EXPERIMENTAL

Remarks and instruments:

Melting points were determined in open-glass capillaries using a Griffin melting point apparatus and are uncorrected.

Follow up of the reactions rates were performed by thin-layer chromatography (TLC) on silica gel (60 GF254) coated glass plates and the spots were visualized by exposure to iodine vapors or UV-lamp at λ 254 nm for few seconds.

Infrared spectra (**IR**) were recorded on Bruker Tensor 37 FT-IR spectrometer at the Central Laboratory Unit, Faculty of Science, Alexandria University.

Nuclear magnetic resonance spectra, **^{1}H-NMR** and **^{13}C-NMR**, were scanned on Jeol spectrophotometer (500 MHz) at Central Laboratory Unit, Faculty of Science, Alexandria University and on a 400 MHz Bruker Avance III FT-NMR Spectrometer at Central Laboratory Unit, Faculty of Pharmacy, Cairo University using deuterated dimethylsulfoxide as a solvent. The data were reported as chemical shifts or δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Signals are indicated by the following abbreviations: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet and *m* = multiplet.

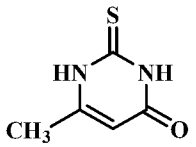
High resolution mass spectra (**HRMS**) were run on AB SCIEX Triple Quad™ 5500 LC/MS/MS at preclinical studies center, City of Scientific Research and Technological Applications, Borg El-Arab, Alexandria.

Electron impact mass spectra (**EIMS**) were run on a gas chromatograph/mass spectrophotometer Shimadzu GCMS/QP-2010 plus (70 eV) at the faculty of Science, Cairo University. Relative intensity % corresponding to the most characteristic fragments were recorded.

Elemental microanalyses were performed at the microanalytical Center, Faculty of Science, Cairo University.

Scheme 1:

6-Methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one, 1

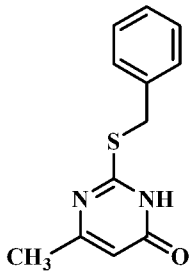


1

Ethyl acetoacetate (35.69 ml, 36.4 g, 0.28 mol) was added to a solution of sodium ethoxide, prepared from sodium (6.5 g, 0.28 g. atom) and absolute ethanol (100 ml). The resulting solution was heated to 60°C, and crystalline thiourea (21.6 g, 0.28 mol) was added at that temperature with vigorous stirring. A suspension formed was refluxed for 3 h. After cooling, the precipitate obtained was filtered off, dissolved in water (500 ml), and acidified with conc. HCl to pH 2. The precipitate obtained was filtered off, washed with cold water, and dried at 100°C for 6 h; yield (23 g, 58%) reported (50%); mp >300°C as reported.[99]

IR (KBr, cm^{-1}): 3115 (NH); 1637 (C=O); 1422, 1384, 1241, 1165 (N-C=S).

2-(Benzylsulfanyl)-6-methylpyrimidin-4(3*H*)-one, 2

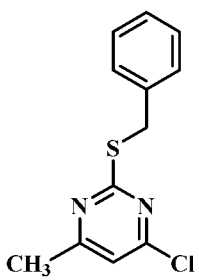


2

Compound **1** (0.14 g, 1 mmol) was added to water (15 ml) containing sodium hydroxide (0.05 g, 1.25 mmol) then benzyl chloride (0.12 ml, 0.13 g, 1 mmol) was added and the reaction mixture was stirred at room temperature overnight. The precipitate obtained was filtered off, washed 3 times with water then twice using diethyl ether, dried and crystallized from ethanol; yield (0.19 g, 83%) reported (78%);[109] mp 186-188°C (reported: 186°C,[109] 183-184 °C).[159]

IR (KBr, cm^{-1}): 3404 (NH); 1647 (C=O); 1572 (C=N); 1225, 1069 (C-S-C).

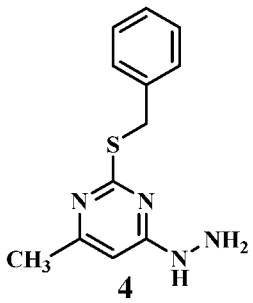
2-(Benzylsulfanyl)-4-chloro-6-methylpyrimidine, 3



3

A mixture of compound **2** (0.23 g, 1 mmol) and phosphorous oxychloride (2.5 ml) was heated under reflux for 1 h then cooled to room temperature and poured dropwise over crushed ice. The oil obtained was extracted with methylene chloride. The extract was dried over anhydrous sodium sulfate for 24 h, filtered, and evaporated to dryness under reduced pressure. A pure oil product was obtained which was used in the following reaction without additional purification; yield (0.2 g, 80%) reported (79%).[109]

2-(Benzylsulfanyl)-4-hydrazinyl-6-methylpyrimidine, 4



4

A mixture of the chloropyrimidine **3** (0.25 g, 1 mmol) and hydrazine hydrate 99% (0.1 ml, 0.1 g, 2 mmol) in absolute ethanol (2 ml) was heated under reflux for 1.5 h, cooled and evaporated on water bath. Crushed ice was added to the residue and the precipitate obtained was filtered off, washed 3 times with water then twice using petroleum ether (40-60), dried and crystallized from methylene chloride/petroleum ether (40-60); yield (0.22 g, 88%); mp 88°C.

Microanalyses for $C_{12}H_{14}N_4S$ (246.33):

	C%	H%	N%	S%
Calcd.	58.51	5.73	22.74	13.02
Found	58.63	5.81	22.90	13.12

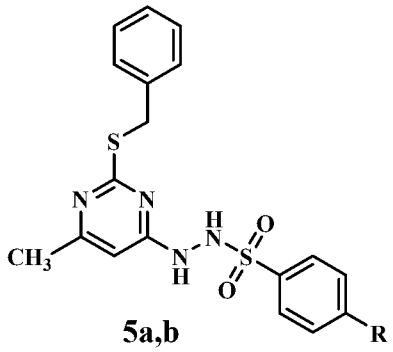
IR (KBr, cm^{-1}): 3349 (NH); 3234, 3183 (NH_2) 1650 (C=N); 1281, 1027 (C-S-C).

^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.19 (s, 3H, CH_3); 4.30 (s, 2H, S-CH_2); 4.33 (s, 2H, NH_2, D_2O-exchangeable); 6.30 (s, 1H, pyrimidine C_5-H); 7.22 (t, *J*=7.3 Hz, 1H, benzyl C_4-H); 7.29 (t, *J*=7.3 Hz, 2H, benzyl $C_{3,5}$-H); 7.41 (d, *J*=7.3 Hz, 2H, benzyl $C_{2,6}$-H); 8.35 (s, 1H, NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.98 (CH_3); 34.20 (S-CH_2); 97.26 (pyrimidine C_5); 127.22 (benzyl C_4); 128.73 (benzyl $C_{2,6}$); 129.42 (benzyl $C_{3,5}$); 139.41 (benzyl C_1); 164.80 (pyrimidine C_6); 165.64 (pyrimidine C_4); 168.67 (pyrimidine C_2).

HRMS (ESI): *m/z* calcd for $C_{12}H_{15}N_4S$: 247.102 $(M+1)^+$; found: 247.687.

N'-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]-4-substituted benzene-sulfonohydrazides, 5a,b



5a,b

A mixture of the hydrazino derivative **4** (0.25 g, 1 mmol) and anhydrous potassium carbonate (0.14 g, 1 mmol) in methylene chloride (5 ml) was stirred at room temperature for 20 minutes then the appropriate sulfonyl chloride (1 mmol) was added and stirring continued for 1.5 h. The reaction mixture was evaporated to dryness on water bath and water (10 ml) was added to the residue and left to stand overnight. The precipitate obtained was filtered off, washed with water, dried and crystallized from the appropriate solvent.

Table 1: Physical and microanalytical data of compounds 5a,b

ID	R	Yield (%)	M.P. (°C) (Cryst. solvent)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
5a	H	89	73-75 Ethanol/H_2O	$C_{18}H_{18}N_4O_2S_2$ (386.49)	**C**	55.94	56.08
					H	4.69	4.73
					N	14.50	14.62
					S	16.59	16.67
5b	CH_3	54	155-157 Methylene chloride/petroleum ether (40-60)	$C_{19}H_{20}N_4O_2S_2$ (400.52)	**C**	56.98	57.17
					H	5.03	5.09
					N	13.99	14.16
					S	16.01	16.08

N'-(2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl)benzenesulfonohydrazide, 5a

IR (KBr, cm^{-1}): 3333 (NH); 1585 (C=N); 1329 (SO_2 asym); 1238, 1087 (C-S-C); 1157 (SO_2 sym).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.15 (s, 3H, CH_3); 4.21 (s, 2H, S-CH_2); 6.21 (s, 1H, pyrimidine C_5-H); 7.19 (t, *J*=7.6 Hz, 1H, benzyl C_4-H); 7.25 (t, *J*=7.6 Hz, 2H, benzyl $C_{3,5}$-H); 7.36 (d, *J*=7.6 Hz, 2H,benzyl $C_{2,6}$-H); 7.56 (t, *J*=7.4 Hz, 2H, SO_2-phenyl $C_{3,5}$-H); 7.65 (t, *J*=7.4 Hz, 1H, SO_2-phenyl C_4-H); 7.78 (d, *J*=7.4 Hz, 2H, SO_2-phenyl $C_{2,6}$-H); 9.32, 9.95 (two s, each 1H, 2NH, D_2O-exchangeable).

N'-(2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl)-4-methylbenzenesulfono-hydrazide, 5b

IR (KBr, cm^{-1}): 3237 (NH); 1587 (C=N); 1338 (SO_2 asym); 1284, 1087 (C-S-C); 1159 (SO_2 sym).

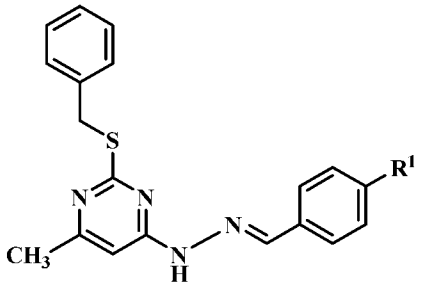
^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.14 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); 4.21 (s, 2H, S-CH_2); 6.19 (s, 1H, pyrimidine C_5-H); 7.19 (t, *J*=7.3 Hz, 1H, benzyl C_4-H); 7.25 (t, *J*=7.3 Hz, 2H, benzyl $C_{3,5}$-H); 7.36 (d, *J*=6.1 Hz, 4H, benzyl $C_{2,6}$-H, SO_2-phenyl $C_{3,5}$-H); 7.65 (d, *J*=6.7 Hz, 2H, SO_2-phenyl $C_{2,6}$-H); 9.26, 9.84 (two s, each 1H, 2NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 21.38 (tolyl CH_3); 23.58 (pyrimidine CH_3); 34.17 (S-CH_2); 98.13 (pyrimidine C_5); 127.49 (benzyl C_4); 128.06 (benzyl $C_{2,6}$); 128.80 (tolyl $C_{2,6}$); 129.40 (benzyl $C_{3,5}$); 130.18 (tolyl $C_{3,5}$); 135.37 (tolyl C_1); 138.75 (benzyl C_1); 144.71 (tolyl C_4); 162.77 (pyrimidine C_6); 166.50 (pyrimidine C_4); 169.08 (pyrimidine C_2).

HRMS (ESI): *m/z* calcd for $C_{19}H_{21}N_4O_2S_2$: 401.111 $(M+1)^+$; found: 401.084.

Scheme 2:

4-[2-(4-Substituted benzylidene)hydrazinyl]2-(benzylsulfanyl)-6-methyl-pyrimidines, 6a-c



6a-c

A mixture of the hydrazino derivative **4** (0.25 g, 1 mmol) and the appropriate aldehyde (1 mmol) in absolute ethanol (2 ml) was heated under reflux for 0.5-2.5 h then cooled to room temperature. The precipitate obtained was filtered off, washed with ethanol, dried and crystallized from the appropriate solvent.

For **6a**, reaction mixture was concentrated to its half volume then 2 drops water was added and mixture was heated till solution return clear again and left overnight. The precipitate obtained was filtered off, washed with water, dried and crystallized from the appropriate solvent.

Table 2: Physical and microanalytical data of compounds 6a-c

ID	R^1	Yield (%)	M.P. (°C) (Cryst. solvent)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
6a	H	58	103-104 Ethanol/H_2O	$C_{19}H_{18}N_4S$ (334.44)	**C**	68.23	68.35
					H	5.42	5.51
					N	16.75	16.89
					S	9.59	9.68
6b	Cl	47	167-169 Ethanol/H_2O	$C_{19}H_{17}ClN_4S$ (368.88)	**C**	61.86	62.03
					H	4.65	4.63
					N	15.19	15.41
					S	8.69	8.82
6c	Br	82	194-196 Methylene chloride/petroleum ether (40-60)	$C_{19}H_{17}BrN_4S$ (413.33)	**C**	55.21	55.27
					H	4.15	4.19
					N	13.55	13.72
					S	7.76	7.84

4-(2-Benzylidenehydrazinyl)-2-(benzylsulfanyl)-6-methylpyrimidine, 6a

IR (KBr, cm^{-1}): 3442 (NH); 1576 (C=N); 1229, 1029 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3); 4.33 (s, 2H, S-CH_2); 6.80 (s, 1H, pyrimidine C_5-H); 7.20 (t, *J*=7.5 Hz, 1H, benzyl C_4-H); 7.27 (t, *J*=7.5 Hz, 2H, benzyl $C_{3,5}$-H); 7.34-7.42 (m, 5H, benzyl $C_{2,6}$-H, benzylidene $C_{3,4,5}$-H); 7.69 (d, *J*=6.9 Hz, 2H, benzylidene $C_{2,6}$-H); 8.08 (s, 1H, =CH); 11.38 (s, 1H, NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 24.04 (CH_3); 34.26 (S-CH_2); 98.28 (pyrimidine C_5); 127.04 (benzyl C_4); 127.44 (benzyl $C_{2,6}$); 128.84 (benzyl $C_{3,5}$); 129.30 (benzylidene $C_{3,5}$); 129.45 (benzylidene $C_{2,6}$); 130.05 (benzylidene C_4); 134.82 (benzylidene C_1); 138.94 (benzyl C_1); 143.60 (=CH-); 166.84 (pyrimidine C_6); 169.26 (pyrimidine C_4); 171.36 (pyrimidine C_2).

HRMS (ESI): *m/z* calcd for $C_{19}H_{19}N_4S$: 335.133 $(M+1)^+$; found: 335.069.

2-(Benzylsulfanyl)-4-[2-(4-chlorobenzylidene)hydrazinyl]-6-methylpyrimidine, 6b

IR (KBr, cm^{-1}): 3455 (NH); 1574 (C=N); 1264, 1090 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3); 4.32 (s, 2H, S-CH_2); 6.81 (s, 1H, pyrimidine C_5-H); 7.20 (t, *J*=7.7 Hz, 1H, benzyl C_4-H); 7.27 (t, *J*=7.7 Hz, 2H, benzyl $C_{3,5}$-H); 7.41 (d, *J*=7.7 Hz, 2H, benzyl $C_{2,6}$-H); 7.45 (d, *J*=8.4 Hz, 2H, benzylidene $C_{3,5}$-H); 7.71 (d, *J*=8.4 Hz, 2H, benzylidene $C_{2,6}$-H); 8.05 (s, 1H, =CH); 11.45 (s, 1H, NH, D_2O- exchangeable).

HRMS (ESI): *m/z* calcd for $C_{19}H_{18}ClN_4S$: 369.094 $(M+1)^+$; found: 368.893.

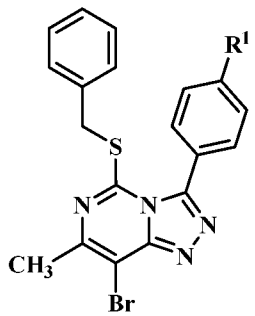
2-(Benzylsulfanyl)-4-[2-(4-bromobenzylidene)hydrazinyl]-6-methylpyrimidine, 6c

IR (KBr, cm^{-1}): 3438 (NH); 1579 (C=N); 1264; 1124 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3); 4.32 (s, 2H, S-CH_2); 6.81 (s, 1H, pyrimidine C_5-H); 7.20 (t, *J*=7.2 Hz, 1H, benzyl C_4-H); 7.27 (t, *J*=7.2 Hz, 2H, benzyl $C_{3,5}$-H); 7.41 (d, *J*=7.2 Hz, 2H, benzyl $C_{2,6}$-H); 7.58 (d, *J*=8.4 Hz, 2H, benzylidene $C_{3,5}$-H); 7.64 (d, *J*=8.4 Hz, 2H, benzylidene $C_{2,6}$-H); 8.04 (s, 1H, =CH); 11.46 (s, 1H, NH, D_2O-exchangeable).

HRMS (ESI): *m/z* calcd for $C_{19}H_{18}BrN_4S$: 413.044 $(M+1)^+$; found: 412.727.

3-(4-Substituted phenyl)-5-(benzylsulfanyl)-8-bromo-7-methyl-[1,2,4]-triazolo[4,3-*c*]pyrimidines, 7a-c



7a-c

To a stirred mixture of the appropriate hydrazone derivative **6a-c** (1 mmol) and anhydrous sodium acetate (0.25 g, 3 mmol) in glacial acetic acid (2 ml), bromine (0.1 ml, 0.32 g, 2 mmol) was added. The reaction mixture was stirred at room temperature for 1 h then poured over crushed ice. The precipitate obtained was filtered off, washed with water, dried and crystallized from the appropriate solvent.

Table 3: Physical and microanalytical data of compounds 7a-c

ID	R^1	Yield (%)	M.P. (°C) (Cryst. solvent)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
7a	H	50	191-192 Ethanol/H_2O	$C_{19}H_{15}BrN_4S$ (411.32)	**C**	55.48	55.70
					H	3.68	3.72
					N	13.62	13.80
					S	7.79	7.86
7b	Cl	98	163-165 Ethanol/H_2O	$C_{19}H_{14}BrClN_4S$ (445.76)	**C**	51.19	51.34
					H	3.17	3.14
					N	12.57	12.74
					S	7.19	7.41
7c	Br	82	132-134 Methylene chloride/petroleum ether (40-60)	$C_{19}H_{14}Br_2N_4S$ (490.21)	**C**	46.55	46.71
					H	2.88	2.87
					N	11.43	11.52
					S	6.54	6.67

5-(Benzylsulfanyl)-8-bromo-7-methyl-3-phenyl-[1,2,4]triazolo[4,3-*c*]pyrimidine, 7a

IR (KBr, cm^{-1}): 1593 (C=N); 1302, 1038 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.55 (s, 3H, CH_3); 4.39 (s, 2H, S-CH_2); 7.18 (t, *J*=7.1 Hz, 1H, benzyl C_4-H); 7.23 (t, *J*=7.1 Hz, 2H, benzyl $C_{3,5}$-H); 7.31(d, *J*=7.1 Hz, 2H, benzyl $C_{2,6}$-H); 7.49 (t, *J*=6.9 Hz, 2H, phenyl $C_{3,5}$-H); 7.58 (t, *J*=6.9 Hz, 1H, phenyl C_4-H); 7.62 (d, *J*=6.9 Hz, 2H, phenyl $C_{2,6}$-H).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 22.54 (CH_3); 36.10 (S-CH_2); 100.73 (triazolopyrimidine C_8); 127.14 (benzyl C_4); 127.93 (phenyl $C_{2,6}$); 128.21 (benzyl $C_{2,6}$); 128.87 (benzyl $C_{3,5}$); 129.76 (phenyl $C_{3,5}$); 131.43 (phenyl C_4); 131.90 (phenyl C_1); 136.58 (benzyl C_1); 147.16 (triazolopyrimidine C_{8a}); 148.13 (triazolopyrimidine C_3); 148.60 (triazolopyrimidine C_7); 149.39 (triazolopyrimidine C_5).

EIMS m/z (relative abundance %): 412 [$M^{+\bullet}$+2] (14.29); 182 (13.48); 165 (17.30); 138 (23.14); 135 (14.29); 119 (24.95); 111 (20.72); 109 (26.56); 99 (47.08); 95 (27.16); 90 (43.66); 83 (100); 69 (50.30); 68 (23.14); 67 (33.60); 57 (40.24); 52 (60.97).

5-(Benzylsulfanyl)-8-bromo-3-(4-chlorophenyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]-pyrimidine, 7b

IR (KBr, cm^{-1}): 1593 (C=N); 1242, 1033 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.55 (s, 3H, CH_3); 4.42 (s, 2H, S-CH_2); 7.19 (t, *J*=7.5 Hz, 1H, benzyl C_4-H); 7.24 (t, *J*=7.5 Hz, 2H, benzyl $C_{3,5}$-H); 7.34 (d, *J*=7.5 Hz, 2H, benzyl $C_{2,6}$-H); 7.58 (d, *J*=8.4 Hz, 2H, chlorophenyl $C_{3,5}$-H); 7.68 (d, *J*=8.4 Hz, 2H, chlorophenyl $C_{2,6}$-H).

EIMS m/z (relative abundance %): 448 [$M^{+\bullet}$+4] (13.62); 446 [$M^{+\bullet}$+2] (38.21); 444 [$M^{+\bullet}$] (33.89); 428 (27.24); 418 (26.25); 410 (29.24); 389 (29.57); 378 (40.53); 369 (26.91); 354 (96.01); 337 (31.89); 325 (36.88); 319 (37.54); 246 (38.54); 205 (45.18); 159 (100); 113 (67.11).

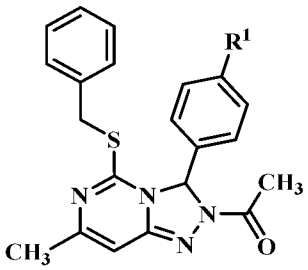
5-(Benzylsulfanyl)-8-bromo-3-(4-bromophenyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]-pyrimidine, 7c

IR (KBr, cm^{-1}): 1596 (C=N); 1300, 1067 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.55 (s, 3H, CH_3); 4.42 (s, 2H, S-CH_2); 7.19 (t, *J*=7.4 Hz, 1H, benzyl C_4-H); 7.24 (t, *J*=7.4 Hz, 2H, benzyl $C_{3,5}$-H); 7.34 (d, *J*=7.4 Hz, 2H, benzyl $C_{2,6}$-H); 7.60 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{3,5}$-H); 7.72 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{2,6}$-H).

EIMS m/z (relative abundance %): 492 [$M^{+\bullet}$+4] (13.62); 490 [$M^{+\bullet}$+2] (38.21); 488 [$M^{+\bullet}$] (33.89); 487 (10.39); 383 (45.10); 381 (28.48); 319 (10.64); 259 (11.48); 226 (10.06); 183 (10.64); 131 (12.77); 109 (10.99); 102 (36.46); 92.10 (14.66); 91 (100); 89 (11.51); 80 (14.94); 65 (40.01).

1-[3-(4-Substituted phenyl)-5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo-[4,3-*c*]pyrimidin-2(3*H*)-yl]ethanones, 8a-c



8a-c

A suspension of the appropriate hydrazone derivative **6a-c** (1 mmol) in acetic anhydride (1 ml) was heated under reflux with stirring for 4-6 h then the reaction mixture was cooled to room temperature, poured over crushed ice and left for 30 min. The precipitate obtained was filtered off, washed with water, dried and crystallized from the appropriate solvent.

Table 4: Physical and microanalytical data of compounds 8a-c

ID	R^1	Yield (%)	M.P. (°C)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
8a	H	61	98-100 Ethanol/H_2O	$C_{21}H_{20}N_4OS$ (376.48)	**C**	67.00	67.14
					H	5.35	5.38
					N	14.88	15.01
					S	8.52	8.58
8b	Cl	82	136-137 Ethanol/H_2O	$C_{21}H_{19}ClN_4OS$ (410.92)	**C**	61.38	61.33
					H	4.66	4.69
					N	13.63	13.80
					S	7.80	7.93
8c	Br	85	160-162 Methylene chloride/petroleum ether (40-60)	$C_{21}H_{19}BrN_4OS$ (455.37)	**C**	55.39	55.62
					H	4.21	4.28
					N	12.30	12.39
					S	7.04	7.11

1-[5-(Benzylsulfanyl)-7-methyl-3-phenyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl]-ethanone, 8a

IR (KBr, cm^{-1}): 3438 (NH); 1684 (C=O); 1611 (C=N); 1268, 1032 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.44 (s, 3H, CH_3); 2.48 (s, 3H, $COCH_3$); 4.30 (s, 2H, S-CH_2); 7.13-7.18 (m, 3H, benzyl $C_{3,4,5}$-H); 7.21 (s, 1H, triazolopyrimidine C_3-H); 7.33 (d, *J*=7.6 Hz, 2H, benzyl $C_{2,6}$-H); 7.41-7.42 (m, 3H, phenyl $C_{3,4,5}$-H); 7.69-7.71 (m, 3H, triazolopyrimidine C_8-H, phenyl $C_{2,6}$-H).

1-[5-(Benzylsulfanyl)-3-(4-chlorophenyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl]ethanone, 8b

IR (KBr, cm^{-1}): 1700 (C=O), 1608 (C=N); 1259, 1039 (C-S-C).

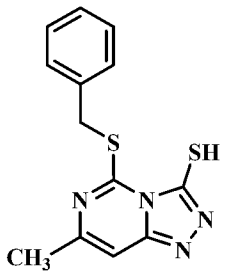
^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm):2.44 (s, 3H, CH_3); 2.48 (s, 3H, $COCH_3$); 4.30 (s, 2H, S-CH_2); 7.13-7.19 (m, 3H, benzyl $C_{3,4,5}$-H); 7.20 (s, 1H, triazolopyrimidine C_3-H); 7.33 (dd, *J*=7.65,1.5 Hz, 2H, benzyl $C_{2,6}$-H); 7.48 (d, *J*=8.78 Hz, 2H, chlorophenyl $C_{2,6}$-H); 7.68 (s, 1H, triazolopyrimidine C_8-H); 7.72 (d, *J*=8.78 Hz, 2H, chlorophenyl $C_{3,5}$-H).

1-[5-(Benzylsulfanyl)-3-(4-bromophenyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl]ethanone, 8c

IR (KBr, cm^{-1}): 1697 (C=O); 1616 (C=N), 1261, 1066 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.43 (s, 3H, CH_3); 2.48 (s, 3H, $COCH_3$); 4.30 (s, 2H, S-CH_2); 7.13-7.19 (m, 3H, benzyl $C_{3,4,5}$-H); 7.19 (s, 1H, triazolopyrimidine C_3-H); 7.33 (d, *J*=6.9 Hz, 2H, benzyl $C_{2,6}$-H); 7.61 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{2,6}$-H); 7.65 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{3,5}$-H); 7.66 (s, 1H, triazolopyrimidine C_8-H).

5-(Benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidine-3-thiol, 9



9

A mixture of the hydrazino derivative **4** (0.25 g, 1 mmol) and potassium hydroxide (0.06 g, 1 mmol) in ethanol (5 ml) and carbon disulfide (2 ml) was heated under reflux for 3 h then the reaction mixture was concentrated by evaporation to half its volume, cooled to room temperature, poured over crushed ice and acidified using dilute HCl. The obtained white precipitate was filtered off, washed with water, dried and crystallized from ethanol/H_2O; yield (0.26 g, 90%); mp 198-200°C.

Microanalyses for $C_{13}H_{12}N_4S_2$ (288.39):

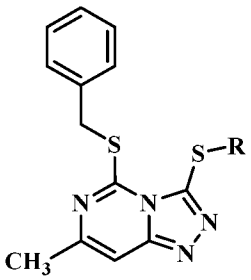
	C%	H%	N%	S%
Calcd.	54.14	4.19	19.43	22.24
Found	54.21	4.25	19.67	22.41

IR (KBr, cm^{-1}): 3438 (NH); 2773 (SH); 1624 (C=N); 1265, 1098 (C-S-C); 1170 (C=S).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.28 (s, 3H, CH_3); 4.32 (s, 2H, S-CH_2); 6.93 (s, 1H, triazolopyrimidine C_8-H); 7.24 (t, *J*=7.3 Hz, 1H, benzyl C_4-H); 7.30 (t, *J*=7.3 Hz, 2H, benzyl $C_{3,5}$-H); 7.44 (d, *J*=7.3 Hz, 2H, benzyl $C_{2,6}$-H); 14.33 (s, 1H, SH, D_2O-exchangeable).

HRMS (ESI): *m/z* calcd for $C_{13}H_{13}N_4S_2$: 289.058 $(M+1)^+$; found: 289.060.

3-Substituted sulfanyl-5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]-pyrimidines, 10a,b



10a,b

A mixture of the sulfanyl derivative **9** (0.29 g, 1 mmol) and anhydrous potassium carbonate (0.14 g, 1 mmol) in dry DMF (1 ml) was stirred at room temperature for 30 minutes then benzyl chloride (0.12 ml, 0.13 g, 1 mmol) or methyl iodide (0.06 ml, 0.14 g, 1 mmol) was added and stirring was continued for 4 h then the reaction mixture was poured over crushed ice and left to stand overnight. The precipitate obtained was filtered off, washed with water, dried and crystallized from ethanol/H_2O.

Table 5: Physical and microanalytical data of compounds 10a,b

ID	R	Yield (%)	M.P. (°C)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
10a	CH_3	70	137-139	$C_{14}H_{14}N_4S_2$ (302.42)	**C**	55.60	55.74
					H	4.67	4.70
					N	18.53	18.71
					S	21.21	21.28
10b	$C_6H_5CH_2$	77	95-97	$C_{20}H_{18}N_4S_2$ (378.51)	**C**	63.46	63.69
					H	4.79	4.87
					N	14.80	14.89
					S	16.94	17.13

5-(Benzylsulfanyl)-7-methyl-3-(methylsulfanyl)-[1,2,4]triazolo[4,3-*c*]pyrimidine, 10a

IR (KBr, cm^{-1}): 1611 (C=N); 1270, 1073 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3); 2.65 (s, 3H, S-CH_3); 4.54 (s, 2H, S-CH_2); 7.24-7.26 (m, 2H, triazolopyrimidine C_8-H, benzyl C_4-H); 7.31 (t, *J*=7.7 Hz, 2H, benzyl $C_{3,5}$-H); 7.47 (d, *J*=7.7 Hz, 2H, benzyl $C_{2,6}$-H).

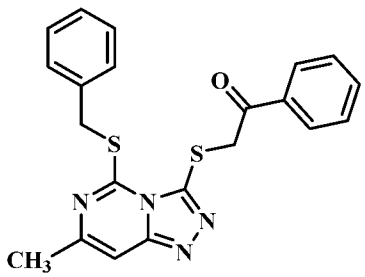
3,5-Bis(benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidine, 10b

IR (KBr, cm^{-1}): 1612 (C=N); 1260, 1070 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3); 4.40 (s, 2H, S-CH_2); 4.50 (s, 2H, S-CH_2); 7.17-7.26 (m, 7H, triazolopyrimidine C_8-H, 5 benzyl-H_s, benzyl C_4-H); 7.31 (t, *J*=7.65 Hz, 2H, benzyl $C_{3,5}$-H); 7.46 (d, *J*=7.65 Hz, 2H, benzyl $C_{2,6}$-H).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.15 (CH_3); 36.28 (two S-CH_2); 103.72 (triazolopyrimidine C_8); 128.05 (two benzyl C_4); 128.87 (benzyl $C_{2,6}$); 128.95 (benzyl $C_{2,6}$); 129.59 (benzyl $C_{3,5}$); 129.97 (benzyl $C_{3,5}$); 136.59 (benzyl C_1); 136.65 (benzyl C_1); 140.23 (triazolopyrimidine C_3); 149.08 (triazolopyrimidine C_{8a}); 151.01 (triazolopyrimidine C_7); 152.21 (triazolopyrimidine C_5).

2-{[5-(Benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-3-yl]-sulfanyl}-1-phenylethanone, 11



11

A mixture of the sulfanyl derivative **9** (0.29 g, 1 mmol) and phenacyl bromide (0.2 g, 1 mmol) in absolute ethanol (2 ml) was heated under reflux for 1 h then cooled to room temperature. The precipitate obtained was filtered off, washed with ethanol, dried and crystallized from ethanol; yield (0.24 g, 59%); mp 123-125°C.

Microanalyses for $C_{21}H_{18}N_4OS_2$ (406.52):

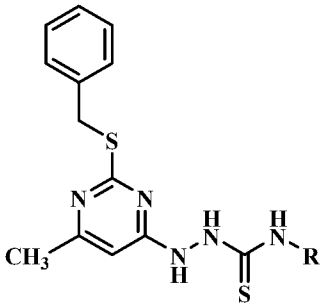
	C%	H%	N%	S%
Calcd.	62.04	4.46	13.78	15.78
Found	62.34	4.52	14.01	15.89

IR (KBr, cm^{-1}): 1680 (C=O); 1609 (C=N); 1244, 1068 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.48 (s, 3H, CH_3); 4.53 (s, 2H, S-CH_2); 4.94 (s, 2H, S-CH_2); 7.23 (t, *J*=7.15 Hz, 1H, benzyl C_4-H); 7.28-7.31 (m, 3H, triazolopyrimidine C_8-H, benzyl $C_{3,5}$-H); 7.44 (d, *J*=7.15 Hz, 2H, benzyl $C_{2,6}$-H); 7.51 (t, *J*=7.33 Hz, 2H, CO-phenyl $C_{3,5}$-H); 7.65 (t, *J*=7.33 Hz, 1H, CO-phenyl C_4-H); 8.01 (d, *J*=7.33 Hz, 2H, CO-phenyl $C_{2,6}$-H).

Scheme 3:

2-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]-*N*-allyl (or phenyl) hydrazine-1-carbothioamides, 12a,b



12a,b

A mixture of the hydrazino derivative **4** (0.25 g, 1 mmol) and the appropriate isothiocyanate (1 mmol) in absolute ethanol (2 ml) was stirred at room temperature overnight. The precipitate obtained was filtered off, washed with petroleum ether (40-60), dried and crystallized from methylene chloride/petroleum ether (40-60).

Table 5: Physical and microanalytical data of compounds 12a,b

ID	R	Yield (%)	M.P. (°C)	Mol. Formula (M.wt.)	Microanalyses %		
					El.	Calcd.	Found
12a	CH_2-CH=CH_2	71	104-106	$C_{16}H_{19}N_5S_2$ (345.49)	**C**	55.62	55.82
					H	5.54	5.61
					N	20.27	20.41
					S	18.56	18.63
12b	C_6H_5	76	125-126	$C_{19}H_{19}N_5S_2$ (381.52)	**C**	59.81	60.05
					H	5.02	5.09
					N	18.36	18.49
					S	16.80	16.94

***N*-Allyl-2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazine-1-carbothioamide, 12a**

IR (KBr, cm^{-1}): 3194 (NH); 1586 (C=N); 1493, 1359, 1233, 1116 (N-C=S); 1284, 1124 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.22 (s, 3H, CH_3); 4.05 (t, *J*=5.4 Hz, 2H, allyl CH_2); 4.29 (s, 2H, S-CH_2); 4.99 (dd, *J*=10.7, 1.5 Hz, 1H, =CH_{cis}); 5.03 (d, *J*=17.5 Hz, 1H, =CH_{trans}); 5.75-5.78 (m, 1H, -CH=); 6.05 (s, 1H, pyrimidine C_5-H); 7.19 (t, *J*=7.3 Hz, 1H, benzyl C_4-H); 7.26 (t, *J*=7.3 Hz, 2H, benzyl $C_{3,5}$-H); 7.38 (d, *J*=7.3 Hz, 2H, benzyl $C_{2,6}$-H); 8.31, 9.19, 9.44 (three s, each 1H, 3NH, D_2O-exchangeable).

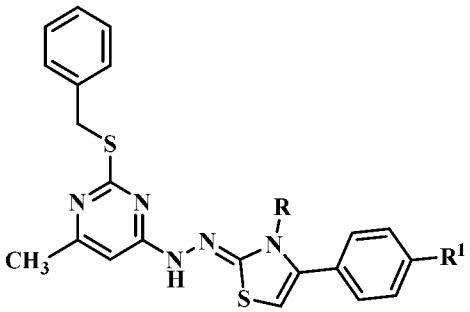
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.56 (CH_3); 34.27 (S-CH_2); 45.98 (allyl CH_2); 99.31 (pyrimidine C_5); 115.76 (allyl =CH_2); 127.47 (benzyl C_4); 128.85 (benzyl $C_{2,6}$); 129.44 (benzyl $C_{3,5}$); 134.74 (-CH=); 138.84 (benzyl C_1); 164.47 (pyrimidine C_6); 166.65 (pyrimidine C_4); 169.51 (pyrimidine C_2); 187.88 (C=S).

2-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]-*N*-phenylhydrazine-1-carbothioamide, 12b

IR (KBr, cm^{-1}): 3184 (NH); 1585 (C=N); 1493, 1367, 1230, 1085 (N-C=S); 1281, 1117 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 2.25 (s, 3H, CH_3); 4.32 (s, 2H, S-CH_2); 6.16 (s, 1H, pyrimidine C_5-H); 7.11 (t, *J*=7.6 Hz, 1H, NH-phenyl C_4-H); 7.18 (t, *J*=7.3, 1H, benzyl C_4-H): 7.24 (t, *J*=7.3 Hz, 2H, benzyl $C_{3,5}$-H); 7.27 (t, *J*=7.6 Hz, 2H, NH-phenyl $C_{3,5}$-H); 7.39 (d, *J*=7.3, 2H, benzyl $C_{2,6}$-H); 7.44 (d, *J*=7.6 Hz, 2H, NH-phenyl $C_{2,6}$-H); 9.32, 9.75, 9.85 (three s, each 1H, 3NH, D_2O-exchangeable).

4-(4-Substituted phenyl)-2-{2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]-hydrazono}-3-allyl (or phenyl)-2,3-dihydrothiazoles, 13a-f



13a-f

A mixture of the appropriate thiosemicarbazide **12a,b** (1 mmol) and anhydrous sodium acetate (0.12 g, 1.5 mmol) and the appropriate phenacyl bromide (1 mmol) in absolute ethanol (2 ml) was heated under reflux 6-12 h. The precipitate obtained after cooling (**13b,c**) or after concentration, addition of 2 drops water and leaving to stand overnight (**13a,d-f**) was filtered off, washed with ethanol, dried and crystallized from methylene chloride/petroleum ether (40-60).

Table 6: Physical and microanalytical data of compounds 13a-f

ID	R	R^1	Yield (%)	M.P. (°C)	Mol. Formula (M.wt.)	Microanalyses %		
						El.	Calcd.	Found
13a	CH_2-CH=CH_2	H	85	104-106	$C_{24}H_{23}N_5S_2$ (445.60)	**C**	64.69	64.91
						H	5.20	5.25
						N	15.72	15.94
						S	14.39	14.57
13b	CH_2-CH=CH_2	Br	97	135-136	$C_{24}H_{22}BrN_5S_2$ (524.50)	**C**	54.96	55.13
						H	4.23	4.27
						N	13.35	13.51
						S	12.23	12.46
13c	CH_2-CH=CH_2	CH_3	45	104-106	$C_{25}H_{25}N_5S_2$ (459.63)	**C**	65.33	65.51
						H	5.48	5.44
						N	15.24	15.38
						S	13.95	14.12
13d	C_6H_5	H	56	69-71	$C_{27}H_{23}N_5S_2$ (481.64)	**C**	67.33	67.49
						H	4.81	4.88
						N	14.54	14.72
						S	13.32	13.48
13e	C_6H_5	Br	63	192-193	$C_{27}H_{22}BrN_5S_2$ (560.53)	**C**	57.85	58.04
						H	3.96	4.02
						N	12.49	12.68
						S	11.44	11.51
13f	C_6H_5	CH_3	37	154-156	$C_{28}H_{25}N_5S_2$ (495.66)	**C**	67.85	68.02
						H	5.08	5.17
						N	14.13	14.32
						S	12.94	13.06

3-Allyl-2-{2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-4-phenyl-2,3-dihydrothiazole, 13a

IR (KBr, cm^{-1}): 3450 (NH); 1570 (C=N); 1271, 1079 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.17 (s, 3H, CH_3); 4.29 (s, 2H, S-CH_2); 4.31 (d, *J*=4.6 Hz, 2H, C$\underline{H_2}$-CH=CH); 4.92 (d, *J*=16.8 Hz, 1H, CH_2-CH=C$\underline{H}_{trans}$); 5.12 (d, *J*=10 Hz, 1H, CH_2-CH=C$\underline{H}_{cis}$); 5.79-5.85 (m, 1H, CH_2-C$\underline{H}$=CH); 6.15 (s, 1H, pyrimidine C_5-H); 6.25 (s, 1H, thiazole C_5-H); 7.19 (t, *J*=7.1 Hz, 1H, benzyl C_4-H); 7.26 (t, *J*=7.1 Hz, 2H, benzyl $C_{3,5}$-H); 7.40 (d, *J*=7.1 Hz, 4H, benzyl $C_{2,6}$-H, 4-phenylthiazole $C_{2,6}$-H); 7.44-7.45 (m, 3H, 3-phenylthiazole $C_{3,4,5}$-H); 9.56 (s, 1H, NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.99 (CH_3); 34.19 (S-CH_2); 47.68 (allyl CH_2); 97.92 (pyrimidine C_5); 101.69 (thiazole C_5); 116.76 (allyl =CH_2); 127.34 (benzyl C_4); 128.77 (benzyl $C_{2,6}$); 129.04 (phenyl $C_{2,6}$); 129.24 (phenyl $C_{3,5}$); 129.47 (benzyl $C_{3,5}$); 129.84 (phenyl C_4); 130.88 (phenyl C_1); 133.10 (allyl –CH=); 139.21 (thiazole C_4); 141.09 (benzyl C_1); 159.67 (thiazole C_2); 165.80 (pyrimidine C_6); 168.66 (pyrimidine C_4); 170.76 (pyrimidine C_2).

3-Allyl-2-{2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-4-(4-bromophenyl)-2,3-dihydrothiazole, 13b

IR (KBr, cm^{-1}): 3439 (NH); 1610 (C=N); 1270, 1072 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.17 (s, 3H, CH_3); 4.29 (s, 2H, S-CH_2); 4.32 (d, *J*=4.6 Hz, 2H, C$\underline{H_2}$-CH=CH); 4.93 (d, *J*=17.5, 1H, CH_2-CH=C$\underline{H}_{trans}$); 5.12 (d, *J*=9.9 Hz, 1H, CH_2-CH=C$\underline{H}_{cis}$); 5.79-5.85 (m, 1H, CH_2-C$\underline{H}$=CH); 6.15 (s, 1H, pyrimidine C_5-H); 6.3 (s, 1H, thiazole C_5-H); 7.19 (t, *J*=7.63 Hz, 1H, benzyl C_4-H); 7.26 (t, *J*=7.63 Hz, 2H, benzyl $C_{3,5}$-H); 7.36 (d, *J*=7.65 Hz, 2H, bromophenyl $C_{2,6}$-H); 7.40 (d, *J*=7.65 Hz, 2H, bromophenyl $C_{3,5}$-H); 7.65 (d, *J*=7.63 Hz, 2H, benzyl $C_{2,6}$-H); 9.56 (s, 1H, NH, D_2O-exchangeable).

3-Allyl-2-{2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-4-(4-methylphenyl)-2,3-dihydrothiazole, 13c

IR (KBr, cm^{-1}): 3451 (NH); 1570 (C=N); 1272, 1064 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.16 (s, 3H, pyrimidine CH_3); 2.32 (s, 3H, *p*-tolyl CH_3); 4.29 (s, 4H, S-CH_2, C$\underline{H_2}$-CH=CH); 4.92 (d, *J*=17.6, 1H, CH_2-CH=C$\underline{H}_{trans}$); 5.12 (d, *J*=9.9 Hz, 1H, CH_2-CH=C$\underline{H}_{cis}$); 5.79-5.84 (m, 1H, CH_2-C$\underline{H}$=CH); 6.14 (s, 1H, pyrimidine C_5-H); 6.18 (s, 1H, thiazole C_5-H); 7.19 (t, *J*=6.9 Hz, 1H, benzyl C_4-H); 7.24-7.29 (m, 6H, benzyl $C_{3,5}$-H, 4-phenylthiazole-H); 7.40 (d, *J*=6.9 Hz, 2H, benzyl $C_{2,6}$-H); 9.53 (s, 1H, NH, D_2O-exchangeable).

2-{2-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-3,4-diphenyl-2,3-dihydrothiazole, 13d

IR (KBr, cm^{-1}): 3440 (NH); 1621 (C=N); 1285, 1071 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.16 (s, 3H, CH_3); 4.27 (d, *J*=6.9 Hz, 2H, S-CH_2); 6.03 (s, 1H, pyrimidine C_5-H); 6.50 (s, 1H, thiazole C_5-H); 7.01 (t, *J*=7.1 Hz, 1H, 3-phenylthiazole C_4-H); 7.13 (t, *J*=7.1 Hz, 1H, benzyl C_4-H); 7.18 (d, *J*=7.1 Hz, 2H, benzyl $C_{2,6}$-H); 7.21 (d, *J*=6.9 Hz, 2H, 3-phenylthiazole $C_{2,6}$-H); 7.23-7.26 (m, 4H, benzyl $C_{3,5}$-H, 3-phenylthiazole $C_{3,5}$-H); 7.30 (t, *J*=6.9 Hz, 3H, 4-phenylthiazole $C_{3,4,5}$-H); 7.38 (d, *J*=7.7 Hz, 2H, 4-phenylthiazole $C_{2,6}$-H); 9.55 (s, 1H, NH, D_2O-exchangeable).

2-{2-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-4-(4-bromophenyl)-3-phenyl-2,3-dihydrothiazole, 13e

IR (KBr, cm^{-1}): 3438 (NH); 1560 (C=N); 1285, 1071 (C-S-C).

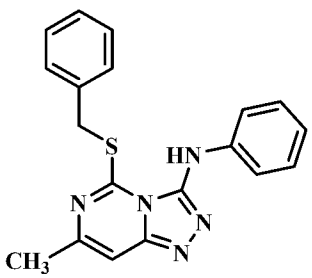
^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.16 (s, 3H, CH_3); 4.28 (s, 2H, S-CH_2); 6.03 (s, 1H, pyrimidine C_5-H); 6.57 (s, 1H, thiazole C_5-H); 7.07 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{2,6}$-H); 7.18 (t, *J*=7.2 Hz, 1H, 3-phenylthiazole C_4-H); 7.23-7.29 (m, 3H, benzyl $C_{3,4,5}$-H); 7.34-7.39 (m, 6H, 3-phenylthiazole $C_{2,3,5,6}$-H, benzyl $C_{2,6}$-H); 7.42 (d, *J*=8.4 Hz, 2H, bromophenyl $C_{3,5}$-H); 9.56 (s, 1H, NH, D_2O-exchangeable).

2-{2-[2-(Benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-3-phenyl-4-(4-methylphenyl)-2,3-dihydrothiazole, 13f

IR (KBr, cm^{-1}): 3451 (NH); 1554 (C=N); 1283, 1070 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.15 (s, 3H, pyrimidine CH_3); 2.18 (s, 3H, *p*-tolyl CH_3); 4.27 (s, 2H, S-CH_2); 6.02 (s, 1H, pyrimidine C_5-H); 6.43 (s, 1H, thiazole C_5-H); 6.98-7.03 (m, 4H, *p*-tolyl-H_s); 7.17-7.20 (m, 2H, benzyl C_4-H, 3-phenylthiazole C_4-H); 7.23-7.26 (m, 4H, benzyl $C_{2,3,5,6}$-H); 7.33 (t, *J*=7.1 Hz, 2H, 3-phenylthiazole $C_{3,5}$-H); 7.38 (d, *J*=7.1 Hz, 2H, 3-phenylthiazole $C_{2,6}$-H); 9.53 (s, 1H, NH, D_2O-exchangeable).

5-(Benzylsulfanyl)-7-methyl-*N*-phenyl-[1,2,4]triazolo[4,3-*c*]-pyrimidin-3-amine, 14



14

A mixture of the thiosemicarbazide **12b** (0.38 g, 1 mmol) and freshly prepared yellow mercuric oxide (0.22 g, 1 mmol) in absolute ethanol (15 ml) was heated under reflux with stirring for 18 h then the reaction mixture was filtered while hot and the filtrate was concentrated to 2 ml and crushed ice was added and left to stand overnight at room temperature. The precipitate obtained was filtered off, washed with water, dried and crystallized from ethanol/H_2O; yield (0.2g, 57%); mp 144-145°C.

Microanalyses for $C_{19}H_{17}N_5S$ (347.44):

	C%	H%	N%	S%
Calcd.	65.68	4.93	20.16	9.23
Found	65.89	5.01	20.34	9.42

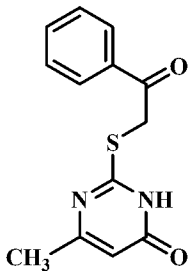
IR (KBr, cm^{-1}): 3218 (NH); 1606 (C=N); 1245, 1074 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3); 4.44 (s, 2H, S-CH_2); 6.69 (d, *J*=7.8 Hz, 2H, benzyl $C_{2,6}$-H); 6.78 (t, *J*=7.8 Hz, 1H, benzyl C_4-H); 7.14 (t, *J*=7.8 Hz, 2H, benzyl $C_{3,5}$-H); 7.20-7.21 (m, 2H, triazolopyrimidine C_8-H, NH-phenyl C_4-H); 7.26 (t, *J*=7.1 Hz, 2H, NH-phenyl $C_{3,5}$-H); 7.38 (d, *J*=7.1 Hz, 2H, NH-phenyl $C_{2,6}$-H); 8.54 (s, 1H, NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.16 (CH_3); 34.68 (S-CH_2); 103.17 (triazolopyrimidine C_8); 115.44 (NH-phenyl $C_{2,6}$); 120.58 (NH-phenyl C_4); 127.93 (benzyl C_4); 128.87 (benzyl $C_{2,6}$); 129.63 (benzyl $C_{3,5}$); 129.83 (NH-phenyl $C_{3,5}$); 136.78 (benzyl C_1); 143.06 (NH-phenyl C_1); 145.15 (triazolopyrimidine C_3); 148.76 (triazolopyrimidine C_{8a}); 149.93 (triazolopyrimidine C_7); 151.01 (triazolopyrimidine C_5).

Scheme 4:

6-Methyl-2-[(2-oxo-2-phenylethyl)sulfanyl]pyrimidin-4(3*H*)-one, 15

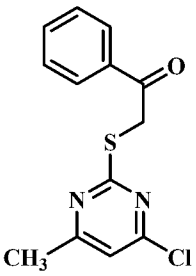


15

Phenacyl bromide (0.2 g, 1 mmol) was added to a solution of compound **1** (0.14 g, 1 mmol) and sodium hydroxide (0.05 g, 1.25 mmol) in water (15 ml). The reaction mixture was stirred at room temperature overnight and the precipitate obtained was filtered off, washed 3 times with water then twice using diethyl ether, dried and crystallized from benzene; yield (0.23 g, 88%) reported (70%); mp 178-180°C (reported: 179-181°C).[152]

IR (KBr, cm^{-1}): 3375 (NH); 1697 (C=O); 1639 (C=N); 1208, 1032 (C-S-C).

2-[(4-Chloro-6-methylpyrimidin-2-yl)sulfanyl]-1-phenylethanone, 16



16

A mixture of pyrimidinone **15** (0.26 g, 1 mmol) and phosphorous oxychloride (5 ml) was heated under reflux for 1 h, cooled to room temperature and poured dropwise over crushed ice. The precipitate obtained was filtered off, washed with water, dried and crystallized from methylene chloride/petroleum ether (40-60); yield (0.24 g, 86%); mp 68-70°C.

Microanalyses for $C_{13}H_{11}ClN_2OS$ (278.76):

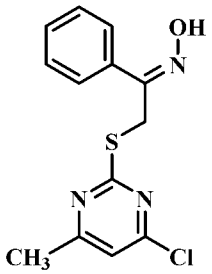
	C%	H%	N%	S%
Calcd.	56.01	3.98	10.05	11.50
Found	56.17	3.93	10.17	11.47

IR (KBr, cm^{-1}): 1695 (C=O); 1589 (C=N); 1295, 1127 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d$_6$, δ ppm): 2.25 (s, 3H, CH_3); 4.74 (s, 2H, S-CH_2); 7.23 (s, 1H, pyrimidine C_5-H); 7.53 (t, *J*=7.7 Hz, 2H, phenyl $C_{3,5}$-H); 7.64 (t, *J*=7.7 Hz, 1H, phenyl C_4-H); 8.02 (d, *J*=7.7 Hz, 2H, phenyl $C_{2,6}$-H).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.61 (CH_3); 38.71 (S-CH_2); 116.80 (pyrimidine C_5); 128.71 (phenyl $C_{3,5}$); 129.22 (phenyl $C_{2,6}$); 133.92 (phenyl C_4); 136.71 (phenyl C_1); 160.20 (pyrimidine C_4); 170.32 (pyrimidine C_6); 170.94 (pyrimidine C_2); 194.09 (C=O).

2-[(4-Chloro-6-methylpyrimidin-2-yl)sulfanyl]-1-phenylethanone oxime, 17



17

A mixture of ethanone **16** (0.28 g, 1 mmol), anhydrous sodium acetate (0.18 g, 2.2 mmol) and hydroxylamine hydrochloride (0.14 g, 2 mmol) in absolute ethanol (2 ml) was heated on water bath (60-70°C) for 1 h then the reaction mixture was cooled to room temperature. The precipitate obtained was filtered off, washed using 60% aqueous ethanol then water, dried and crystallized from methylene chloride/petroleum ether (40-60); yield (0.26 g, 90%); mp 146-148°C.

Microanalyses for $C_{13}H_{12}ClN_3OS$ (293.77):

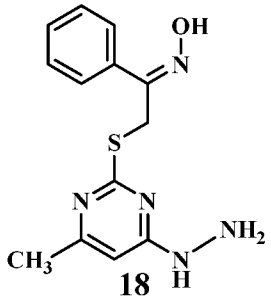
	C%	H%	N%	S%
Calc.	53.15	4.12	14.3	10.91
Found	53.28	4.14	14.42	10.99

IR (KBr, cm^{-1}): 3258 (OH); 1629 (C=N); 1259, 1054 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3); 4.46 (s, 2H, S-CH_2); 7.27 (s, 1H, pyrimidine C_5-H); 7.35-7.38 (m, 3H, phenyl $C_{3,4,5}$-H); 7.69-7.70 (m, 2H, phenyl $C_{2,6}$-H); 11.83 (s, 1H, OH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.64 (CH_3); under DMSO (S-CH_2); 116.84 (pyrimidine C_5); 126.46 (phenyl $C_{2,6}$); 128.96 (phenyl $C_{3,5}$); 129.68 (phenyl C_4); 134.90 (phenyl C_1); 153.20 (pyrimidine C_4); 160.41 (C=N); 170.63 (pyrimidine C_6); 170.95 (pyrimidine C_2).

2-[(4-Hydrazinyl-6-methylpyrimidin-2-yl)sulfanyl]-1-phenylethanone oxime, 18



18

A mixture of chloropyrimidine **17** (0.29 g, 1 mmol) and hydrazine hydrate 99% (0.1 ml, 0.1 g, 2 mmol) in absolute ethanol (2 ml) was heated under reflux for 1.5 h, cooled to room temperature and poured over crushed ice. The precipitate obtained was filtered off, washed with water, dried and triturated several times using diethyl ether then crystallized from ethyl acetate; yield (0.18 g, 62%); mp 155-157°C.

Microanalyses for $C_{13}H_{15}N_5OS$ (289.36):

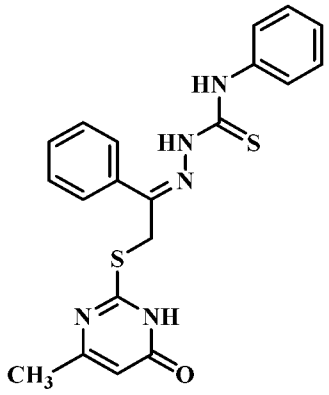
	C%	H%	N%	S%
Calcd.	53.96	5.23	24.2	11.08
Found	54.12	5.32	24.37	11.21

IR (KBr, cm^{-1}): 3424 (OH); 3314, 3169 (NH); 1591 (C=N); 1239, 1054 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.16 (s, 3H, CH_3); 4.33 (s, 2H, NH_2, D_2O-exchangeable); 4.41 (s, 2H, S-CH_2); 6.33 (s, 1H, pyrimidine C_5-H); 7.31-7.36 (m, 3H, phenyl $C_{3,4,5}$-H); 7.72-7.73 (m, 2H, phenyl $C_{2,6}$-H); 8.37 (s, 1H, NH, D_2O-exchangeable); 11.64 (s, 1H, OH, D_2O-exchangeable).

HRMS (ESI): *m/z* calcd. for $C_{13}H_{16}N_5OS$: 290.108 $(M+1)^+$; found: 290.051.

2-{2-[(4-Methyl-6-oxo-1,6-dihydropyrimidin-2-yl)sulfanyl]-1-phenylethylidene}-*N*-phenylhydrazine-1-carbothioamide, 19



19

A mixture of pyrimidinone **15** (0.26 g, 1 mmol) and phenyl thiosemicarbazide (0.17 g, 1 mmol) in absolute ethanol (2 ml) was heated under reflux for 1 h then cooled to room temperature. The precipitate obtained was filtered off, washed with ethanol, dried and crystallized from DMF/ethanol; yield (0.21 g, 51%); mp 173-174°C.

Microanalyses for $C_{20}H_{19}N_5OS_2$ (409.53):

	C%	H%	N%	S%
Calcd.	58.66	4.68	17.10	15.66
Found	58.84	4.65	17.21	15.92

IR (KBr, cm^{-1}): 3436 (NH); 1662 (C=O); 1594 (C=N); 1542, 1363, 1213, 1119 (N-C=S); 1214, 1119 (C-S-C).

^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.33 (s, 3H, CH_3); 4.67 (s, 2H, S-CH_2); 6.03 (s, 1H, pyrimidine C_5-H); 7.19 (t, *J*=7.7 Hz, 1H, NH-phenyl C_4-H); 7.34 (t, *J*=7.7 Hz, 2H, NH-phenyl $C_{3,5}$-H); 7.40-7.41 (m, 3H, benzylidene $C_{3,4,5}$-H); 7.50 (d, *J*=7.7 Hz, 2H, NH-phenyl $C_{2,6}$-H); 7.89 (d, *J*=6.9 Hz, 2H, benzylidene $C_{2,6}$-H); 10.14, 10.67, 12.63 (three s, each 1H, 3NH, D_2O-exchangeable).

^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 18.38 (CH_3); under DMSO (S-CH_2); 103.96 (pyrimidine C_5); 127.42 (NH-phenyl $C_{2,6}$); 128.65 (phenyl $C_{2,6}$); 128.81 (phenyl $C_{3,5}$); 129.05 (NH-phenyl $C_{3,5}$); 129.28 (NH-phenyl C_4); 130.07 (phenyl C_4); 134.15 (phenyl C_1); 136.38 (NH-phenyl C_1); 154.20 (pyrimidine C_4); 159.64 (C=N); 163.51 (pyrimidine C_2); 166.49 (pyrimidine C_6); 175.84 (C=S).

BIOLOGICAL SCREENING

1- CYTOTOXICITY SCREENING

The cytotoxicity screening of the target compounds was performed in *Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Borg El-Arab, Alexandria, Egypt.*

Experimental:

All newly synthesized compounds were subjected to *in vitro* cytotoxicity assay to assess their cytotoxic effects on human peripheral blood mononuclear cells (PBMCs) using neutral red uptake assay as described by Borenfreund and Puerner.[160] This assay depends on the fact that neutral red dye can be incorporated into the lysosomes of living cells[161] providing a quantitative assay to the cytotoxic effects.

Cytotoxicity assay involved three main steps. First, isolation of PBMCs from freshly collected blood sample, then incubating PBMCs with the tested compounds and finally, measuring cells viability using neutral red uptake assay.

1. The PBMCs were isolated by density gradient centrifugation technique as described by Boyum.[162] Blood samples were freshly collected into heparinized sterile tubes and diluted using equal volume of RPMI-1640 medium containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and then layered over equal volume of Ficoll-Pague™ Plus (Fischer Bioreagents, USA) (lymphocyte Separation Medium LSM) and centrifuged at 2000 rpm for 30 minutes with no acceleration or break at room temperature. The buffy mononuclear cell layer was collected using sterile Pasteur pipette into 50 ml sterile Falcon tube and washed twice in phosphate buffered saline (PBS) (Sigma, USA) using centrifugation at 1650 rpm for five minutes. The isolated PBMCs viability was determined by hemocytometer count using the trypan blue exclusion method.[163]

   The PBMCs were re-suspended at 1 x 10^6 cells/ml in RPMI-1640 medium containing 25 mM HEPES buffer, 4mM L-Glutamine and 10% heat-inactivated fetal bovine serum (FBS). The appropriate number of cells for the experiment was chosen to be 1 x 10^5 cells/well thus 100 µl of the prepared cells suspension, containing 1 x 10^5 cells, were pipetted into wells of polystyrene 96-well plates.

2. A 1 mg/ml stock solution of each compound was prepared in RPMI-1640 medium containing least amount of DMSO and sterilized by filtration using a 0.2 µm syringe filter. The desired concentrations (10, 5, 2.5, 1.25 and 0.625 µg/ml) were prepared using serial dilution method in RPMI culture media. Compound wells were prepared by adding 100 µl of the previously prepared concentrations to a suspension of 1×10^5 PBMCs in 100 µl culture media. Parallel concentrations of the solvent were prepared to be used as controls. 5-FU was used as a positive control. Control wells were prepared by adding 100 µl culture media to a suspension of 1×10^5 PBMCs in 100 µl culture media. Blank wells contained 200 µl of culture media only (without cells or compound solution). Each set of samples was pipetted in duplicate. The plate was then gently shaken then incubated at 37 ºC, 5% CO_2 for 72 h.

3. After incubation, the plate was centrifuged at 2000 rpm for ten minutes. The media were discarded by suction. Neutral red working solution (80 µg /ml) stain (Bio Basic Inc., Canada) was prepared, and 100 µl of this solution was added to each well, then the plate was gently shaken. Followed by incubation at 37 ºC in humidified 5% CO_2 for 3 h, and then centrifuged (2000 rpm for ten minutes). Excessive dyes were discarded, and the stained cells were washed 3 timed using PBS then fixed with 100 µl fixation solution (0.5% formalin with 1% calcium chloride (Sigma, USA)) for one minute. Cells were destained using 100 µl destaining solution (50% ethanol with 1% glacial acetic acid (Sigma, USA)) for five minutes by shaking. The stain intensity was measured using automated microplate reader spectrophotometer adjusted at λ=540 nm. viable cell fraction was calculated according to the following equation:

   Cell viability (%) = [(At-Ab)/ (Ac-Ab)] × 100

   Where, At = Absorbance value of test compound
   Ab = Absorbance value of blank
   Ac = Absorbance value of control

The results were interpreted to calculate the lethal concentration that kills 50% of cells (LC_{50}) using GraphPad InStat3.0 software.[164] The results of *in vitro* cytotoxicity assay are listed in table 7.

Table 7: Results of *in vitro* cytotoxicity assay

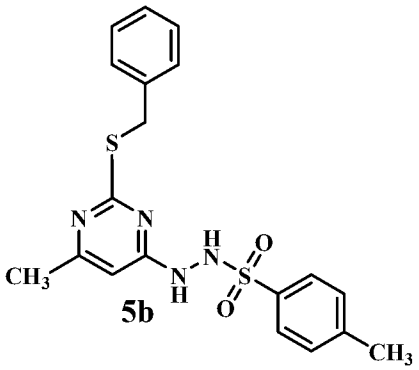
Compound ID	LC_{50} (µg/ml)[a]	Compound ID	LC_{50} (µg/ml)
4	25.83 ± 2.4	**11**	**10.19 ± 1.4**
5a	21.14 ± 0.7	**12a**	34.71 ± 3.9
5b	**12.23 ± 0.1**	**12b**	16.72 ± 2.5
6a	29.38 ± 1.9	**13a**	19.29 ± 0.5
6b	52.48 ± 6.2	**13b**	19.07 ± 3.9
6c	14.08 ± 0.1	**13c**	19.66 ± 0.7
7a	26.50 ± 1.4	**13d**	17.83 ± 0.2
7b	15.26 ± 0.5	**13e**	**11.49 ± 0.8**
7c	24.31 ± 1.3	**13f**	**120.28 ± 2.4**
8a	41.10 ± 5.6	**14**	24.71 ± 0.2
8b	15.43 ± 0.5	**16**	35.65 ± 1.9
8c	**5.23 ± 0.1**	**17**	**95.42 ± 3.6**
9	16.93 ± 1.6	**18**	37.89 ± 0.0
10a	14.58 ± 1.5	**19**	55.70 ± 1.1
10b	22.52 ± 2.5	**5-FU**	16.05 ± 1.5

(a) Data were expressed as the means of two determinations ± SEM.

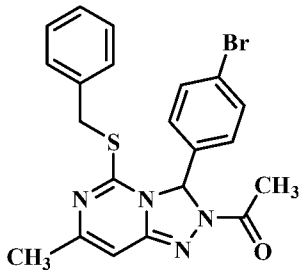
Results and Discussion:

Inspection of the recorded cytotoxicity screening results listed in table 7 revealed that majority of the tested compounds exerted a remarkable cytotoxic effect on PBMCs with LC_{50} ranging from 5.23 to 120.28 µg/ml.

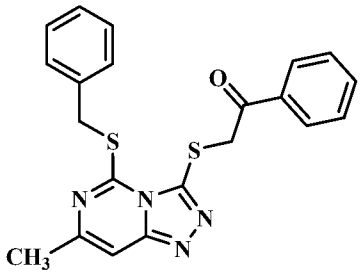
Compounds **5b, 8c, 11** and **13e** exerted the highest cytotoxic effect with LC_{50} values ranging from 5.23 to 12.23 µg/ml which are higher than standard, 5-FU.



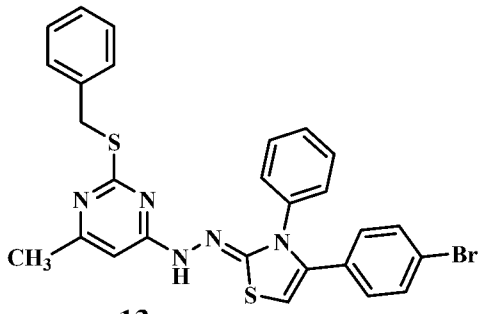
5b



8c

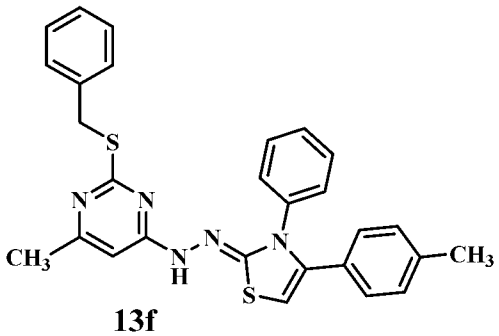


11

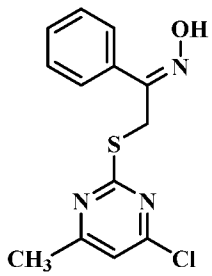


13e

The lowest cytotoxic effect was detected in case of compounds **13f** and **17** with LC_{50} values 120.28 and 95.42 µg/ml, respectively.



13f



17

2- ANTICANCER SCREENING

The anticancer screening of the target compounds was performed in *Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Borg El-Arab, Alexandria, Egypt.*

Experimental:

All newly synthesized compounds were screened for their potential anticancer activity against four human cancer cell lines, MCF-7 (breast), HepG2 (liver), CaCo-2 (colon), A549 (lung) cancer cell lines using neutral red uptake assay as described by Borenfreund and Puerner[160] and compared with the well-known anticancer drug 5-FU.

Both MCF-7 and HepG2 cancer cells were grown in RPMI-1640 medium (Lonza, USA), while A549 and CaCo-2 cancer cells were grown in DMEM (Lonza, USA). Both media were supplemented with 10% heat-inactivated FBS (Lonza, USA), 200 µM L-glutamine (Lonza, USA) and 25 µM HEPES (Lonza, USA) to form the culture media.

Cells were maintained at 37 °C in a humidified air incubator containing 5% CO_2 and were subcultured for two weeks before assay. Cells viability were assessed using trypan blue exclusion method.[163]

Anticancer assay involved three main steps. First, the cancer cells suspension preparation, then incubating cancer cells with the tested compounds and finally, measuring cells viability using neutral red uptake assay.

1. Cancer cells were washed twice in its respective culture media then re-suspended in its respective culture media to form the cancer cells suspension. The appropriate number of cells (seeding cell density) for the experiment was chosen to be 3 x 10^3 cells/well (100 µl of the prepared suspension) for MCF-7 and HepG2 cells and 4 x 10^3 cells/well (100 µl of the prepared suspension) for A549 and CaCo-2 cells thus 100 µl of the prepared cells suspension were pipetted into wells of polystyrene 96-well plates and left to adhere on the plate wells at 37 ºC, 5% CO_2 and 95% humidity for 24 h.

2. A 1 mg/ml stock solution of each compound was prepared in RPMI-1640 medium containing least amount of DMSO and sterilized by filtration using a 0.2 µm syringe filter. The desired concentrations (10, 5, 2.5, 1.25 and 0.625 µg/ml) were prepared using serial dilution. Compound wells were prepared by adding 100 µl of the previously prepared concentrations to a 100 µl of cancer cells suspension. Parallel concentrations of the solvent were prepared to be used as controls. 5-FU was used as a positive control. Control wells were prepared by adding 100 µl culture media to a 100 µl of cells suspension. Blank wells contained 200 µl of culture media only (without cells or compound solution). Each set of samples was pipetted in duplicate. The plate was gently shaken, then incubated at 37 ºC, 5% CO_2 for 72 h.

3. After incubation, Cancer cells viability was measured using neutral red uptake assay as described previously.

The results were interpreted to calculate the concentration causing 50% cancer cell death (IC_{50}) of each compound using GraphPad InStat3.0 software.[164] The results of *in vitro* anticancer screening are listed in table 8.

Table 4: Anticancer activities (IC_{50} in µg/ml) of the tested compounds on some human cancer cell lines

Compound ID	IC_{50} (µg/ml)[a] MCF-7	IC_{50} (µg/ml) HepG2	IC_{50} (µg/ml) CaCo-2	IC_{50} (µg/ml) A549
4	16.86 ± 0.5	43.38 ± 2	59.08 ± 7.4	85.01 ± 0.7
5a	13.84 ± 1.6	22.35 ± 3.2	54.67 ± 1.0	30.53 ± 0.3
5b	26.79 ± 1.6	47.03 ± 1.9	35.91 ± 0.1	54.14 ± 3.7
6a	11.82 ± 2	39.68 ± 4	31.52 ± 2.9	20.00 ± 2.2
6b	23.23 ± 0	39.11 ± 6.2	35.31 ± 4.0	48.44 ± 2.3
6c	13.89 ± 2	39.87 ± 0.5	32.06 ± 2.2	33.07 ± 0.9
7a	12.23 ± 2.6	51.95 ± 3.2	23.98 ± 0.6	21.72 ± 0.8
7b	33.4 ± 0.7	17.63 ± 0	23.1 ± 1.7	29.37 ± 1.4
7c	11.63 ± 1.4	26.86 ± 2.2	11.32 ± 0.2	28.83 ± 1.3
8a	37.18 ± 0.5	7.71 ± 1.4	20.91 ± 0.3	24.85 ± 3.1
8b	19.17 ± 2.1	89.43 ± 0.7	32.78 ± 1.1	27.36 ± 0.4
8c	14.41 ± 1.7	110.90 ± 9.7	21.03 ± 0.5	27.90 ± 2.9
9	12.13 ± 1.7	47.38 ± 5.5	25.74 ± 0.4	53.73 ± 2.0
10a	19.12 ± 2.8	12.76 ± 1.9	34.69 ± 1.4	105.04 ± 7.5
10b	20.93 ± 2.1	13.92 ± 0.6	13.58 ± 1.2	14.75 ± 2.1
11	15.79 ± 2.2	86.11 ± 6.5	31.16 ± 3.5	18.35 ± 2.4
12a	38.26 ± 1.1	24.26 ± 2	62.77 ± 6.1	34.45 ± 1.6

Table 8. (*Continued*)

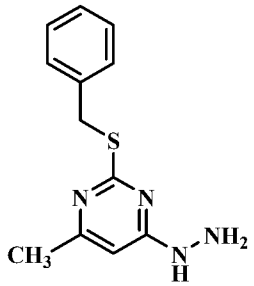
Compound ID	IC_{50} (µg/ml) MCF-7	IC_{50} (µg/ml) HepG2	IC_{50} (µg/ml) CaCo-2	IC_{50} (µg/ml) A549
12b	29.92 ± 1.1	40.51 ± 1.2	56.40 ± 0.6	35.61 ± 0.3
13a	34.32 ± 0.6	29.32 ± 0.6	18.36 ± 3.0	115.01 ± 5.1
13b	41.18 ± 7.9	33.94 ± 3.8	20.69 ± 3.2	38.12 ± 1.4
13c	22.18 ± 3.6	23.43 ± 0.3	11.14 ± 0.4	53.78 ± 3.3
13d	48.18 ± 3.8	25.56 ± 3	13.14 ± 0.1	35.97 ± 0.7
13e	22.1 ± 4.4	14.99 ± 0.2	7.71 ± 0.1	51.17 ± 2.8
13f	36.56 ± 0.9	18.34 ± 0.8	8.68 ± 0.2	31.70 ± 1.7
14	16.37 ± 3.6	72.25 ± 3.6	32.98 ± 5.8	54.75 ± 7.4
16	12.54 ± 0.3	79.34 ± 1.3	18.70 ± 1.5	38.66 ± 0.7
17	8.88 ± 1.3	121.89 ± 3.2	55.97 ± 0.7	36.73 ± 2.1
18	31.5 ± 2.9	78.19 ± 9.9	37.10 ± 0.8	57.23 ± 5.9
19	15.77 ± 1.7	114.88 ± 5.2	28.21 ± 0.3	112.60 ± 7.8
5-FU	8.93 ± 1.8	2.60 ± 0.4	4.01 ± 1.2	0.33 ± 0.05

[a] Data are expressed as the means of two determinations ± SEM.

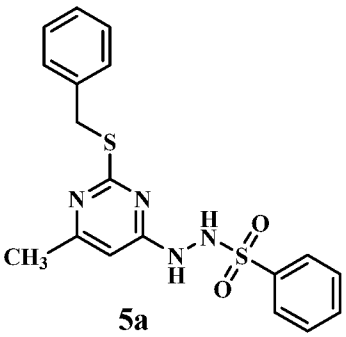
Results and Discussion:

It has been reported that IC_{50} values less than 100 µg/ml reflected a potential anticancer activity, while values between 100 and 1000 µg/ml indicated a moderate anticancer activity.[165]

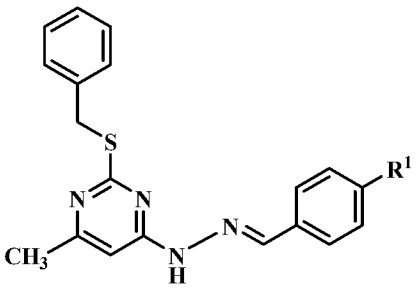
The anticancer screening results revealed that many compounds showed significant activity against MCF-7 cell line. Compound **17** was as active as 5-FU against MCF-7 cell line (IC_{50} 8.88 µg/ml). Compounds **4**, **5a**, **6a,c**, **7a,c**, **8c**, **9**, **11**, **14**, **16** and **19** exhibited half the activity of 5-FU (IC_{50} 11.63-16.86 µg/ml) while other tested compounds showed moderate activity with IC_{50} ranging from 19.12-48.18 µg/ml.



4

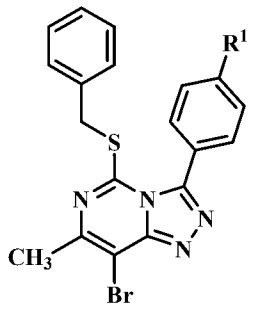


5a



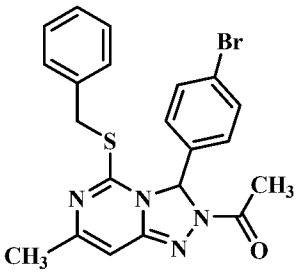
6a,c

6a: R^1=H, **6c:** R^1= Br

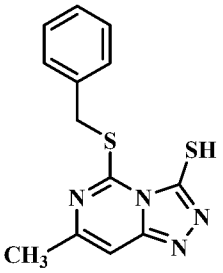


7a,c

7a: R^1=H, **7c:** R^1= Br



8c



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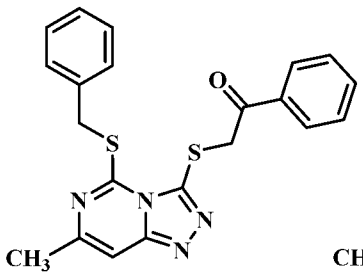

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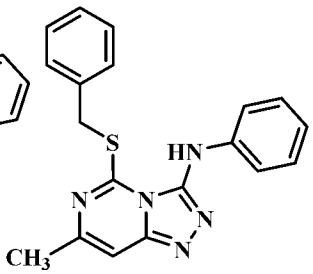

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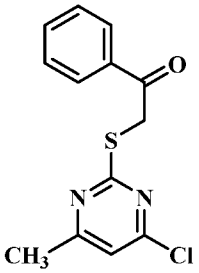

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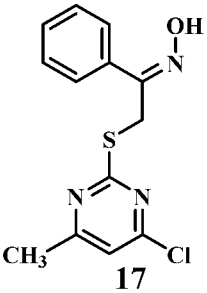

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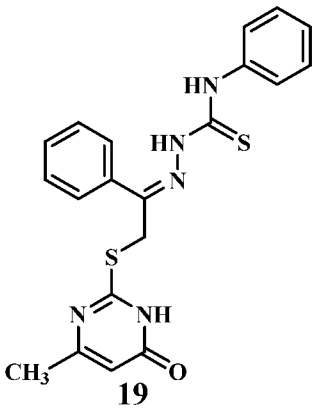
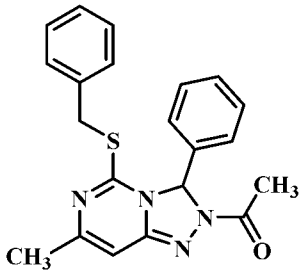
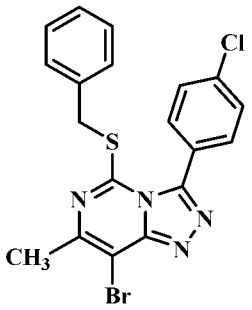


19

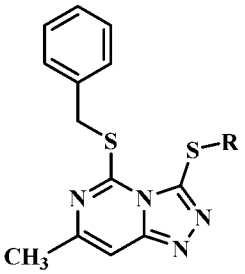
On the other hand, compound **8a** was the most active against HepG2 cell line (IC_{50} 7.71 µg/ml) compared with 5-FU (IC_{50} 2.6 µg/ml) while compounds **7b**, **10a,b** and **13e,f** exerted good activity (IC_{50} 17.63, 12.76, 13.92, 14.99 and 18.34 µg/ml, respectively).



8a

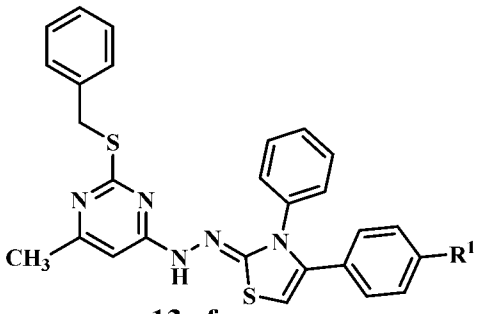


7b



10a,b

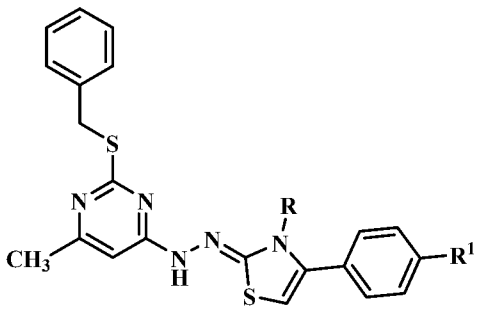
10a: R=CH_3, **10b:** R=$C_6H_5CH_2$



13e,f

13e: R^1=Br, **13f:** R^1=CH_3

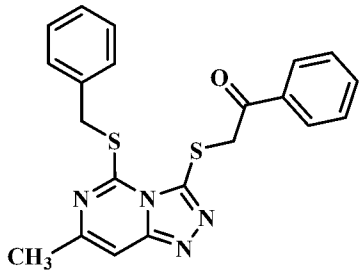
In addition, compounds **13e,f** were the most active against CaCo-2 cell line (IC_{50} 7.71 and 8.68 µg/ml, respectively) exhibiting nearly half the activity of 5-FU (IC_{50} 4.01 µg/ml) while compounds **7c**, **10b**, **13a,c,d** and **16** displayed considerable activity (their IC_{50} values were 11.32, 13.58, 18.36, 11.14, 13.14 and 18.70 µg/ml, respectively). The other tested compounds showed moderate activity with IC_{50} ranging from 20.69 to 62.77 µg/ml.



13a,c,d

13a: R=-CH_2-CH=CH_2, R^1=H
13c: R=-CH_2-CH=CH_2, R^1=CH_3
13d: R=C_6H_5, R^1=H

Furthermore, compounds **10b** and **11** were the most active against A549 cell line (IC_{50} 14.75 and 18.35 µg/ml, respectively) while the other tested compounds except **10a**, **13a** and **19** displayed moderate activity (IC_{50} 20.00-85.01 µg/ml).



11

It is worth mentioning that compounds **6a-c**, **7b,c**, **8a**, **10b** and **13b,d,f** exhibited potent and broad spectrum anticancer activity with IC_{50} values ranging from 7.71 to 48.44 µg/ml.

One of the most important criteria of an anticancer agent is its selectivity and ability to discriminate between cancer and normal cells. In order to assess the selectivity of the active newly synthesized compounds, selectivity index (SI) was calculated for the tested compounds against the four cancer cell lines. SI is a measure of the selectivity of the drug candidate towards cancer cells rather than normal cells (SI = LC_{50} on normal cells/ IC_{50} on cancer cells). It was also reported that compounds with SI values higher than 3 could be considered as highly selective.[165] SI values for the tested compounds are recorded in table 9.

Interestingly, compound **13f** could be considered as a promising anticancer lead compound as it showed a remarkable anticancer effect over the four cancer cell lines and a safer effect on PBMCs normal cells with selectivity indices ranging from 3.29 to 13.86. Moreover, the concentration that killed 100% of cancer cells (IC_{100}) was also calculated for this compound (using GraphPadInStat3.0 software),[164] and was found to be 66.63 ± 1.9, 39.69 ± 0.6, 21.24 ± 0.25 and 65.06 ± 2.2 µg/ml for MCF-7, HepG2, Caco-2 and A549 cancer cells, respectively. These values were also much lower than the LC_{50} of this compound on PBMCs (120.28 ± 2.4 µg/ml) which indicates that compound **13f** was highly selective towards these four cancer cell lines.

In addition, compound **8a** showed high SI against HepG2 cell line while compounds **17** and **19** showed high SI against MCF-7 cell line.

Table 5: SI values of the tested compounds

Compound ID	SI	SI	SI	SI
	MCF-7	HepG2	CaCo-2	A549
4	1.53	0.60	0.44	0.30
5a	1.53	0.95	0.39	0.69
5b	0.46	0.26	0.34	0.23
6a	2.49	0.74	0.93	1.47
6b	2.26	1.34	1.49	1.08
6c	1.01	0.35	0.44	0.43
7a	2.17	0.51	1.11	1.22
7b	0.46	0.87	0.66	0.52
7c	2.09	0.91	2.15	0.84
8a	1.11	**5.33**	1.97	1.65
8b	0.80	0.17	0.47	0.56
8c	0.36	0.05	0.25	0.19
9	1.40	0.36	0.66	0.32
10a	0.76	1.14	0.42	0.14
10b	1.08	1.62	1.66	1.53
11	0.65	0.12	0.33	0.56
12a	0.91	1.43	0.55	1.01

Table 9. (*Continued*)

Compound ID	SI	SI	SI	SI
	MCF-7	HepG2	CaCo-2	A549
12b	0.56	0.41	0.30	0.47
13a	0.56	0.66	1.05	0.17
13b	0.46	0.56	0.92	0.50
13c	0.89	0.84	1.76	0.37
13d	0.37	0.70	1.36	0.50
13e	0.52	0.77	1.49	0.22
13f	**3.29**	**6.56**	**13.86**	**3.79**
14	1.51	0.34	0.75	0.45
16	2.84	0.45	1.91	0.92
17	**10.75**	0.78	1.70	2.60
18	1.20	0.48	1.02	0.66
19	**3.53**	0.48	1.97	0.49
5-FU	1.80	6.17	4.00	48.64

Structure-activity relationship:

Structure-activity correlation reveals that thiourea moiety incorporated in both pyrimidine or triazolopyrimidine rings exhibited potential anticancer activity.

It is clearly noticeable that benzylidene hydrazino moiety in compounds **6a-c** showed remarkable improvement in anticancer effect over the unsubstituted hydrazino derivative **4**.

Introducing the thiosemicarbazide moiety in compounds **12a,b** improved the activity against A549 cancer cell line while decreased the activity against MCF-7 cancer cell line.

For compounds **13a-f**, the allyl substitution at position-3 generally improved anti-cancer activity over the phenyl substitution.

Benzyl substitution in case of compound **10b** showed improvement in anticancer activity against the four cancer cell lines over the unsubstituted **9** or methyl substituted **10a** analogues.

3- ANTIOXIDANT SCREENING

The antioxidant screening of the target compounds was performed in *Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Borg El-Arab, Alexandria, Egypt.*

Experimental:

All newly synthesized compounds were screened for their *in vitro* antioxidant activity using DPPH free radical scavenging assay which was originally described by Blois.[166]

DPPH is a stable free radical with a delocalized spare electron showing an absorption band in methanol at 520 nm yielding a deep violet color. In presence of an antioxidant, DPPH is reduced with consequent loss of the violet color.[167]

The DPPH scavenging activity of the test compounds was measured according to the method described by Nahar *et al.*[168] Briefly, 100 µl of different concentrations of the test compounds (2.5, 5, 10, 20, 40 and 80 µg/ml) were pipetted into a 96-well flat-bottomed plate. Next, 100 µl of 100 µM DPPH methanolic solution were added to each well and the plate was incubated protected from light at room temperature for 30 minutes. The absorbance of the solution was measured at 517 nm. Trolox (water-soluble analogue of vitamin E) and DMSO were used as positive control and blank, respectively. The percentage of DPPH scavenging activity was calculated according to the following equation:

$$\% \text{ of DPPH scavenging} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test sample.

Dose-response curve was plotted between % DPPH free radical scavenging activity and the drug concentration. Linear regression analysis was carried out for calculating concentration showing 50% free radical scavenging activity (EC_{50}). The results of *in vitro* antioxidant screening are listed in table 10.

Table 6: Results of *in vitro* antioxidant assay.

Compound ID	EC_{50} (µg/ml)[a]	Compound ID	EC_{50} (µg/ml)
4	**25.38**	**8a**	1015.01
5a	**33.53**	**8b**	9992.26
5b	**29.66**	**8c**	5424.96
6a	936.15	**9**	**46.29**
6b	1222.83	**10a**	513.86
6c	7646.62	**10b**	432.20
7a	888.97	**11**	192.38
7b	404.17	**12a**	**23.64**
7c	405.34	**12b**	**12.04**

Table 10. (*Continued*)

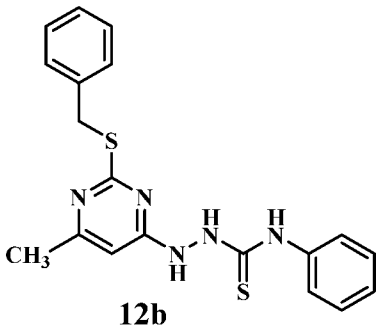
Compound ID	EC_{50} (µg/ml)	Compound ID	EC_{50} (µg/ml)
13a	**12.07**	**14**	82.07
13b	**25.39**	**16**	204.79
13c	**14.77**	**17**	1093.77
13d	**13.56**	**18**	**15.48**
13e	**46.49**	**19**	67.63
13f	**45.23**	**trolox**	20.42

(a) Data are expressed as the means of three determinations

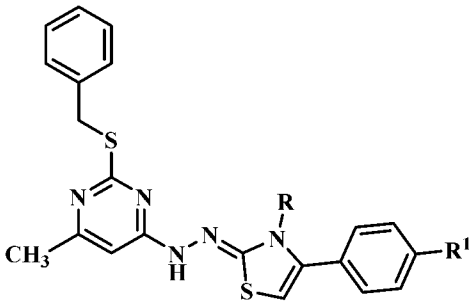
Results and discussion:

Investigating the antioxidant screening results revealed that some of the tested compounds showed strong to weak antioxidant activity.

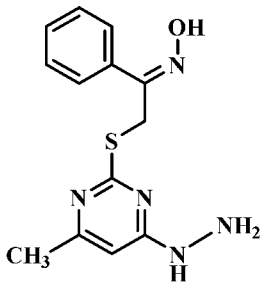
Compounds **12b**, **13a,c,d** and **18** showed antioxidant activity (EC_{50} ranging from 12.04 to 15.48 µg/ml) higher than that of the standard trolox (EC_{50} 20.42 µg/ml).



12b



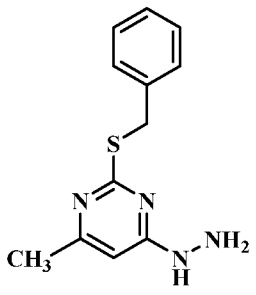
13a,c,d



18

13a: R = -CH_2-CH=CH_2, R^1 = H
13c: R = -CH_2-CH=CH_2, R^1 = CH_3
13d: R=C_6H_5, R^1 = H

Compounds **4**, **5a,b**, **12a** and **13b** showed moderate antioxidant activity with EC_{50} ranging from 23.64 to 33.53 µg/ml.



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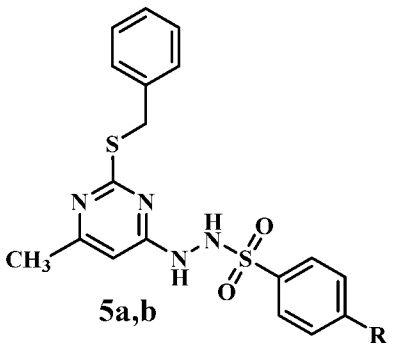
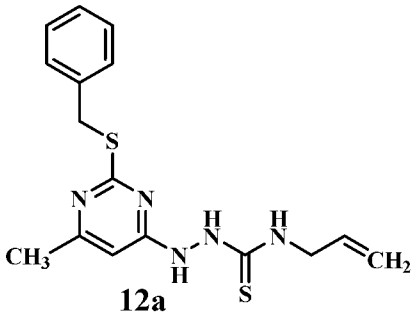
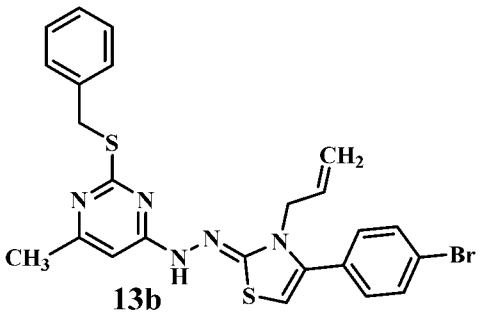


5a,b

5a: R=H, **5b:** R=CH_3

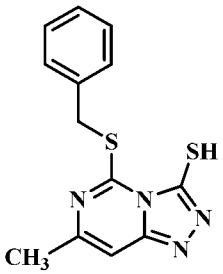


12a

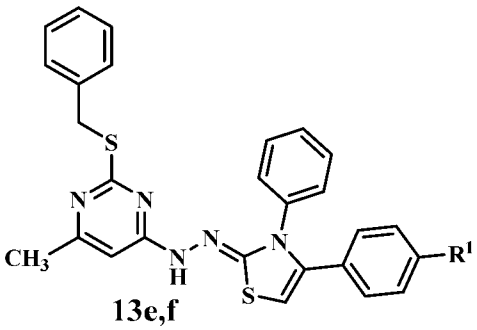


13b

Compounds **9** and **13e,f** showed weak antioxidant activity with EC_{50} ranging from 45.23 to 46.49 µg/ml.



9



13e,f

13e: R^1=Br, 13f: R^1=CH_3

Structure-activity relationship:

The role of antioxidant is to scavenge free radical. One important mechanism through which this is achieved is by donating hydrogen to free radicals resulting in its reduction to an unreactive species because addition of hydrogen would remove the odd electron feature which is responsible for radical reactivity.[169]

In general, all tested compounds with antioxidant activity contained NH or SH functions which might explain their activity by hydrogen atom donating ability.

Compound **12b** with phenyl thiosemicarbazide moiety showed the highest activity. Replacement of the phenyl ring with allyl side chain in **12a** decreased the antioxidant activity.

Cyclization of allyl thiosemicarbazide moiety in **12a** into 4-substituted phenyl-1,3-thiazole increased the antioxidant activity in case of unsubstituted **13a** or 4-methyl substituted **13c** but had no significant difference in 4-bromo substitution **13b**.

Cyclization of the phenyl thiosemicarbazide moiety in **12b** into the corresponding phenyl-1,3-thiazole increased the antioxidant activity in case of unsubstituted **13d** but decreased the activity in 4-bromo or 4-methyl derivatives **13e,f**.

Hydrazino derivative **4** showed moderate activity. Insertion of oxime function beside the hydrazine moiety in compound **18** increased antioxidant activity.

Alkylation of compound **9** abolished antioxidant activity which might be explained by the removal of SH function.

In case of sulfonamide derivatives **5a,b**, presence of methyl group at position-4 didn't show significant difference in antioxidant activity.

4- ANTIMICROBIAL SCREENING

The antimicrobial screening of the target compounds was performed in Pharmaceutical Microbiology Department, Faculty of Pharmacy, Alexandria University.

Experimental:

All newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 6538P) and *Bacillus subtilis* (ATCC 19659) as representatives of Gram-positive; *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027) as representatives of Gram-negative bacteria. These compounds were also evaluated for their *in vitro* antifungal activity against *Candida albicans* (ATCC 2091). Their inhibition zones were measured using the cup diffusion technique.[170] Further evaluation was then carried out on the potential active compounds to determine their minimal inhibitory concentration (MIC) using the two-fold serial dilution method.[171] Ciprofloxacin and ampicillin were used as standard antibacterial agents while clotrimazole was used as antifungal reference. DMSO was used as a blank and showed no antimicrobial activity.

Inhibition zone measurement

Antimicrobial tests were carried out using the agar well diffusion method[170] using 100 µl of suspension containing 1 x 10^8 CFU/ml of pathological tested bacteria and 1 x 10^6 CFU/ml of fungi spread on nutrient agar. After the media had cooled and solidified, wells (8 mm in diameter) were made in the solidified agar and loaded with 75µl of test compound solution; prepared by dissolving 10 mg of the tested compounds in 10 ml of DMSO. The inculated plates were then incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compounds. Ciprofloxacin and ampicillin (1 mg/ml) were used as standard for antibacterial activity while clotrimazole (1 mg/ml) was used as standard for antifungal activity. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standards. The observed zones of inhibition in millimeters (mm) of tested compounds against the pathological strains are listed in table 11.

Minimal inhibitory concentration (MIC) measurement

The antimicrobial activity of the active compounds was then evaluated using the two fold serial dilution technique.[171] Two fold serial dilutions of the test compound solutions were prepared using the proper nutrient broth. Each 5 ml received 0.1 ml of the appropriate inoculums and incubated at 37°C for 24 h for bacteria and 48 h at 28 °C for fungi. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC). The MIC values of the active compounds are listed in table 12.

Table 7: Results of *in vitro* antimicrobial assay

Compound ID	Inhibition zone diameter (mm)				
	Gram positive bacteria		Gram negative bacteria		Yeast
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*
4	-[a]	-	-	-	-
5a	-	-	-	-	-
5b	-	-	-	-	-
6a	-	-	-	-	-
6b	-	-	-	-	-
6c	-	-	-	-	-
7a	4	-	-	-	-
7b	-	-	-	-	-
7c	-	-	-	-	-
8a	4	-	-	-	-
8b	-	-	-	-	-
8c	**13**	-	-	-	-
9	**13**	-	-	-	-
10a	4	2	-	-	-
10b	**7**	-	-	-	-
11	-	-	-	-	-

Table 11. (*Continued*)

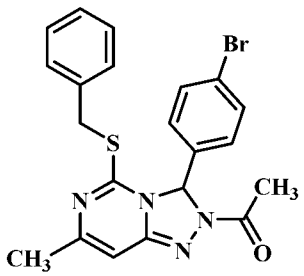
Compound ID	Inhibition zone diameter (mm)				
	Gram positive bacteria		Gram negative bacteria		Yeast
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*
12a	5	-	-	-	-
12b	3	-	-	-	-
13a	3	-	-	-	1
13b	-	-	-	-	-
13c	-	-	-	-	-
13d	4	-	-	-	-
13e	3	-	-	-	1
13f	3	-	2	-	1
14	-	-	-	-	-
16	-	-	-	-	-
17	-	2	-	2	-
18	-	-	-	2	-
19	-	-	-	2	-
ciprofloxacin	18	15	13	12	-
ampicillin	29	25	NT[b]	26	-
clotrimazole	NT	NT	NT	NT	23

a (-) No activity.
b (NT) Not tested.

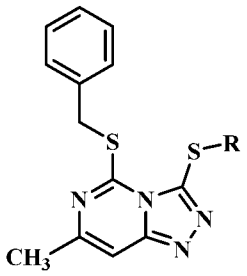
Results and discussion:

Inspection of the recorded antimicrobial screening data listed in table 11 revealed that majority of the tested compounds lacked any significant antibacterial or antifungal activity against the tested pathological strains.

Compounds **8c**, **9** and **10b** showed potential antibacterial activity against *S. aureus* with inhibition zone diameters ranging from 7 to 13 mm compared with ciprofloxacin and ampicillin with inhibition zone diameters 18 and 29 mm, respectively.



8c



9, 10b

9: R= H, 10b: R= CH_2-C_6H_5

Compounds **8c**, **9**, **10a** were selected to be potential lead antibacterial compounds and were subjected to MIC measurements against *S. aureus*. The MIC values of the tested and reference compounds are listed in table 12.

Table 12: MIC values (µg/ml) against *S. aureus*

Compound ID	MIC (µg/ml)
8c	400
9	200
10b	200
ciprofloxacin	20
ampicillin	10

Conclusion:

As a part of an ongoing research program on heterocyclic chemistry hoping to identify new chemical entities suitable for development as new class of chemotherapeutic agents, this study deals with the design and synthesis of novel pyrimidine and triazolopyrimidine derivatives that incorporate thiourea moiety. The synthesized compounds were tested for their potential cytotoxic effect on PBMCs.

The cytotoxicity screening results showed that majority of the tested compounds exerted a remarkable cytotoxic effect on PBMCs. Compounds **5b, 8c, 11** and **13e** exerted the highest cytotoxic effects that are comparable to the standard 5-FU while compounds **13f** and **17** exhibited the lowest cytotoxic effects.

In addition, all newly synthesized compounds were evaluated for their anticancer, antioxidant and antimicrobial activity.

The anticancer screening results revealed that majority of the tested compounds showed potential *in vitro* anticancer activity against the four cancer cell lines with IC_{50} values less than 100 µg/ml. Compound **17** showed good safety profile and displayed the same growth inhibitory activity as 5-FU against MCF-7 cell line. Compound **13f** was the most safe and displayed broad spectrum of anticancer activity while compound **8a** was the most active against HepG2 cell line (IC_{50} 7.71 µg/ml). Compound **13e** was the most active against CaCo-2 cell line (IC_{50} 7.71 µg/ml). In addition, compound **10b** was the most active against A549 (IC_{50} 14.75 µg/ml). Moreover, Compounds **6a-c, 7b,c, ^a, 10b** and **13b,d,f** exhibited potent and broad spectrum of anticancer activity.

In general, all tested compounds with antioxidant activity contained NH or SH functions which might explain their activity by hydrogen atom donating ability. Compounds **12b**, **13a**, **13c**, **13d** and **18** showed antioxidant activity higher than that of standard, trolox. Compounds **4**, **5a,b**, **12a** and **13b** showed moderate activity while compounds **9**, **13e** and **13f** showed weak antioxidant activity.

The antimicrobial screening results showed that majority of the tested compounds lacked any significant antibacterial or antifungal activity against the tested pathological strains except compounds **8c**, **9** and **10b** which exhibited potential antibacterial activity against *Staphylococcus aureus*.

The aforementioned biological screening results revealed that such series of compounds could be considered as structural leads that deserve further derivatization and investigation which might be of value to achieve new structural candidate that displayed dual antioxidant and anticancer effects.

MOLECULAR MODELING

1- *In silico* physico-chemical properties and toxicity prediction

Molecular property prediction is becoming a useful tool in the generation of molecules with the correct parameters to be useful drug candidates. Drug design and lead optimization benefits from the ability to predict physical properties such as lipophilicity and solubility, as well as molecular properties such as topological polar surface area (TPSA) and number of H-bond donors and acceptors to build activity prediction tool which predicts drug likeness.[172]

1. LogP

The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water, is a well-established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values cause poor absorption or permeation. It has been shown that for compounds to have a reasonable propability of being well absorbed, their logP values must not be greater than 5. The distribution of logP values of more than 3000 drugs in the market underlines this fact and is represented in figure 25.[173]

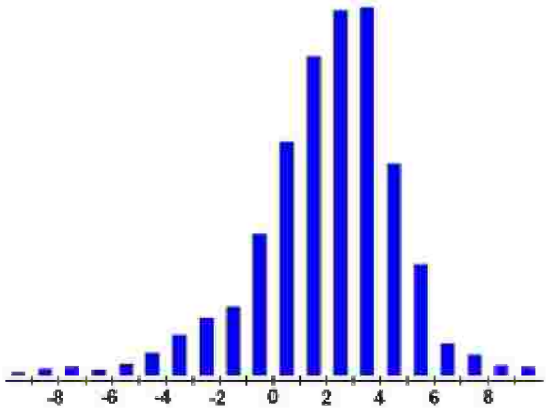


Figure 25: Distribution of logP values of commercial drugs

2. Aqueous solubility (logS)

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds. The diagram shown in figure 26 shows that more than 80% of the drugs in the market have an estimated logS values greater than -4.[173]

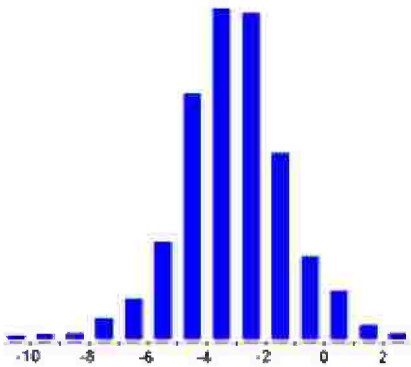


Figure 26: Distribution of logS values of commercial drugs

3. Molecular weight

Optimizing compounds with high activity on a biological target almost often goes along with increased molecular weights. However, compounds with higher weights are less likely to be absorbed and therefore to ever reach the place of action. Thus, trying to keep molecular weights as low as possible should be the desire of every drug development. The diagram shown in figure 27 reveals that more than 80 % of all commercial drugs have a molecular weight below 450.[173]

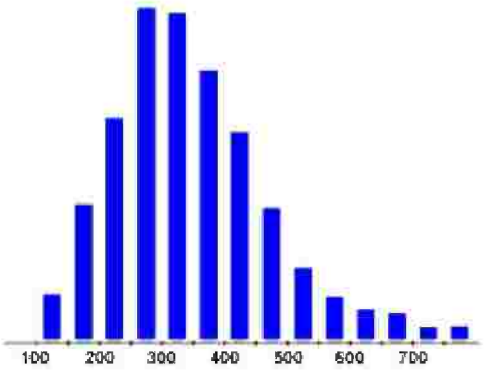


Figure 27: Distribution of molecular weight values of commercial drugs

4. TPSA

It has been reported that the total sum of all the polar regions of a molecule's surface correlates well with various bioavailability related properties, such as intestinal absorption, and blood brain barrier penetration. The TPSA values for most known drugs are less than 120 $Å^2$.[174]

5. Fragment based drug likeness

Drug likeness is a qualitative concept used in drug design and it generally means that molecule contain functional groups and/or have physical properties consistent with the majority of known drugs.[175]

The diagram shown in figure 28 reveals the distribution of drug likeness values calculated for 15000 non-drug like chemicals (shown in blue) and 3300 commercial drugs (shown in red). It indicates that about 80% of the known drugs have a positive drug likeness value while almost all of the non-drug like chemicals had negative values.[173]

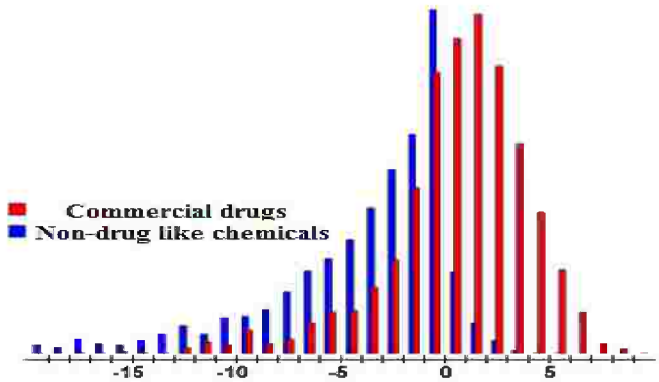


Figure 28: Distribution of drug likeness values of commercial drugs and non-drug like chemicals

A positive Drug likeness value states that a molecule contains predominantly fragments (building blocks) which are frequently present in commercial drugs.

6. Drug Score

The drug score combines drug likeness, logP, logS, molecular weight and toxicity risks in one value that may be used to judge the compound's overall potential to qualify for a drug.[173]

7. Toxicity Risk Assessment

The toxicity risk predictor locates fragments within the compound under investigation which indicate a potential toxicity risks for mutagenicity, tumorigenicity and/or reproductive side effects.[173]

It is worthy to mention that absence of any toxicity alert does not mean the molecule will not display toxicity; rather, an alert for a toxic substructure may not have been compiled yet. Conversely, the presence of an alert does not necessarily mean that a molecule will display toxicity either, since other properties determined by its overall structure are not considered in this type of analysis.[176]

8. The Lipinski rule of five[177]

The medicinal chemist Christopher Lipinski and his colleagues analysed the physico-chemical properties of more than 2,000 drugs and candidate drugs in clinical trials, and concluded that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches the following criteria:

- Its molecular weight is less than 500.
- The compound's lipophilicity, expressed as logP, is less than 5.
- The number of groups in the molecule that can donate hydrogen atoms to hydrogen bonds is less than 5.
- The number of groups that can accept hydrogen atoms to form hydrogen bonds is less than 10.

The Lipinski criteria are widely used to predict not only the absorption of compounds, as Lipinski originally intended, but also overall drug likeness.

Experimental:

All the newly synthesized compounds were subjected to physical and molecular properties prediction tool provided by Osiris Property Explorer[173] which is a knowledge based activity prediction tool able to predict drug likeness, drug score and undesired properties of novel compounds based on chemical fragment data of available drugs and non-drugs as reported. TPSA values and number of H-bond donors and acceptors were calculated using Molinspiration online software.[178] The results of physical and molecular properties and toxicity prediction are listed in table 13.

Table 8: *In silico* physico-chemical properties and toxicity prediction results

Compound ID	Mol. Wt.	logP	TPSA	Solubility	H-bond donors	H-bond acceptors	Drug likeness	Drug-score %	Toxicity risks[a]			
									Mutagenic	tumorigenic	Irritant	Reproductive effect
4	246.33	3.01	89.13	-3.5	3	4	-7.05	25	(+)	(-)	(+)	(+)
5a	386.49	3.72	117.6	-3.02	2	6	-9.35	38	(+)	(+)	(+)	(+)
5b	400.52	4.07	117.6	-3.36	2	6	-8.31	35	(+)	(+)	(+)	(+)
6a	334.44	6.32	75.47	-5.24	1	4	-1.03	26	(+)	(+)	(+)	(+)
6b	368.88	6.84	75.47	-5.97	1	4	-0.02	25	(+)	(+)	(+)	(+)
6c	413.33	6.96	75.47	-6.07	1	4	-2.95	16	(+)	(+)	(+)	(+)
7a	411.32	4.62	68.38	-8.37	0	4	-5.72	9	(-)	(+)	(+)	(+/-)
7b	445.76	5.23	68.38	-9.1	0	4	-5.16	7	(-)	(+)	(+)	(+/-)
7c	490.22	5.34	68.38	-9.2	0	4	-6.2	7	(-)	(+)	(+)	(+/-)
8a	376.48	3.59	73.57	-5.22	0	4	-0.85	38	(+)	(+)	(+)	(+)
8b	410.92	3.85	73.57	-5.61	0	4	**1.29**	**46**	(+)	(+)	(+)	(+)
8c	455.37	3.97	73.57	-5.71	0	4	-1.4	28	(+)	(+)	(+)	(+)
9	288.39	2.43	107.1	-6.26	1	5	-3.28	29	(+)	(+)	(+)	(+)
10a	302.42	2.97	93.68	-6.01	0	5	-0.91	37	(+)	(+)	(+)	(+)
10b	378.51	4.45	96.68	-7.59	0	5	-1	25	(+)	(+)	(+)	(+)
11	406.52	4.02	110.7	-7.55	0	6	-0.35	29	(+)	(+)	(+)	(+)
12a	345.49	4	119.2	-4.43	3	4	-4.65	12	(-)	(+)	(+)	(-)
12b	381.52	5.04	119.2	-5.43	3	4	-3.83	14	(+)	(+)	(+)	(-)
13a	445.60	7.58	104	-6.26	1	5	-2.09	15	(+)	(+)	(+)	(+)

Table 13. (*Continued*)

Compound ID	Mol. Wt.	log *P*	TPSA	Solubility	H-bond donors	H-bond acceptors	Drug likeness	Drug-score %	Toxicity risks[a]			
									Mutagenic	tumorigenic	Irritant	Reproductive effect
13b	524.50	8.3	104	-7.1	1	5	-4.12	10	(+)	(+)	(+)	(+)
13c	459.63	7.92	104	-6.61	1	5	-3.31	13	(+)	(+)	(+)	(+)
13d	481.64	8.4	104	-7.6	1	5	**0.11**	16	(+)	(+)	(+)	(+)
13e	560.53	9.12	104	-8.43	1	5	-1.92	10	(+)	(+)	(+)	(+)
13f	495.66	8.74	104	-7.94	1	5	-1.1	13	(+)	(+)	(+)	(+)
14	347.44	4.15	80.41	-7.24	1	4	-1.44	26	(+)	(+)	(+)	(+)
16	278.76	2.97	68.15	-3.64	0	4	**0.3**	**64**	(+)	(+)	(+)	(+)
17	293.77	3.59	83.67	-4.02	1	5	**0.29**	**59**	(+)	(+)	(+)	(+)
18	289.36	3.2	121.7	-3.84	4	6	-2.64	25	(+)	(-)	(+)	(+)
19	409.53	3.24	135.2	-6.12	3	5	-0.67	12	(-)	(+)	(+)	(-)

[(a)]Ranking as (+) no bad effect, (+/-) medium bad effect, (-) bad effect.

Results and Discussion:

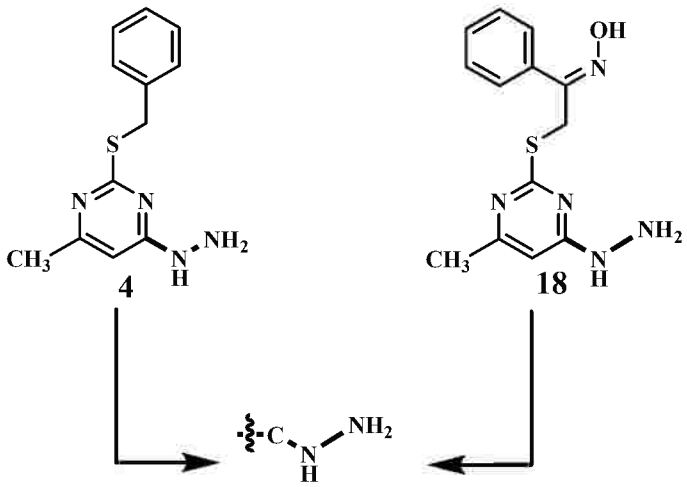
All tested compounds have between 4 and 6 H-bond acceptors, and 4 or fewer H-bond donors. The TPSA values of all molecules except **18** and **19** are well below the "drug like" value of 120 $Å^2$. Compounds **4**, **5a,b**, **16** and **18** showed the best solubility profile with LogS values greater than -4.

Compounds **8b**, **13d**, **16** and **17** have positive Drug likeness values, ranging from 0.11 to 1.29. Compounds **8b**, **16** and **17** showed the highest drug score with values 46, 64 and 59%, respectively.

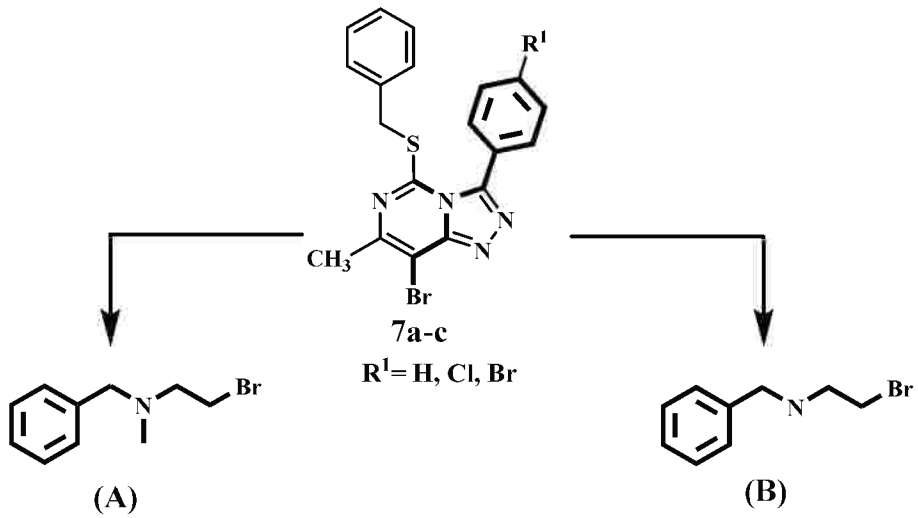
In addition, compounds **4**, **5a,b**, **7a**, **8a-c**, **9-12a**, **14** and **16-19** obey the Lipinski rule of five.

Investigating the toxicity risks of the tested compounds revealed that:

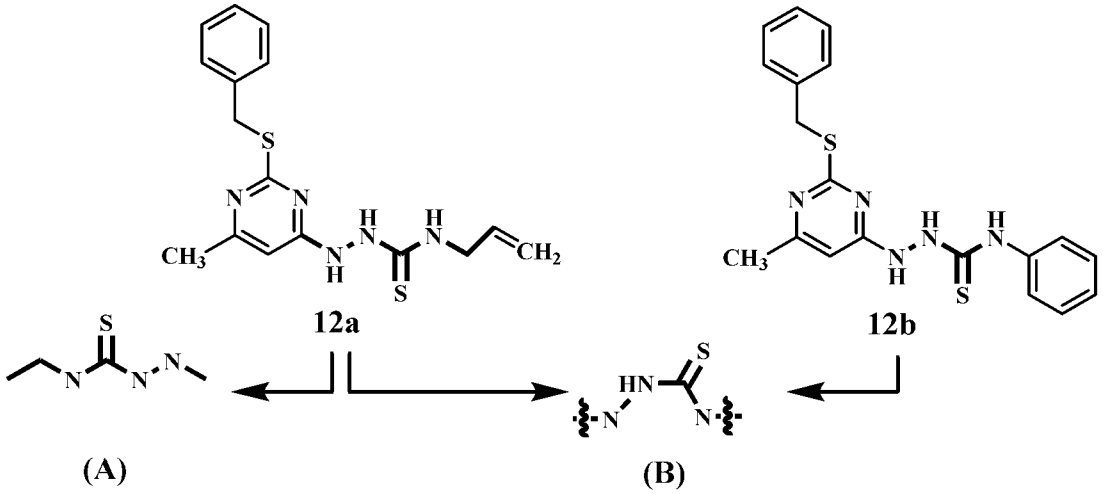
- Compounds **5a,b, 6a-c, 8a-c, 9, 10a,b, 11, 13, 14, 16** and **17** showed high safety profile.
- On the other hand, both compounds **4** and **18** showed high risk for tumorigenicity due to presence of the following fragment.



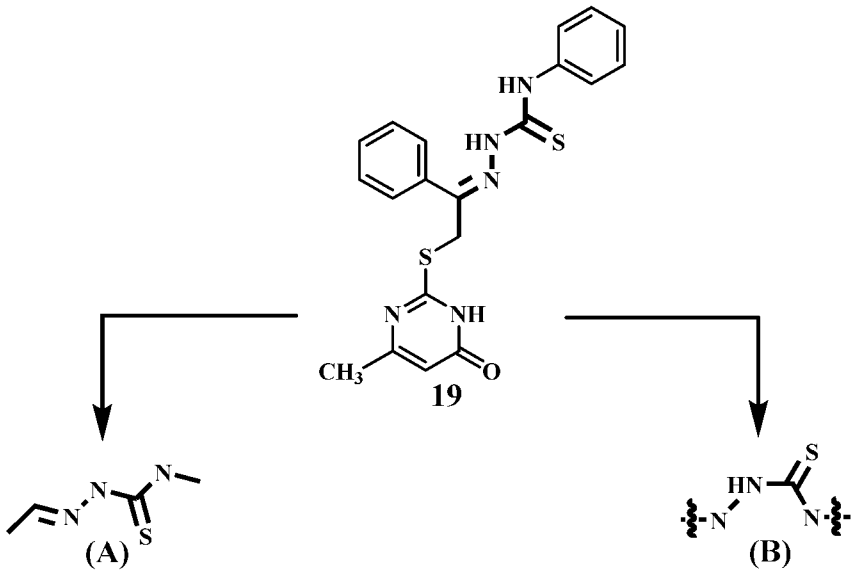
- Compounds **7a-c** showed high risk for mutagenicity due to presence of fragment (**A**) and showed medium risk for mutagenicity and reproductive side effects due to presence of fragment (**B**).



- Compound **12a** showed high risk for mutagenicity due to presence of fragment (**A**) while the 2 compounds **12a,b** showed high risk for reproductive side effects due to presence of fragment (**B**).



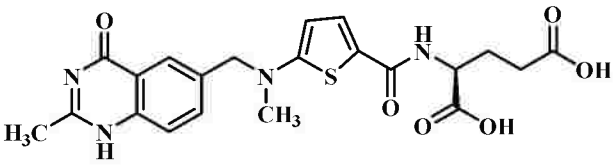
- Compound **19** showed high risk for mutagenicity due to presence of fragment (**A**) and showed high risk for reproductive side effects due to presence of fragment (**B**).



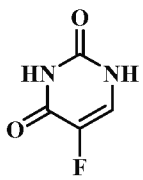
2- Anticancer docking study

Thymidylate synthase (TS) is a key enzyme that plays a crucial role in the early stages of DNA synthesis and repair. It catalyzes the conversion by methylation of 2`-deoxyuridine-5`-monophosphate (dUMP) to 2`-deoxythymidine-5`-monophosphate (dTMP) with 5,10-methylene tetrahydrofolate (CH_2THF) as the methyl donor. dTMP is then converted to deoxythymidine triphoshphate (dTTP) and finally incorporated in the DNA.[179]

Because of its critical importance in DNA replication and repair, this enzyme has served as a target of many chemotherapeutic anticancer agents. It is inhibited by analogues of the folate-based inhibitors, such as raltitrexed (RTX), and the nucleotide-based inhibitors, such as 5-fluoro-dUMP (FdUMP), which is the active metabolite of 5-FU.[179]



RTX



5-FU

FdUMP binds to the nucleotide-binding site of TS and forms a stable complex with TS and CH_2THF, blocking access of dUMP to the nucleotide-binding site and inhibiting dTMP synthesis. This results in deoxynucleotide triphosphate (dNTP) pool imbalances and increased levels of deoxyuridine triphosphate (dUTP), both of which cause DNA damage (Figure 29). [180]

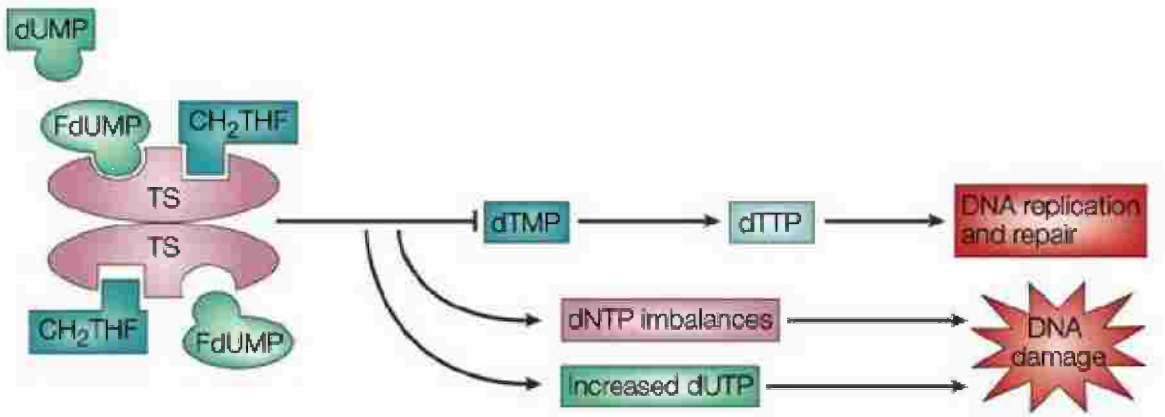
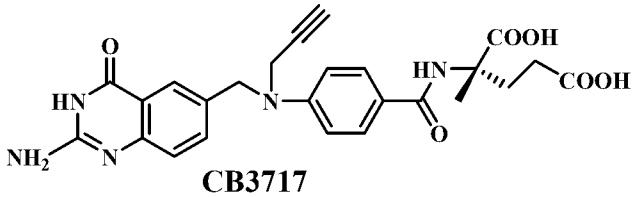
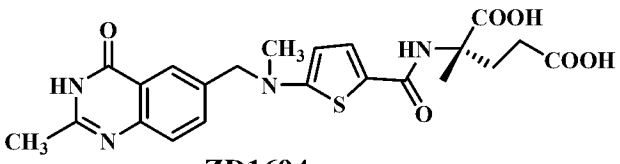


Figure 29: mechanism of action of 5-FU

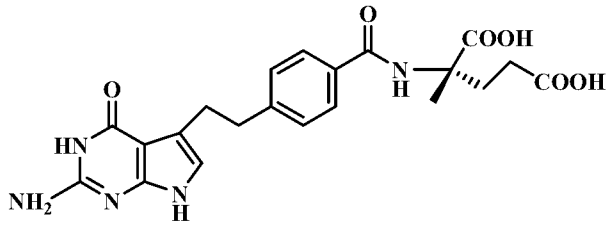
Several fused pyrimidine derivatives have been reported as TS inhibitors and have entered clinical trials as anticancer drugs, notable among them are CB3717 (PDDF),[181] ZD1694 (Tomudex)[182] and LY231514 (Alimta).[183] Alimta has been approved for treatment of lung cancer.[184]



CB3717

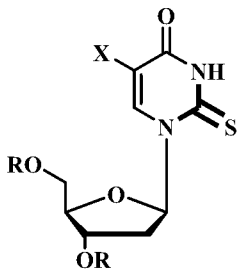


ZD1694



LY231514

The pyrimidine nucleoside and nucleotide analogues **LXVII** with the thiourea moiety incorporated in the pyrimidine nucleus had been reported to possess TS inhibitory effect and exhibited marked antitumor activity.[185]



LXVII

R= H, H_2PO_3

X= H, F

Experimental:

The docking study was performed using the Molecular Operating Environment (**MOE**) software.[186] The crystal structure of human TS bound to dUMP and the anticancer drug RTX (PDB ID code: 1HYV) was downloaded from Protein Data Bank (PDB) website. The targeted compounds were constructed in MOE using the builder module, and collected in a database. The database were prepared by using the option "Protonate 3D" to add hydrogens, calculate partial charges and minimize energy (using Force Field MMFF94x).

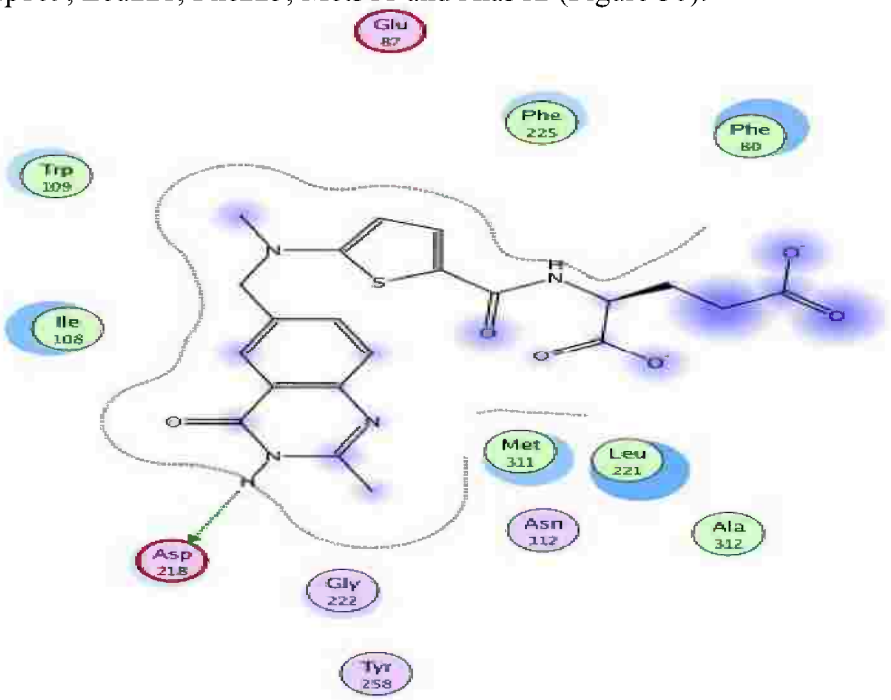
In addition, the protein structure was prepared by deleting the repeating chains, dUMP, water molecules and any surfactants. Hydrogens were also added to the atoms of the receptor and the partial charges were calculated. The active site of TS was generated using the MOE-Alpha Site Finder, and then the ligands were docked within this active site using the MOE dock. MOE was also used to calculate the best score between the ligands and the enzymes' binding sites. Scoring was determined using alpha HB as scoring function. The resulted database contained the score between the ligands' conformers and the enzyme binding sites in kcal/mol. Thirty conformers of each compound were retained with best score by default.

To confirm the credibility of docking results, the pose selection method[186] was used to validate the adopted docking protocol in which TS co-crystallized ligand (RTX) was drawn in MOE, prepared as the targeted compounds (hydrogens addition, partial charges calculation and energy minimization), and then docked into the active site of the protein using the same protocol. The conformer with the best score was superimposed on the original conformation and orientation of the co-crystallized ligand from the co-crystal structure acquired from the (PDB) using PyMOL software.[171] The Root mean square deviation (RMSD) between the original and docked conformer was calculated by PyMOL and was 1.18 Å. It was reported that values less than 1.5 or 2 Å were a sign of a successful and reliable docking protocol.[186]

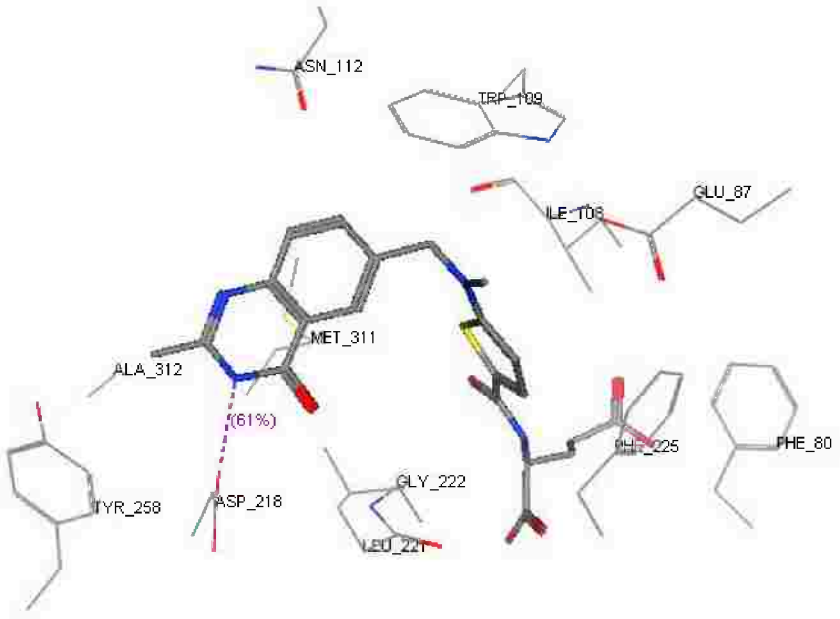
Results and discussion:

The obtained anticancer screening results revealed that compounds **6a-c**, **7b,c**, **8a**, **10b** and **13b,d,f** exhibited the most potent and broad spectrum anticancer activity with IC_{50} values ranging from 7.71 to 48.44 μg/ml against the four cancer cell lines. These results promoted us to perform molecular docking studies to understand the possible binding modes of the active compounds inside TS active site.

Concerning the co-crystallized ligand (RTX), several interactions were considered to be responsible for its observed affinity. One nitrogen of the dihydroquinazoline nucleus acted as a hydrogen bond donor with Asp218 (61%) in addition to hydrophobic interactions with Phe80, Ile108, Trp109, Leu221, Phe225, Met311 and Ala312 (Figure 30).



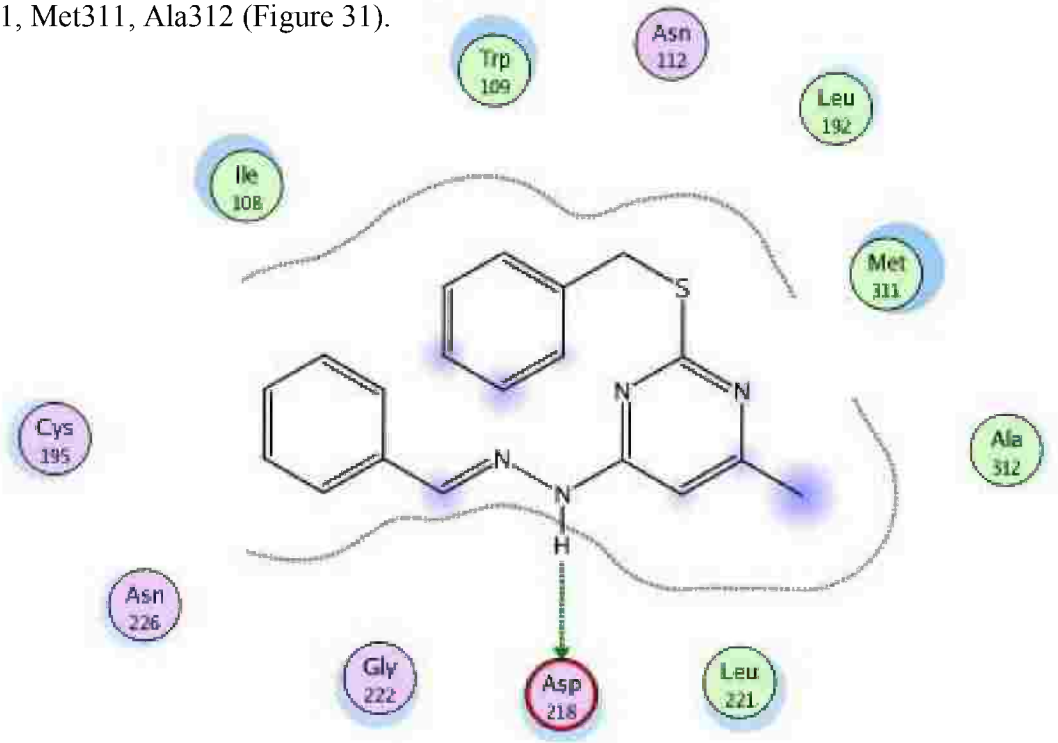
2D ligand (RTX)-enzyme interaction



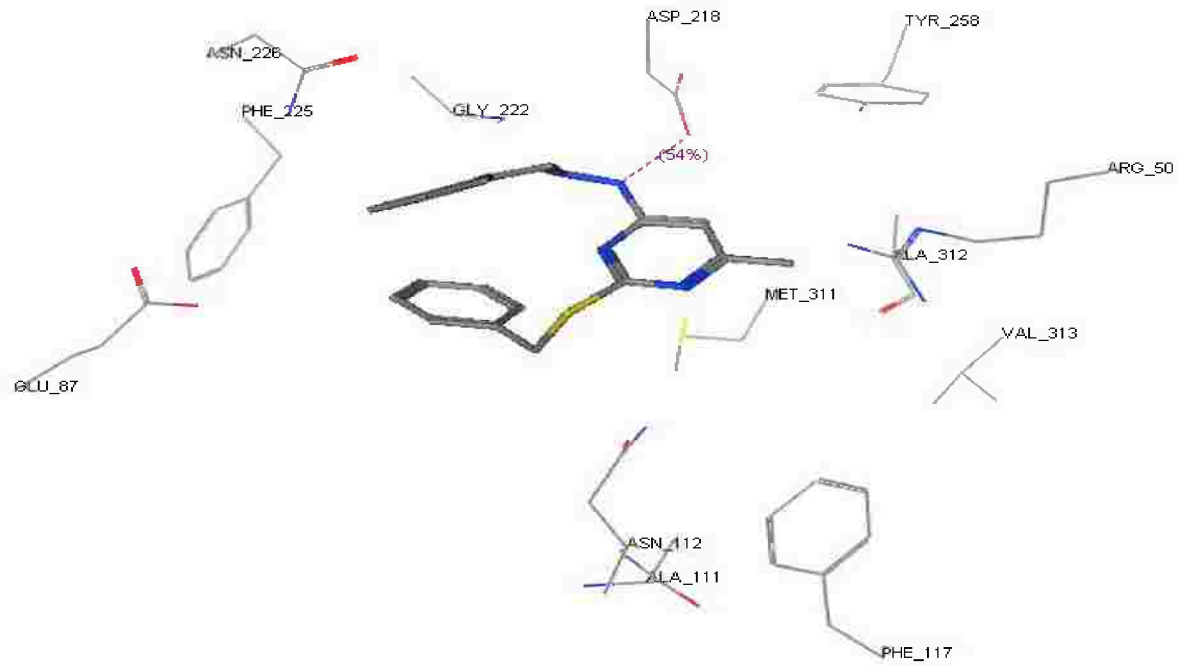
3D ligand (raltitrexed)-enzyme interaction

Figure 30: Mode of binding of raltitrexed inside TS active site

For compound **6a**, NH of the hydrazone function acted as a hydrogen bond donor with Asp218 (54%). Additional hydrophobic interactions were observed with Ile108, Trp109, Leu192, Leu221, Met311, Ala312 (Figure 31).



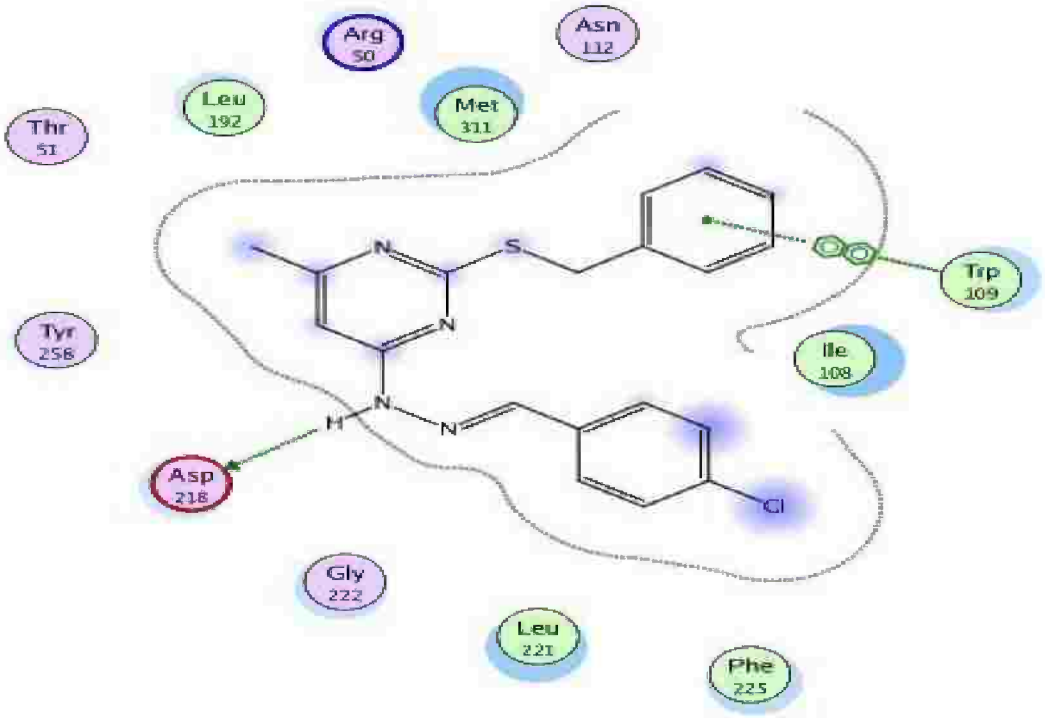
2D ligand (**6a**)-enzyme interaction



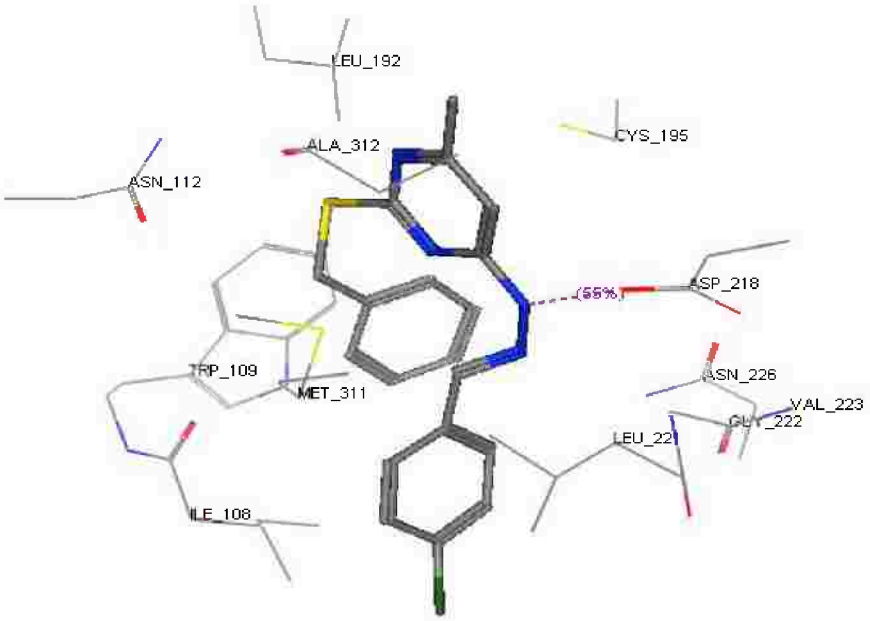
3D ligand (**6a**)-enzyme interaction

Figure 31: Mode of binding of 6a inside TS active site

As well for compound **6b**, NH of the hydrazone function acted as a hydrogen bond donor with Asp218 (55%) in addition to an arene-arene interaction between the benzyl side chain and Trp109. Hydrophobic interactions were also observed with Ile108, Trp109, Leu192, Leu221, Phe225, Met311 (Figure 32).



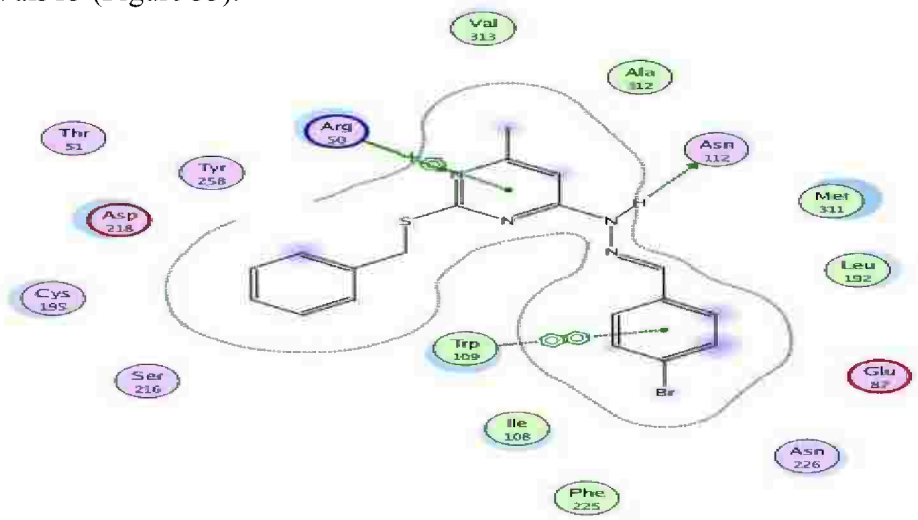
2D ligand (**6b**)-enzyme interaction



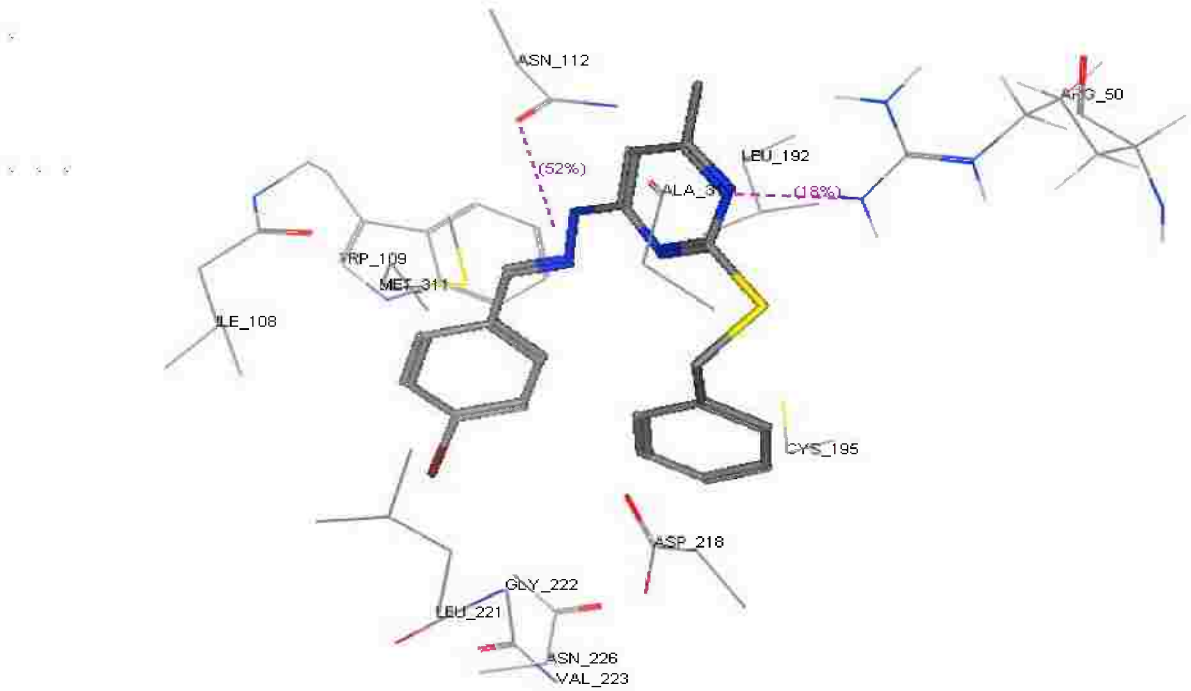
3D ligand (**6b**)-enzyme interaction

Figure 32: Mode of binding of 6b inside TS active site

Compound **6c** participated a hydrogen bonding between NH of the hydrazone function and Asn112 (52%). It also displayed arene-cation interaction (18%) between the pyrimidine ring and Arg50 in addition to an arene-arene interaction between the benzylidene nucleus and Trp109. Hydrophobic interactions were also observed with Ile108, Trp109, Leu192, Phe225, Met311, Ala312 and Val313 (Figure 33).



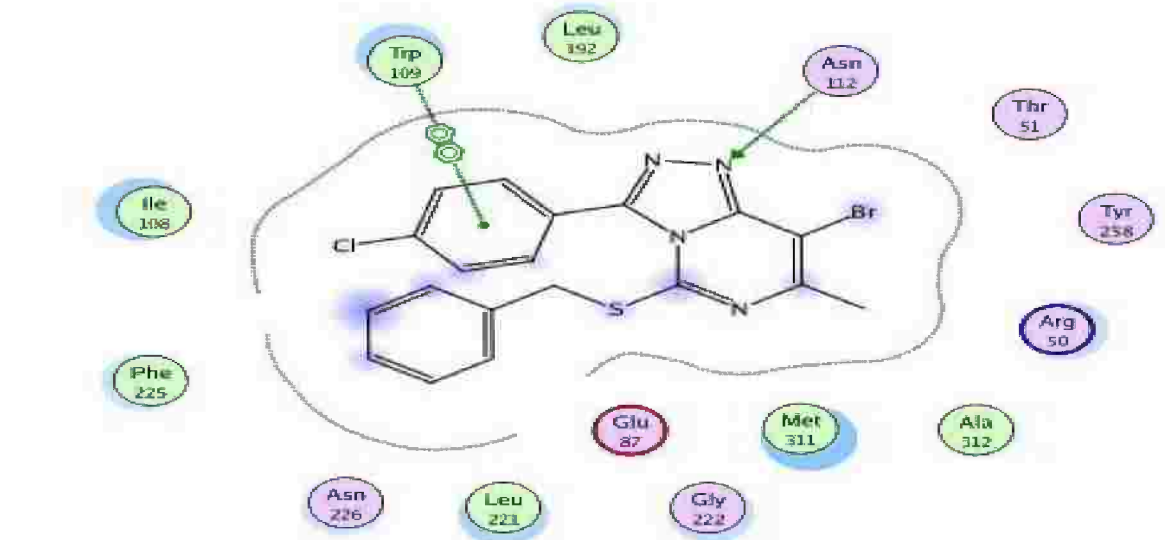
2D ligand (**6c**)-enzyme interaction



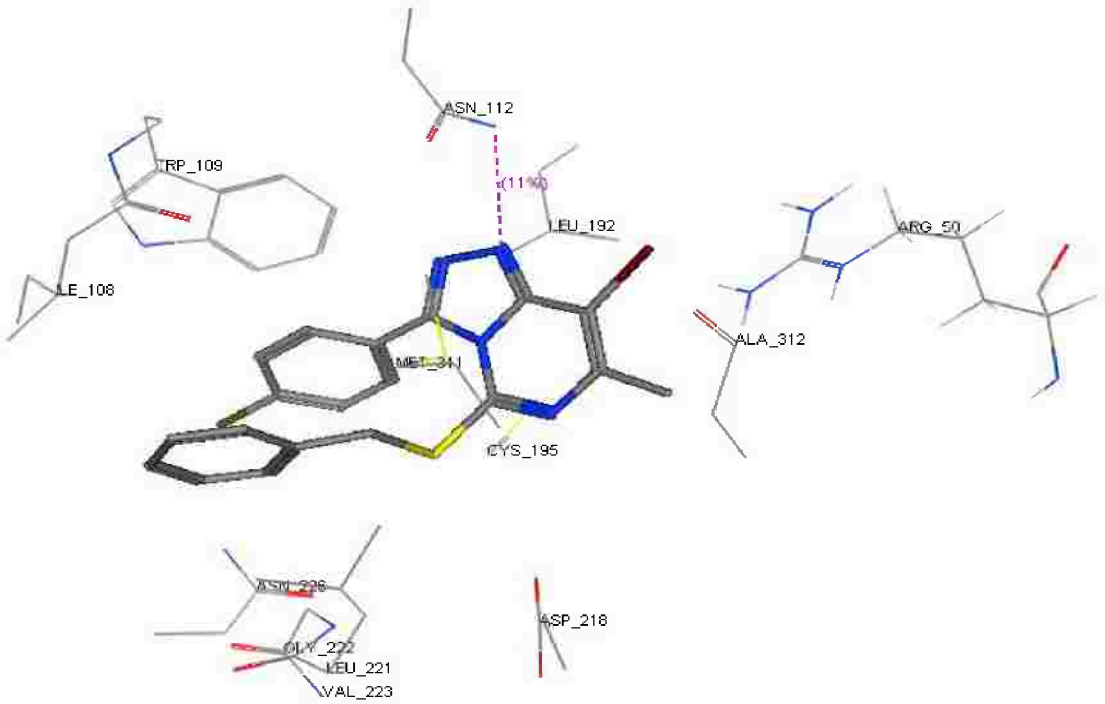
3D ligand (**6c**)-enzyme interaction

Figure 33: Mode of binding of 6c inside TS active site

Whereas, compound **7b** contributed hydrogen bonding between one nitrogen of the triazolopyrimidine nucleus and Asn112 (11%) in addition to an arene-arene interaction between the *p*-chlorophenyl ring and Trp109. Hydrophobic interactions were also observed with Ile108, Trp109, Leu192, Leu221, Phe225, Met311 and Ala312 (Figure 34).



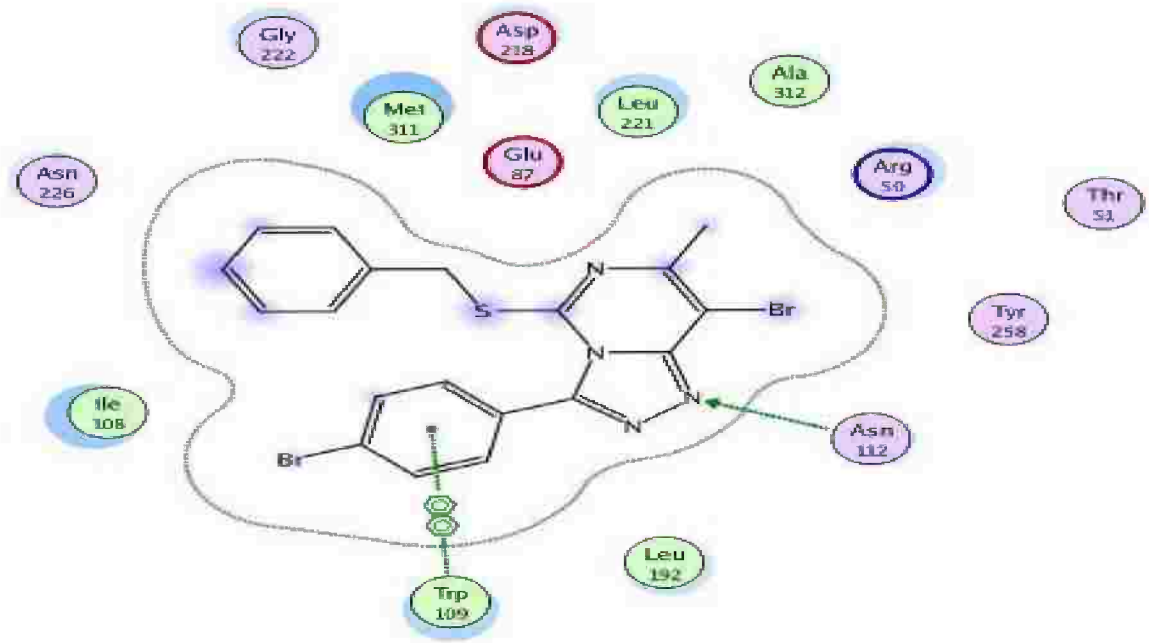
2D ligand (**7b**)-enzyme interaction



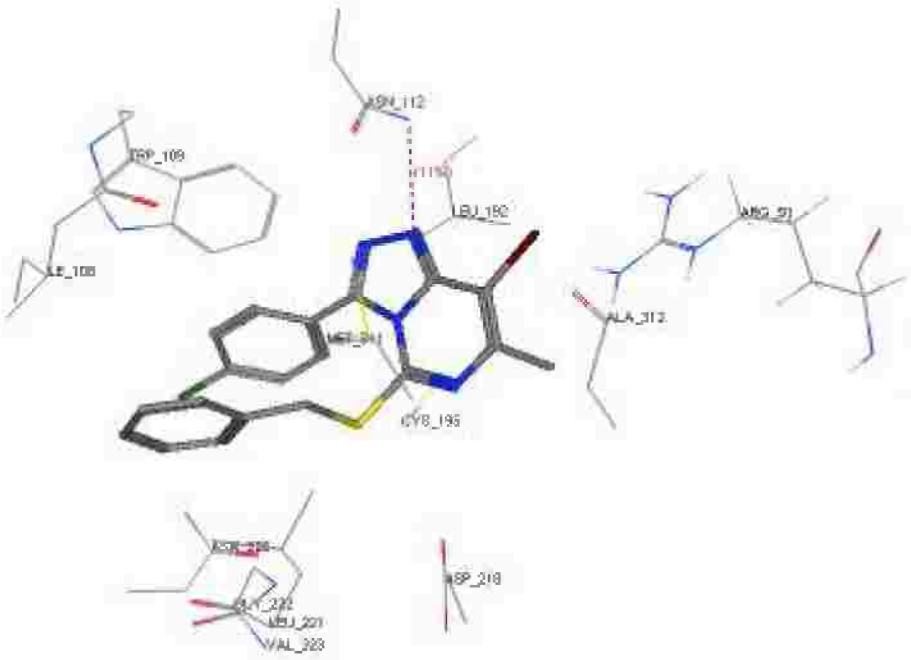
3D ligand (**7b**)-enzyme interaction

Figure 34: Mode of binding of 7b inside TS active site

As for compound **7c**, a hydrogen bonding was observed between one nitrogen of the triazolopyrimidine nucleus and Asn112 (11%) in addition to an arene-arene interaction between the *p*-bromophenyl ring and Trp109. Hydrophobic interactions were also observed with Ile108, Trp109, Leu192, Leu221, Met311 and Ala312 (Figure 35).



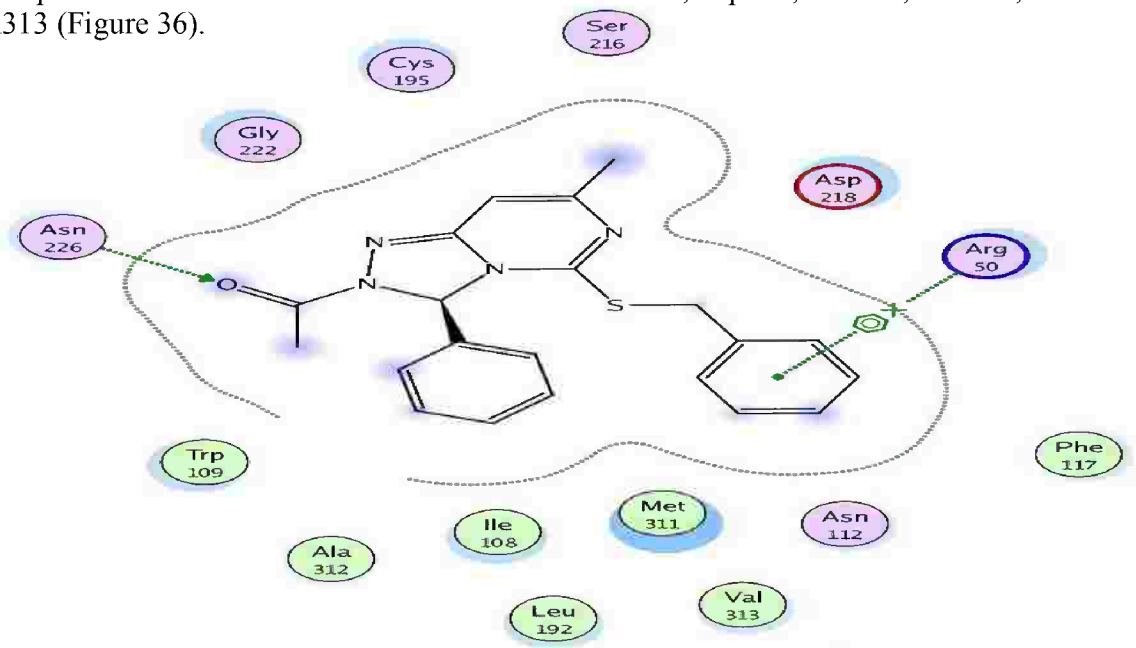
2D ligand (**7c**)-enzyme interaction



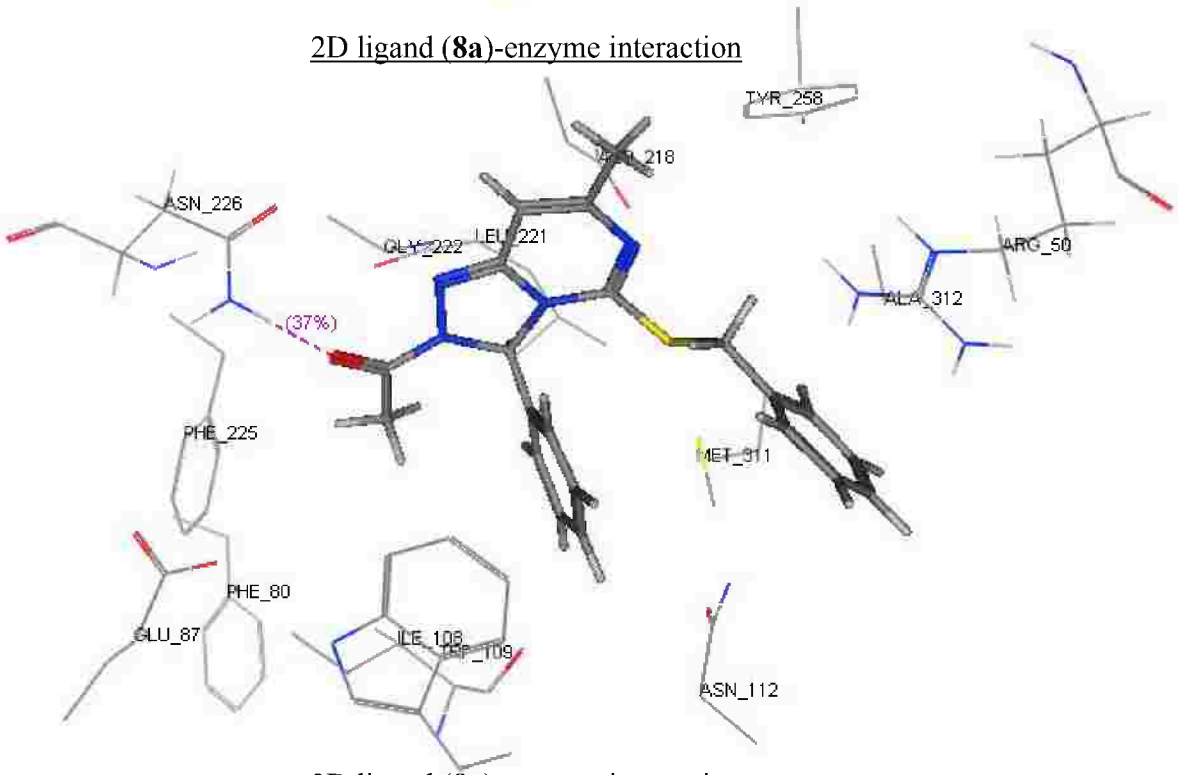
3D ligand (**7c**)-enzyme interaction

Figure 35: Mode of binding of 7c inside TS active site

Compound **8a** demonstrated a hydrogen bonding between the carbonyl oxygen and Asn226 (37%) in addition to an arene-cation interaction between the benzyl ring and Arg50. Hydrophobic interactions were also observed with Ile108, Trp109, Phe117, Leu192, Ala312 and Val313 (Figure 36).



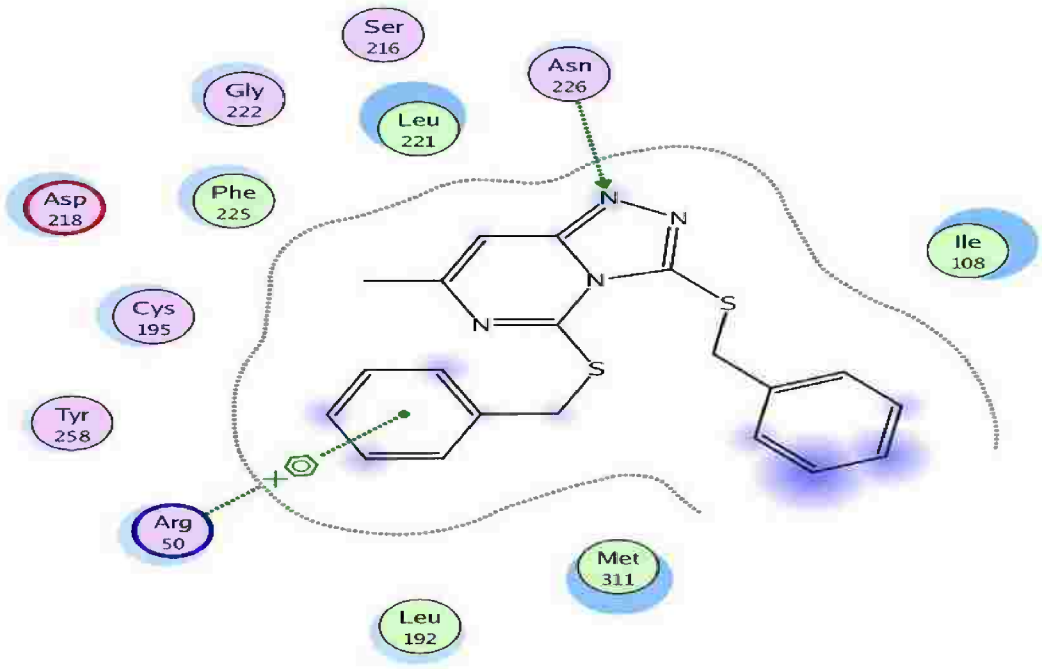
2D ligand (**8a**)-enzyme interaction



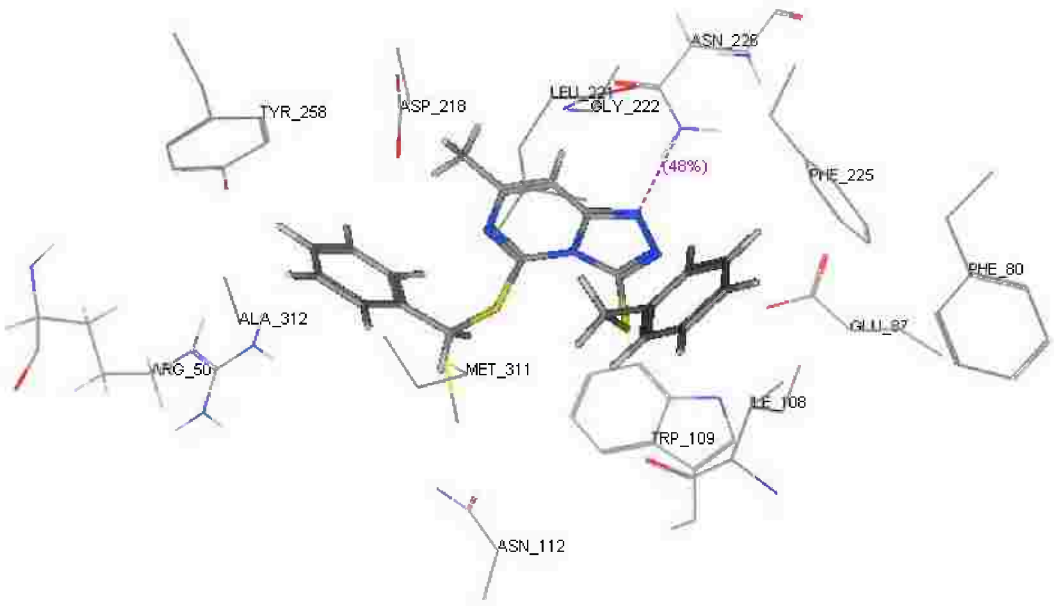
3D ligand (**8a**)-enzyme interaction

Figure 36: Mode of binding of 8a inside TS active site

Whereas, compound **10b** showed a hydrogen bonding between one nitrogen of the triazolopyrimidine nucleus and Asn226 (48%) in addition to an arene-cation interaction between the benzyl ring and Arg50. Hydrophobic interactions were also observed with Ile108, Leu192, Leu221, Phe225 and Met311 (Figure 37).



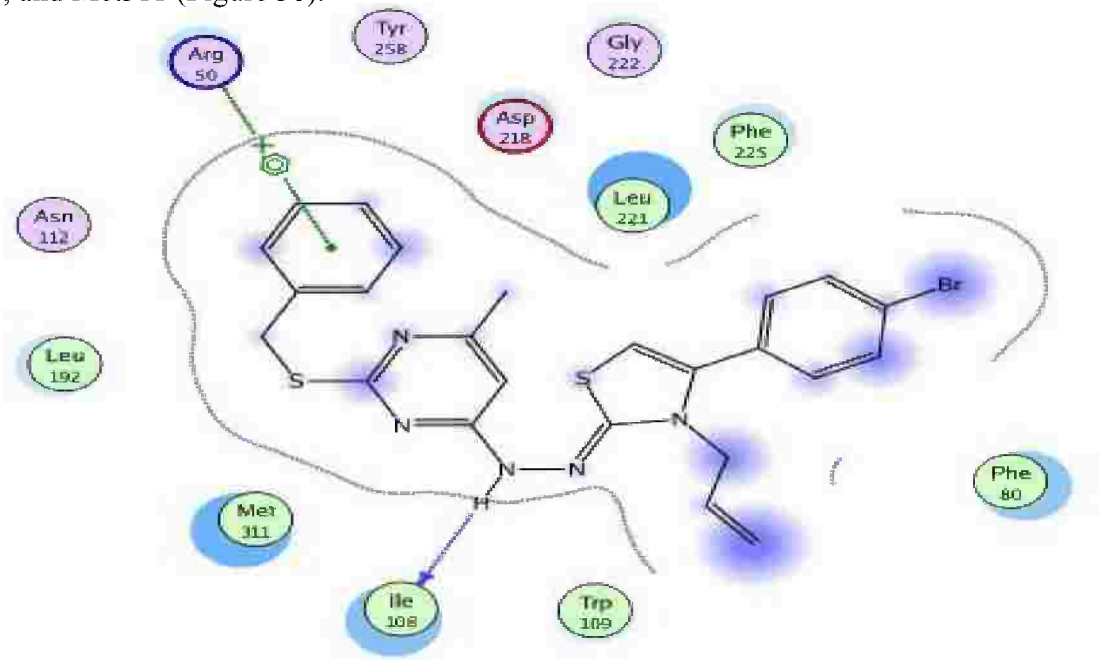
2D ligand (**10b**)-enzyme interaction



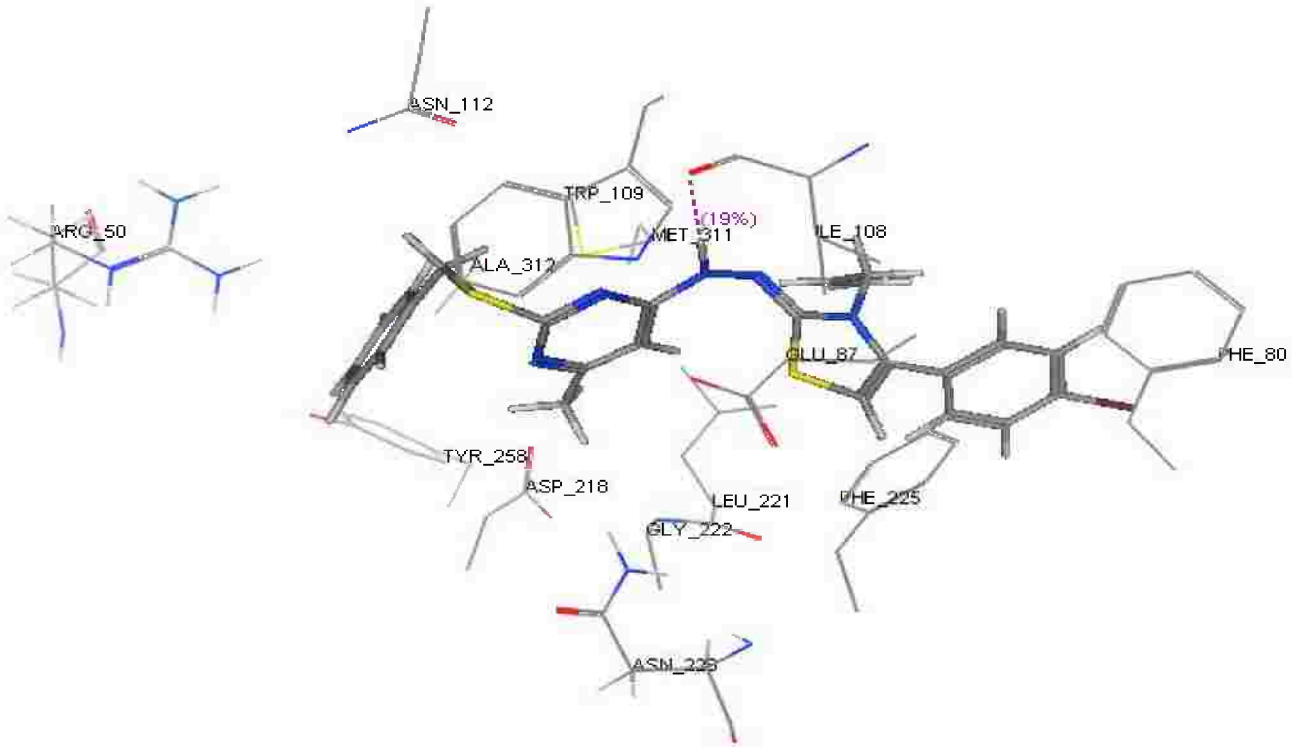
3D ligand (**10b**)-enzyme interaction

Figure 37: Mode of binding of 10b inside TS active site

In compound **13b**, a hydrogen bond was detected between NH of the hydrazone function and Ile108 (19%) in addition to an arene-cation interaction between the benzyl ring and Arg50. Hydrophobic interactions were also observed with Phe80, Ile108, Trp109, Leu192, Leu221, Phe225, and Met311 (Figure 38).



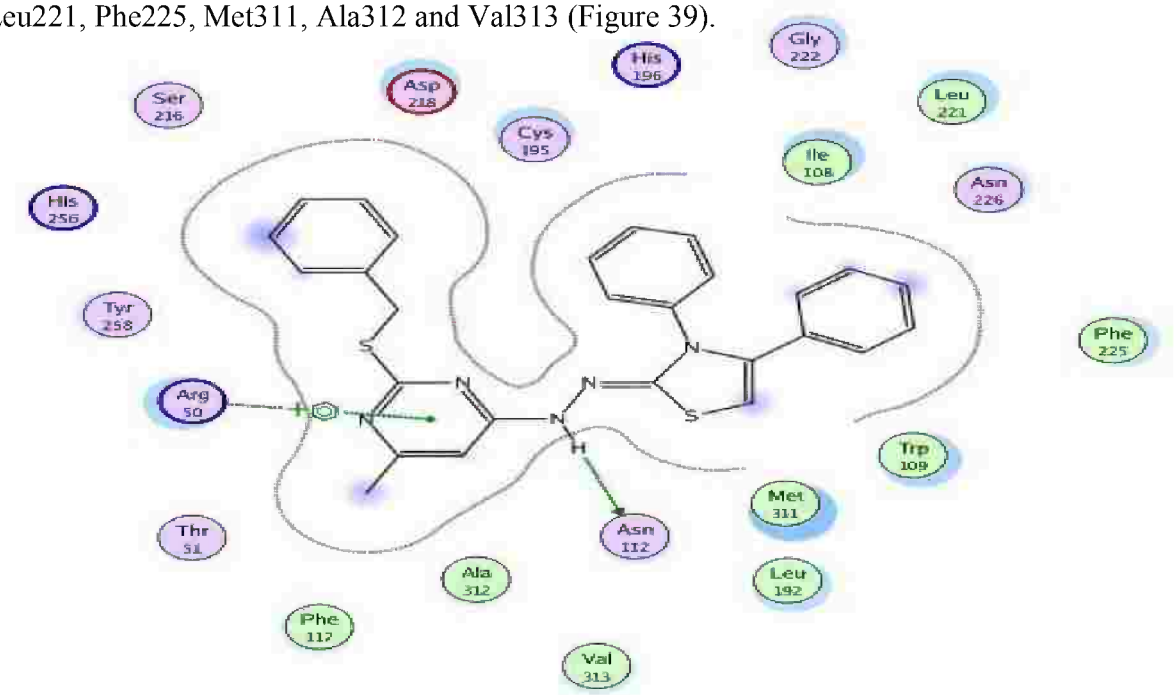
2D ligand (**13b**)-enzyme interaction



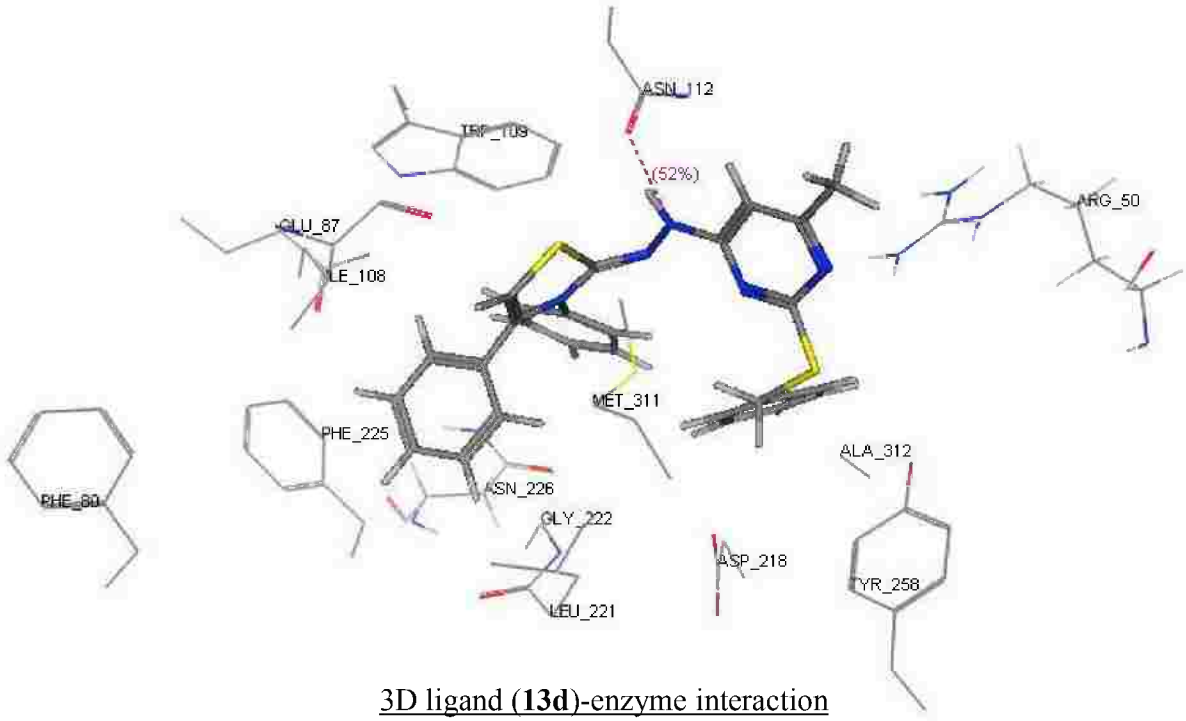
3D ligand (**13b**)-enzyme interaction

Figure 38: Mode of binding of 13b inside TS active site

As for compound **13d**, it displayed a hydrogen bonding between NH of the hydrazone function and Asn112 (52%) in addition to an arene-cation interaction between the benzyl ring and Arg50. Hydrophobic interactions were also observed with Ile108, Trp109, Phe117, Leu192, Leu221, Phe225, Met311, Ala312 and Val313 (Figure 39).



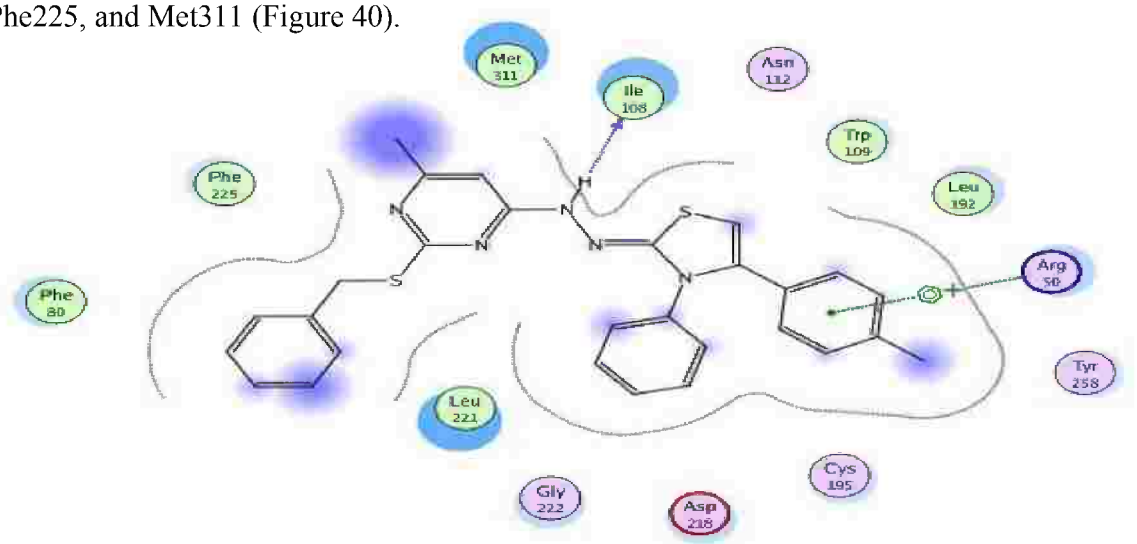
2D ligand (**13d**)-enzyme interaction



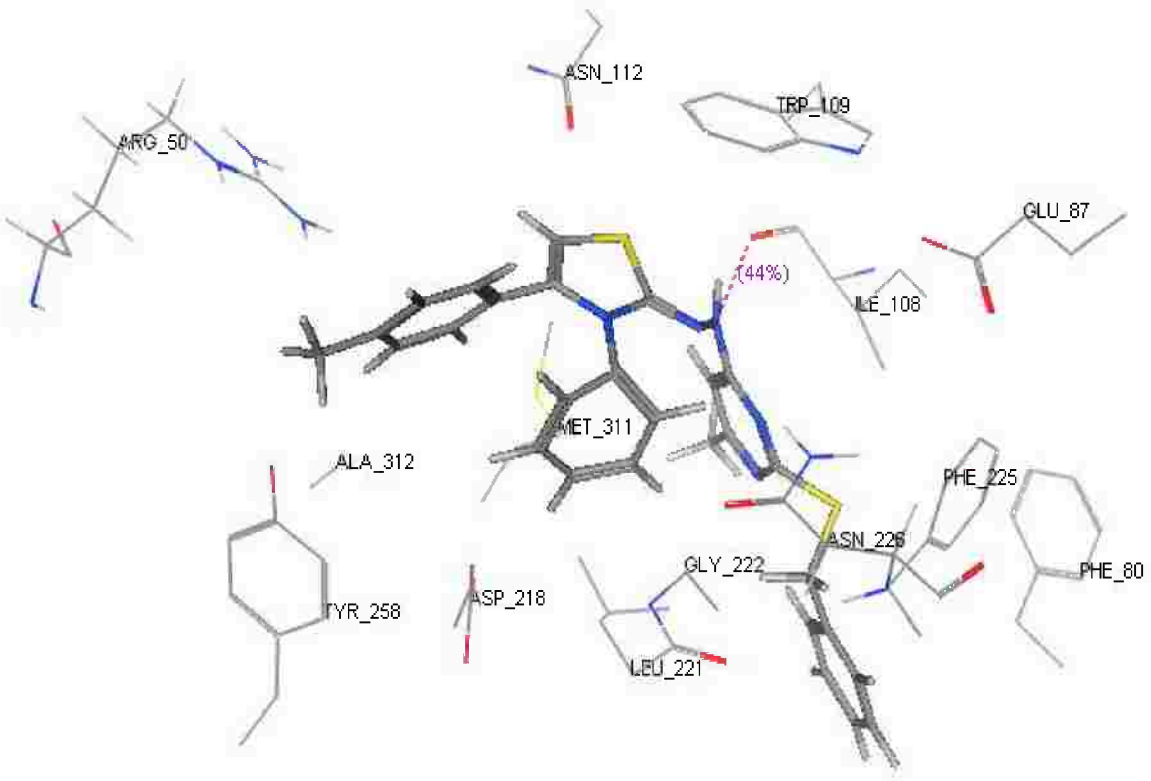
3D ligand (**13d**)-enzyme interaction

Figure 39: Mode of binding of 13d inside TS active site

Compound **13f**, a hydrogen bond was detected between NH of the hydrazone function and Ile108 (19%) in addition to an arene-cation interaction between the benzyl ring and Arg50. Hydrophobic interactions were also observed with Phe80, Ile108, Trp109, Leu192, Leu221, Phe225, and Met311 (Figure 40).



2D ligand (**13f**)-enzyme interaction



3D ligand (**13f**)-enzyme interaction

Figure 40: Mode of binding of 13f inside TS active site

Conclusion:

In conclusion, modeling studies revealed that all docked compounds (**6a-c**, **7b,c, 8a**, **10b** and **13b,d,f**) showed good fitting into TS active site. The binding mode is relatively similar to that of ligand RTX. They displayed hydrogen bonding interactions with residue(s) Ile108, Asn112, Asp218 and Asn226 and hydrophobic interactions with residue(s) Phe80, Ile108, Trp109, Leu192, Leu221, Phe225, Met311, Ala312 and Val313. They displayed one or two hydrogen bonds and 5-7 hydrophobic interactions. While compounds **6b,c** and **7b,c** displayed additional arene-arene interactions with Trp109. In addition, compounds **6c**, **8a**, **10b** and **13b,d,f** showed arene-cation interactions with Arg50.

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ENGLISH SUMMARY

Thiourea moiety, either in open form or incorporated in heterocyclic rings, was found in many therapeutic agents. Several substituted thiourea derivatives were proved to possess various biological activities including anticancer, antiviral, antioxidant, antimicrobial, antiparasitic, anti-inflammatory, CNS and antithyroid activities.

Pyrimidine derivatives have a great biological and medicinal significance, its chemotherapeutic efficacy is related to their ability to inhibit vital enzymes responsible for DNA biosynthesis. Large array of pyrimidine derivatives possess a variety of pharmacological effects such as anticancer, antiviral, antimicrobial and antiparasitic effects.

In addition to their biological importance, pyrimidine derivatives are valuable for the preparation of fused ring compounds, such as triazolopyrimidines. 1,2,4-Triazolopyrimidine is one of the important ring systems that has drawn the attention for its diverse biological activities.

Motivated by these facts and as a continuation of our research on heterocyclic chemistry aiming to find new structure leads which might be of value for development of new more potent chemotherapeutic agents, with higher safety profile, the present investigation was directed to design, synthesize and biologically investigate a new series of thiourea derivatives.

The target compounds comprised a series of substituted pyrimidines and triazolopyrimidine derivatives that incorporate thiourea moiety within their structures or linked to it with different atoms spacer. Substitution patterns were varied in order to achieve structure-activity relationship knowledge.

The present thesis comprises the following chapters:

Chapter I: Introduction

It represents a brief literature survey on biologically active substituted thiourea derivatives, either in open form or incorporated in various heterocyclic rings, exhibiting anticancer, antiviral, antimicrobial, antioxidant, antiparasitic, anti-inflammatory, CNS and antithyroid activities, focusing on the recent researches.

Chapter II: Research objectives

It clarifies the goal of the present work and the rational upon which the newly suggested compounds were designed.

Chapter III: Discussion

It discusses the basic concepts of the experimental methods adopted for the synthesis of the designed compounds referring to available literature. It also investigates the structural elucidation of the synthesized compounds by elemental analyses and various spectroscopic techniques (IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra).

It includes the following four schemes:

Scheme 1:

In this scheme, ethyl acetoacetate was condensed with thiourea to produce 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **1** which was S-alkylated with benzyl chloride to give the corresponding 2-(benzylsulfanyl)-6-methylpyrimidin-4(3*H*)-one **2.** Chlorination of the latter using phosphorous oxychloride afforded the corresponding 2-(benzylsulfanyl)-4-chloro-6-methylpyrimidine **3** which was then reacted with hydrazine hydrate to yield 2-(benzylsulfanyl)-4-hydrazinyl-6-methylpyrimidine **4**. *N'*-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]-4-substituted benzenesulfonohydrazides **5a,b** were obtained from the reaction between the hydrazino compound **4** and 4-substituted benzenesulfonyl chlorides.

Scheme 2:

It deals with the reaction of the key intermediate hydrazino derivative **4** with aromatic aldehydes and carbon disulphide to yield the corresponding 4-[2-(4-substituted benzylidene)hydrazinyl]-2-(benzylsulfanyl)-6-methylpyrimidines **6a-c** and 5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidine-3-thiol **9**, respectively.

Oxidative cyclization of **6a-c** with bromine in the presence of anhydrous sodium acetate afforded the corresponding 3-(4-substituted phenyl)-5-(benzylsulfanyl)-8-bromo-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidines **7a-c**. In addition, cyclocondensation of **6a-c** with acetic anhydride gave the respective 1-[3-(4-substituted phenyl)-5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl]ethanones **8a-c**.

In addition, S-alkylation of **9** using methyl iodide, benzyl chloride and phenacyl bromide yielded the corresponding 3-substituted sulfanyl-5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo-[4,3-*c*]pyrimidines **10a,b** and 2-{[5-(benzylsulfanyl)-7-methyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-3-yl]sulfanyl}-1-phenylethanone **11**, respectively.

Scheme 3:

It deals with reaction of the key intermediate hydrazino derivative **4** with phenyl or allyl thiosemicarbazides to afford the corresponding 2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]-*N*-allyl (or phenyl)hydrazine-1-carbothioamides, **12a,b** which underwent cyclocondensation with substituted phenacyl bromides and yellow mercuric oxide to afford the target 2-{2-[2-(benzylsulfanyl)-6-methylpyrimidin-4-yl]hydrazono}-4-(4-substituted phenyl)-3-allyl (or phenyl)-2,3-dihydrothiazoles **13a-f** and 5-(benzylsulfanyl)-7-methyl-*N*-phenyl-[1,2,4]triazolo[4,3-*c*]pyrimidin-3-amine **14**, respectively.

Scheme 4:

It deals with S-alkylation of the key intermediate **1** with phenacyl bromide to afford 6-methyl-2-[(2-oxo-2-phenylethyl)sulfanyl]pyrimidin-4(3*H*)-one **15** which underwent chlorination using phosphorous oxychloride to yield 2-[(4-chloro-6-methylpyrimidin-2-yl)-sulfanyl]-1-phenylethanone **16**. Reacting the latter with hydroxylamine hydrochloride gave 2-[(4-chloro-6-methylpyrimidin-2-yl)sulfanyl]-1-phenylethanone oxime **17** which was then reacted with hydrazine hydrate to afford 2-[(4-hydrazinyl-6-methylpyrimidin-2-yl)sulfanyl]-1-phenylethan-

one oxime **18**. In addition, reacting **15** with phenyl thiosemicarbazide afforded 2-{2-[(4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)sulfanyl]-1-phenylethylidene}-*N*-phenylhydrazine-1-carbothioamide **19**.

Chapter IV: Experimental

This chapter illustrates the detailed practical procedures adopted for the synthesis and purification of the intermediates as well as the target compounds throughout the thesis. In addition, it includes the physical characters, microanalyses, IR and ^{1}H-NMR spectra for all new derivatives as well as ^{13}C-NMR and mass spectra for some representative examples.

Chapter V: Biological screening

This chapter describes the biological investigation of all newly synthesized compounds in the following aspects:

I- *In vitro* cytotoxicity screening

The results revealed that majority of the tested compounds exerted a remarkable cytotoxic effect on PBMCs. Compounds **5b, 8c, 11** and **13e** exerted the highest cytotoxic effects that are comparable to the standard 5-FU while compounds **13f** and **17** exhibited the lowest cytotoxic effects.

II- *In vitro* anticancer screening

All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against four human cancer cell lines (MCF-7, HepG-2, CaCo-2 and A549) using neutral red uptake assay. The results revealed that majority of the tested compounds showed potential *in vitro* anticancer activity against the four cancer cell lines with IC_{50} values less than 100 µg/ml. Compound **17** showed good safety profile and displayed the same growth inhibitory activity as 5-FU against MCF-7 cell line. Compound **13f** which was the most safe and displayed broad spectrum of anticancer activity while compound **8a** was the most active against HepG2 cell line (IC_{50} 7.71 µg/ml). Compound **13e** was the most active against CaCo-2 cell line (IC_{50} 7.71 µg/ml). In addition, compound **10b** was the most active against A549 (IC_{50} 14.75 µg/ml). On the other hand, compounds **6a-c, 7b,c, 8a, 10b** and **13b,d,f** exhibited potent and broad spectrum of activity.

III- *In vitro* antioxidant screening

All the newly synthesized compounds were screened for their *in vitro* antioxidant activity using DPPH free radical scavenging assay. The results showed that in general, all tested compounds with antioxidant activity contained NH or SH function which might explain their activity by hydrogen atom donating ability. Compounds **12b**, **13a**, **13c**, **13d** and **18** showed antioxidant activity higher than that of standard, trolox. Compounds **4**, **5a, 5b**, **12a** and **13b** showed moderate activity while compounds **9**, **13e** and **13f** showed weak antioxidant activity.

IV- *In vitro* antimicrobial screening

All the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The results showed that majority of the tested compounds

lacked any significant antibacterial or antifungal activity against the tested pathological strains. Compounds **8c**, **9**, **10b** exhibited potential antibacterial activity against *Staphylococcus aureus*.

Chapter VI: Molecular modeling

This chapter includes:

I- *In silico* physico-chemical properties and toxicity prediction:

It involves prediction of physical and molecular properties of all the newly synthesized compounds in order to predict its overall drug likeness. It also involves *in silico* toxicity prediction which predicts the potential toxicity risks of the synthesized compounds for mutagenicity, tumorigenicity and/or reproductive side effects.

II- Anticancer docking study:

It explains the docking procedure of compounds **6a-c**, **7b,c**, **8a**, **10b** and **13b,d,f** within the binding site of human thymidylate synthase enzyme using Molecular Operating Environment (MOE Dock 2008) software

References

In this part, 186 cited references, dealing with the planning and elaboration of the different aspects for the present investigation, have been listed according to their appearance throughout the text.

Arabic summary

It includes a brief Arabic summary.

الملخص العربى

يزخر التراث العلمى بالعديد من الحقائق العلمية التى تبين أن مركبات الثيوبوريا و مشتقاتها لها تطبيقات عديدة فى مجال الكيمياء الصيدلية و ذلك لأن مجموعة الثيوريا توجد فى العديد من المركبات ذات فعالية بيولوجية كمضادات للسرطان، والفيروسات، والميكروبات، والالتهابات. كما تم اثبات فعاليتها كمضادات للأكسده و كمؤثرات عقلية لها القدرة على تثبيط نشاط الجهاز العصبى المركزى .

و لقد عرفت المركبات التى تحتوى على مشتقات حلقة البيريميدين بفاعليتها الكبيرة كمضادات للسرطان بناء على ما ذكر فى العديد من المراجع العلمية وذلك لما لها من قدرة على تثبيط العديد من الانزيمات المهمة لتصنيع الحمض النووى داخل الخلايا. كما تم اثبات فعالية مشتقات حلقة البيريميدين كمضادات للفيروسات والميكروبات.

كما أن لمشتقات حلقة البيريميدين أهميه بالغه فى تحضير مشتقات البيريميدين المدمجه و خاصة مشتقات حلقة البيريميدين المدمجة مع حلقة التريازول والتى تم اثبات فعاليتها كمضادات للسرطان و الميكروبات فى العديد من الأبحاث العلمية.

وفى ضوء ما تقدم وفى سبيل البحث عن مركبات جديدة ملائمه للتطوير الى أدوية أكثر فعالية وأقل سمية، فقد استهدف هذا البحث تصميم، وتشييد مشتقات مستحدثة من الثيوبوريا بهدف دراسة التأثير البيولوجى لتلك المركبات كمضادات للسرطان والبكتيريا والفطريات وايضا كمضادات للأكسده. و تتكون هذه المركبات من مجموعة الثيوبوريا الموجوده ضمن حلقة البيريميدين أو حلقة البيريميدين المدمجة مع حلقة التريازول.

و تنقسم الرساله لهذه الابواب الرئيسيه التاليه:

الباب الأول: المقدمه

يتضمن عرضا موجزا لما تواتر فى التراث العلمى عن مشتقات الثيوبوريا الموجودة كسلسلة مفتوحة أو الموجودة ضمن الحلقات الغير متجانسة والتى ثبتت فعاليتها ضد الأورام الخبيثه والفيروسات والميكروبات و الإلتهابات و كمضادات للأكسدة.

الباب الثانى: أهداف البحث

يناقش هذا الجزء الأسس العلمية التى بنى على أساسها برنامج البحث و قد استهدف تصميم و تشييد مشتقات الثيوبوريا الموجودة ضمن حلقة البيريميدين أو حلقة التريازولوبيريميدين و ذلك بغرض الحصول على مركبات مستحدثة ذات فعاليه أكثر تأثيرا و أقل سمية فى مجال مضادات الأورام الخبيثه و البكتيريا و الفطريات وكذلك مضادات الأكسدة. و المركبات المستهدفه صممت من مشتقات البيريميدين والتريازولوبيريميدين التى تحتوى على مجموعة الثيوبوريا مدمجة فى النظام الحلقى للمركبات. كذلك بعض المركبات متصلة بوحدة من الثيوبوريا بالإضافة إلى الأخرى المدمجة فى النظام الحلقى. ولقد أختيرت المجموعات المستبدلة للوصول إلى دراسة العلاقة بين التركيب البنائى والتركيب البيولوجى المستهدف.

الباب الثالث: المناقشه

يتضمن هذا الباب شرحا وافيا للطرق المعملية المختلفة المتبعة فى المراجع العلمية لتشييد المركبات الأولية، الوسيطة والنهائية التى استهدفها البحث. كما يعرض إثبات التركيب البنائى للمركبات المشيدة باستخدام التحليل الدقي لعناصرها و دراسة أطياف الأشعة تحت الحمراء و الرنين النووى المغناطيسى لذرة الهيدروجين. و كذلك دراسة طيف الكتلة والرنين النووى المغناطيسى لذرة الكربون لبعض المركبات المختارة.

و يحتوى هذا الباب على أربعة مخططات تبين سير التفاعلات للوصول الى المركبات المستهدفة.

المخطط الاول:

يتضمن هذا المخطط مناقشة تحضير ٢-بنزيل سلفانيل-٦-ميثيل بيريميدين-4-ون (٢) وتفاعله مع أوكسى كلوريد الفوسفورثم هيدرازين هيدرات من اجل تحضير ٢-بنزيل سلفانيل-٤-هيدرازنيل-٦-ميثيل بيريميدين (٤). كما يشمل أيضا طريقة تحضير 'N-[(٢-بنزيل سلفانيل)-٦-ميثيل بيريميدين-٤-يل]بنزين سلفونواميدات(**٥أ،ب**).

المخطط الثانى:

يتضمن هذا المخطط مناقشة تحضير ٢-(بنزيل سلفانيل)-٤-[٢-(٤-مستبدل البنزيليدين(هيدرازينيل)]-٦-ميثيل البريميدات (**٦أ،ب**) ثم حولقتها باستخدام البرومين أو أندريد الخليك. كما يصف هذا المخطط تحضير ٥-(بنزيل سلفانيل)-٧-ميثيل-[١،٢،٤]ترايازولوبيريميدين-٣-ثيول (٩) ثم ألكلة الكبريت فى هذا المركب بإستخدام يوديد الميثيل أو كلوريد البنزيل أو بروميد الفينيثل.

المخطط الثالث:

يوضح هذا المخطط الطريقة المستخدمة لتحضير ٢-[٢-(بنزيل سلفانيل)-٦-ميثيل بيريميدين-٤-يل]-N-أليل أو فينيل هيدرازين-١-كاربوثيوأميد (**١٢أ،ب**) ثم حولقة هذه المركبات باستخدام مشتقات بروميد الفينيثيل او أوكسيد الزئبق الأصفر.

المخطط الرابع:

يصف هذا المخطط الطريقة المستخدمة لتحضير ٦-ميثيل-٢-[(٢-اوكسو-٢-فينيل ايثيل)سلفانيل]بيريميدين-٤-ون (١٥) و تفاعله مع أوكسى كلوريد الفوسفورثم الهيدروكسيل أمين هيدروكلوريد ثم هيدرازين هيدرات من أجل تحضير ٢-[(٤-هيدرازينيل-٦-ميثيل بيريميدين-٢-يل)سلفانيل]-١-فينيل إيثانون أوكسيم (١٨).

كما يبين المخطط طريقة تحضير مركب ٢-{٢-[(٤-ميثيل-٦-أوكسو-١،٦-داى هيدروبيريميدين-٢-يل)سلفانيل]-١-فينيل ايثيليدين}-N-فينيل هيدرازين-١-كاربوثيوأميد (١٩).

الباب الرابع: التجارب المعمليه

يتضمن هذا الباب وصفا تفصيليا للتجارب المعملية التى أتبعت لتحضير كافة المركبات الوسيطة والنهائية التى استهدفها البحث و رصدا علميا للخواص الطبيعية قرين كل مركب. هذا و قد تم إثبات التركيب البنائى للمركبات الجديدة باستخدام التحليل الدقي لعناصرها و بدراسة أطياف الأشعه تحت الحمراء و الرنين النووى المغناطيسى لذرة الهيدروجين. و كذلك دراسة طيف الكتله والرنين النووى المغناطيسى لذرة الكربون لبعض المركبات المختارة.

الباب الخامس: التقييم البيولوجى

و يحتوى هذا الباب على ما يلى:

١. **فحص السمية الخلوية**:

يشتمل هذا القسم على وصف للطريقة المستخدمة فى تقييم السمية الخلوية الخاصة بالمركبات المصنعة حديثا على خلايا الدم أحادية النواة. و قد أظهرت النتائج أن المركبات **٥ب،٨ج،١١،١٣ه** لها أعلى تأثير سمى والذى يعادل تأثير المركب المعيارى ٥-فلوروبوراسيل بينما المركبان **١٣و،١٧** فكان لهما أقل تأثير سمى على الخلايا.

٢. **إختبار الفعاليه ضد الاورام السرطانيه**

يشتمل هذا القسم على وصف للطريقة المستخدمة فى تقييم فعالية المركبات المصنعة حديثا ضد اربعة أنواع من الخلايا السرطانيه وهى سرطان الثدى، الكبد، القولون و الرئه. و قد أظهرت النتائج أن معظم المركبات لها تأثير كمضادات للسرطان. مركب ١٧ له تأثير آمن على خلايا الدم أحادية النواة بينما له تأثير مثبط لنمو خلايا سرطان الثدى. مركب **١٣أ** له تأثير مثبط لنمو الخلايا السرطانيه الأربعه وكذلك هو آمن على خلايا الدم أحادية النواة. المركب ٨أ كان المركب صاحب أعلى تأثير مثبط لنمو خلايا سرطان الكبد بينما **١٠ب** فكان المركب صاحب أعلى

تأثير مثبط لنمو خلايا سرطان الرئه. جدير بالذكر أن المركبات **٦أ-ج، 7ب،ج، ٨أ، ١٠ب، ١٣ب،د،و** لها أعلى فاعليه ضد الأربعة أنواع من الخلايا السرطانية المستخدمة فى التجربة.

٣. إختبار الفعاليه كمضادات للأكسده

يشتمل هذا القسم على وصف للطريقة المستخدمة فى تقييم فعالية المركبات المصنعة حديثا كمضادات للأكسدة. و قد أظهرت النتائج أن المركبات **١٢ب، ١٣أ،ج،د، ١٨** لها تأثير اقوى من الترولوكس كما اظهرت النتائج أن المركبات **٤، ٥أ،ب، ١٢أ، ١٣ب** لها تاثير متوسط بينما المركبات **٩، ١٣هـ،و** فكان لها تأثير ضعيف مقارنة بالترولوكس.

٤. إختبار الفعاليه ضد البكتيريا و الفطريات

يشتمل هذا القسم على وصف للطرق المستخدمة فى تقييم فعالية المركبات المصنعة حديثا ضد بعض انواع البكتيريا الموجبة والسالبة الجرام و الفطريات. و قد أظهرت النتائج أن غالبية المركبات لم تظهر اية فاعلية ضد أنواع البكتيريا والفطريات المستخدمة فى التجربة ما عدا المركبات **٨ج، ٩، ١٠ب** التى كان لها تأثير متوسط على البكتيريا الكروية العنقودية الذهبية (ستافيلوكوكس أيريس).

الباب السادس: النمذجه الجزيئيه

يدرس هذا الباب حساب بعض المعايير الكيموفيزيائية المنتقاة للمركبات وذلك للتنبؤ بامكانية تطويرها كأدوية وكذلك توقع السمية طويلة المدى لهذه المركبات. كما يدرس هذا الباب مدى قدرة بعض المركبات (**٦أ،ج،٧ب،ج،٨أ،١٠ب،١٣ب،د،و**) على الاتحاد الجزيئى مع النموذج المقترح لانزيم (TS) فى الجيب الخاص للتعرف عليها كموانع للانزيم باستخدام برنامج الحاسوب لتصميم الأدوية (MOE).

المراجع العلميه

يشتمل هذا الباب على سرد لجميع المراجع و الدوريات التى تم الاستعانة بها أثناء التخطيط لهذا البحث و كذلك أثناء إجراء التجارب المعملية و قد بلغ عددها ١٨٦ و هى مرتبه ترتيبا علميا طبقا لورودها فى فحوى الرسالة.

الملخص باللغة الانجليزية

يتضمن ملخصا للرساله باللغة الانجليزية.

الملخص باللغه العربية

يتضمن ملخصا موجزا للرساله باللغة العربية.

تشييد وتقييم بيولوجى لبعض مركبات جديدة غير متجانسة الحلقة تحتوي على وحدة الثيويوريا

رسالة

مقدمة إلى

قسم الكيمياء الصيدلية

كلية الصيدلة - جامعة الإسكندرية

ضمن متطلبات درجة

الماجستير

فى

العلوم الصيدلية

(كيمياء صيدلية)

من

أشرف محمد عمر الصغير

بكالوريوس فى العلوم الصيدلية- ٢٠١٠

كلية الصيدلة

جامعة الإسكندرية

ج.م.ع

٢٠١٥

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٢٠١٥

تشييد وتقييم بيولوجى لبعض مركبات جديدة غير متجانسة الحلقة تحتوي على وحدة الثيوبوريا

رسالة
مقدمة إلى
قسم الكيمياء الصيدلية
كلية الصيدلة - جامعة الإسكندرية

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(كيمياء صيدلية)

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