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Design, synthesis, molecular docking of new thiopyrimidine-5-carbonitrile derivatives and their cytotoxic activity against HepG2 cell line

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ABSTRACT

A series of new thiopyrimidine derivatives were synthesized *via* the reaction of ethyl cyanoacetate with thiourea and the appropriate aldehydes namely, 3-methoxy-benzaldehyde, 2, 5-dimethoxy-benzaldehyde and 3,5-dimethoxy-benzaldehyde to give the corresponding pyridine thiones **1a-c**. Compounds **1a-c** were then chlorinated to give the corresponding chloro compounds **2a-c**, which then underwent variant cyclocondensation reactions to afford different cyclized compounds **3-10**. On the other hand, **1a-c** were condensed with monochloroacetic acid and different aldehydes to give **11-14**. Some of the new derivatives were selected for cytotoxicity evaluation against HepG2 cell line in comparison to 5-FU as a reference drug. Among all tested compounds, compound **4a** was the most potent with IC_{50} value of 13.18 µM. Furthermore, a docking study of the most active compounds was carried out with thymidylate synthase enzyme. Structures of all new compounds were elucidated by their correct elemental analysis and spectral data.

INTRODUCTION

Despite the recent improvement achieved in cancer treatment strategies during the last 3 decades, resulting in increasing the survival rate and better quality of life for cancer patients, cancer is still incurable in most cases. Recent statistical studies have shown that incidence of cancer continues to increase (Parkin 2001; Parkin *et al.,* 2005; Ferlay *et al.,* 2007). Estimations show that in year 2002, there were 10.9 million new cancer cases, 6.7 million cancer related deaths and 24.6 million persons living with cancer within 5 years of diagnosis(Parkin *et al.,* 2005) . In 2010, Global status report on non-communicable diseases show that the number of cancer-related deaths in 2008 have increased to approximately 7.6 million person worldwide, making cancer disease the second cause of death after cardiovascular disease(Alwan 2011). Traditional anti-cancer drugs such as alkylating agents, antimetabolites, topoisomerase inhibitors, and anti-microtubule agents are targeting DNA synthesis and cell division. Even though these drugs show efficacy, their lack of selectivity for tumor cells over normal cells usually lead to severe adverse effects such as bone marrow suppression, cardiac, hepatic, and renal toxicities which limit their use.

In recent years, thiouracil derivatives have drawn great attention for their anti-tumor activity. For example, 5-substituted-2-thiouracil derivatives were found to inhibit DNA synthesis (Singh *et al.,* 1998; Cocco *et al.,* 2001; Fathalla *et al.,* 2002). Moreover, a literature survey revealed that the thiouracil carbonitrile ring system has occupied a marked position in the design and synthesis of novel chemotherapeutic agents with remarkable antitumor and antimicrobial activities (Ram *et al.,* 1987; Wyrzykiewicz *et al.,* 1993; Kamalakannan and Venkappayya 2002; Ibrahim and El-Metwally 2010). It is well established that uracil derivatives exert their anti-cancer activity through inhibition of folate metabolism, which is considered as an important target for the development of new anticancer agents due to its role in the biosynthesis of nucleic acid precursors (Chan *et al.,* 2006; Hawser *et al.,* 2006).

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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(Blakley ; MacKenzie 1984). Based on all these findings, this work aims to design and synthesis of a new series of thiouracil carbonitrile derivatives as expected anti-cancer agents. The cytotoxic activities of some of the new compounds were screened against HepG2 (human liver carcinoma cell line). Moreover docking study of the most active new derivatives against thymidylate synthase 3D X-ray crystal structure was performed in an attempt to understand their possible mechanism of action.

RESULT AND DISCUSSION

The study began with synthesis of the essential building block 1a-c by reaction of thiourea and ethyl cyanoacetate with the appropriate aldehydes namely 3-methoxy benzaldehyde, 2,5-dimethoxy benzaldehyde and 3,5-dimethoxy benzaldehyde to give 6-substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carbonitriles 1a-c. (Fathalla *et al.,* 2009) (Scheme1.)

Compounds 1a-c were allowed to react with phosphorus pentachloride and phosphorus oxychloride to give 4-chloro-6-(substituted)-2-thioxo-1,2-dihydropyrimidine-5-carbonitriles 2a-c. Compounds 2a-c underwent variant substitution and cycliztion reactions (Scheme 2). First compounds 2a-c were allowed to react with hydrazine hydrate to give 4-hydrazinyl-6-(substituted)-2-thioxo-1,2-dihydropyrimidine-5-carbonitriles (3a-c). On the other hand compounds 2a-c reacted with the primary amines namely 4-nitrophenyl amine, 4-amonoanyipyrine, *4*-chlorophenyl amine to give 4-[(substituted)amino]-6-(substituted)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (4a-c), (5a-c), (6a-c). Furthermore compounds 2a-c were allowed to react with glycine to give 7-(substituted)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-*f*]pyrimidine-8-carbonitrile 7a-c. In addition compounds 2a-c reacted with acetohydrazide to give 7-substituted-3-methyl-5-thioxo-5,6-dihydro[1,2,4]-triazolo[4,3-*f*]pyrimidine-8-carbonitrile (8a-c). Compounds 2a-c also reacted with sodium azide to give 7-(substituted)-5-thioxo-5,6- dihydrotetrazolo[1,5-*f*]pyrimidine-8-carbonitrile (9a-c). Finally compound 2a-c reacted with anthranilic acid to give 3-(substituted)-10-oxo-1-thioxo-2,10-dihydro-1*H*-pyrimido[6,1-*b*]quinazoline-4-carbonitriles (10a-c).

In a different route compound 1a-c reacted with chloroacetic acid to give 7-(substituted)-3,5-dioxo-3,5-dihydro-2*H*-thiazolo [3,2-*a*]pyrimidine -6- carbonitrile 11a-c. Compounds 11a-c were then reacted with different aldehydes namely *p*-nitrobenzaldehyde *p*-flourobenzaldehyde and *p*-chlorobenzaldehyde to give 2-(substituted)-7-(substituted)- 3,5-dioxo-3,5- dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carbonitrile 12a-c, 13a-c, 14a-c. Alternatively, the same products 12a-c, 13a-c, 14a-c were preprapred and identified via one pot reaction between of compounds 1a-c, monochloroacetic acid and the same different aldehydes respectively (Scheme 3). All new compounds structure was proved via elemental analysis, mass spectroscopic data, IR and 1H NMR.

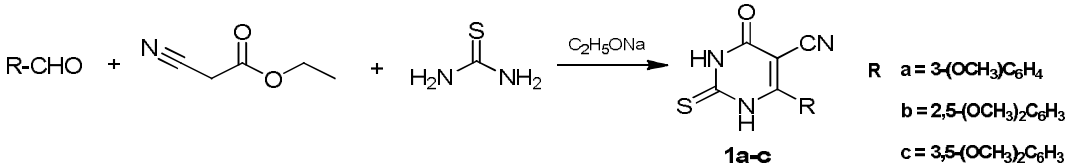
Chemotoxicity

Due to the structure similarity between 5-FU (Figure 1) and thiouracil derivatives, 5-FU was selected as a standard reference anticancer agent. The chemosensitivity responses of cell lines to 5-FU and analogues are presented in (Table 1) Following a 48 h exposure. All selected novel compounds induced significant antiproliferative activity against HepG2 liver cancer cells compared to that induced by 5-FU. Of all 5-FU analogues evaluated, compound 4a was found to be the most potent with IC_{50} value of 13.18 µM. This value was almost 3-fold less than that measured for 5-FU (38.44 µM). Three other compounds (6b, 6c and 13b) also exhibit higher antiproliferative activity than 5-FU with a ratio of 1.75, 1.34 and 2.63-fold, respectively. Compounds 5a, 5b, 5c and 9 showed similar toxicity compared to 5-FU. Whereas, compound 4b was the only member of the group that induced significantly less activity than 5-FU with an IC50 value of 58.49 µM.

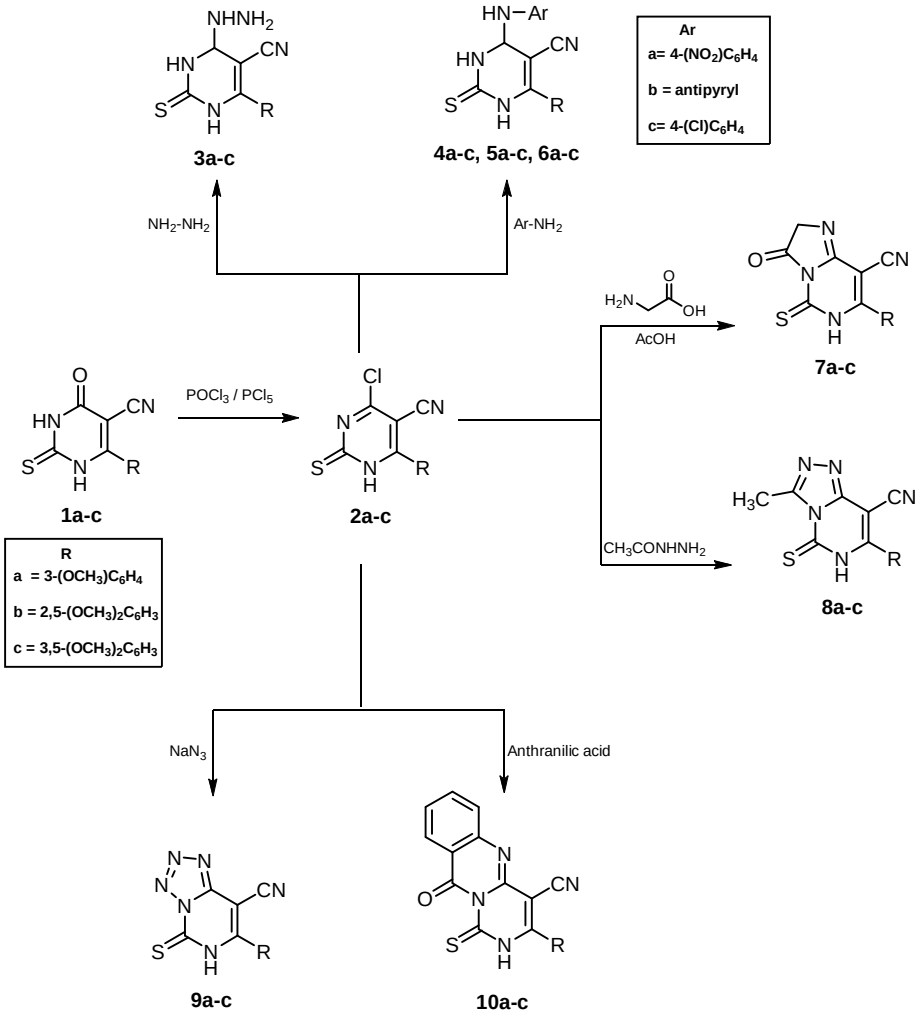
Table 1: Cytotoxic activity of the newly synthesized selected derivatives against liver HepG2 cancer cell lines in comparison to the traditional anticancer drug 5-fluorouracil (5-FU)

Compound	IC_{50} (µM)	Ratio (5-FU IC_{50}/test compound IC_{50})
5-FU	38.44	1
4a	13.18	0.34
4b	58.49	1.52
5a	39.08	1.02
5b	36.25	0.94
5c	39.61	1.03
6b	21.92	0.57
6c	28.58	0.74
9a	42.00	1.09
13b	14.61	0.38

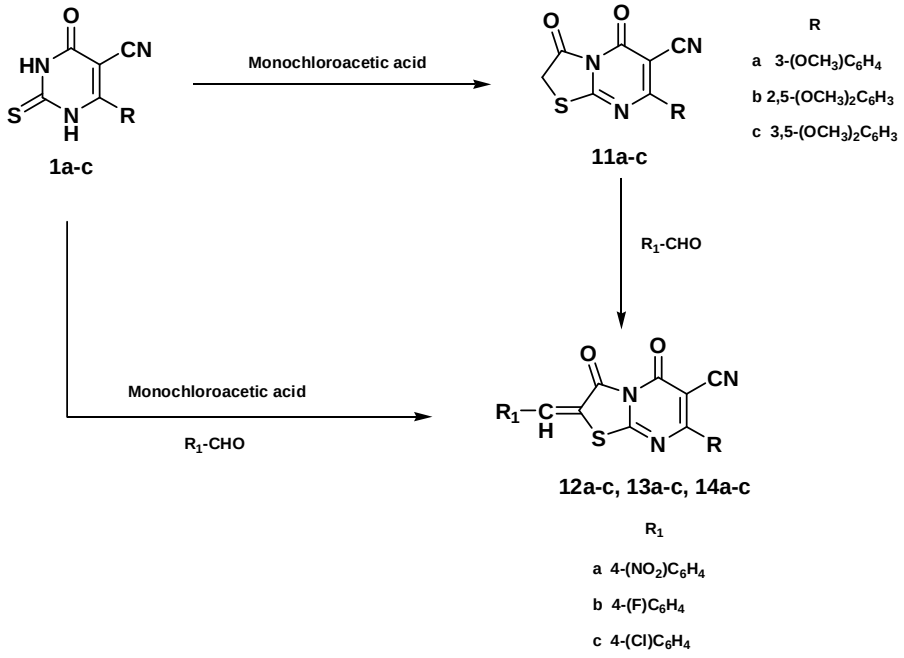
Similarity in structures between 5-FU and thiouracil derivatives may suggest possible similarity in the mechanism of action between these drugs. 5-FU induces cell kill mainly via destructing DNA synthesis by inhibiting Thymidylate synthase which is the enzyme catalyses the conversion of deoxy-uridylate to deoxy-thymidylate(Longley *et a.,* 2003). Molecular docking comparing the binding affinity of 5-FU and thiouracil derivatives was therefore conducted.



(Scheme1.)



(Scheme2.)



(Scheme3.)

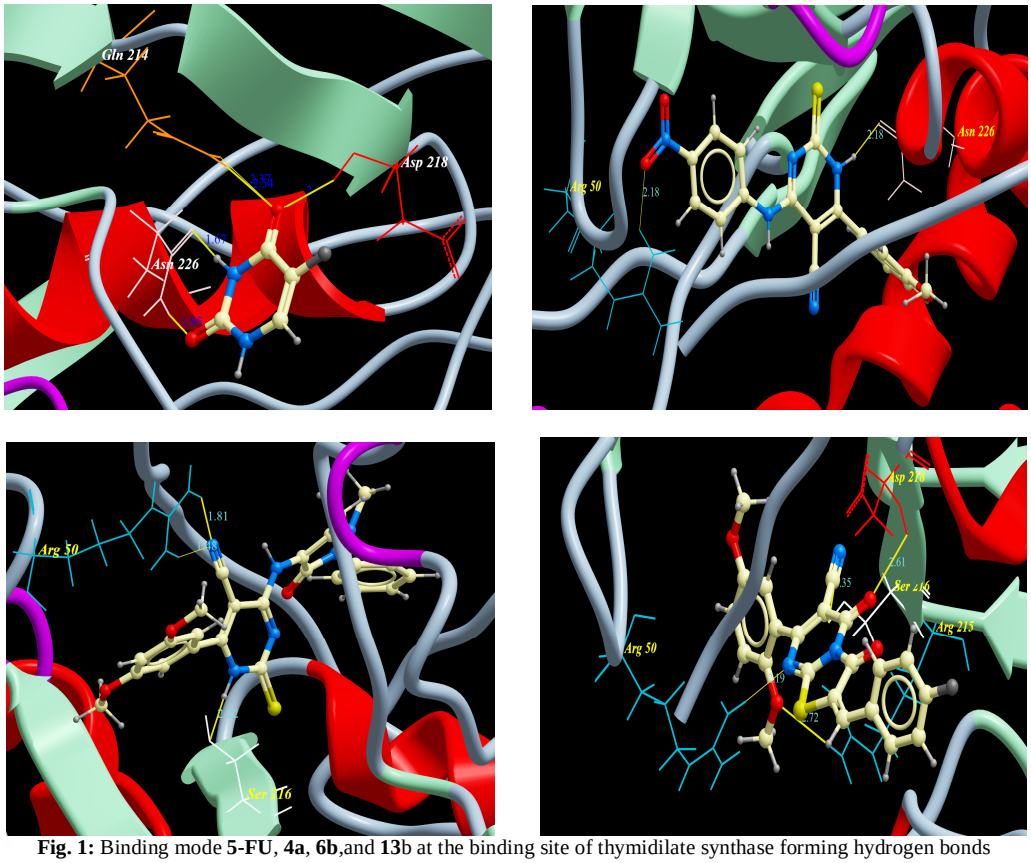


Fig. 1: Binding mode **5-FU**, **4a**, **6b**,and **13**b at the binding site of thymidilate synthase forming hydrogen bonds

Molecular Docking

Based on the fact that 5-fluorouracil derivatives and their structurally related compounds like thiouracil carbonitrile derivatives, are well known to inhibit thymidylate synthase. We decided to carry out a molecular docking study of the highest biologically active three newly synthesized thiouracil carbonitrile derivatives into the binding site of thymidylate synthase (PDB id 1JU6) which was retrieved from PDB bank http://www.rcsb.org/pdb using 5-FU as a reference for docking results. The docking results revealed that, the highest binding compound to TS was 6b with binding energy of -88.52 Kcal/mol. Compound 6b formed three hydrogen bonds with the amino acid residues of thymidilate synthase, between N of the cyano group of thiopyrimidine ring of the ligand and H-12 and H-21 of the amino acid Arg-50 as well as H-1 of the thiopyrimidine ring and Oxygen-9 of the amino acid Ser-216 of the enzyme. The results for binding energy for all tested compounds and 5-FU the reference drug are shown in (Table 2). On the other hand, compounds 4a, 6c and 13b showed strong binding affinity towards thymidilate synthase ranging from -70.45 to -78.37 Kcal/mol, forming hydrogen bonds with different amino acid residues of the target enzyme. The best poses for compounds 5-FU, 4a, 6b and 13b interacting with thymidylate synthase 3D-structure are illustrated in (fig. 1).

All tested compounds formed hydrogen bond with a common amino acid Arg-50 which indicates that this particular amino acid is essential in fitting the tested thiopyrimidine derivatives to the active binding site of thymidylate synthase.

In addition, another important amino acid Asn-226 forms hydrogen bond with compounds 4a, 6c and 5-FU (the reference drug). Furthermore compounds 6c and 13b were found to form hydrogen bond with the amino acid Asp-218 similar to the reference drug 5-FU.

Table 2: The docking energy scores of compounds 5-FU, 4a, 6b, 6c and 13b with the amino acid residues of the target enzyme thymidilate synthase forming hydrogen bonds

Cpd. No.	Docking score (Kcal/mol)	No. of Hydrogen bonds	Amino acid residues forming hydrogen bonds in A^0
5-FU	-34.69	5	Q214 he21 -- m M o2 : 2.40 Q214 he22 -- m M o2 : 2.49 D218 hn -- m M o2 : 1.53 N226 hd22 -- m M o1 : 1.78 N226 od1 -- m M h2 : 1.73
4a	-73.11	2	R50 hh11 -- m M o2 : 2.18 N226 od1 -- m M h1 : 2.18
6b	-88.52	3	R50 hh12 -- m M n3 : 1.48 R50 hh21 -- m M n3 : 1.81 S216 og -- m M h1 : 2.22
6c	-70.54	3	R50 hh12 -- m M n1 : 2.48 D218 hn -- m M n3 : 1.79 N226 hd22 -- m M o1 : 2.24
13b	-78.37	4	R50 hh12 -- m M n1 : 2.19 R215 hh21 -- m M o4 : 2.72 S216 hg -- m M n3 : 2.35 D218 hn -- m M o1 : 2.61

Form the above data we can conclude that, the new thiopyrimidine derivatives exerts their anti-tumor activity probably via similar mechanism of action to that of 5-FU based on the presence of

common amino acid residues which are bonded to the thiopyriminde ring common to all new compounds. In addition the fact that thiopyrimidine ring itself is considered as an analogue to pyrimidine ring in 5-FU. Support the possible similarity of the mechanism of action of both 5-FU and the new thiopyrimidine derivatives. Of course, this conclusion remains as an assumption only and needs to be verified *via* experimental data.

The ability of thiouracil derivatives to show strong binding affinity towards thymidilate synthase suggest the possible ability to inhibit TS enzyme, thus, possible similarity between the mechanism of action of these compounds and 5-FU. However, it is worth mentioning that thiouracil compounds exhibit different pattern of binding by forming hydrogen bonds with amino acids different from those involved in 5-FU binding. This may be important since thymidylate synthase gene polymorphism shown to influence the activity of 5-FU and linked to poor therapeutic responses(Parkin 2001; Pullarkat *et al.*, 2001).

Experimental

Chemistry

All melting points were uncorrected and determined by the Electro-thermal IA 9100 melting point apparatus. The infra-red (IR) spectra were recorded using potassium bromide disc technique on Schimadzu 435 IR Spectrophotometer at the microanalytical unit, National Research Centre. The proton nuclear magnetic resonance (^{1}H NMR) spectra were performed on Varian Gemini 300 MHz Spectrophotometer using tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) are given using parts per million scale (ppm) at the micro analytical unit, National Research Centre. Mass Spectra were recorded on Hewlett Packard 5988 Spectrometer using CI, EI or FAB ionization techniques at the micro analytical unit, National Research Centre. Elemental microanalyses were carried out at micro analytical unit, Cairo University and the results were within ±0.3 from the theoretical values. All reactions were monitored by TLC using pre-coated Aluminum sheet silica gel Merck 60 F 254 and were visualized by UV lamp. Chemical naming, calculation of molecular weight (M.wt.) of new compounds were performed by ChemBioDraw 12 software.

General procedure for the preparation of the pyrimidine - thiones 1a-c

A mixture of thiourea (0.1 mol), ethyl cyanoacetate (0.1 mol) and the appropriate aldehydes namely 3-methoxybenzaldehyde, 2,5-dimethoxy-benzaldehyde and 3,5-dimethoxy-benzaldehyde were stirring in sodium ethoxide solution for 48 h and then the reaction mixtures were poured in ice-water, then acidification. The precipitate was filtered off, dried then crystallized from methanol to give compounds 1a-c.

6-(3-Methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine -5- carbonitrile (1a)

M.p. 215-217°C (CH_3OH), yield (95%). Analysis: for $C_{12}H_9N_3O_2S$, M.W (259.28). Calcd: %C, 55.59; H, 3.50; N, 16.21. Found: %C, 59.53; H, 3.45; N, 16.29. IR: 3390, 3190 (2NH), 3190 (CH aromatic), 2219 (CN), 1670 , 1650 (C=C), 1270 (C=S). ^{1}H NMR: 3.80 (3H, s, OCH_3), 6.7-7.5 (4H,m, CH aromatic),12.8,1313 (2H, 2NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 259 (12%).

6-(2,5-Dimethoxyphenyl)-4- oxo-2- thioxo-1,2,3,4-tetrahydropyrimidine -5-carbonitrile (1b)

M.p.137-140°C (CH_3OH), yield (90%). Analysis: for $C_{13}H_{11}N_3O_3S$, M.W (289.31). Calcd: % C, 53.97; H, 3.83; N, 14.52. Found: % C, 53.92; H, 3.87; N, 14.48. IR: 3429, 3290 (2 NH), 3073(CH aromatic), 2221 (CN), 1730 , 1650 (C=C), 1155(C=S). ^{1}H NMR: 3.59, 3.68 (6H, s, $2OCH_3$), 6.87-8.28 (3H, m, CH aromatic) and 11.20, 11.70 (2H, s, 2 NH, exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 289 (15%).

6-(3,5-Dimethoxyphenyl)-4- oxo-2- thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1c)

M.p. 130-132°C (CH_3OH), yield (94%). Analysis: for $C_{13}H_{11}N_3O_3S$, M.W (289.31). Calcd: % C, 53.97; H, 3.83; N, 14.52. Found: % C, 54.02; H, 3.79; N, 14.58. IR: 3420, 3285 (2 NH), 3060 (CH aromatic), 2212 (CN), 1745, 1645 (C=C) 1160 (C=S). ^{1}H NMR: 3.81, 3.87 (6H, s, 2 OCH_3), 6.43-6.91 (3H, m, aromatic-H), 10.25, 11.80 (2H, s, 2 NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 291 (100%), 59(37.9), 95(15.4), 164(39.5), 208(74.5).

General Procedure for the Preparation of the 4-chloropyrimidines 2a-c

A mixture of 1a-c (0.01 mol) and phosphorus pentachloride (0.01 mol) in phosphorus oxychloride (20 ml) was heated on a steam bath for 3 h. Then the reaction mixture poured gradually onto crushed ice. The precipitate was filtered off, dried to give compounds 2a-c.

4-Chloro-6-(3-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile

M.p.180-182^{0}C , yield (92%). Analysis: for $C_{12}H_8ClN_3OS$ M.w (277.73). Calcd: % C, 51.90, H, 2.90, N, 15.13. Found: % C, 51.85, H, 2.92, N, 15.18. IR: 3367 (NH), 3066 (CH aromatic), 2210 (CN), 1650 (C=C), 1157(C=S). ^{1}H NMR: 3.8 (3H, s, OCH_3), 7.1-7.4 (4H, m,CH aromatic) and 11.8 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 277(16%).

4-Chloro-6-(2,5-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine -5-carbonitrile (2b)

M.p. 210-212^{0}C, yield (96%). Analysis: for $C_{13}H_{10}ClN_3O_2S$ M.w (307.76). Calcd: % C, 50.73, H, 3.28, N, 13.65 .Found: % C, 50.80, H, 3.33, N, 13.58. IR: 3521(NH), 3178 (CH aromatic), 2202(CN), 1616 (C=C), 1122(C=S). ^{1}H NMR: 3.8, 3.9 (6H, s, $2OCH_3$), 7.3-7.5 (3H, m, CH aromatic) and 10 (1H, s, NH, exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 307.5 (23%).

4-Chloro-6-(3,5-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine -5-carbonitrile (2c)

M.p.143-145^{0}C, yield (96%). Analysis: for $C_{13}H_{10}ClN_3O_2S$, M.w (307.76). Calcd: % C, 50.73, H, 3.28, N, 13.65. Found: % C, 50.66, H, 3.21, N, 13.70. IR: 3479 (NH), 3074 (CH aromatic), 2221(CN), 1631 (C=C), 1110 (C=S).^{1}H NMR: 3.8, 3.9 (6H, s, 2OCH_3), 7.7-8.2 (3H, m, CH aromatic) and 10.5 (1H, s, NH, exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 307.5 (23%).

General Procedure for the Preparation of Compounds 3a-c

A mixture of 2a-c (0.01 mol) and hydrazine hydrate 100% (0.01 mol) in methanol (10 ml) was stirred for 8 h. The precipitate was filtered off, dried then crystallized from acetic acid to give compounds 3a-c.

4-Hydrazinol -6-(3-methoxyphenyl)-2- thioxo-1,2-dihydropyrimidine -5-carbonitrile (3a)

M.p. 158-160^{0}C , yield (65%). Analysis: for $C_{12}H_{11}N_5OS$, M.w (273.31). Calcd: % C, 52.73, H, 4.06, N, 25.62. Found: % C, 52.78, H, 4.10, N, 25.50. IR: 3423, 3335 (NH2), 3193 (NH), 2927 (CH aromatic), 2212 (CN), 1681 (C=C), 1110 (C=S). ^{1}H NMR: 3.7 (3H, s, OCH_3), 3.75 (1H, s, NH exchangeable with D_2O), 7-7.2 (4H, m, CH aromatic), 13.16, 13.24 (2H, s, NH_2 exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 273 (13%).

4-Hydrazinol-6- (2,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (3b)

M.p. 270-272^{0}C , yield (66%). Analysis: for $C_{13}H_{13}N_5O_2S$, M.w (303.34). Calcd: % C, 51.47, H, 4.32, N, 23.09. Found: % C, 51.40, H, 4.27, N, 23.13. IR: 3352, 3205 (NH_2), 3062 (NH), 2935 (CH aromatic), 2206 (CN), 1589 (C=C), 1141 (C=S). ^{1}H NMR: 3.5 (1H, s, NH exchangeable with D_2O), 3.8-3.9 (6H, s, 2 OCH_3), 7.2-7.5 (3H, m, CH aromatic), 9-12.2 (2H, s, NH_2 exchangeable with D_2O) MS: (m/z) M^+ at m/z ≈ 303 (11%).

4-Hydrazinol-6- (3,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine -5-carbonitrile (3c)

M.p. 253-255^{0}C, yield (70%). Analysis: for $C_{13}H_{13}N_5O_2S$, M.w (303.34). Calcd: % C, 51.47, H, 4.32, N, 23.09. Found: % C, 51.52, H, 4.41, N, 23.03. IR: 3410, 3255 (NH), 3015 (CH aromatic), 2206 (CN), 1653 (C=C), 1162 (C=S). ^{1}H NMR: 3.6 (1H, s, NH exchangeable with D_2O), 3.7-3.8 (6H, s, 2 OCH_3), 7.01-7.10 (3H, m, CH aromatic), 9.1- 10.2 (2H, s, NH_2 exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 303 (11%).

General Procedure for the Preparation of Compounds 4a-c, 5a-c, 6a-c

A mixture of 2a-c (0.01 mol) and the primary amines namely 4-nitrophenyl amine, 4-aminoantipyrine, 4-chlorophenyl amine (0.02 mol) in methanol (30 ml) containing a few drops of pyridine was refluxed for 8-12 h. The solid obtained after cooling was poured on ice / water-containing HCl, filtered off, air dried on suction, and crystallized from acetic acid to give compounds 4a-c, 5a-c, 6a-c.

4-(4-Nitrophenylamino)-6- (3-methoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (4a)

M.p. 170-172^{0}C, yield (84%). Analysis: for $C_{18}H_{13}N_5O_3S$, M.w (376.43). Calcd: % C, 56.98, H, 3.45, N, 18.46. Found: % C, 57.03, H, 3.3, N, 18.4. IR: 3336, 3255 (2NH), 3043 (CH aromatic), 2202 (CN), 1639 (C=C), 1154 (C=S). ^{1}H NMR: 3.6 (1H, s, NH exchangeable with D_2O), 3.7 (3H, s OCH_3), 6.9-7.1 (8H, m, CH aromatic), 10.3 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 376 (12%).

4-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro- 1H-pyrazol-4-yl) amino)-6-(3-methoxyphenyl)-2-thioxo- 1,2-dihydropyrimidine-5-carbonitrile (4b)

M.p. 163-165^{0}C , yield (80%). Analysis: for $C_{23}H_{20}N_6O_2S$, M.W (444.51). Calcd: % C, 62.15, H, 4.54, N, 18.91. Found: % C, 62.08, H, 4.59, N, 18.87. IR: 3420, 3285 (2NH), 3060 (CH aromatic), 2212 (CN), 1654 (C=O), 1596 (C=C), 1157 (C=S). ^{1}H NMR: 2.4 (3H, s, C-CH_3), 3.3 (1H, s, NH exchangeable with D_2O), 3.31(6H, s, 2 OCH_3), 3.7 (3H, s, N-CH_3) 6.7-7.8 (9H, m, CH-aromatic), 11.8 (1H, s, NH exchangeable with D_2O).

4-(4-Chlorophenylamino)-6-(3-methoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (4c)

M.p. 159-161^{0}C , yield (76%). Analysis: for $C_{18}H_{13}ClN_4OS$, M.w (368.84). Calcd: % C, 58.61, H, 3.55, N, 15.19. Found: % C, 58.55, H, 3.60, N, 15.22. IR: 3371, 3218 (2NH), 3065 (CH aromatic), 2211 (CN), 1656 (C=C) 1060 (C=S). ^{1}H NMR: 3.69 (1H, s, NH exchangeable with D_2O), 3.77(6H, s, 2 OCH_3), 6.5-8.1 (7H, m, CH aromatic), 10.5 (1H, s, 1NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 369 (67%), 367(100%), 215(8%), 213(24%), 79(9%).

4-(4-Nitrophenylamino)-6-(2,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (5a)

M.p. 147-150^{0}C , yield (82%). Analysis: for $C_{19}H_{15}N_5O_4S$, M.w (409.42). Calcd: % C, 55.74, H, 3.69, N, 17.11. Found: % C, 55.69, H, 3.73, N, 17.06. IR: 3323, 3200 (2NH), 3082 (CH aromatic), 2214 (CN), 1600 (C=C) 1157 (C=S). ^{1}H NMR: 3.7- 3.9 (6H, s, 2 OCH_3), 6.7-7.5 (7H, m, CH aromatic), 8.5-9.7 (2H, s, 2NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 409 (15%).

4-(antipyryl-4-ylamino)-6-(2,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (5b)

M.p. 155-157^{0}C, yield (77%). Analysis: for $C_{24}H_{22}N_6O_3S$, M.W (474.53). Calcd: % C, 60.75, H, 4.67, N, 17.71. Found: % C, 60.69, H, 4.59%, N, 17.78. IR: 3323, 2958 (2NH), 2935 (CH aromatic), 2210 (CN), 1600 (C=C) 1161 (C=S) ^{1}H NMR: 3.7- 3.9 (6H, s, 2OCH_3), 6.5-7.1 (8H, m, CH aromatic),

8.5-9.9 (2H, s, 2NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 474 (45%), 460 (100%), 250 (28.5%), 235 (28%), 57 (18%).

4-(4-Chlorophenylamino)-6-(2,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (5c)

M.p. 165-168^{0}C, yield (75%). Analysis: for $C_{19}H_{15}ClN_4O_2S$, M.W (398.87). Calcd: % C, 57.21, H, 3.79, N, 14.05. Found: % C, 57.27, H, 3.74, N, 15.11. IR: 3320, 3274 (2 NH), 3089 (CH aromatic), 2225 (CN), 1647 (C=C) 1157 (C=S). ^{1}H NMR: 3.7- 3.8 (6H, s, 2 OCH_3), 6.7-7.5 (7H, m, CH aromatic), 8.7- 9.9 (2H, s, 2NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 400.5 (67%),398.5 (100%), 372 (9%), 370 (27%), 282 (6.3%), 280 (20%), 133 (6.4%), 131 (20%).

4-(4-Nitrophenylamino)-6- (3,5-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (6a)

M.p. 173-175^{0}C, yield (78%). Analysis: for $C_{19}H_{15}N_5O_4S$, M.W (409.42). Calcd: % C, 55.74, H, 3.69, N, 17.11. Found: % C, 55.79, H, 3.62, N, 17.04. IR: 3371, 3218 (2 NH), 3001 (CH aromatic), 2218 (CN), 1597 (C=C), 1037 (C=S). ^{1}HNMR: 3.3 (1H, s ,NH exchangeable with D_2O), 3.7-3.8 (6H, s, 2 OCH_3), 7.08-7.6 (7H, m, CH aromatic), 10 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 409 (13%).

4-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl- 1H-pyrazol-4-ylamino)-6- (3,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (6b)

M.p. 225-227^{0}C, yield (76%). Analysis: for $C_{24}H_{22}N_6O_3S$, M.W (474.53). Calcd: % C, 60.75, H, 4.67, N, 17.71. Found: % C, 60.81, H, 4.71, N, 17.68. IR: 3301, 3263 (2 NH), 3074 (CH aromatic), 2214 (CN), 1666 (C=O), 1608 (C=C), 1091 (C=S). ^{1}H NMR: 2.46 (3H, s, C-CH_3), 3.3 (3H, s, N-CH_3), 3.77 (1H, s , NH exchange able with D_2O), 3.58- 3.77 (6H, s, 2 OCH_3), 6.5-7.4 (8H, m, CH aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 474 (43.7%), 460 (100%), 250 (27.5%), 245 (28%), 57 (19%).

4-(4-Chlorophenylamino)-6-(3,5-dimethoxyphenyl) -2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (6c)

M.p. 194-196^{0}C, yield (86%). Analysis: for $C_{19}H_{15}ClN_4O_2S$, M.W (398.87). Calcd: % C, 57.21, H, 3.79, N, 14.05. Found: % C, 57.26, H, 3.83, N, 14.09. IR: 3301.9, 3263.3 (NH), 3074.3 (CH aromatic), 2214 (CN), 1666.4 (C=C) 1172 (C=S). ^{1}H NMR: 2.46 (3H, s, C-CH_3), 3.38 (1H, s, NH exchangeable with D_2O), 3.79 (6H, s, 2 OCH_3), 3.82 (3H, s, N-CH_3) 6.5-7.9 (7H, m, CH aromatic), 9.9 (1H, s, 1NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 398.5 (22%).

General Procedure for the Preparation of Compounds (7a-c)

A mixture of 2a-c (0.01 mol) and glycine (0.01 mol) in n-butanol (30 ml) was heated under refluxed for 3 h. The soild separated was refluxed with anhydrous acetic acid (5 ml) for 2 h. The precipitate obtained after cooling was filtered off, dried then crystallized from acetic acid to give compounds (7a-c).

7-(3-Methoxyphenyl)-3-oxo-5-thioxo-2,3,5,6- tetrahydroimidazo [1,2-f]pyrimidine-8-carbonitrile (7a)

M.p. 234-236^{0}C, yield (74%). Analysis: for $C_{14}H_{10}N_4O_2S$, M.W (298.32). Calcd: % C, 56.37, H, 3.38, N, 18.7. Found: % C, 56.31, H, 3.32, N, 18.85. IR: 3420 (NH), 3095 (CH aromatic), 2211 (CN), 1766 , 1655 (C=C) 1160 (C=S). ^{1}H NMR: 3.76 (3H, s, OCH_3), 3.8 (1H, s, NH exchangeable with D_2O), 4.1 (2H, s, N-CH_2), 6.7-8.1 (4H, m, CH-aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 298 (18%).

7-(2,5-Dimethoxyphenyl) -3-oxo-5- thioxo-2,3,5,6-tetrahydroimidazo [1,2-f]pyrimidine-8-carbonitrile (7b)

M.p. 212-214^{0}C , yield (72%). Analysis: for $C_{15}H_{12}N_4O_3S$, M.W (328.35). Calcd: % C, 54.87, H, 3.68, N, 17.06. Found: % C, 54.81, H, 3.75, N, 17.13. IR: 3425 (NH), 3095 (CH aromatic), 2212 (CN), 1765 , 1655 (C=C) 1160 (C=S). ^{1}H NMR: 3.76 - 3.84 (6H, s, 2 OCH_3), 3.9 (1H, s, NH exchangeable with D_2O), 3.9 (2H, s, N-CH_2), 6.5-7.9 (4H, m, CH-aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 328 (41%).

7-(3,5-Dimethoxyphenyl) -3-oxo-5- thioxo-2,3,5,6-tetrahydroimidazo [1,2-f]pyrimidine-8-carbonitrile (7c)

M.p. 245-247^{0}C, yield (79%). Analysis: for $C_{15}H_{12}N_4O_3S$, M.W (328.35). Calcd: % C, 54.87, H, 3.68, N, 17.06. Found: % C, 54.93, H, 3.61, N, 17.11. IR: 3430 (NH), 3195 (CH aromatic), 2209 (CN), 1768 , 1655 (C=C) 1160 (C=S). ^{1}H NMR: 3.76, 3.84 (6H, s, 2 OCH_3), 3.89 (1H, s, NH exchangeable with D_2O), 4.2 (2H, s, N-CH_2), 6.3-7.8 (3H, m, CH-aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 328 (42%).

General Procedure for the Preparation of Compounds (8a-c)

A mixture of 2a-c (0.01mol) and acetohydrazide (0.01 mol) in (30 ml) butanol was heated under reflux for 48 h. The solid obtained after cooling was filtered off, dried under suction and crystallized from acetic acid to give compounds 8a-c.

7-(3-Methoxyphenyl)-3- methyl-5-thioxo-5, 6-dihydro[1,2,4] triazolo[4,3-f]pyrimidine-8-carbonitrile (8a)

M.p. 200-203^{0}C, yield (70%). Analysis: for $C_{14}H_{11}N_5OS$, M.W (297.34). Calcd: % C, 56.55, H, 3.73, N, 23.55. Found: % C, 56.61, H, 3.70, N, 23.48. IR: 3410 (NH), 3190 (CH aromatic), 2210 (CN), 1265 (C=S). ^{1}H NMR: 2.4 (3H, s, C-CH_3), 3.89 (3H, s, OCH_3), 6.5-7.4 (4H, m, CH- aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 297 (21%).

7-(2,5-Dimethoxyphenyl)-3-methyl-5-thioxo-5,6-dihydro [1,2,4] triazolo[4,3-f]pyrimidine-8-carbonitrile (8b)

M.p. 215-217^{0}C, yield (69%). Analysis: for $C_{15}H_{13}N_5O_2S$, M.W (327.36). Calcd: % C, 55.03, H, 4.00, N, 21.39. Found: % C, 55.11, H, 4.06, N, 21.32. IR: 3426 (NH), 3190 (CH aromatic), 2214 (CN), 1265 (C=S). ^{1}H NMR: 2.3 (3H, s, C-CH_3), 3.7 - 3.8 (6H, s, 2 OCH_3), 6.9-7.8 (3H, m, CH- aromatic),

10.4 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈327 (19%).

7-(3,5-Dimethoxyphenyl)-3 -methyl-5- thioxo-5,6-dihydro [1,2,4]triazolo[4,3-f]pyrimidine-8-carbonitrile (8c)

M.p. 237-240^{0}C, yield (68%)). Analysis: for $C_{15}H_{13}N_5O_2S$, M.W (327.36). Calcd: % C, 55.03, H, 4.00, N, 21.39. Found: % C, 54.98, H, 4.08, N, 21.47. IR: 3410 (NH), 3190 (CH aromatic), 2209 (CN), 1265 (C=S). ^{1}H NMR: 2.5 (3H, s, C-CH_3), 3.7-3.89 (6H, s, 2 OCH_3), 6.9-7.6 (3H, m, CH- aromatic), 11.2 (1H, s, NH exchangeable with D_2O); MS :(m/z) M^+ at m/z ≈ 327 (19%).

General Procedure for the Preparation of Compounds (9a-c)

A mixture of 2a-c (0.01 mol) and sodium azide (0.05 mol) in (30 ml) glacial acetic acid was refluxed for 3 h. The solid obtained after cooling was filtered off, dried under suction and crystallized from acetic acid to give compounds 9a-c.

7-(3-Methoxyphenyl) -5-thioxo-5, 6-dihydrotetrazolo[1,5-f]pyrimidine -8-carbonitrile (9a)

M.p. 258-260^{0}C, yield (74%). Analysis: for $C_{12}H_8N_6OS$, M.W (284.30). Calcd: % C, 50.70, H, 2.84, N, 29.56. Found: % C, 50.65, H, 2.93, N, 29.51. IR: 3430 (NH), 3190 (CH aromatic), 2214 (CN), 1595 (C=C), 1252 (C=S). ^{1}H NMR: 3.79 (1H, s, NH exchangeable with D_2O), 3.80 (3H, s, OCH3), 5.1-7.49 (4H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 264 (31%).

7-(2,5-Dimethoxyphenyl) -5-thioxo-5,6-dihydrotetrazolo[1,5-f]pyrimidine-8-carbonitrile (9b)

M.p. 207-210^{0}C, yield (78%). Analysis: for $C_{13}H_{10}N_6O_2S$, M.W (314.32). Calcd: % C, 49.67, H, 3.21, N, 26.74. Found: % C, 49.72, H, 3.28, N, 26.68. IR: 3421 (NH), 3228 (CH aromatic), 2214 (CN), 1596 (C=C), 1203 (C=S). ^{1}H NMR: 3.8 (6H, s, 2 OCH_3), 6.7-7.5 (3H, m, CH aromatic), 10 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈314 (12%).

7-(3,5-Dimethoxyphenyl)- 5-thioxo-5, 6-dihydrotetrazolo[1,5-f]pyrimidine-8-carbonitrile (9c)

M.p. 273-275^{0}C, yield (75%). Analysis: for $C_{13}H_{10}N_6O_2S$, M.W (314.32). Calcd: % C, 49.67, H, 3.21, N, 26.74. Found: % C, 49.59, H, 3.17, N, 26.82. IR: 3425 (NH), 2935 (CH aromatic), 2214 (CN), 1600 (C=C), 1203 (C=S). ^{1}H NMR: 3.8 (6H, s, 2 OCH_3), 6.6-6.9 (3H, m, CH aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 314 (22%).

General Procedure for the Preparation of Compounds (10a-c)

A mixture of 2a-c (0.01 mol) and anthranilic acid (0.015 mol) in (30 ml) n-butanol was heated under reflux for 12 h. The solid obtained after cooling was crystallized from DMF / Water to give compounds 10a-c.

3-(3-Methoxyphenyl)-10-oxo-1-thioxo-2,10-dihydro- 1H-pyrimido [6,1-b]quinazoline-4-carbonitrile (10a)

M.p. 243-245^{0}C, yield (66%). Analysis: for $C_{19}H_{12}N_4O_2S$, M.W (360.39). Calcd: % C, 63.32, H, 3.36, N, 15.55. Found: % C, 63.29, H, 3.31, N, 26.63. IR: 3436 (NH), 3943 (CH aromatic), 1685 , 2221 (CN), 1604 (C=C), 1234 (C=S). ^{1}H NMR: 3.8 (3H, s, OCH_3), 6.2-8.03 (8H, m, CH aromatic), 10.2 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 360 (22%).

3-(2,5-Dimethoxyphenyl) -10-oxo-1-thioxo-2,10-dihydro-1H-pyrimido[6,1-b]quinazoline-4-carbo -nitrile (10b)

M.p. 282-285^{0}C, yield (71%). Analysis: for $C_{20}H_{14}N_4O_3S$, M.w (390.42). Calcd: % C, 61.53, H, 3.61, N, 14.35. Found: % C, 61.49, H, 3.68, N, 14.29. IR: 3371(NH), 3224 (CH aromatic), 1698, 2214(CN), 1604(C=C), 1249 (C=S). ^{1}H NMR: 3.7-3.89 (6H, s, 2 OCH_3), 6.5-8.1(7H, m, CH- aromatic), 11.7 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 390 (15%).

3-(3,5-Dimethoxyphenyl)- 10-oxo-1-thioxo-2,10-dihydro-1H-pyrimido[6,1-b]quinazoline-4-carbo -nitrile (10c)

M.p. 240-242^{0}C, yield (75%). Analysis: for $C_{20}H_{14}N_4O_3S$, M.W (390.42). Calcd: % C, 61.53, H, 3.61, N, 14.35. Found: % C, 61.62, H, 3.58, N, 14.42. IR: 3174 (NH), 3001 (CH aromatic), 1681 , 1593 (C=C), 2214 (CN), 1203 (C=S). ^{1}H NMR: 3.7-3.9 (6H, s, 2 OCH_3), 6.5-8.2 (7H, m, CH- aromatic), 9.8 (1H, s, NH exchangeable with D_2O); MS: (m/z) M^+ at m/z ≈ 390 (14%).

General Procedure for the Preparation of Compounds (11a-c)

A mixture of 1a-c (0.01 mol), monochloroacetic acid (0.01 mol) in glacial acetic acid (20 ml) and acetic anhydride (10 ml) was heated under refluxed for 5-9 h. The precipitate was filtered off, dried on suction then crystallized from acetic acid to give compounds 11a-c.

7-(3-Methoxyphenyl)-3,5-dioxo-3,5-dihydro- 2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (11a)

M.p. 275-278^{0}C, yield (83%). Analysis: for $C_{14}H_9N_3O_3S$ M.W (299.30). Calcd: % C, 56.18, H, 3.03, N, 14.04. Found: % C, 56.13, H, 3.07, N, 14.11. IR: 3430 (NH), 2985 (CH aromatic), 2223 (CN), 1767, 1651 (CO of thiazole), 1265 (C=S). ^{1}H NMR: 3.72 (2H, s, -CH_2), 3.89 (3H, s, -OCH_3), 7.1-7.3 (4H, m, CH- aromatic); MS: (m/z) M^+ at m/z ≈ 290 (16%).

7-(2,5-Dimethoxyphenyl)-3,5-dioxo-3,5-dihydro- 2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (11b)

M.p. 263-265^{0}C, yield (85%). Analysis: for $C_{15}H_{11}N_3O_4S$, M.W (329.33). Calcd: % C, 54.71, H, 3.37, N, 12.76. Found: % C, 54.79, H, 3.42, N, 12.69. IR: 3420 (NH), 2979 (CH aromatic), 2212.5 (CN), 1745, 1687.1 (CO of thiazole), 1265 (C=S). ^{1}H NMR: 3.75 (2H, s, -CH_2), 3.81-3.9 (6H, s, 2 OCH_3),

7.0-7.34 (3H, m, CH- aromatic); MS: (m/z) M^+ at m/z ≈ 332 (100%), 304 (6.3%), 255 (11.3%), 77 (11.2%), 51 (8.5%).

7-(3,5-Dimethoxyphenyl)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (11c)

M.p. 246-248^{0}C, yield (76%). Analysis: for $C_{15}H_{11}N_3O_4S$, M.W (329.33). Calcd: % C, 54.71, H, 3.37, N, 12.76. Found: % C, 54.77, H, 3.32, N, 12.71. IR: 3424 (NH), 2980 (CH aromatic), 2214.5 (CN), 1767 , 1682.1 (CO of thiazole), 1265 (C=S). ^{1}H NMR: 3.70 (2H, s, -CH_2), 3.84-3.87 (6H, s, 2 OCH_3), 7.0-7.34 (3H, m, CH- aromatic); MS: (m/z) M^+ at m/z ≈ 330 (36.1 %), 306 (45.1%), 194 (100%), 151 (19.3%), 51(10.7%).

General Procedure for the Preparation of Compounds 12a-c, 13a-c, 14a-c

Method A

A mixture of 11a-c (0.01 mol) and different aldehydes namely, 4-nitrobenzaldehyde, 4-fluoro -benzaldehyde and 4-chlorobenzaldehyde (0.02 mol) in ethanol (30 ml) and few drops of acetic acid was refluxed for 5-8 h. The solid obtained after cooling was filtered off, dried on suction and crystallized from acetic acid to give compounds 12a-c, 13a-c, and 14a-c.

Method B

A mixture of 1a-c (0.01 mol), monochloroacetic acid (0.01 mol) and different aldehydes namely namely, 4-nitrobenzaldehyde, 4-fluoro -benzaldehyde and 4-chlorobenzaldehyde (0.02 mol) in glacial acetic acid (30 ml) and acetic anhydride (10ml) was refluxed for 8-12 h. The solid obtained after cooling was crystallized from acetic acid to give compounds 12a-c, 13a-c, 14a-c.

2-(4-Nitrobenzylidene)-7- (3-methoxyphenyl)-3,5-dioxo-3,5-dihydro -2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (12a)

M.p. 185-188^{0}C, yield (83%). Analysis: for $C_{21}H_{12}N_4O_5S$, M.W (432.41). Calcd: % C, 58.33, H, 2.80, N, 12.96. Found: % C, 58.38, H, 2.84, N, 12.91. IR: 3373 (NH), 2980 (CH aromatic), 2223 (CN), 1767 , 1697 (CO of thiazole), 1252 (C=S). ^{1}H NMR: 3.8 (3H, s, OCH_3), 6.9 (1H, d, CH=C), 7.1-8.4 (8H, m, CH aromatic)

2-(4-Fluorobenzylidene)-7-(3-methoxyphenyl)-3, 5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine -6-carbonitrile (12b)

M.p. 237-240^{0}C, yield (87%). Analysis: for $C_{21}H_{12}FN_3O_3S$, M.W (405.4). Calcd: % C, 62.22, H, 2.98, N, 10.37. Found: % C, 62.28, H, 2.94, N, 10.33. IR: 3413 (NH), 2990 (CH aromatic), 2217 (CN), 1772 , 1686 (CO of thiazole), 1249 (C=S). ^{1}HNMR: 3.8 (3H, s, OCH_3), 7.2(1H, s, CH=C), 7.2-8.2 (8H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 405 (22%).

2-(4-Chlorobenzylidene)- 7-(3-methoxyphenyl)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine -6-carbonitrile (12c)

M.p. 271-273^{0}C, yield (75%). Analysis: for $C_{21}H_{12}N_3O_3S$, M.W (421.86). Calcd: % C, 59.79, H, 2.87, N, 9.96. Found: % C, 59.71, H, 2.82, N, 9.89. IR: 3388 (NH), 2980 (CH aromatic), 2227 (CN), 1761 , 1700 (CO of thiazole), 1256 (C=S). ^{1}HNMR: 3.8 (3H, s, OCH_3), 7.2 (1H, s, CH=C), 7.25-8.25 (7H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 421 (8%), 423 (24%).

2-(4-Nitrobenzylidene)-7-(2,5-dimethoxyphenyl)-3,5- dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyramid- ine -6-carbonitrile (13a)

M.p. 217-220^{0}C, yield (72%). Analysis: for $C_{22}H_{14}N_4O_6S$, M.W (462.43). Calcd: % C, 57.14, H, 3.05, N, 12.12. Found: %C, 57.09, H, 3.13, N, 12.04. IR: 3124 (NH), 3070 (CH aromatic), 2221 (CN), 1740 , 1693 (CO of thiazole). ^{1}HNMR: 3.7-3.8 (6H, s, 2OCH_3), 7.09(1H, s, CH=C), 7.7-8.27 (8H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 464 (100%), 359 (18%), 232 (24.5%), 105(20%), 77(17%).

2-(4-Fluorobenzylidene)-7-(2,5-dimethoxyphenyl) -3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimi- dine-6-carbonitrile (13b)

M.p. 228-230^{0}C, yield (83%). Analysis: for $C_{22}H_{14}FN_3O_4S$, M.W (435.43). Calcd: % C, 60.68, H, 3.23, N, 9.65. Found: % C, 60.61, H, 3.19, N, 9.71. IR: 3326 (NH), 2800 (CH aromatic), 2225 (CN), 1693 , 1681 (CO of thiazol), 1284 (C=S). ^{1}HNMR: 3.81-3.89 (6H, s, 2OCH_3), 7.1 (1H, s, CH=C), 7.4-8.02 (7H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 435 (21%).

2-(4-Chlorobenzylidene)- 7-(2,5-dimethoxyphenyl)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimi- dine-6-carbonitrile (13c)

M.p.205-207^{0}C, yield (77%). Analysis: for $C_{22}H_{14}ClN_3O_4S$, M.W (451.88). Calcd: % C, 58.47, H, 3.12, N, 9.30. Found: % C, 58.41, H, 3.09, N, 9.36. IR: 3426 (NH), 2980 (CH aromatic), 2215 (CN), 1702 , 1681(CO of thiazol), 1260 (C=S). ^{1}H NMR: 3.7-3.81(6H, s, 2OCH_3), 7.1(1H, s, CH=C), 7.5-8.42 (7H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 451 (12%), 453 (37%).

2-(4-Nitrobenzylidene)-7- (3,5-dimethoxyphenyl)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyramid- ine-6-carbonitrile (14a)

M.p. 197-200^{0}C, yield (72%). Analysis: for $C_{22}H_{14}N_4O_6S$, M.W (462.43). Calcd: % C, 57.14, H, 3.05, N, 12.12. Found: % C, 57.19, H, 3.12, N, 12.18. IR: 3367 (NH), 2943 (CH aromatic), 2221 (CN), 1714 , 1689 (CO of thiazole), 1234 (C=S). ^{1}HNMR: 3.81-3.83 (6H, s, 2OCH_3), 7.2 (1H, s, CH=C), 7.2-8.2 (7H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 462 (22%).

2-(4-Fluorobenzylidene)-7- (3,5-dimethoxyphenyl)-3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimi- dine-6-carbonitrile (14b)

M.p.163-165^{0}C, yield (81%). Analysis: for $C_{22}H_{14}FN_3O_4S$, M.W (435.43). Calcd: % C, 60.68, H, 3.23, N, 9.65. Found: % C, 60.72, H, 3.18, N, 9.59. IR: 3386 (NH), 3035 (CH aromatic), 2216.8 (CN), 1724 , 1698 (CO of thiazole), 1235(C=S). ^{1}HNMR: 3.86-3.88 (6H, s, 2OCH_3), 7.2 (1H, s, CH=C), 7.3-8.4(7H, m, CH aromatic); MS: (m/z) M^+ at m/z ≈ 437 (100%), 422 (72%), 346 (48%), 273 (20%), 217 (17%), 93 (25%), 77 (12%).

2-(4-Chlorobenzylidene)-7-(3,5-dimethoxyphenyl) -3,5-dioxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimi -dine-6-carbonitrile (14c)

M.p. 177-180^{0}C, yield (85%). Analysis: for $C_{22}H_{14}ClN_3O_4S$, M.W (451.88). Calcd: % C, 58.47, H, 3.12, N, 9.30. Found: % C, 58.52, H, 3.19, N, 9.35. IR: 3487 (NH), 3089 (CH aromatic), 2225 (CN),1700 , 1693 (CO of thiazole). ^{1}HNMR: 3.31-3.77 (6H, s, 2 OCH_3), 6.71 (1H, s, CH=C), 6.7-7.78 (7H, m, CH-aromatic); MS: (m/z) M^+ at m/z ≈ 451 (22%), 453 (67%).

Chemosensitivity Assay

The ability of thiouracil derivatives to inhibit the proliferation of liver cancer cells (HepG2) was evaluated using SRB assay as previously described(Skehan *et al.*, 1990). Briefly, cells ($1x10^4$ cells/well) were left to adhere overnight in a 96-well plate at 37°C in humidified atmosphere containing 5% CO_2 prior to exposure to a suitable range of drug concentrations for 48 h. cells were then fixed in ice-cold TCA (10% w/v), washed in five times in ice-cold water and left to dry in air. Cells were then stained for 1 h in sulfo-Rhodamine-B stain (0.4 w/v in 1% acetic acid). Excess stain was removed by acetic acid (1%) before the attached stain was solubilized in Tris base (10 mM). The absorbance of the resulting solution was measured at 515 nm using a multiwall spectrophotometer. Survival and chemosensitivity were expressed as IC_{50} values. All experiments were performed in triplicates.

Molecular Docking

All docking studies were performed using 'Internal Coordinate Mechanics [Molsoft ICM 3.5-0a]. A set novel thiopyrimidine derivatives, were compiled by us using ChemDraw. 3D structures were constructed using Chem 3D ultra 12.0 software [Molecular Modeling and Analysis, Cambridge Soft Corporation, USA (2010)]. The selected compounds were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Job Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (* .mol). The thymidylate synthase (PDB id 1JU6) which was retrieved from PDB bank http://www.rcsb.org/pdb. All bound waters ligands and cofactors were removed from the protein prior to the docking process.

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