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SYNTHESIS, ANTITUMOR ACTIVITY AND MOLECULAR DOCKING STUDY OF NOVEL BENZOFURAN-2-YL PYRAZOLE PYRIMIDINE DERIVATIVES

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Abstract: A new series of (benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl) pyrimidine derivatives were synthesized from 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-carbaldehyde (**1**) through different routes of cyclocondensation reactions. Condensation of **1** with active methylene compounds afforded compounds **2-8**. The cyclization of **2** with chloroacetic acid, *ortho* substituted benzoic acid and/or ethanolamine gave compounds **9-12**. Also condensation of **2** with hydrazine hydrate followed by cyclocondensation afforded corresponding triazines and pyrazole derivatives **18-27**. Some docking studies of the newly prepared compounds as thymidylate synthase inhibitors have been done. Also the cytotoxic activity of some of the prepared compounds as a representative examples was evaluated against HEPG2 (human liver carcinoma cell line) in comparison with 5-fluorouracil (5-Fu).

Keywords: pyrimidin-2-thione, benzofuranoyl-pyrazoles, thiazolidinone, triazole, thymidylate synthase inhibitor, Autodock Vina, antitumor activity

The synthesis of dihydropyrimidine derivatives have been attracting extensive attention as a wide range of such compounds played an important role in the field of medicinal chemistry as antiviral (1), antibacterial (2), antimalarial (3), antihypertensive (4) and anti-inflammatory (5) agents. In addition, pyrimidine derivatives form the basis of a large number of pharmacological products with anticancer and protein kinase inhibitory activity (6).One of the most famous pyrimidine derivatives is 5-florouracil (5-Fu), considered as one of the most important drugs for the treatment of colorectal, head and neck, pancreatic and breast carcinomas (7). Furthermore, recent studies showed that benzofuran ring system fused with heterocyclic moieties exhibit a wide spectra of pharmacological activities and especially antitumor activity (8–10). Moreover, pyrazole derivatives have been reported to act as antitumor agents against various types of carcinomas such as lung, breast cancer and leukemia (11, 12).

Folate metabolism is considered as an important target for the development of new anticancer agents due to its role in the biosynthesis of nucleic acid precursors (13, 14). The inhibition of folate-dependent enzymes such as thymidylate synthase, which catalyzes the reductive methylation of deoxyuridylate (dUMP) to thymidylate (dTMP) has also been recognized as an interesting target for drug discovery (15, 16). Classical antitumor agents that prevent this pathway have a disadvantage that they need an active transport mechanism to enter the cells, which can cause tumor resistance if impaired (17, 18). Also, recent preclinical experiments on human liver carcinoma cell lines (HEPG2) revealed that at higher doses of antifolate a loss of thymidylate synthase inhibition occurs and cytotoxic effects are preserved (19).

Based on all these findings, this work aims to design a new series of compounds structurally containing benzofuranyl-pyrazole ring system conjugated with pyrimidine derivatives as a trial to develop a new thymidylate synthase inhibitor that could be selective and may be a good target for drug discovery. For this target, some docking of the newly synthesized compounds was done using Autodock Vina (20). Moreover the cytotoxic activities of new five selected compounds were screened against HEPG2 (human liver carcinoma cell line).

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EXPERIMENTAL

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Microanalytical Laboratory, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, on Vario Elementar analyzer and were found within ± 0.5% of the theoretical values. Infrared spectra were recorded on a FT/IR-6100, Fourier transform, infrared spectrometer (Japan) using KBr disc technique. ^{1}H-NMR spectra were determined using a JEOl EX-270 NMR spectrometer and measured in δ scale using TMS as an internal standard. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected using UV lamp at λ_{254} nm. The chemical names given for the prepared compounds are according to the IUPAC system.

3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**)

This compound was prepared according to reported method in our previously published paper (21).

6-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2,3,4-tetrahydro-4-oxo-2-thioxopyrimidine-5-carbonitrile (**2**)

A mixture of compound **1** (1.44 g, 0.005 mole), ethyl cyanoacetate (0.57 mL, 0.005 mole) and thiourea (0.38 g, 0.005 mole) in sodium ethoxide (20 mL) was stirred for one hour at room temperature. The reaction mixture was poured into ice cold water and acidified by hydrochloric acid; the solid separated was filtered and recrystallized to give compound **2**.

1-(4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidin-5-yl)ethanone (**3**) and ethyl 4-(3-benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrmidine-5-carboxylate (**4**)

General procedure: A mixture of compound **1** (0.58 g, 0.002 mole), thiourea (0.16 g, 0.002 mole), acetylacetone and/or ethyl acetoacetate (0.002 mole) in absolute ethanol (15 mL) containing few drops of concentrated hydrochloric acid was refluxed for 4 h. The formed precipitate was filtered, washed with water several time, dried and recrystallized to give compounds **3** and **4**, respectively.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,4,5,6,7,8- hexahydroquinazoline-2(1*H*)-thione (**5**) and 4-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,4-dihydro-1*H*-indeno[1,2-d]pyrimidine-(5*H*)-thione (**6**)

General procedure: A mixture of compound **1** (0.58 g, 0.002 mole), thiourea (0.16 g, 0.002 mole), cyclohexanone and/or 2-indanone (0.0025 mole) in absolute ethanol (15 mL) containing few drops of concentrated hydrochloric acid was refluxed for 3 h. After cooling the formed precipitate was filtered, washed several times with water, dried and recrystallized to give compounds **5** and **6**, respectively.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2-dihydro-6-(1,2,3,4-tetrahydronaphthalen-7-yl)-2-oxopyridine-3-carbonitrile (**7**)

A mixture of compound **1** (0.58 g, 0.002 mole), ethyl cyanoacetate (0.23 mL, 0.002 mole), anhydrous ammonium acetate (1.24 g, 0.016 mole) and 6-acetyl-1,2,3,4-tetrahydronaphthalene (0.5 mL, 0.002 mole) in n-butanol (10 mL) was heated under reflux for 3 h. The formed precipitate was filtered, dried and recrystallized to give compound **7**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2-dihydro-6-(1,2,3,4-tetrahydronaphthalen-7-yl)-2-iminopyridine-3-carbonitrile (**8**)

A mixture of compound **1** (0.58 g, 0.002 mole), malononitrile (0.14 g, 0.002 mole), anhydrous ammonium acetate (1.24 g, 0.016 mole) and 6-acetyl-1,2,3,4-tetrahydronaphthalene (0.5 mL, 0.002 mole) in absolute ethanol (10 mL) was refluxed for 3 h. The formed precipitate on cooling was filtered, dried and recrystallized to give compound **8**.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,5-dihydro-3,5- dioxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (**9**)

A mixture of compound **2** (0.42 g, 0.001 mole), chloroacetic acid (0.1 g, 0.001 mole) and anhydrous sodium acetate (0.33 g, 0.004 mole) in glacial acetic acid (10 mL) and acetic anhydride (2 mL) was refluxed for 3 h. The reaction mixture was cooled, poured into ice cold water, the formed precipitate was filtered and recyrstallized to give compound **9**.

2-(4-Methoxybenzylidene)-7-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,5-dihydro-3,5-dioxo-2*H*-thiazolo[3,2-a] pyrimidine-6-carbonitrile (**10a**),

7-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,5-dihydro-3,5-dioxo-2-((thiophen-2-yl) methylene)-2*H*-thiazolo[3,2 a]pyrimidine-6-carbonitrile (**10b**) and 2-((1*H*-indol-3-yl) methylene)-7-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-3,5-dihydro-3,5-dioxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (**10c**)

General procedure: A mixture of compound **9** (0.46 g, 0.001 mole) and an appropriate aldehyde namely; p-anisaldehyde, 2-thiophenecarboxaldehyde or indole-3-carboxaldehyde (0.001 mole) in acetic acid (10 mL) was refluxed for 8 h. The reaction mixture was cooled, poured into ice cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized to give compounds **10a-c,** respectively.

2-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-6,11-dihydro -4,6 -dioxo-4*H*-pyrimido[2,1-b]quinazoline-3-carbonitrile (**11a**), 2-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-6-hydro-4,6-dioxo-4*H* -pyrimido[2,1-b]benzo[1,3]oxazine-3-carbonitrile (**11b**) and 2-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-6-hydro-4,6-dioxo-4*H* -pyrimido[2,1-b]benzo[1,3]thiazine-3-carbonitrile (**11c**)

General procedure: A mixture of compound **2** (0.42 g, 0.001 mole) and *ortho* substituted benzoic acid namely: anthranilic acid, salicylic acid or thiosalicylic acid (0.001 mole) in 2% sodium ethoxide (20 mL) was refluxed for 10 h. Then cooled, poured into ice cold water and acidified by diluted hydrochloric acid. The formed precipitate was filtered, washed several times with water, dried and recrystallized from to give compounds **11a-c**, respectively.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)1,2,3,5-tetrahydro-5-oxoimidazo[1,2-a]pyrimidine-6-carbonitrile (**12**)

A mixture of compound **2** (0.42 g, 0.001 mole) and ethanolamine (0.07 mL, 0.001 mole) in isopropyl alcohol (10 mL) was refluxed for 6 h. Then poured into ice cold water and acidified by diluted hydrochloric acid. The formed precipitate was filtered and recrystallized to give compound **12**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-2- (methylthio)-6-oxopyrimidine-5-carbonitrile (**13**)

A mixture of compound **2** (2.0 g, 0.005 mole) and iodomethane (0.72 mL, 0.005 mole) in 2% sodium ethoxide (20 mL) was refluxed for 3 h. The reaction mixture was cooled, poured into ice cold water and acidified by diluted hydrochloric acid. The formed precipitate was filtered and recrystallized to give compound **13**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-2-hydrazinyl-1,6-dihydro-6-oxopyrimidine-5-carbonitrile (**14**)

Method A: A mixture of compound **13** (1.28 g, 0.003 mole) and hydrazine hydrate 98% (0.15 mL, 0.003 mole) was refluxed for 3 h. The formed precipitate was filtered and washed with ethanol, dried and recrystallized to give compound **14**.

Method B: A mixture of compound **2** (2.0 g, 0.005 mol) and hydrazine hydrate 98% (0.3 mL, 0.005 mole) was refluxed for 2 h. The formed precipitate was filtered and washed with ethanol, dried and recrystallized to give compound **14**.

6-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-4-chloro-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile (**15**)

A mixture of compound **2** (2.1 g, 0.005 mole) and phosphorus pentachloride (1.04 g, 0.025 mole) in phosphorus oxychloride (15 mL) was heated on water bath for 6 h, then cooled and poured into ice cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized to give compound **15**.

6-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-4-hydrazinyl-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile (16)

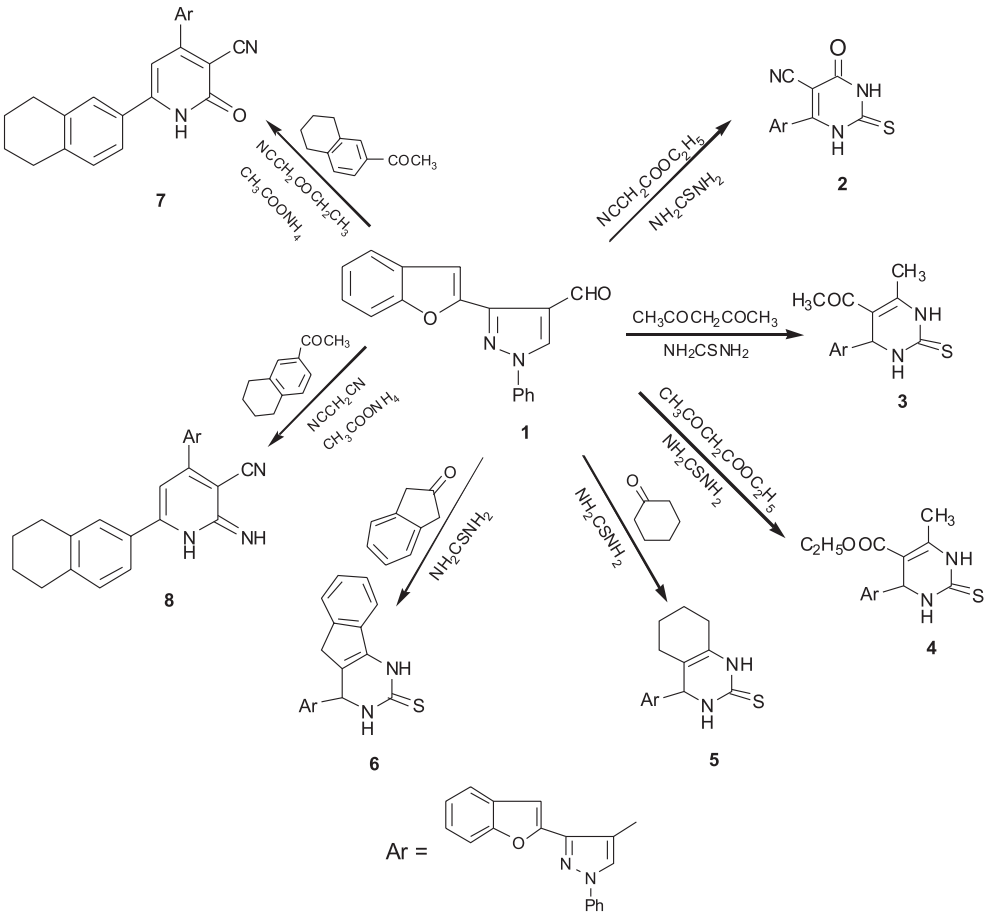
Hydrazine hydrate (99%) (0.1 mL, 0.002 mole) was added to compound **15** (0.86 g, 0.002 mole) in ethanol (15 mL) and stirred at room temperature. After complete addition, stirring was continuous overnight. The formed precipitate was filtered and recrystallized to give compound **16**.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-5,6-dihydro-5-thioxotetrazolo[1,5-f]pyrimidine-8-carbonitrile (**17**)

A mixture of compound **15** (0.86 g, 0.002 mole) and sodium azide (0.14 g, 0.002 mole) in acetic acid (15 mL) was refluxed for 6 h. The reaction mixture was cooled and poured into ice cold water. The formed precipitate was filtered, dried and recrystallized to give compound **17**.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,2,3,5-tetrahydro-3,5-dioxo[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (**18**)

A mixture of compound **14** (0.41 g, 0.001 mole) and ethyl chloroformate (0.10 mL, 0.001 mole) in pyridine (10 mL) was refluxed for 6 h.



Scheme 1.

Then it was cooled and poured into ice cold water containing few drops of hydrochloric acid. The formed precipitate was filtered, washed several times with water, dried and recrystallized to give compound **18**.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,5-dihydro-5-oxo [1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (**19**)

A mixture of compound **14** (0.41 g, 0.001 mole) and triethylorthoformate (1 mL, 0.001 mole) in absolute ethanol (10 mL) was refluxed for 8 h. The reaction mixture was concentrated; the formed precipitate was filtered, dried and recrystallized to give compound **19**.

7-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,5-dihydro-3-methyl-5-oxo[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (**20**)

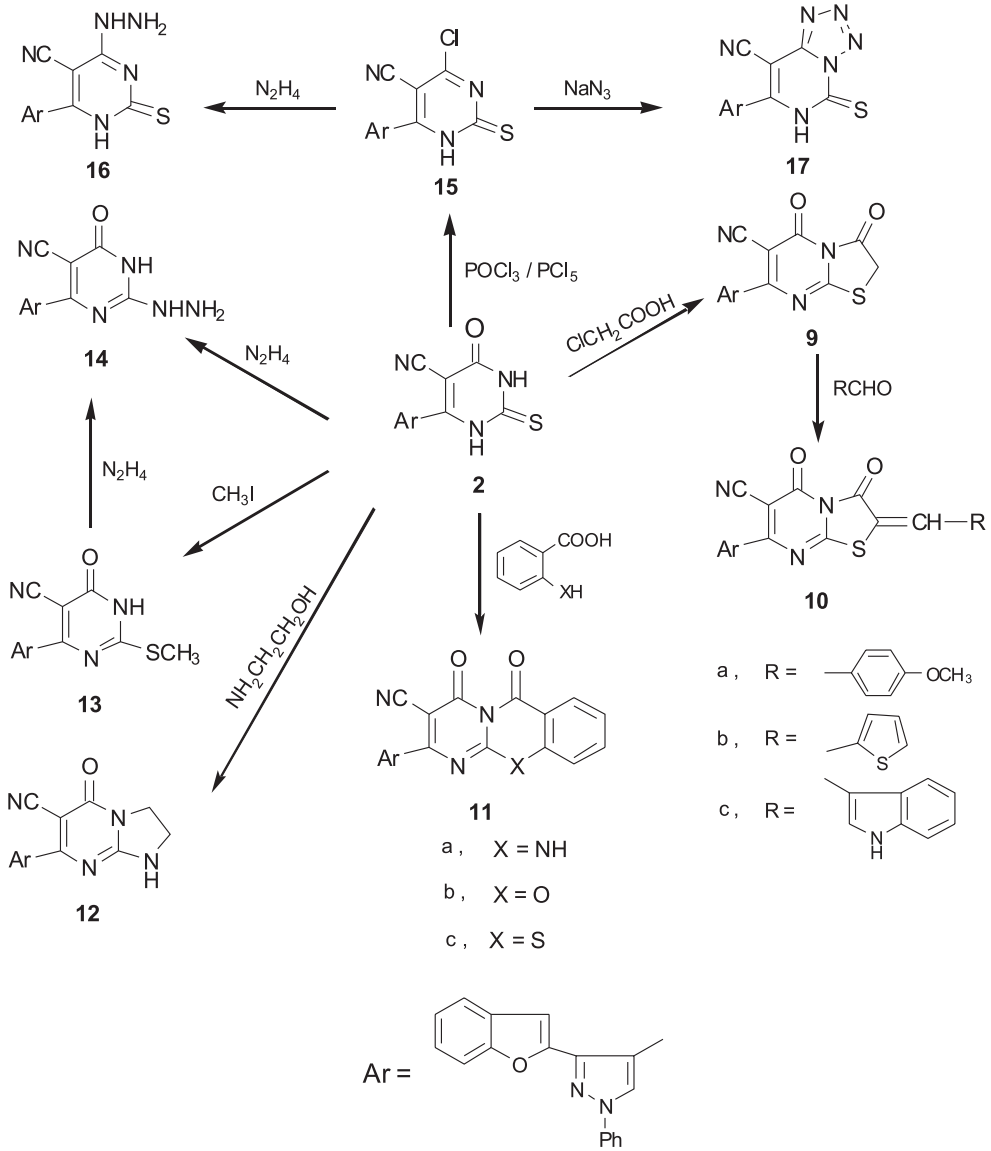
A mixture of compound **14** (0.41 g, 0.001 mole) and acetic anhydride (0.1 mL, 0.001 mole) in acetic acid (10 mL) was refluxed for 6 h. The reaction mixture was cooled and poured into ice cold water. The formed precipitate was filtered, dried and recrystallized to give compound **20**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-6-oxo-2-(3,5-dioxopyrazolidin-1-yl)pyrimidine-5-carbonitrile (**21**)

A mixture of compound **14** (0.41 g, 0.001 mole) and diethyl malonate (0.16 mL, 0.001 mole) in acetic acid (10 mL) was refluxed for 8 h. Then it was cooled and poured into ice cold water. The formed precipitate was filtered, dried and recrystallized to give the compound **21**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-2-(4,5-dihydro-3-methyl-5-oxopyrazol-1-yl)-6-oxopyrimidine-5-carbonitrile (**22**)

A mixture of compound **14** (0.41 g, 0.001 mole) and ethyl acetoacetate (0.13 mL, 0.001 mole) in acetic acid (10 mL) was refluxed for 6 h. The



Scheme 2.

reaction mixture was cooled and poured into ice cold water. The formed precipitate was filtered, dried and recrystallized to give compound **22**.

4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-oxopyrimidine-5-carbonitrile (**23**)

A mixture of compound **14** (0.41 g, 0.00 mole) and acetylacetone (0.1 mL, 0.001 mole) in acetic acid (15 mL) was refluxed for 6 h. The reaction mixture was cooled and poured into ice cold water. The formed precipitate was filtered, dried and recrystallized to give compound **23**.

8-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3,4,6-tetrahydro-3,4,6-trioxo-1*H*-primido[2,1-c][1,2,4]triazine-7-carbonitrile (**24**)

A mixture of compound **14** (0.41 g, 0.001 mole) and diethyl oxalate (0.15 mL, 0.001 mole) in absolute ethanol (15 mL) was refluxed for 8 h. The precipitate formed on cooling was filtered, dried and recrystallized to give compound **24**.

Table 1. Physical and analytical data of all new compounds **2-26b**

Comp. no.	M.p. (°C) (Cryst. solvent)	Yield %	Mol. formula (Mol. Wt.)	Analysis (%) Calcd./Found			
				C	H	N	S
2	124–125 (Benzene)	83	$C_{22}H_{13}N_5O_2S$ (411.44)	64.22 64.53	3.18 3.41	17.02 16.99	7.79 7.99
3	235–236 (Ethanol)	53	$C_{24}H_{20}N_4O_2S$ (428.51)	66.27 66.43	4.70 4.48	13.07 13.39	7.48 7.24
4	191–192 (Ethanol)	51	$C_{25}H_{22}N_4O_3S$ (458.53)	65.48 65.72	4.84 4.67	12.22 12.53	6.99 7.31
5	219–220 (Ethanol)	52	$C_{25}H_{22}N_4OS$ (426.53)	70.40 70.69	5.20 5.37	13.14 13.28	7.52 7.81
6	258–259 (Ethanol)	89	$C_{28}H_{20}N_4OS$ (460.55)	73.02 73.33	4.38 4.61	12.17 12.28	6.96 7.23
7	> 300 (Acetic acid)	80	$C_{33}H_{24}N_4O_2$ (508.57)	77.93 77.58	4.76 4.42	11.02 11.32	
8	224–225 (Ethanol)	65	$C_{33}H_{25}N_5O$ (507.58)	78.09 77.88	4.96 4.72	13.80 13.51	
9	132–133 (Benzene)	71	$C_{24}H_{13}N_5O_3S$ (451.46)	63.85 63.72	2.90 3.11	15.51 15.31	7.10 7.23
10a	281-283 (Ethanol)	59	$C_{32}H_{19}N_5O_4S$ (569.59)	67.48 67.32	3.36 3.41	12.30 12.51	5.63 5.72
10b	160–161 (Isopropanol)	92	$C_{29}H_{15}N_5O_3S_2$ (545.59)	63.84 63.62	2.77 2.54	12.84 12.59	11.75 11.48
10c	112–113 (Isopropanol)	71	$C_{33}H_{18}N_6O_3S$ (578.60)	68.50 68.83	3.14 3.23	14.52 14.34	5.54 5.31
11a	115–116 (Ethanol)	80	$C_{29}H_{16}N_6O_3$ (496.48)	70.16 70.32	3.25 3.52	16.93 16.67	
11b	121–122 (Ethanol)	83	$C_{29}H_{15}N_5O_4$ (497.46)	70.02 70.32	3.04 3.21	14.08 14.36	
11c	142–143 (Ethanol)	94	$C_{29}H_{15}N_5O_3S$ (513.53)	67.83 67.44	2.94 2.61	13.64 13.82	6.24 5.99
12	128–129 (Benzene)	61	$C_{24}H_{16}N_6O_2$ (420.42)	68.56 68.67	3.84 3.62	19.99 19.73	
13	200–201 (Ethanol)	97	$C_{23}H_{15}N_5O_2S$ (425.46)	64.93 64.71	3.55 3.91	16.64 16.22	7.54 7.31
14	Method A 256–258 Method B 258–260 (Acetic acid)	31 75	$C_{22}H_{15}N_7O_2$ (409.40)	64.54 64.72	3.69 3.55	3.95 23.71	
15	223–225 (Ethanol)	91	$C_{22}H_{12}ClN_5OS$ (429.88)	61.47 61.23	2.81 2.96	16.29 16.53	7.46 7.31
16	280–282 (Ethanol)	44	$C_{22}H_{15}N_7OS$ (425.11)	62.10 62.41	3.55 3.99	23.04 23.32	7.54 7.78
17	199–200 (Ethanol)	78	$C_{22}H_{12}N_8OS$ (436.45)	60.54 60.84	2.77 2.95	25.67 25.32	7.35 7.53
18	186–187 (Ethanol)	81	$C_{23}H_{13}N_7O_3$ (435.39)	63.45 63.72	3.01 3.22	22.52 22.24	
19	226–228 (Isopropanol)	69	$C_{23}H_{13}N_7O_2$ (419.40)	65.87 65.61	3.12 3.45	23.38 23.58	
20	159–160 (Ethanol)	95	$C_{24}H_{15}N_7O_2$ (433.42)	66.51 66.83	3.49 3.56	22.62 22.87	
21	182-184 (Ethanol)	76	$C_{25}H_{15}N_7O_4$ (477.43)	62.89 62.52	3.17 3.33	20.54 20.41	

Table 1. cont.

Comp. no.	M.p. (°C) (Cryst. solvent)	Yield %	Mol. formula (Mol. Wt.)	Analysis (%) Calcd./Found			
				C	H	N	S
22	218-219 (Ethanol)	67	$C_{26}H_{17}N_7O_3$ (475.46)	65.68 65.87	3.60 3.24	20.62 20.17	
23	200-202 (Ethanol)	78	$C_{27}H_{19}N_7O_2$ (473.49)	68.49 68.79	4.04 3.68	20.71 20.96	
24	215-216 (Ethanol)	52	$C_{24}H_{13}N_7O_4$ (463.40)	62.20 62.54	2.83 2.51	21.16 21.48	
25a	199-200 (Ethanol)	54	$C_{25}H_{20}N_8O_2S$ (496.54)	60.47 60.52	4.06 4.28	22.57 22.81	6.46 6.81
25b	225-226 (Ethanol)	88	$C_{29}H_{26}N_8O_2S$ (550.63)	63.26 63.57	4.76 4.33	20.35 20.21	5.82 5.61
25c	203-205 (Ethanol)	80	$C_{29}H_{20}N_8O_2S$ (544.59)	63.96 63.59	3.70 4.11	20.58 20.86	5.89 6.21
25d	199-200 (Ethanol)	76	$C_{30}H_{22}N_8O_2S$ (558.61)	64.50 64.79	3.97 3.61	20.06 20.32	5.74 5.96
26a	201-202 (Acetic acid)	51	$C_{26}H_{17}N_7O_4$ (491.46)	63.54 63.91	3.49 3.11	19.95 20.18	
26b	223-225 (Acetic acid)	48	$C_{29}H_{16}N_8O_4$ (540.49)	64.44 64.21	2.98 2.62	20.73 20.38	

1-(4-(3-(Benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-5-cyano-1,6-oxopyrimidin-2-yl)-4-substituted thiosemicarbazides (**25a**–**d**)

General procedure: A mixture of compound **14** (0.41 g, 0.001 mole) and an appropriate substituted thiosemicarbazide namely: ethylthiosemicarbazide, cyclohexylthiosemicarbazide**,** phenylthiosemicarbazide or o-tolylthiosemicarbazide (0.001 mole) in dry benzene (20 mL) was refluxed for 6 h. After cooling, the formed precipitate was filtered and recrystallized to give compounds **25a-d**, respectively.

2-(2,5-Dioxopyrrolidin-1-ylamino)-4-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-6-oxopyrimidine-5-carbonitrile (**26a**) and 2-(5,7-dioxo-5*H*-pyrrolo[3,4-b]pyridine-6(7*H*)-ylamino)-4-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1,6-dihydro-6-oxopyrimidine-5-carbonitrile (**26b**)

General procedure: A mixture of compound **14** (0.41 g, 0.001 mole), and an appropriate acid anhydride namely: succinic anhydride or 2,3-pyridinedicarboxylic anhydride (0.001 mole) in acetic acid (10 mL) was refluxed for 6 h. The formed precipitate was filtered, dried and recrystallized to give compounds **26a-b**, respectively.

Antitumor activity

Materials and Methods

The aim of the present study was to illustrate the effect of some newly synthesized compounds on the human liver carcinoma cell line (HEPG2) in comparison with 5-fluorouracil (5-FU) in a trial to get more effective and less toxic agents. Human tumor cell lines were obtained frozen in liquid nitrogen (–180°C) from the American Type Culture Collection. RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA). The medium was prepared and used for culturing and maintenance of the human tumor cell lines. The prepared medium was kept in a refrigerator (4°C) and checked at regular intervals for contamination. Before the use, the medium was warmed at 37°C in a water bath and supplemented with penicillin/streptomycin and FBS. Experiments were set up using the Sulforhodamine-B (SRB) assay according to Skehan et al. (22). Different concentrations of the compounds tested (0, 5, 12.5, 25 and 50 μg mL^{-1}) were added to the cell monolayer. Each concentration was evaluated three times (each dose was incubated with the cells in three different wells). Monolayer cells were incubated with the compounds for 48 h at 37°C.

Table 2. Spectral data of the newly synthesized compounds

Compd.no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS [m/z (%)]
2	3220, 3127 (NH, NH), 2226 (C≡N), 1729 (C=O), 1148 (C=S)	($CDCl_3$): 7.26–7.98 (m, 10H, Ar-H, benzofuran protons), 8.62 (s, 1H, pyrazole ring), 9.15, 10.25 (s, s, 1H, 1H, NH, NH exchangeable by D_2O	411 [M^+] (21), 378 [M^+–SH] (9), 77 [C_6H_5] (100)
3	3320, 3188 (NH, NH), 1720 (C=O), 1181 (C=S)	(DMSO-d_6): 2.14 (s, 3H, CH_3), 2.33 (s, 3H, CH_3CO), 5.80 (s, 1H, thioxopyrimidine ring), 7.28–7.97 (m, 10H, Ar-H, benzofuran protons), 8.33 (s, 1H, pyrazole ring), 9.24, 10.20 (s, s, 1H, 1H, NH, NH exchangeable by D_2O)	428 [M^+] (2), 386 [M^+–CH_2CO] (2), 62 [C_5H_2] (100)
4	3402, 3282 (NH, NH), 1745 (C=O, ester), 1173 (C=S)	(DMSO-d_6): 0.84 (t, 3H, $\mathbf{CH_3}CH_2$), 2.30 (s, 3H, CH_3), 3.83 (q, 2H, $CH_3\mathbf{CH_2}$), 5.74 (s, 1H, thioxopyrimidine ring), 7.26–7.89 (m, 10H, Ar-H, benzofuran protons), 8.43 (s, 1H, pyrazole ring), 9.40, 10.19 (s, s, 1H, 1H, NH, NH exchangeable by D_2O)	459 [M^++1] (25), 457 [M^+–1] (19), 77 [C_6H_5] (38), 44 [C_3H_8] (100)
5	3399, 3192 (NH, NH), 2929 (CH_2, cyclohexane), 1063 (C=S)	(DMSO-d_6): 1.60, 1.72 (m, m, 4H, 4H, cyclohexane ring), 5.50 (s, 1H, thioxopyrimidine ring), 7.25–7.98 (m, 10H, Ar-H, benzofuran protons), 8.49 (s, 1H, pyrazole ring), 9.40, 10.19 (s, s, 1H, 1H, NH, NH exchangeable by D_2O)	428 [M^++2] (4), 63 [C_5H_3] (100)
6	3397, 3140 (NH, NH), 2989 (CH_2), 1193 (C=S)	(DMSO-d_6): 3.61 (m, 2H, CH_2 indanone ring), 6.16 (s, 1H, thioxopyrimidine ring), 6.89–7.89 (m, 14H, Ar-H, benzofuran protons, indanone ring), 8.70 (s, 1H, pyrazole ring), 9.05, 10.74 (1 s, s, H, 1H, NH, NH exchangeable by D_2O)	460 [M^+] (2), 415 [M^+–HCS] (3), 63 [C_5H_3] (100)
7	3138 (NH), 2930 (CH_2 of tetrahydronaphthalene),	(DMSO-d_6): 1.70, 2.72 (m, m, 4H, 4H, tetrahydronaphthalene), 7.14–7.95 (m, 14H, 2217(C≡N), 1646 (C=O) Ar-H, benzofuran protons, tetrahydronaphthalene ring, pyridine ring), 8.00 (s, 1H, pyrazole ring), 9.08 (s, 1H, NH, exchangeable by D_2O)	508 [M^+] (12), 77 [C_6H_5] (100)
8	3436, 3202 (NH, NH), 2929 (CH_2 of tetrahydronaphthalene), 2217(C≡N), 1599 (C=N)	(DMSO-d_6): 1.79, 2.74 (m, m, 4H, 4H, tetrahydronaphthalene), 7.19–7.95 (m, 14H, Ar-H, benzofuran protons, tetrahydronaphthalene ring, pyridine ring), 8.02 (s, 1H, pyrazole ring), 9.60 (s, 1H, NH, exchangeable by D_2O)	507 [M^+] (15), 77 [C_6H_5] (100)
9	2222 (C≡N), 1757(C=O), 1692 (C=O, thiazole ring)	($CDCl_3$): 3.69 (s, 2H, thiazole ring), 7.25–7.94 (m, 10H, Ar-H, benzofuran protons), 8.28 (s, 1H, pyrazole ring)	451 [M^+] (3), 77 [C_6H_5] (100)
10a		($CDCl_3$): 3.88 (s, 3H, OCH_3), 7.25–7.90 (m, 15H, Ar-H, benzofuran protons, methylene protons), 8.69 (s, 1H, pyrazole ring)	569 [M^+] (2), 77 [C_6H_5] (100)
10b		($CDCl_3$): 7.25–7.85 (m, 14 H, Ar-H, benzofuran protons, methylene proton), 8.70 (s, 1H, pyrazole ring)	545 [M^+] (2), 313 [$C_{20}H_{15}N_3O$] (39), 273 [$C_{18}H_{13}N_2O$] (100)
10c		($CDCl_3$): 7.25–8.30 (m, 16H, Ar-H, benzofuran protons, indole protons), 8.69 (s, 1H, pyrazole ring), 10.05 (s, 1H, NH-indole exchangeable by D_2O)	578 [M^+] (10), 77 [C_6H_5] (100)
11a	3210 (NH), 1720, 1695 (2 C=O), 2222 (C≡N)	(DMSO-d_6): 7.26–7.98 (m, 14H, Ar-H, benzofuran protons), 8.45 (s, 1H, pyrazole ring), 9.20 (s, 1H, NH exchangeable by D_2O)	497 [M^++1] (4), 77 [C_6H_5] (100)
11b	2228 (C≡N), 1724, 1698 (2 C=O)		499 [M^++2] (3), 423 [M^+–C_6H_2] (58), 395 [423–CO] (100)
11c	2221 (C≡N), 1720, 1692 (2 C=O)		513 [M^+] (5), 439 [M^+–C_6H_2] (23), 411 [439–CO] (57), 395 [411–NH_2] (100)

Table 2. cont.

Compd.no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS [m/z (%)]
12		($CDCl_3$): 3.42 (m, 2H, CH_2-imidazole ring) 3.65 (m, 2H, CH_2 imidazole ring), 7.17–7.95 (m, 10H, Ar-H, benzofuran protons), 8.52 (s, 1H, pyrazole ring), 10.12 (s, 1H, NH exchangeable by D_2O)	423 [M^++3] (26), 394 [M^+–C_2H_2] (3), 395 [394+1] (21), 273 [$C_{18}H_{13}N_2O$] (66), 77 [C_6H_5] (100)
13	3128 (NH), 2223 (C≡N), 1667 (C=O), 1309 (S-CH_3)	(DMSO-d_6): 2.25 (s, 3H, S-CH_3), 7.25–7.98 (m, 10H, Ar-H, benzofuran protons), 8.63 (s, 1H, pyrazole ring), 9.21 (s, 1H, NH exchangeable by D_2O)	25 [M^+] (13), 423 [M^+ –2] (14), 4 378 [M^+–SCH_3] (14), 77 [C_6H_5] (100)
14	Broad band centered at 3282 ($NHNH_2$), 3115 (NH), 2196 (C≡N), 1667 (C=O)	(DMSO-d_6): 7.24–7.64 (m, 10H, Ar-H, benzofuran protons), 7.93 (s, 1H, pyrazole ring), 9.00 (s, 1H, NH exchangeable by D_2O), 10.18 (s, 3H, $NHNH_2$ exchangeable by D_2O)	409 [M^+] (6), 410 [M^++1] (31), 394 [M^+–NH] (25), 379 [M^+ –N_2H_2] (68), 77 [C_6H_5] (100)
15	NH), 2221 (C≡N), 1214 (C=S)	(DMSO-d_6): 7.25–7.92 (m, 10H, Ar-H, benzofuran protons), 8.61 (s, 1H, pyrazole ring), 9.20 (s, 1H, NH exchangeable by D_2O)	429, 431[M^+] (7, 25), 430, 432 [M^++1] (9, 30), 77 [C_6H_5] (100)
16	Broad band centered at 3319 ($NHNH_2$), 3171 (NH), 2213 (C≡N), 1069 (C=S)		425 [M^+] (5), 263 [$C_{17}H_{15}N_2O$] (100)
17	3241 (NH), 2222 (C≡N), 1148 (C=S)		455 [$M^{+\cdot}$–1] (10), 77 [C_6H_5] (100)
18	Broad band centered at 3223 (NH, NH), 2215 (C≡N), 1661, 1596 (2C=O)	(DMSO-d_6): 7.31–7.94 (m, 11H, Ar-H, benzofuran protons, pyrazole ring), 9.16 (s, 1H, NH exchangeable by D_2O)	434 [M^+ –1] (54), 433 [M^+–2] (46), 77 [C_6H_5] (77), 51 [C_4H_3] (100)
19	3251 (NH), 2198 (C≡N), 1673 (C=O)	(DMSO-d_6): 7.26–7.98 (m, 12H, Ar-H, benzofuran protons, pyrazole ring, triazole ring), 9.02 (s, 1H, NH exchangeable by D_2O)	419 [M^+] (11), 77 [C_6H_5] (100)
20	3262 (NH), 2216 (C≡N), 1673 (C=O)	(DMSO-d_6): 2.78 (s, 3H, CH_3), 7.30–7.96 (m, 11H, Ar-H, benzofuran protons, pyrazole ring), 9.10 (s, 1H, NH exchangeable by D_2O)	433 [M^+] (29), 438 [M+1] (13), 77 [C_6H_5] (100)
21	3225 (NH), 2216 (C≡N), 1671 (C=O)	($CDCl_3$): 2.75 (s, 2H, CH_2), 7.04–7.70 (m, 11H, Ar-H, benzofuran protons, pyrazole ring), 8.77 (s, 1H, NH exchangeable by D_2O)	477 [M^+] (10), 77 [C_6H_5] (100)
22	3122 (NH), 2210 (C≡N), at 1667 (C=O)	(DMSO-d_6): 1.90 (s, 3H, CH_3), 2.25 (s, 2H, CH_2), 7.26–7.99 (m, 10H, Ar-H, benzofuran protons), 8.39 (s, 1H, pyrazole ring), 9.17 (s, 1H, NH exchangeable by D_2O)	475 [M^+] (13), 77 [C_6H_5] (100)
23	3119 (NH), 2212 (C≡N), 1705 (C=O)	(DMSO-d_6): 2.25 (s, 6H, 2 CH_3), 5.36 (s, 1H, CH), 7.29–7.90 (m, 10H, Ar-H, benzofuran protons), 8.76 (s, 1H, pyrazole ring), 9.15 (s, 1H, NH exchangeable by D_2O)	473 [M^+] (14), 77 [C_6H_5] (100)
24	Broad band centered at 3239 (NH), 2207 (C≡N), 1664 (C=O)	(DMSO-d_6): 7.31–7.57 (m, 10H, Ar-H, benzofuran protons), 7.92 (s, 1H, pyrazole ring), 9.01 (s, 1 H, NH exchangeable by D_2O)	465 [M^++2] (39), 77 [C_6H_5] (77), 53 [C_4H_5] (100)
25a	3274, 3152 (NH), 2207 (C≡N), 1671 (C=O), 1214 (C=S)	(DMSO-d_6): 1.03 (t, 3H, CH_2**CH_3**), 3.60 (q, 2H, **CH_2**CH_3), 7.30–7.92 (m, 10H, Ar-H, benzofuran protons), 8.33 (s, 1H, pyrazole ring), 8.59, 9.01, 9.11, 10.50 (s, 4H, 4NH exchangeable by D_2O)	496 [M^+] (25), 51 [C_4H_3] (100)

Table 2. cont.

Compd.no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS [m/z (%)]
25b		(DMSO-d_6): 1.24–1.90 (m, 11H, cyclohexyl), 7.35–7.93 (m, 11H, Ar-H, benzofuran, protons, pyrazole ring), 8.59, 8.97, 9.13, 11.43 (s, 4H, 4(NH) exchangeable by D_2O)	548 [M^+–2] (4), 43 [C_3H_7] (100)
25c	3300, 3210, 3116 (4NH), 2208 (C≡N), 1666 (C=O), 1196 (C=S)	(DMSO-d_6): 7.37–7.91 (m, 16H, Ar-H, benzofuran protons, pyrazole ring), 8.72, 9.29, 9.93, 11.88 (s, 4H, 4NH exchangeable by D_2O)	546 [M^++2] (33), 547 [M^++3] (67), 77 [C_6H_5] (100)
25d	3312, 3139 (NH), 2199 (C≡N), 1666(C=O)	(DMSO-d_6): 2.28 (s, 3H, CH_3), 7.26–7.87 (m, 15H, Ar-H, benzofuran protons, pyrazole ring), 8.70, 9.25, 9.80, 11.83 (s, 4H, 4 NH exchangeable by D_2O)	558 [M^+] (20), 106 [C_7H_8N] (94), 51 [C_4H_3] (100)
26a	Broad band centered at 3116 (2 NH), 2210 (C≡N), 1708 (C=O), 1615 (C=O)	(DMSO-d_6): 3.10 (s, 4H, -CH_2CH_2-), 7.35–7.96 (m, 10H, Ar-H, benzofuran protons), 8.75 (s, 1H, pyrazole ring), 9.12, 9.44 (s, 2H, 2NH exchangeable by D_2O)	491 [M^+] (14), 77 [C_6H_5] (100)
26b		DMSO-d_6): 7.34–7.95 (m, 10H, Ar-H, benzofuran protons), 8.73, 9.12, 9.42 (m, 4H, pyridine ring, pyrazole ring), 11.93, 12.63 (s, 1H, 1H, 2NH exchangeable by D_2O)	540 [M^+] (11), 77 [C_6H_5] (100)

Table 3. Cytotoxic activity of the selected compounds on human liver carcinoma cell line (HEPG2) compared to 5-fluorouracil (5-Fu).

Compd. no.	Cell Line
	HEPG2
	IC_{50} (μg/mL)
9	6.48
14	11.8
18	15.6
21	9.53
25b	11.5
5-Fluorouracil	27.7

IC_{50} = dose of the compound which reduces survival to 50%

Molecular docking

Preparation of protein and ligands for docking

The crystal structure of thymidylate synthase complexed with Raltitrexed® was downloaded from protein data bank (http://www.pdb.org/pdb/home/home.do) with pdb code = 1HVY and the site in which the inhibitor was complexed was identified and the surrounding important residues such as Tyr 258, Asp 218, Leu 221, Gly 222, Asn 226, PHE 225, PHE 80, Glu 87, IIe 108, TRP 109, Ala 312, Asn 112 and Leu 192 were recognized. All compounds were drawn using Chem3D ultra 8.0 software. Both the protein and ligands were saved in pdbqt format. Docking was performed as in the Autodock Vina manual and the results were visualized using Autodock tools. Binding affinities were calculated and the highly ranked compounds were selected and docking was repeated for these selected compounds to confirm their affinity.

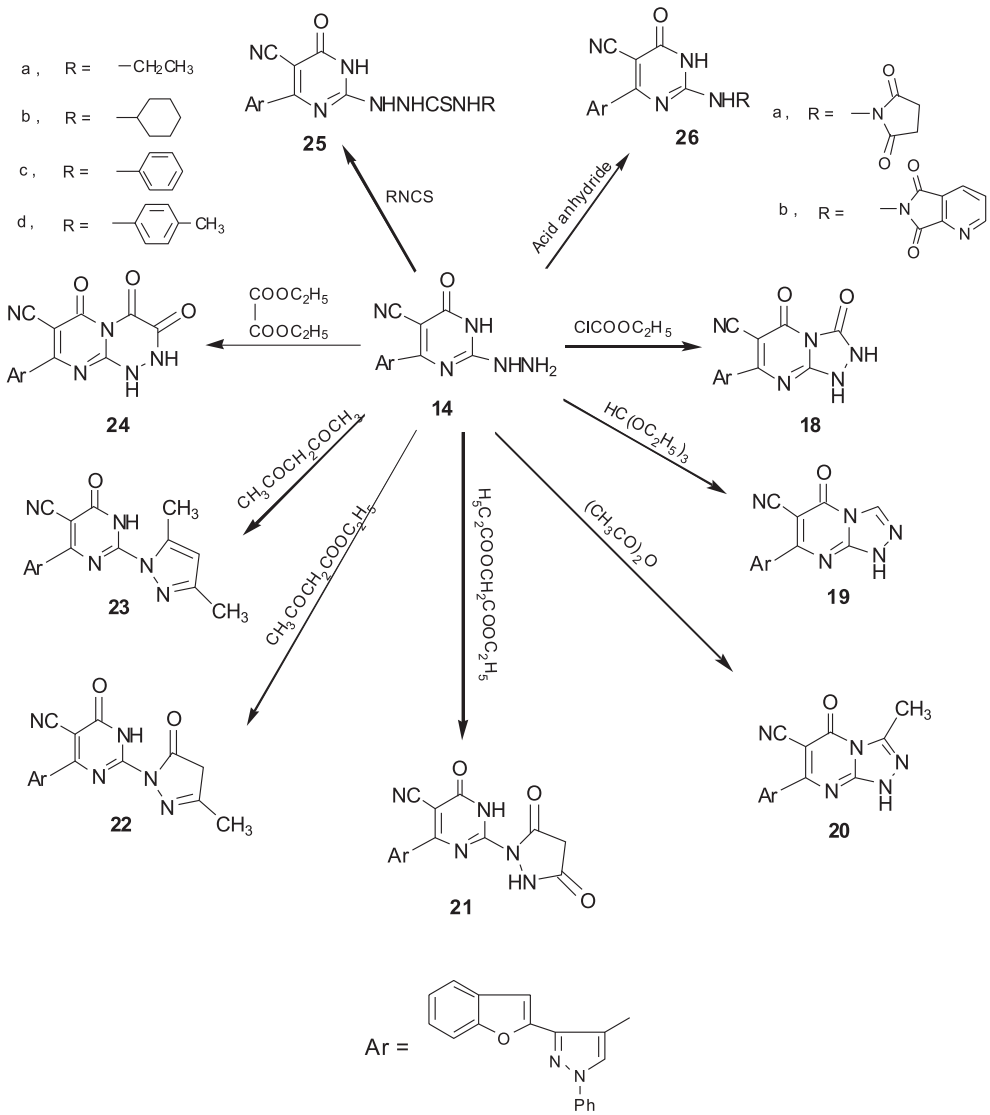
Building of Grid box

The grid maps representing the native ligand in the actual docking target site were calculated. The grid box dimensions were chosen to be sufficiently large to include all the previously mentioned residues that are surrounding the complexes inhibitor.

RESULTS AND DISCUSSION

Chemistry

During our drug discovery program (23, 24) and in continuation of the recent studies concerning synthesis and cytotoxic screening of 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-carbaldehyde (**1**) (21), it was of interest to introduce compound **1** as useful starting material for future synthesis. Condensation



Scheme 3.

of **1** with active methylene compounds namely: ethyl cyanoacetate, acetylacetone and/or ethyl acetoacetate in the presence of thiourea gave the corresponding 2-thioxopyrimidine derivatives **2-6**. Also one pot reaction (25) of **1** with 6-acetyl-1,2,3,4-tetrahydronaphthalene, ethyl cyanoacetate and/or malononitrile in excess anhydrous ammonium acetate afforded compounds **7** and **8**, respectively. (Scheme 1)

Furthermore, refluxing of compound **2** with chloroacetic acid in the presence of acetic acid anhydride and glacial acetic acid afforded compound **9**. The presence of an active methylene group in compound **9** was established by condensation with different aromatic aldehydes namely: p-anisaldehyde, 2-thiophene carboxaldehyde and/ or indole-3-carboxaldehyde in acetic acid to give compounds **10a-c**, respectively. Compound **2** was condensed with *ortho* substituted benzoic acids namely: anthranilic acid, salicylic acid and/or thiosalicylic acid in sodium ethoxide yielding compounds **11a-c**, respectively. The displacement reaction of the mercapto group of **2** was achieved by refluxing with ethanolamine in isopropyl alcohol to give compound **12**, whereas alkylation of **2** with iodomethane in sodium ethoxide gave **13**, which condensed with hydrazine hydrate (99%) in absolute ethanol afforded **14**. Another route for synthesis of compound **14** was

Table 4. Docking results of the synthesized compounds

Compound	Affinity Kcal/mol	Interactions of the best mode			
		Interacted moiety	Main Residue	Distance Å	Angle
2	–9.0	**CN group**	NH_2 of Lys 308	2.07	21.4
3	–8.9	C=O	NH_2 of Lys 308	2.48	114.4
4	–9.6	-NH	C=O of lle 108	2.64	82.0
5	–8.9	No action			
6	–9.0	-NH	C=O of Leu 221	2.96	110
7	–9.9	C=O	NH_2 of PHE 225	2.82	121.5
8	–9.8	CN group	NH_2 of Lys 308	3.01	19.9
9	–10.4	C=N	-NH_2 of Asp 218	2.03	13.8
10a	–9.1	No action			
10b	–9.5	C=O	NH_2 of Leu 221	2.17	43.5
10c	–9.0	C=N	NH_2 of PHE 80	2.40	134.4
11a	–9.4	No action			
11b	–9.2	No action			
11c	–9.0	No action			
12	–9.1	C=N	NH_2 of Glu 87	2.91	134.9
13	–9.5	CN group	NH_2 of PHE 225	2.57	29.6
14	–10.7	NH_2 of –NH-NH_2	-C=O of Asp 218	2.58	39.2
15	–9.2	CN group	NH_2 of Lys 308	3.23	133.8
16	–9.6	-NH_2	-C=O of Gly 222	2.72	93.1
17	–9.0	No action			
18	–10.5	CN group	NH_2 of Lys 308	2.52	86.1
19	–9.3	CN group	NH_2 of Lys 308	2.66	112.8
20	–9.6	No action			
21	–10.4	CN group -C=O	NH_2 of Lys 308 NH_2 of Leu 221	2.66 3.16	118.2 102.7
22	–8.9	No action			
23	–9.7	No action			
24	–9.5	-C=O	NH_2 of PHE 80	2.44	121.6
25a	–9.3	No action			
25b	–10.3	-C=O of pyrimidinone	-NH2 of Asp 218	2.49	91.3
25c	–8.9	No action			
25d	–8.7	No action			
26a	–8.7	No action			
26b	–8.2	No action			

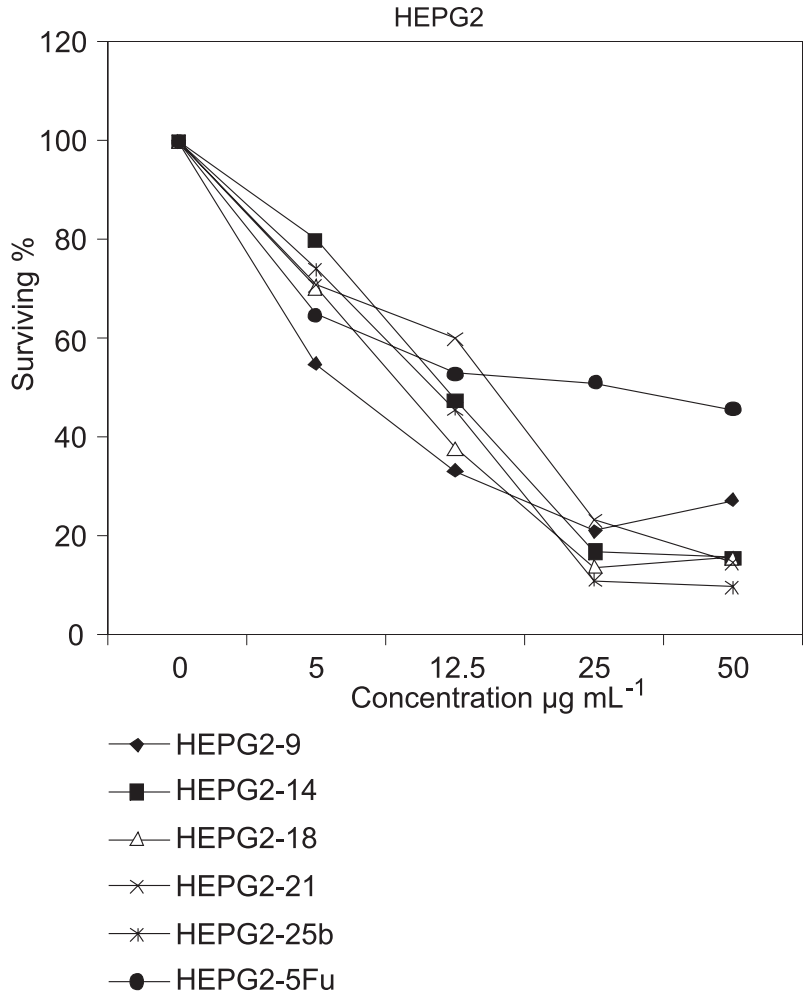


Figure 1. Cytotoxicity screening of compounds **9**, **14**, **18**, **21**, **25b** against HEPG2

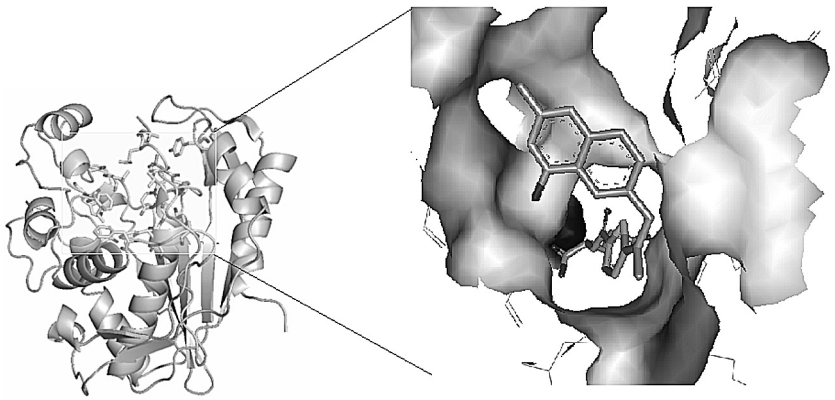


Figure 2. The crystal structure of thymidylate synthase showing the site of action of the complexed inhibitor which was used for docking.

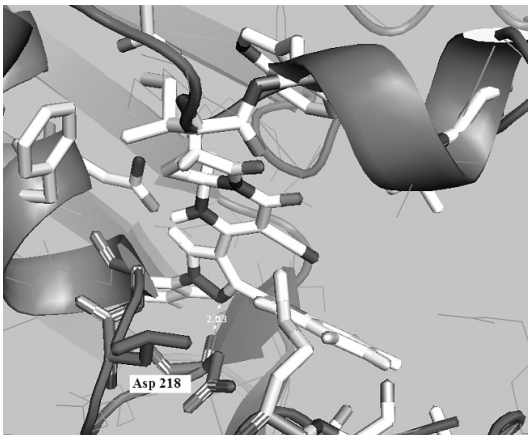


Figure 3. Hydrogen bond formation of compound **9** with Asp 218

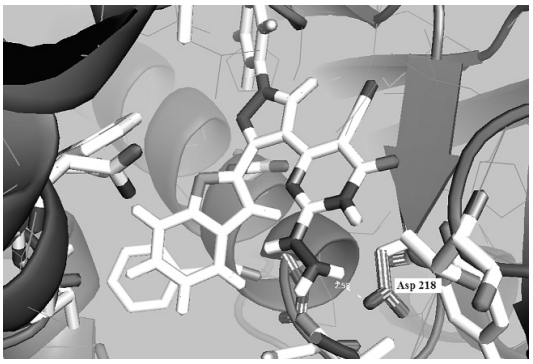


Figure 4. Hydrogen bond formation of compound **14** with Asp 218.

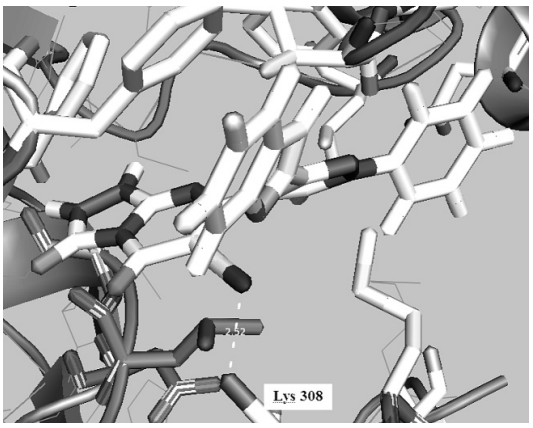


Figure 5. Compound **18** interaction and formation of hydrogen bond with Lys 308

succeeded by refluxing of **2** with hydrazine hydrate (99%) in absolute ethanol. Heating a mixture of compound **2** with phosphorus pentachloride/phosphorus oxychloride in a water bath yielded carbonitrile **15**, which was condensed with hydrazine hydrate (99%) in absolute ethanol at room temperature or sodium azide in refluxing acetic acid giving **16** and **17**, respectively. (Scheme 2)

In addition, the reported anticancer activity of triazole (26, 27), pyrazole (28) and triazine (29, 30) derivatives against different types of carcinoma cell line prompted us to introduce these ring systems in our parent compound. Accordingly, cyclocondensation of compound **14** with ethyl chloroformate, triethylorthoformate or acetic anhydride afforded carbonitriles **18**, **19** and **20**, respectively. The reflux of **14** with active methylene compounds namely: diethyl malonate, ethyl acetoacetate and/or acetylacetone in acetic acid afforded compounds **21**–**23**, respectively. Further, cyclocondensation of **14** with diethyl oxalate in refluxing ethanol yielded carbonitrile **24**. Conjugated N-N-S- tridentates ligand system of thiosemicarbazide ($RNH\text{-}CS\text{-}NH\text{-}NH_2$) seems essential for anticancer activity and display antiproliferative activity on different tumors cell line (31). Thus, we aimed to introduce such thiosemicarbazide side chain into our parent compound *via* reaction of **14** with thiosemicarbazide derivatives namely: ethyl, cyclohexyl, phenyl or p-tolyl thiosemicarbazides in dry benzene to give **25a-d**, respectively. For incorporation of pyrrolidinedione moiety, which exihibt antiproliferative activity toward murine leukemia and human colon carcinoma (32, 33), into the key structure, compound **14** was allowed to react with different acid anhydrides namely: succinic acid anhydride and/or 2,3-pyridinedicarboxylic acid anhydride yielding compounds **26a** and **26b**, respectively (Scheme 3).

Antitumor activity

Five of the newly synthesized compounds (**9**, **14**, **18**, **21**, **25b**) were chosen for screening of their biological cytotoxicity against human liver carcinoma cell line (HEPG2). According to Table 3 and Figure 1, it is obvious that all of the mentioned compounds are highly active against human liver carcinoma cell line (HEPG2) as compared to 5-fluorouracil (5-Fu). In case of derivative **9** (IC_{50} 6.48 μg/mL) which contain thiazolidinone ring, it showed about 4-fold higher activity than of the reference 5-Fu. The cytotoxic activity decreased slightly to 3-fold higher than 5-Fu in case of derivative **21** (IC_{50} 9.53 μg/mL) which contain the pyrazolidinedione nucleus. Furthermore, a decrease was observed in case of compounds **14**, **18** and **25b** (IC_{50} 11.8, 15.6, 11.5 μg/mL, respectively) where the benzofuran pyrazole moiety was attached to 2-hydrazinyloxopyrimidine, triazolopyrimidine and thiosemicarbazide pyrimidine moieties. From the above discussion, we

can conclude that the newly synthesized moieties attached to benzofuran pyrazolo pyrimidine moiety are important for antitumor activity and especially thiazolidinone ring of compound **9**.

Molecular modeling of TS

Autodock Vina (20) is a new program for drug discovery. It provides a fast and highly accurate molecular docking. By obtaining the calculated binding affinities we can rank the compounds and select those that are expected to be of good activity.

In the present work we used the crystal structure of thymidylate synthase complexed with Raltitrexed inhibitor in which the glutamate moiety was not found to have any role in the interactions of the complexed ligand as supposed for most of antifolate or well known thymidylate synthase inhibitors. Instead of that we found that the nitrogen atom in position 3 of 2-methyl-3,4-dihydro-4-quinazolinone moiety was the main key for hydrogen bond formation with –C=O group of Asp 218 residue with distance = 2.63 Å.

This can show the important role of the pyrimidine ring in the proposed activity. In this work we synthesized a number of compounds that contain substituted pyrimidine ring, thiopyrimidine or fused pyrimidine ring system with thiazolone ring system as in compound **9.** All these compounds were docked into the same site of the complexed ligand that was determined using pdbsum (http://www.ebi.ac.uk/thornton-srv/ databases/pdbsum/). According to the docking results, there are five compounds with high affinity, namely: **9**, **14**, **18**, **21 and 25b** with affinities of: –10.4, –10.7, –10.5, –10.4 and –10.3 kcal/mole, respectively. We found that the shared functional groups in the compounds can have common interaction pattern. For example; the N atom of –C=N bond in the pyrazole ring was found interacting with -NH_2 group of Asp 218 as in **9. I**t has been shown also another hydrogen bonds formation with –NH_2 of PHE 80 as found for compound **10c,** -NH_2 of PHE 225 as for compound **13**, and –NH_2 of Glu 87 as found in compound **12.**

On the other hand, the common cyano group in most of the compounds has shown a fixed figure, which was responsible for the formation of hydrogen bond with -NH_2 of Lys 308. Benzofuran ring system did not show any observed interactions. The –C=O groups in the 1,6-dihdro-2-pyrimidine ring of compounds **7, 10b** and **21** were found interacting with -NH_2 of PHE 225, -NH_2 of Leu 221 and -NH_2 of Leu 221, respectively. The results of the docking together with the measured affinities, distances, and bond angles are shown in Table 4.

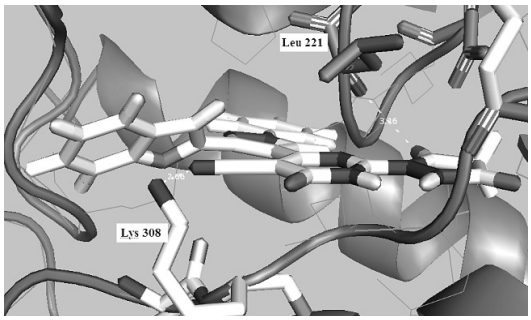


Figure 6. Compound **21** shows a single conformation that interacts with two residues (Lys 308 and Leu 221).

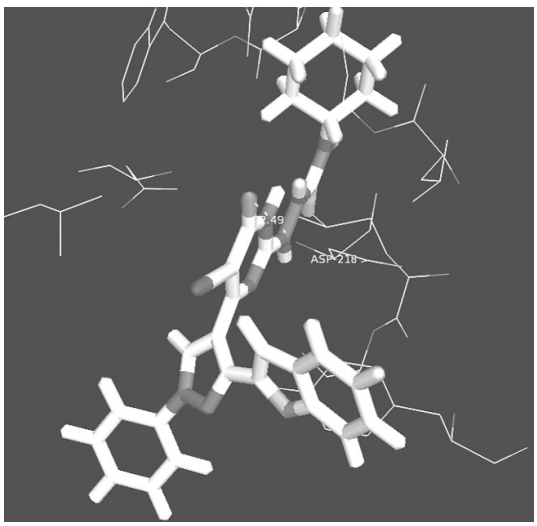


Figure 7. Compound **25b** hydrogen bond between –C=O group and –NH_2 of Asp 218.

An explanation for the activity of the tested compounds could be referred to their interactions. For example; compound **9** has shown a hydrogen bond formed between the N atom of -C=N bond in pyrazole ring with the -NH_2 group of Asp 218 residue (2.03 Å), which is the main residue found in the interaction profile of Raltitrexed inhibitor (Fig. 3).

Also, compound **14** interacted by its -NH_2 group of the hydrazine side chain with Asp 218, but with its -C=O of the carboxylate moiety (2.58 Å) (Fig. 4).

Compound **18** has two interactions with two different conformations: the first is the formation of hydrogen bond between the cyano group and -NH_2 of Lys 308 (2.52 Å) and the second between -NH of triazole ring with –C=O of Gly 222 (2.84 Å). Also, compound **21** has two interactions but, within one conformation that was found forming hydrogen bond by its -CN group with -NH_2 of Lys 308 (2.66

Å) and by its -C=O (found in the dioxotetrahydro-1H-pyrazol ring with -NH_2 of Leu 221 (3.16 Å) (Figs. 5, 6).

Finally, compound **25b** has a conformation that poses a hydrogen bond formed between the -C=O group of pyrimidinone ring and -NH_2 group of Asp 218 (2.49 Å). That could confirm that the interaction with such residue may be the key reason for the activity of the tested compounds as **25b** is the third compound found forming hydrogen bond with Asp 218 (Fig. 7).

CONCLUSION

This study includes the preparation of 32 derivatives, which contain benzofuran pyrazolo pyrimidine as backbone skeleton attached to various aromatic and/or heterocyclic ring systems such as thiazolidinone, quinazoline, triazole, pyrazole, triazine, pyrrolidine and side chain as thiosemicarbazide derivatives. All of the compounds were autodocked for the inhibition of the enzyme thymidylate synthase *via* Autodock Vina. The study showed that most of the derivatives exhibited high affinities towards the enzyme thymidylate synthase except compounds **5**, **10a**, **11a-c**, **17**, **22**, **23**, **25a**, **25c**,**d** and **26a**,**b**. In order to specify the autodock study, five of the compounds: **9**, **14**, **18**, **21** and **25b** were studied for cytotoxicity against human liver carcinoma cell line (HEPG2) and all showed higher activity than that of 5-fluorouracil. This indicates that the heterocyclic rings thiazolidinone, pyrazolidinedione, triazole and side chain as hydrazinyl, in thiosemicarbazide derivatives are essential for the antitumor activity.

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