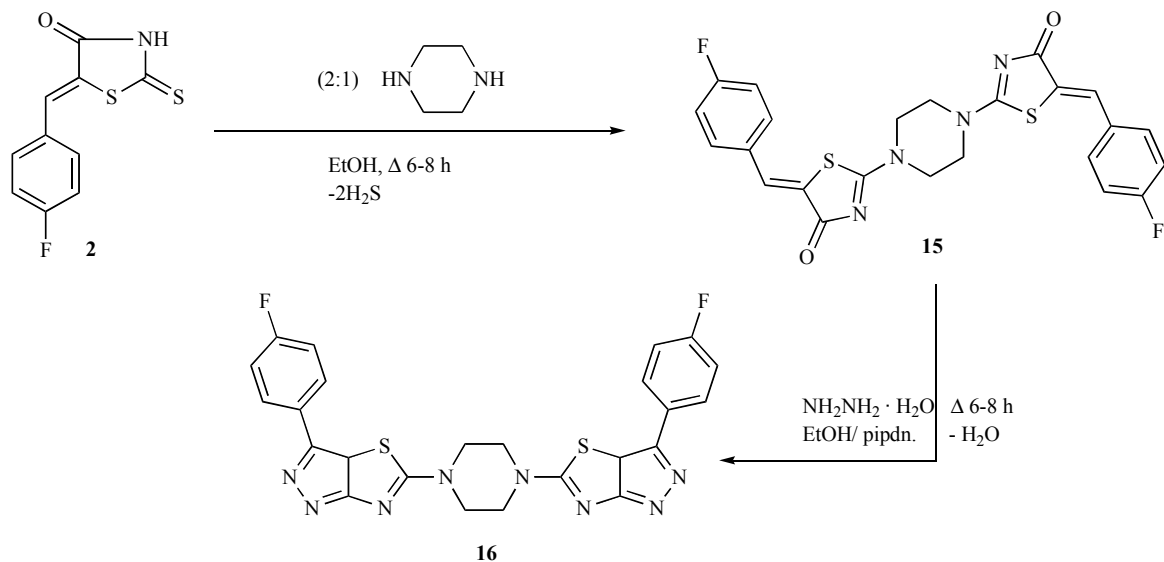
Fig. (2). Formation of compound **11** from **2**.

Scheme 4. Formation of compounds **13** and **14** from **2**.

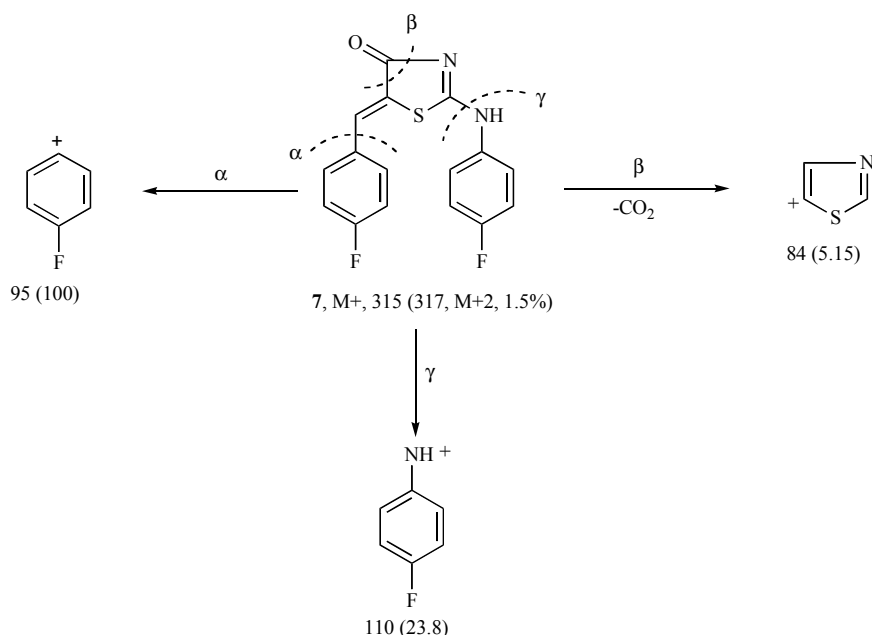**Scheme 5.** Formation of compounds **15** and **16** from **2**.

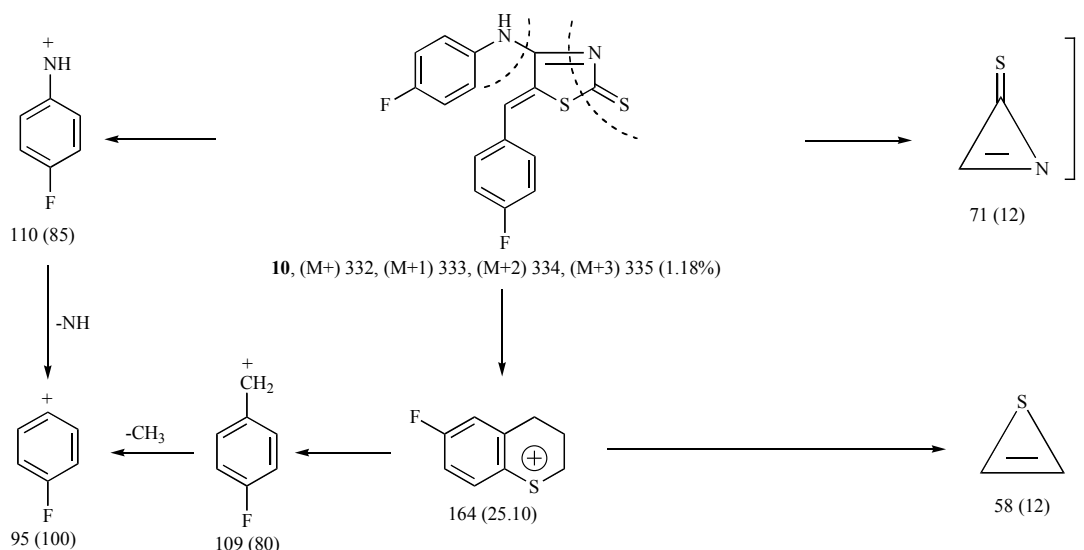**Fig. (3).** Mass fragmentation pattern of compound **7**.

Fig. (4). Mass fragmentation pattern of compound **10**.

1690 cm^{-1} attributed to C=O of acid and C=O of thiazole nucleus. Its interesting to note that FT-IR spectrum of compound **15** showed lack of SH and / or NH groups, with the presence $\bar{\upsilon}$ at 1700, 1610 and 1230 cm^{-1}, for C=O, C=C, and C-F groups, respectively, while the spectrum of **16** showed lack of C=O, C=C, NH, and SH groups. Both the compounds **15** and **16** exhibited only $\bar{\upsilon}$ at 2985, and 2880 cm^{-1} for stretching and $\bar{\upsilon}$ at 1488, and 1440 cm^{-1} for bending of CH$_2$, for piperazine, respectively.

^{1}H NMR spectra of the new compounds give more indications about their structures.

Therefore, ^{1}H NMR spectra of compounds **3-5** showed resonated signals δ at 7.6 and 2.4 for the exo (C=CH-Ar) and endo N-CH$_2$, while the spectra of compound **6-8** recorded δ at 8.2 ppm for NH bonded with aromatic protons at 7-8 ppm.

Compound **6** showed δ at 7.8 and 5.1 ppm attributed to NH, OH, and CH$_2$ protons. Also, ^{1}H NMR spectra of compounds **10-12**

indicate the existence of NH protons with aryl protons at δ 8.5-8.1 and 7.6-7.4 (m & m) ppm. Both the compounds **13** and **14** showed δ at 2.5 ppm for aliphatic protons. Only ^{1}H NMR spectra of compounds **15** and **16** exhibited resonated signals at δ 2.90∼2.51 ppm for N-CH$_2$, and aryl-H with lack of both SH and NH protons. All the compounds recorded δ at 7.5 and 7.4 (d,d) ppm being attributed to CH-F protons. The interaction can occur only in "Z" geometry. Thus, the exocyclic -CH=C- was deduced from recorded signals at higher chemical shift at δ7.6 ppm [28].

Also, ^{13}C NMR spectra of compounds **3-5** showed resonated signals at δ 180, 170∼165, 146 and 142 ppm for C=S, C=O, C-F, C=C respectively, while that of **6-8**, there was lack of C=S carbons. ^{13}C NMR spectra of both **10** and **12** exhibited signals at δ 180, 146, 144, and 142-140 ppm which were attributed to C=S, C-F, C-Cl, C=N respectively, and aryl carbons at 133-128 ppm; while the spectra of compound **11** showed a resonated signal at 180 ppm for C=S. Mass fragmentation pattern of some compounds showed a molecular ion peak, which, under the fragmentation process pro-
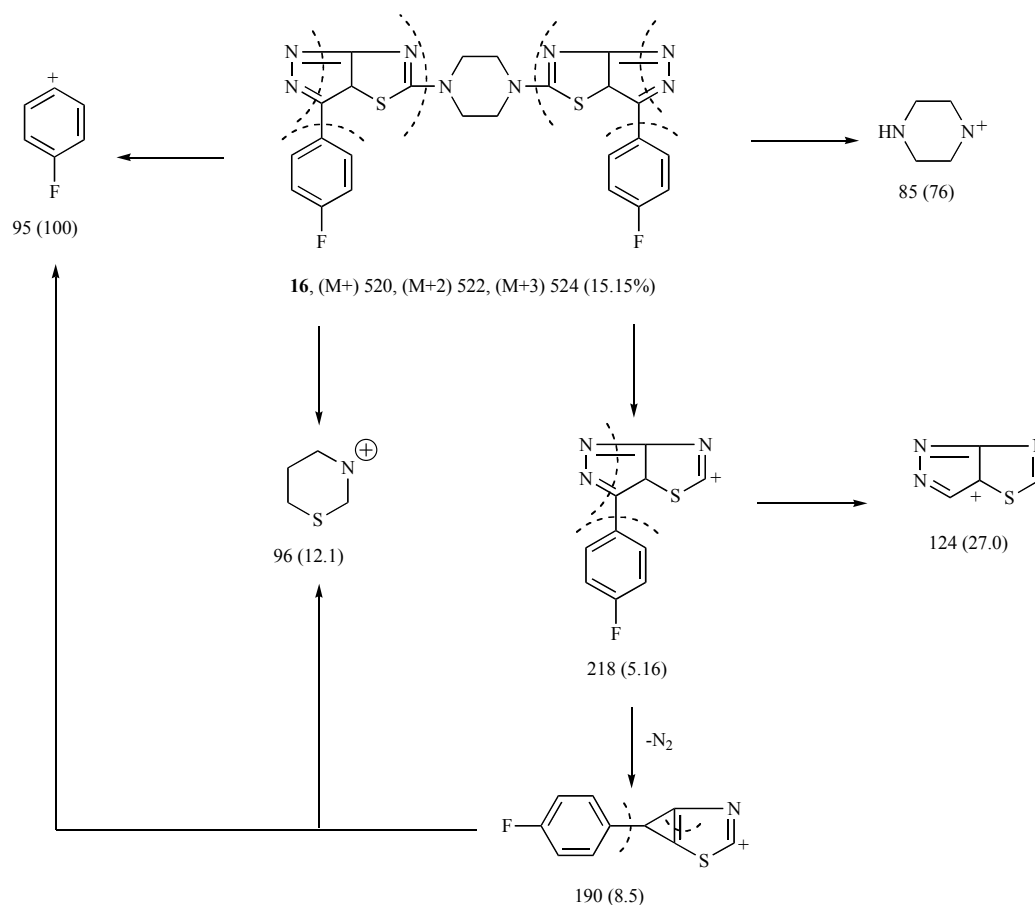
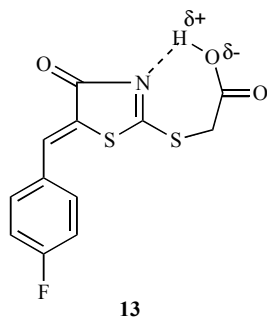**Fig. (5).** Mass fragmentation pattern of compound **16**.

Fig. (6). H-bonding in compound **13**.

duced the base peak m/e 95 attributed to 4-fluorophenyl ions shown in Figs. (**3-5**). The pathway fragment steps indicated the relationship between the common base peak which had a significant role in the biodynamic effects.

It is interesting that ^{13}C NMR spectrum of compound **13**, showed lack of C-OH proton, due to the H-bonding formed as shown in Fig. (**6**).

Finally, ^{19}F NMR spectra of all the synthesized compounds 2-16 showed resonated signals as d,d at -119.75 and -123.16 ppm for the fluorine bonded to the aromatic nucleus.

3.2. Biological Evaluation

3.2.1. Antifungal Activity

All the obtained compounds were evaluated as fungicidal probes against *Candida albicans* and *Aspergillus Fumigatus* fungi

Table 1.   **The biological activity as antifungal of the new fluorinated-substituted rhodanine.**

Compd. No.	C. Albicans (µg/ml)	A. Fumigatus (µg/ml)
3	16	14
4	15	12
5	16	13
6	14	10
7	15	11
8	20	18
9	12	10
10	19	17
11	12	11
12	15	10
13	18	18
14	19	19
15	10	8
16	8	7
Ketoconazole	18	16

by using DMSO as the solvent (1mg/ml) and Ketoconazole as the standard drug. The inhibition was recorded by measuring the diameter inhibition zones at the end of 48 h according to the reported method (Table **1**) [25].

From the results, we can conclude that all the fluorinated systems exhibited an antifungal activity. Higher effects of compounds were observed towards the two tested fungi as: **14 > 13**, **8 > 7 > 6** and **10 > 12 > 11 > 3 > 5 > 4**.

Also, the result obtained indicated a particular activity more than the standard drug used in the order **8 > 10 > 14 > 5 > 3**, while compounds 1,4-disubstituted piperazine **15** and **16** exhibited a lethal activity.

3.2.2. The Enzymatic Effects on Cellobiase of Some Fungi

The efficiency of the synthesized fluorinated systems obtained towards the activity of cellobiase produced enzyme as thermophilic fungi namely, *Thermomyces lanuginosus* and *Chaetomium Thermophilum was* evaluated according to the reported method [26]. In DMF, which was used as a solvent, the enzyme solution and the substrate (cellobiase) was added and the solution was then dissolved in citrate-phosphate buffer at pH 5 and incubated at 40ºC for 1 h. The freed reducing sugar was evaluated colorimetrically at 540 nm as an induction for the enzymatic activity [27].

Table 2. The efficiency of new compounds on cellobiase activity produced by thermophilic fungi[*].

Compd. No.	Amount of Glucose (mg/ml)	
	C. Thermophilum	*T. lanuginosus*
5	4.6	5.2
8	8.0	8.8
10	7.8	8.0
13	6.2	7.0
14	7.0	7.7

[*]Blank: 0.97 and 0.80 mg/ml-without substance DMF. DMF: 0.7 and 0.72 mg/ml.

The data is recorded in Table **2**. Only compounds **5**, **8**, **10**, **13** and **14** showed higher activity against the two tested fungi, which showed the activity of reducing sugar in the order **8 > 10 >14 >13 > 5**. The relationship between the structure and activity showed that the presence of a sulfa drug, fluoroaniline, and alkylthia of 5-(4'-fluorobenzylidene) -2-thioxo-thiazol-5-one Skelton enhanced the antifungal activity.

3.2.3. CDK2 Inhibition of Tumor Cells (DNA Damage)

Cyclin dependent-kinases are a family of serine/ threonine kinases, that strongly control the mammalian cell-cycle by binding to cyclins. Thus, CDK/ cyclin complexes are involved in the DNA repair when they are presented in the inhibition state in response to DNA damage [28-31].

Also, the presence of fluorine atom in various hetero-cyclic nitrogen/ sulfur and oxygen systems, has a significant effect on the modulation of electronic, lipophilic and steric parameters determing the pharmacodynamic and pharmacokinetic properties [32].

Thus, the new fluorine substituted rhodanines (**2-16**) have been evaluated toward CDK2 in a biochemical assay. The inhibitory concentration (IC_{50}) values were reported according to the methods described above [33]. Olomoucine has been used as a standard, and the results are reported in Table **3**. From the data obtained, we can indicate that:

All the tested compounds showed a very significant CDK2 inhibitory activity. Only, compound **2** showed an Olomoucine effect, while the other tested compounds were observed to be more potent than the standard control. It is interesting to note that compound **10** had a higher enzymatic effect as the strong inhibitor for CDK2, which may be used as a new inhibitor in the future.

Table 3. CDK2 inhibiting activity of fluorinated rhodanine derivatives 2-16.

Compd. No.	$IC_{50} \pm SD$ (μM)*
2	5.0 ± 1.7
4	6.0 ± 1.8
8	3.7 ± 1.7
10	15.0 ± 2.7
14	12.0 ± 1.7
15	12.2 ± 1.5
16	4.1 ± 2.8
Olomoucine	5.0 ± 1.0

*IC_{50} in $\mu M/dm^{-1}$, SD: standard deviation.

4. EXPERIMENTAL

Compound **2** was obtained according to the reported method [24].

4.1. 5-(4-Fluorobenzylidene)-3-(hydroxymethyl)-2-thioxo thiazolidin-4-one (3)

A solution of **2** (1.554 g, 6.5 mmol) and HCHO (0.195 g, 6.5 mmol) in MeOH (40 ml) was refluxed for 2 h. The mixture of was cooled down at room temperature. The precipitated product was filtered off and crystallized from dioxane to give compound **3**. Orange crystals, Yield 75% (25.43 g), M.p: 128-130ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3450-3420(broad, OH), 3060(ArH), 2944, 2880(aliph. CH), 1716(C=O), 1616(C=C), 1594(C=C), 1229(C-F), 1157(C=S), 889, 852, 793(subst. phenyl), 707(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.76(s, 1H, Ar CH=C), 7.66, 7.64(d,d, 2H, CH-F), 7.59-7.28(m, 2H, Ar), 4.59(s, OH), 2.51-2.50(s, CH$_2$-OH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 180(C=S), 173(C=O) 167(C-S), 162(Ar CH=C), 142(C-F), 133.6-127.42(Ar carbons), 66.45(CH$_2$ carbon). Calculated, C$_{11}$H$_8$NFS$_2$O$_2$ (269), %: C, 49.07; H, 2.97; N, 5.20; F, 7.06, S,23.79. Found, %: C, 48.77; H, 2.66; N, 5.01; F, 6.85; S, 23.69.

4.2. Mannish Bases 4 & 5

A mixture of compound **2** (1.554 g, 6.5 mmol), piperidine (0.55 g, 6.5 mmol) and/ or morpholine (0.57 g, 6.5 mmol), and HCHO (0.195 g, 6.5 mmol) in MeOH (40 ml) was refluxed for 2h. The mixture was cooled down at room temperature. The obtained precipitate was filtered off and crystallized from dioxane to give **4** and /or **5**.

4: Yellow crystals, Yield 75% (1.57 g), M.p: 165-167ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3050(Ar CH), 2939, 2862, 2798 (aliph. CH), 1725(C=O), 1599(C=C), 1235(C-F), 1190 (C=S), 880, 853(subst. phenyl), 723(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.62(s, Ar-CH =C),7.60, 7.54(d,d, 2H , CH-F), 7.29-7.27(m, 2H, Ar), 2.4(N-Me) 1.62 & 1.56(CH$_2$ of piperidine). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 180(C=S), 173(C=O), 164(C-S), 162(Ar-CH=C), 146(C-F), 132.32-128.82(aromatic carbons), 50.55, 49.75(N-Me & CH$_2$-CH$_2$ of piperidine). Calculated, C$_{16}$H$_{17}$N$_2$FS$_2$O (336), %: C, 57.14; H, 5.05; N, 8.33; F, 5.65; S, 19.04. Found, %: C, 56.88; H, 4.91; N, 8.11; F, 5.55; S, 18.85.

5: Yellow crystals, Yield 78% (1.65 g), M.p: 163-165ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 2937, 2854(str. CH), 1676(C=O), 1600(C=C), 1448, 1412(bending CH), 1389(NCSN), 1266(C-F), 831, 803(aryl ring). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.6, 7.59(d,d 2H, Ar), 7.52, 7.26(d,d, 2H , Ar), 3.795(s, 2H, CH$_2$), 3.53, 2.46, 1.60, 1.54(CH$_2$ protons). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm):

180.19(C=S), 174.98(C=O), 163(C-S), 162.38(Ar-CH=C), 146(C-F), 132-116(Ar carbons), 66.27-65.42(2H, CH$_2$), 26.16-23.71(2H, CH$_2$). Calculated, C$_{15}$H$_{15}$N$_2$FS$_2$O$_2$ (338), %: C, 53.25; H, 4.43; N, 8.28; F,5.62; S, 18.93. Found, %: C, 53.01; H, 4.13; N, 8.11; F, 5.50; S, 18.78.

4.3. 5-(4-Fluorobenzylidene)-2-[(2-hydroxyethyl)amino] thiazole-4(5*H*)-one (6)

An equimolar amounts of compound **2** and ethanolamine were mixed with in ethanol (40 ml) and refluxed for 6h. The mixture was then cooled down at room temperature. The precipitate obtained was filtered off and crystallized from dioxane to give compound **6**. Yellow crystals, Yield 65% (1.125g), M.p: 233-235ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3500 (OH), 3259(NH), 3050(ArH), 2900, 2869(aliph. CH), 1696(C=O), 1621(C=C), 1594(C=C), 1550(C=N), 1488, 444 (bending CH$_2$), 1228(C-F), 895, 859, 790(subst. phenyl), 751(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.8(s, 1H, NH), 7.47, 7.46(d,d, CH-F), 7.21-7.19(m, 2H, Ar), 5.1(s, 1H , OH), 3.45 , 2.39(CH$_2$-CH$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 163.54(C=O), 162.27(Ar-CH=C), 146(C-F), 142(C=N), 32.5-128.02(Ar carbons), 62.78, 47.48(CH$_2$-CH & CH$_2$-N). Calculated, C$_{12}$H$_{11}$N$_2$FSO$_2$ (266), %: C, 54.13; H, 4.13; N, 10.52; F, 7.14; S, 12.03. Found, %: C, 53.89; H, 4.11; N, 10.35; F, 7.00; S, 11.79.

4.4. 5-(4-Fluorobenzylidene)-2-[(4-fluorophenyl)amino] thiazole-4(5*H*)-one (7)

Compound **2** (1.554 g, 6.5 mmol) and 4-fluoroaniline (0.723 g, 6.5 mmol) were dissolved in EtOH (30 ml) and refluxed for 6h. The mixture was then cooled down at room temperature. The precipitated product was filtered off and crystallized from ethanol to give compound **7**. Orange crystals, Yield 80% (1.64 g), M.p: 225-227ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3150(NH), 3050(ArH), 2854(aliph. CH), 1678(C=O), 1610(C=C), 1590(C=N), 1465, 1413(bending CH$_2$), 1228(C-F), 891, 856, 825(substituted phenyl), 735(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 8.2 (s. 1H, NH), 7.67, 7.61(d,d, 2H, CH-F), 7.39-7.37(m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 170(C=O), 169(Ar-CH=C), 145(C-F), 142(C=N), 133.47-125.79(Ar carbons). Mass spectrum, *m/z* (*I*$_{rel}$, %): 315 (317, M+2, 1.50 %), 110(23.8), 95(100), 84(5.15). Calculated, C$_{16}$H$_{10}$N$_2$F$_2$SO (316), %: C, 60.75; H, 3.16; N, 8.86; F, 12.02; S, 10.12. Found, %: C, 60.55; H, 3.11; N, 8.59; F, 11.83; S, 10.01.

4.5. 4-[(5-(4-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino]benzenesulfon-amide (8)

Compound **2** (1.554 g, 6.5 mmol), and sulfanilamide (1.12 g, 6.5 mmol) were dissolved in EtOH (150 ml) with a small amount of piperidine. The mixture was refluxed for 6h, and then cooled down at room temperature. The precipitated product was filtered off and crystallized from MeOH to give compound **8**. Orange crystals, Yield 70% (1.71g), M.p: 168-170ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3180(NH), 3050(ArH), 2859(aliph. CH), 1690(C=O), 1592(C=C), 1576(C=N), 1465, 1416(bend. CH$_2$), 1314 (NHSO$_2$), 1230(C-F), 910, 826, 794(subst. phenyl), 706(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 8.2(s,1H, NH), 7.67, 7.66(Ar-CH=C), 7.65, 7.63(d, d, 2H, CH-F), 7.46, 7.45(d,d, 2H, 2CH-SO$_2$), 7.40-7.34, 6.89-6.58(each m, 4H , ArH), 2.50(NH$_2$-SO$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 173(C=O), 169(Ar-CH=C), 162(C-F), 152(C=N), 133.46-125.77(Ar carbons). Calculated, C$_{16}$ H$_{12}$ N$_3$FS$_2$O$_3$ (377), %: C, 50.92; H, 3.18; N, 11.14; F, 5.03; S, 16.97. Found, %: C, 50.71; H, 3.01; N, 10.95; F, 4.89; S, 16.77.

4.6. 4-Chloro-5-(4-fluorobenzylidene)thiazole-2(5*H*)-thione (9)

A mixture of **2** (7 g, 29.28 mmol) and POCl$_3$ (15ml) was heated to the fusion for 1h, and then cooled down at room temperature. After that, it was poured onto ice and stirred. The precipitated prod-

uct was filtered off and crystallized from ethyl benzene to give compound **9**. Dark-green crystals, Yield 65% (4.91 g), M.p: 178-180ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3060(ArH), 2854(aliph. CH), 1594(C=N), 1229(C-F), 1192(C=S), 853, 827(subst. phenyl), 707(C-Cl). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.84 (Ar CH=C), 7.78, 7.77(d,d, 2H, CH-F), 7.75-7.05(m , 2H, ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 195(C=S), 169.4(Ar-CH=C), 165(C-F), 162(C=N), 139.28(C-Cl), 133-122.33(Ar carbons). Calculated, C$_{10}$H$_5$NFClS$_2$ (257), %: C, 46.69; H, 1.94; N, 5.44; F, 7.30; S, 24.90; Cl, 13.61. Found, %: C, 46.48; H, 1.69; N, 5.33; F, 7.15; Cl, 13.45; S, 24.79.

4.7. 5-(4-Fluorobenzylidene)-4-[(4-fluorophenyl)amino] thiazole-2(5*H*)-thione (10)

Equimolar amounts of compound **2** and *p*-fluoroaniline were dissolved in DMF (15 ml) and refluxed for 1h. The mixture was then cooled down at room temperature and poured onto ice. The precipitated product was filtered off and crystallized from ethanol to give compound **10**. Dark-brown crystals, Yield 82% (1.73 g), M.p: 179-180ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3151(NH), 3060(ArH), 2880 (aliph. CH), 1603(C=C), 1574(C=N), 1221(C-F), 830, 810(subst. phenyl), 710 (C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 8.26 (s, 1H, NH), 7.7(Ar- CH= C), 7.698, 7.694(d, d, 2H, CH-F), 7.68, 7.63(d,d, 2H, CH-F), 7.61-7.45, 7.37-7.12(each m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 178.89(C=S), 163.07(Ar-CH=C), 159.46(C-F), 157.53(C-F), 155(C=N), 135.65-126.25(Ar carbons). Mass spectrum, *m/z* (*I*$_{rel}$, %): 332(335, M+3, 1.18%); 164(25.10), 110(85.0), 109(80.11), 95(100), 71(12.0), 58(15.1). Calculated, C$_{16}$H$_{10}$N$_2$F$_2$S$_2$ (332), %: C, 57.83; H, 3.02; N, 8.43; F, 11.44; S, 19.27. Found, %: C, 57.61; H, 2.85; N, 8.12; F, 11.08; S,19.11.

4.8. 3-(4-Fluorophenyl)-3,6-dihydro-5*H*-pyrazolo[3,4-*d*] thiazole-5-thione (11)

A solution of compound **2** (1.635 g, 6.34 mmol) and NH$_2$NH$_2$ (0.2 g, 6.34 mmol) was dissolved in DMF (50ml) and refluxed for 6 h. The reaction mixture was cooled down at room temperature, and then poured onto ice. The precipitated product was filtered off and crystallized from EtOH to give compound **11**. Light-brown crystals, Yield 72% (1.24 g), M.p: 133-135ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3180(NH), 3060(ArH), 2880(aliph. CH), 1644(bend. NH), 1607(C=C), 1568(C=N), 1230(C-F), 1150(C=S), 862, 810(subst. phenyl), 716(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 8.5(s, 1H, NH), 7.8(Ar CH=C), 7.3, 7.2(d,d, 2H, CH-F), 7.0-6.5(m, 2H, ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 180(C=S), 147(C-F), 142(C=N), 138(C=C), 133-123(Ar carbons). Calculated, C$_{10}$H$_7$N$_3$FS$_2$ (252), %: C, 47.61; H, 2.77; N, 16.66; F, 7.53; S, 25.39. Found, %: C, 47.42; H, 2.55; N, 16.41; F, 7.11; S, 25.09.

4.9. 2-(4-Chlorophenyl)-3-(4-fluorophenyl)-1,2-dihydro-5*H*-pyrazolo[3,4-*d*]thiazole-5-thione (12)

Compound **2** (1.635 g, 6.34 mmol) and 4-chlorophenyl hydrazine HCl (0.904 g, 6.34 mmol) were dissolved in DMF (50ml) and refluxed for 6h. The mixture was then cooled down at room temperature and onto ice. The precipitated product was filtered off and crystallized from ethanol to give **12**. Black crystals, Yield 78% (1.93g), M.p: 188-190ºC. FT-IR spectrum **ῡ** (cm^{-1}): 3239(NH), 3060(ArH), 2900(aliph. CH), 1596(C=C), 1567 (C=N), 1488(bend. CH), 1225(C-F), 1157(C=S), 890, 823, 786(subst. phenyl), 736(C-F), 664(C-Cl). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 10.47(s, 1H, NH), 7.87, 7.71(d,d, 2H, CH-F), 7.0, 7.69 (d,d, 2H, CH-Cl), 7.63-7.24 & 7.23-6.72(each m, 4H , ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 179.38(C=S), 168(C-S), 160(C=N), 148(C-F), 144(C-N), 137(C-C), 132.95-121.37(Ar carbons). Calculated, C$_{16}$

$H_9N_3FCl\ S_2$ (361), %: C, 53.18; H, 2.49; N, 11.63; F, 5.26; Cl, 9.27; S, 17.72. Found, %: C, 52.98; H, 2.39; N, 11.51; F, 5.20; Cl, 8.89; S, 17.55.

4.10. 2-[(5-(4-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)thio]acetic acid (13)

Compound **2** (4 g, 16.73 mmol) and chloroacetic acid (1.58 g, 16.73 mmol) were dissolved in DMF (25 ml) and refluxed for 1h. The reaction mixture was then cooled down at room temperature poured onto ice. The precipitated product was filtered off and crystallized from ethanol to give compound **13**. Light-yellow crystals, Yield 65% (3.23 g), M.p: 238-240ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3500-3100(broad, OH), 3060(ArH), 2937, 2880(aliph. CH), 1693, 1675(2C=O), 1595(C=C), 1562 (C=N), 1443, 1411(bend. CH$_2$), 1232(C-F), 1001(C-S-C), 827, 793(subst. phenyl), 749 (C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.0(Ar CH=C), 7.697, 7.690(d,d, 2H, CH-F), 7.630-7.360(m, 2H, ArH), 2.50(2H, CH$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 178.91(C-S), 163.0(Ar-CH=C), 161(C-F), 144(C=N), 131.84-128.59(Ar carbons), 49.56(CH$_2$ carbons). Calculated, $C_{16}H_8NFS_2O_3$ (297), %: C, 48.48; H, 2.69; N, 4.71; F, 6.39; S, 21.54. Found, %: C, 48.31; H, 2.43; N, 4.55; F, 6.12; S, 21.40.

4.11. 5-(4-Fluorobenzylidene)-2-(methylthio)thiazol-4(5H)-one (14)

Compound **13** (1.5 g, mmol) and *aq.* K$_2$CO$_3$ (5%, 15 ml) were heated for 1h, and then cooled down followed by neutralization by AcOH to give **14**. Light-yellow crystals, Yield 55% (0.70g), M.p: 173-175ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3050(ArH), 2936(aliph. CH), 1674.9(C=O), 1620(C=C), 1599(C=N), 1467, 1411(bend. CH$_3$), 1224(C-F), 1050(C-S-C), 853, 832, 822(subst. phenyl), 723(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.70(Ar CH=C), 7.69, 7.68(d,d, 2H, CH- F), 7.63-7.53, 7.37-7.16(2H , ArH), 2.51(Me-S). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 184, 179(C-S), 163.52 (Ar-CH=C), 162.70(C-F), 161.55(C=N), 136-121.39(Ar CH), 26.21(CH$_3$). Calculated, $C_{11}H_8NFS_2O$ (253), %: C, 52.17; H, 3.16; N, 5.53; F, 7.50; S, 25.29. Found, %: C, 52.01; H, 2.89; N, 5.28; F, 7.31; S, 25.11.

4.12. 2,2'-(Piperazine-1,4-diyl)bis[5-(4-fluorobenzylidene) thiazol-4(5H)-one] (15)

Compound **2** (4 g, 16.73 mmol) and piperazine (0.721 g, 8.37 mmol) were dissolved in EtOH (70 ml), refluxed for 6-8h, and then cooled down. The precipitated product was filtered off and crystallized from ethanol to give compound **15**. Yellow crystals, Yield 85% (3.53g), M.p: 273-275ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3060(ArH), 2939, 2880(aliph. CH), 1690, 1666(2C=O), 1597(C=C), 1564 (C=N), 1505, 1414(bend. CH$_2$), 1231(C-F), 847, 826, 810(subst. phenyl), 725(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 7.70(Ar- CH=C), 7.696, 7.693, 7.68, 7.65(each d,d , 4H , CH-F), 7.54-7.36 , 7.35-7.17(each m, 4H , ArH), 2.90, 2.89 ,2.86 , 2.55, 2.51(4 CH$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 173.74, 173.2(C=O), 163.11, 161.90(C-F), 131.86-115.95(Ar carbons), 49.91-49.18(CH$_2$). Calculated, $C_{24}H_{18}N_4\ F_2S_2O_2$ (496), %: C, 58.06; H, 3.62; N, 10.88; F, 7.66; S, 12.90. Found, %: C, 57.85; H, 3.41; N, 10.44; F, 7.49; S, 12.78.

4.13. 1,4-Bis(3-(4-fluorophenyl)-3H-pyrazolo[3,4-d]thiazol-5-yl)piperazine (16)

Compound **15** (2.5 g, 5.03 mmol) and hydrazine hydrate (0.503 g, 10.06 mmol) were dissolved in EtOH (45 ml) with a small amount of piperidine, and refluxed for 6-8h. The reaction mixture was cooled down at room temperature, and then poured onto ice. The obtained product was filtered off and crystallized from MeOH to give compound **16**. Orange crystals, Yield 70% (1.835g), M.p: 188-190ºC. FT-IR spectrum $\bar{\upsilon}$ (cm^{-1}): 3080(ArH), 2938,

2880(aliph. CH), 1600(C=C), 1560(C=N), 1221(C-F), 914, 821, 785 (substituted-phenyl), 742(C-F). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 10.27(s, 1H, C$_5$-H of thiazole), 7.8-7.76, 7.40-7.34 (each d,d, 4H, CH-F), 7.31-6.86(m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ (ppm): 172.21(S-CN-N), 161(C-F), 132.8-114.93 (Ar carbons), 47-46.9(CH$_2$), 24.6-23.76(CH). Mass spectrum, *m/z* (I_{rel}, %): 520(524, M+3, 15.15%), 218(5.16), 124(27.0), 190(8.5), 95(100), 96(12.1), 85(70.0). Calculated, $C_{24}H_{18}N_8F_2S_2$ (520), %: C, 55.38; H, 3.46; N, 21.53; F, 7.30; S, 12.30. Found, %: C, 55.18; H, 3.28; N, 21.11; F, 7.15; S, 12.20.

CONCLUSION

Due to the high resistance of fungi towardantibiotics and drugs, the present work designed, synthesized and evaluated them as dual binding site modified fluorine compounds bearing rhodanine drug inhibitors

Thus, in search for new rhodanine derivatives, the recent development of rhodanine *via the* introduction of both fluorine atoms followed by alkyl, amino and hydrazino substituents at C-2, N-3, C=O and CH$_2$ corresponding to **2**, **3**, **4** and **5**-position has been deduced with a hope to obtain highly biologically active probes for their enzymatic effects on the cellobiase activity produced by some fungi and as CDK2 inhibitors of tumor cells.

The importance of novel fluorine substituted rhodanine as modified drugs has been highlighted in this study. The highly bioactive substitutes at positions **2** and **5** have gainedmore attention of the researchers. The study also concluded that both the compounds **2** and **10** can be used as Olomoucine (standard referees).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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