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Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

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To cite this article: Mohamed M. Mohamed, Ali K. Khalil, Eslam M. Abbass & Abeer M. El-Naggar (2017): Design, Synthesis of New Pyrimidine Derivatives as Anticancer and Antimicrobial Agents, Synthetic Communications, DOI: 10.1080/00397911.2017.1332223

To link to this article: http://dx.doi.org/10.1080/00397911.2017.1332223

Design, synthesis of new pyrimidine derivatives as anticancer and antimicrobial agents

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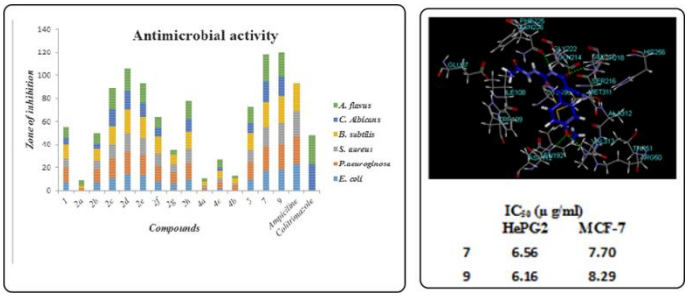
Received 07 March 2017.

Abstract

A new series of 6-aryl-5-cyano thiouracil derivatives were synthesized. Cyanouracil **1** was condensed with monochloroacetic acid and different aldehydes to give thiazolopyrimidine **2**. On the other hand, treatment of cyanouracil **1** with 2-chloro-N-substituted-phenylac etamide afforded **4**. Hydrazinolysis of **6** afforded the hydrazino derivatives **7** which upon reaction with different electrophilic reagents such as acetic anhydride, benzoyl chloride and carbon disulfide yielded pyrimidine derivatives **8-15**. Some of the new derivatives were explored for their antimicrobial activities. Compounds **7** and **9** have a promising activity, relatively equipotent to the reference drug. All of the new synthesized compounds were tested *in vitro* for their anti-proliferative activities against HePG-2 and MCF-7 cell lines. Compounds **7**, **9** and **2d** displayed potent growth inhibitory effect toward the two cell lines more than the standard drug 5-FU. Furthermore, a docking study of the most active compounds was carried out with thymidylate synthase enzyme.

GRAPHICAL ABSTRACT



KEYWORDS: antimicrobial, docking, pyrimidines, thiazolopyrimidines, triazolopyrimidine

INTRODUCTION

Dihydropyrimidines occupy a unique place in medicinal chemistry due to their wide application as drug and drug-intermediates possessing diverse pharmacological activities including antitumor, analgesic, antineoplastic, cardiovascular and antiallergic activities.[1-5] Similarly, the related thiouracil therapeutic derivatives have been known as potential antiviral, antioxidant, anticancer and antimicrobial agents.[6-9] Moreover, 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 and HCV inhibiting activities **Figure 1**. [10-14]

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. [15] 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. [16] In the light of the aforementioned facts, this work aims to design and synthesize of a new series of thiouracil carbonitrile derivatives hoping that they could have some chemical and biological interest.

RESULTS AND DISCUSSION

Chemistry

In connection with our program aiming to the synthesize and evaluate the biological activity of fused heterocycles,[17-19] we have tried to designed and synthesized a novel series of biologically active pyrimidines derivatives and evaluated their anticancer activity. Thus, thioxopyrimidine **1** was condensed with chloroacetic acid and different aromatic aldehydes in a mixture of acetic acid and acetic anhydride in the presence of fused sodium acetate to yield thiazolopyrimidine derivatives **2a-h** (Scheme 1).

The structures of compounds **2a-h** were confirmed by their analytical and spectral data. Thus the IR spectrum of **2** showed the disappearance of NH band and presence of characteristic absorption band corresponding to the vibrational coupling of carbonyl groups around 1769-1671cm^{-1} which elucidate the rout of cyclization, this direction of cyclization may be due to the steric hindrance of anisyl group moiety.[20, 21] ^{1}H-NMR spectra for this group exhibited a singlet signal corresponding to the ethylinic protons at the range 7.07-8.46 ppm. Further evidence was gained from their mass spectra that showed the correct molecular ion peaks beside some of abundant peaks (cf.

experimental). The mechanistic pathway for the transformation of **1** to **2** is represented in (Scheme 2).

Compound **1** was allowed to react with the appropriate 2-chloro-N-substituted-phenylacetamide (**3a-c**) in the presence of potassium carbonate, in dimethylformamide (DMF) to yield the target compounds 2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-aryl) acetamide and 3-((4-chloro-2-methylphenyl)amino)-7-(4-methoxyphenyl)-5-oxo-5H-thiazolo[3, 2-a]pyrimidine-6-carbonitrile (**4a-c**) in good yield. Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in (Scheme 1). The reaction proceeded via nucleophilic substitution reaction followed by cyclization in case of **4c.** IR spectra of **4a,b** revealed the existence of bands in the frequency range 3293-3308 and 1654-1711 cm^{-1} corresponding to NH_2, NH and C=O groups, respectively. ^{1}H-NMR spectra comfirmed the structure of compounds **4a-c.** where spectrum of **4a** showed signals at 4.16, 10.41 ppm corresponding to CH_2 and two NH group, respectively. Meanwhile, **4b** spectrum showed signals at 4.19**,** 10.67, 13.78 ppm corresponding to CH_2, NH and NH_2 groups, respectively. However, ^{1}H-NMR spectrum of **4c** exhibited a singlet signal at 9.63 corresponding to NH which supported the suggested structure. On the other hand, *N*-alkylation of **1** with *p*-toluene sulphonyl chloride produced 6-(4-methoxyphenyl)-4-oxo-2-thioxo-1-tosyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**5)** which was carried out by stirring in dry DMF utilizing potassium carbonate as base catalyst. The IR spectra of compound **5** showed the disappearance of NH bands and the appearance of characteristic absorption bands corresponding to SO_2 at 1167 cm^{-1}**.** The

^{1}H-NMR spectra of compound **5** revealed two singlet signals at 2.69, 13.11 ppm assignable to CH_3 and NH groups respectively. On the other hand, Compound **1** was alkylated with ethylchloroacetate, in the presence of anhydrous potassium carbonate to give the ethyl ester derivative **6.** Analytical and spectral data were in agreement with the proposed structure. The IR spectrum showed signals at 3301, 1735, and 1660 cm^{-1} corresponding to NH, (CO ester) and (CO) amide respectively. The ^{1}H-NMR spectrum of **6** revealed signals at 1.08, 4.05 and 13.7 ppm for CH_3, CH_2 and NH respectively. The structure of compound **6** was established chemically by the reaction with hydrazine hydrate in boiling ethanol afforded 2-hydrazinyl-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **7** (Scheme 1). The hydrazine derivative **7** was also isolated by treating **1** with hydrazine hydrate under the same reaction condition. The structure of compound **7** was corroborated by spectroscopic data.

The IR spectrum showed bands at 3324, 3277 and 1685 cm^{-1} attributed to NH_2, NH and C=O groups respectively. The ^{1}H NMR spectrum of compound **7** showed signals at 3.49 and 8.19 attributed to NH_2, NH and at 10.15 for NH (amide) groups, respectively. A plausible mechanism for the formation of the hydrazine derivative **7** is outlined in (scheme 3). Hydrazino pyrimidines can be considered as key starting precursor for synthesis of new pyrimidine derivatives which might have chemotherapeutic and biological activity. So, the hydrazino derivative **7** was reacted with some electrophilic reagents such as arylidine malononitrile, formic acid, acetic anhydride, cyclopentanone, benzoyl chloride and cabon disulphide. Thus, when the hydrazino derivative **7** was subjected to react with arylidene in pyridine, it afforded the Schiff base product **8**. The

conversion of **7** to **8** could be visualized on the basis of the nucleophilic attack by nitrogen of the hydrazino group at the electron deficiency β- carbon of the arylidene followed by elimination of malononitrile molecule. (C.f. Scheme 5).

IR of **8,** showed bands at 3223, 1658 cm^{-1} corresponding to NH, C=O groups, respectively. ^{1}H-NMR of **8** revealed signals at 3.79, 3.82, 8.10, 12.19 and 12.37 corresponding to 2 OCH_3, olifinic CH and 2NH groups. A strong evidence for the structure of **8** was gained chemically by reacting the hydrazine derivative **7** with *p*-methoxybenzaldehyde under the same condition. Refluxing of hydrazino derivative **7** with acetic anhydride afforded the mono acetyl derivative **9**. The spectral data of compound **9** was in agreement with the assigned structure. IR spectrum displayed bands at 3226, 1630-1660 cm^{-1} corresponding to NH, 2(CO) groups, respectively. ^{1}H-NMR showed signals at 1.89, 10.19 ppm corresponding to CH_3, NH groups, respectively. 2-(2-cyclopentylidenehydrazinyl)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **10** was obtained by refluxing compound **7** with cyclopentanone in ethanol. Its ^{1}H-NMR showed multiplet signals at (1.65-1.96) ppm due to CH_2 groups of cyclopentanone moiety (cf. Experimental part).

However, stirring of 2-Chloro-N-(4-chlorophenyl)acetamide with hydrazino derivative **7** afforded N-(4-chlorophenyl)-2-(2-(5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)hyda-zinyl) acetamide **11.** IR spectrum of **11** showed signals at 3317, 1700, 1649 cm^{-1} corresponding to NH and two C=O groups, respectively. ^{1}H-NMR of **11** showed signals at 3.85, 4.86 and 8.76, 10.63 ppm for OCH_3, CH_2 and 4NH groups,

respectively. Diazotization of compound **7** resulted in the formation of tetrazolopyrimidine derivative **12**. The structure of **12** was elucidated by the spectral data. IR showed absorption bands at 3221 and 1651 cm^{-1} due to NH and CO groups, respectively and disappearance of NH_2 group. In addition to ^{1}H-NMR exhibited signals at 3.83 and 5.96 ppm attributed to OCH_3 and NH groups, respectively.

Treatment of the hydrazino derivative **7** with formic acid afforded triazolopyrimidone derivative **13** (Scheme 6). IR spectrum was in agreement with the predicted structure as it showed the disappearance of NH_2 bands. In addition, ^{1}H-NMR spectrum revealed signals at 9.23 ppm for olefinic CH. Triazolopyrimidone derivative **14** was obtained by stirring **7** with benzoyl chloride in dry DMF with anhydrous potassium carbonate. The structure of compound **14** was characterized by the presence of bands 3267 and 1685cm^{-1} for the amide groups NH and C=O groups. Moreover, ^{1}H-NMR spectra exhibited a singlet band at 8.65 ppm for NH group. Refluxing of compound **7** with carbon disulphide in pyridine afforded triazolopyrimidone derivative **15**. The formation of **15** is assumed to proceed via nucleophilic addition of nucleophilic amino group of the hydrazino moiety to C=S group of carbon disulfide followed by cyclization and elimination of H_2S molecule. (Scheme 7) IR spectrum of **15** exhibited signals at 3421, 1679 due to twoNH and C=O groups, respectively. In addition, an absorption band was revealed at 1452 cm^{-1} corresponding to C=S group. Moreover, ^{1}H NMR showed two singlet signals at 9.30 and 9.80 ppm attributed to two NH groups.

BIOLOGICAL ACTIVITY

Antimicrobial Studies

Some of the newly synthesized compounds **1**, **2**, **4**, **5**, **7**, **9** and **13** were assayed *in vitro* for their antimicrobial activity against two gram negative bacteria: namely, *Pseudomonas aeruginosa* and *Escherichia coli*, and two gram positive bacteria: *Staphylococcus aureus and Bacillis subtilis* and two fungal species, namely: *Aspergillus flavus and Candida albicans*. The fungicide *Colitrimazole* and the bactericides *Ampicillin* were used as references to evaluate the potency of the tested compounds under the same conditions. As a parameter of antimicrobial activity, the values of the zone of inhibition (mm) and % activity index were determined. The results are depicted in **Table 1**, (**Figure 2**).

It has been observed that compounds **2d, 7** and **9** possess a pronounced antimicrobial activity against *Staphylococcus aureus, Bacillis Subtilis*, *Escherichia coli, Candida albicans* and *Aspergillus flavus* compared to the reference drugs, while, compounds **2c, 2e** and **2h** showed exerted moderate activity. Compounds (**1**, **2b**, **2f**, **4c**, **4a**, and **5**) showed low activity. However, compounds **2a**, **4c**, **4b** and **13** were either inactive or weakly active against the tested microorganisms. Hydrazino derivative **7** and acetohydrazide derivative **9** caused a pronounced inhibition effect against Gram-positive (*Staphylococcus aureus, Bacillus cereus*), Gram-negative (*Escherichia coli*) bacteria and fungi (*Candida albicans* and *A. flavus)*. On the other hand, increased antibacterial activity was achieved by cyclization to thiazolopyrimidine. However, cyclization to triazolopyrimidine as in **13** and alkylation in **4(a-c)** either decreased or diminished antimicrobial activity, respectively.

In Vitro Anti-Proliferative Activity

Anticancer activity screening of the newly synthesized compounds was measured *in vitro* utilizing two different human cancer cell lines namely, hepatocellular carcinoma (HePG-2), mammary gland (MCF-7), using sulforhodamine B (SRB) colorimetric assay as described by Skehan et al.[22] Due to the structure similarity between 5-FU (**Figure 3**) 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 2**.

The results obtained indicated that the tested compounds exhibited good, moderate or weak anti-proliferative activities against the tested cell lines. In general, the compounds **7, 9, 2d** and **2e** were found to be the most potent derivatives against the two cell lines. Compound **7** was 0.62 and 0.62 times as active as 5-FU against HepG2 (IC_{50} = 25.5 µM) and MCF-7 (IC_{50} = 23.9 µM), respectively. Compound **9** it was 0.66 and 0.72 times as active as 5-FU against HepG2 (IC_{50} = 25.7 µM) and MCF-7 (IC_{50} = 25.7 µM), respectively. Compound **2d** it was 0.62 and 0.70 times as active as 5-FU against HepG2 (IC_{50} = 24.1 µM) and MCF-7 (IC_{50} = 27.7 µM), respectively. Compound **2e** it was 1.06 and 0.58 times as active as 5-FU against HepG2 (IC_{50} = 40.9 µM) and MCF-7 (IC_{50} = 22.4 µM), respectively.

Compound **2g** it was 0.60 times as active as 5-FU against HepG2 (IC_{50} = 23.4 µM). However, it was possessed moderate anti-proliferative activities against MCF-7 cell. Compound **2h** it was 0.96 times as active as 5-FU against MCF-7 (IC_{50} = 36.9 µM). However, it was possessed moderate anti-proliferative activities against HepG2 cell.

Compound **15** it was 0.90 times as active as 5-FU against HepG2 (IC_{50} = 34.9 µM) and possessed moderate anti-proliferative activities against MCF-7 cell. The compounds **(2a-c), 2f, (4a-c), 6, 5, 8, 10, 11, 12, 13** and **14** were found to possess either moderate or weak anti-proliferative activities against the two cell lines .

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 deoxyuridylate to deoxythymidylate.[23] Molecular docking comparing the binding affinity of 5-FU and thiouracil derivatives was therefore conducted.

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 id1JU6) which was retrieved from PDB bank http://www.rcsb.org/pdb using 5-FU as a reference for docking results. The results of docking study were reported as TS binding free energy (ΔG). The negative values of free energies refer to spontaneity of bindings **Table 3**.

The docking results revealed that, the highest binding compound to TS was **7** with binding energy of -42.23 Kcal/mol. Compound **7** formed six hydrogen bonds with the amino acid residues of thymidilate synthase. Carbonyl group at the 4-position of pyrimidine moiety was anchored by hydrogen bonding interactions with His 256. Cyano group at the 5-position of pyrimidine moiety was involved in a hydrogen bonding interaction with ASP218 between N of the subitituted NH $\underline{N}H_2$ group at the 2-position of thiopyrimidine ring of the ligand and the amino acids Ala312 and Val 313 and as well as between N at $\underline{N}H$-NH_2 of the substituted group at the 2-position of thiopyrimidine ring and the amino acid Try 258 of the enzyme **Figure 4**.

On the other hand, compound **9** exhibited promising TS inhibitory activity (IC_{50} = 25.73± 2.22 µM) and TS inhibition % = 0.72). Introduction of acetyl moiety of the compound **9** is likely important for increasing the affinity for the receptor. It showed hydrogen bonds with the amino acid Asn226, Arg50, Asn112, Gln214, and Asp218. (**Figure 5**).

The proposed binding mode of compound **2d** showed affinity value of -39.19 kcal/mol. It showed one hydrogen bond interaction between S at fused bicyclic system and the amino acid Ser216, Oxygen atom at thiazolidine moiety formed interaction with the amino acid Arg50. In addition, It showed one hydrogen bond interaction between OCH_3 at 4-position of phenyl moiety and Ans226 **Figure 6**.

EXPERIMENTAL PART

Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. The IR spectra were recorded on a Pye-Unicam SP-3-300 infrared spectrophotometer (KBr dicks) and expressed in wave number (cm^{-1}). ^{1}H NMR spectra were run at 400 MHz, on a Varian Mercury VX-300 and BrukerAvance III NMR spectrometer respectively, while ^{13}C NMR spectra were run at 75 MHz. TMS was used as an internal standard in deuterated dimethylsulphoxide (DMSO-*d6*). Chemical shifts (δ) are quoted in ppm. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. All coupling constant (*J*) values are given in hertz. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were performed on CHN analyzer and all compounds were within ± 0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. All reagents and solvents were purified and dried by standard techniques. All the newly synthesized compounds gave satisfactory elemental analysis. Compounds **1** [14], **3(a-c)** [24, 25] are previously reported.

General Procedure For The Preparation Of Compounds 2a-H

A mixture of **1** (10 mmol) and different aldehydes namely, 4-methoxybenzaldehyde, thiophene- carboxaldehyde, 3,4-dimethoxybenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, benz- aldehyde, 3,4-dichlorobenzaldehyde and salycialdehyde (0.01 mol) in a mixture of glacial acetic acid (30 mL)/acetic anhydride (15 mL) in the presence of anhydrous sodium acetate (2 g) was refluxed for 5-8 h. The solution was cooled,

gradually poured onto cold water, and the formed precipitate was washed several times with water, filtered off, and recrystallized from ethanol to afford pure product **2a-h**.

2-(4-Methoxybenzylidene)-7-(4-Methoxyphenyl)-3,5-Dioxo-3,5-Dihydro-2H-Thiazo Lo[3,2-A]Py Rimidine-6-Carbonitrile (2a)

Yield 78 %; yellow crystal; m.p. 291-293 ^{o}C (EtOH); IR (cm^{-1}) *v*: 3040 (CH aromatic), 2845 (CH aliphatic), 2213 (CN), 1751, 1685 (CO). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.14 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.16 (d, 2H, Ar-H, *J* = 7.2 Hz), 7.73 (d, 2H, Ar-H, *J* = 8.8Hz), 8.06 (d, 2H, Ar-H, *J* = 7.2 Hz), 8.12 (s, 1H, CH ethylenic). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 56.3 (2), 99.5, 115.2 (4), 118.6 (2), 128.7 (2), 129.2, 130.2, 133.8, 133.4, 145.4, 161.7 (2), 166.7, 167.6, 168.8, 170.3; **MS (*m/z*):** 417 (M^{+}, 19%), 164 (100 %); Anal. Calcd for: $C_{22}H_{15}N_3O_4S$ (417.44): C, 63.30; H, 3.62; N, 10.07; Found: C, 63.07; H, 3.50; N, 9.96 %.

General Procedure For The Preparation Of Compounds 4a-C

To a suspension of (**1**) (0.01 mol) in dry DMF (20 ml) containing anhydrous K_2CO_3 (0.01 mol), 2‑chloro‑N‑substituted‑phenylacetamide (0.01 mol) (**3a-c**) was added. The reaction mixture was stirred at room temperature for 8-10 h, coold, poured onto crushed ice, and then acidified with dilute acetic acid. The obtained precipitate was filtered, washed with H_2O, dried, and recrystallized from ehanol to afforded **4a-c**.

N-(4-Chlorophenyl)-2-(5-Cyano-4-(4-Methoxyphenyl)-6-Oxo-1,6-Dihydropyrimidin-2-Ylthio) Acetamide (4a)

Yield 77 %; white crystal; m.p. 268-270 ^{o}C (EtOH); IR (cm^{-1}) *v*: 3308 (NH), 3083 (CH aromatic), 2841 (CH aliphatic), 2222 (CN), 1683 (CO amide), 1654 (CO amide), ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 3.78 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 6.83 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.34 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.54 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.88 (d, 2H, Ar-H, *J* = 8.0 Hz), 10.41 (br. s, 2H, 2NH); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 35.4, 55.4, 91.4, 113.6 (2), 116.1, 120.4 (2), 126.9, 127, 128.7 (2), 130.8 (2), 127.9, 161, 162, 164.9, 165.3, 166.1; **MS (*m/z*):** 426 (M^+, 25 %), 428 (M^++2, 8.32 %), 126 (100 %); Anal. Calcd for: $C_{20}H_{15}ClN_4O_3S$ (426.88): C, 56.27; H, 3.54; N, 13.12; Found: C, 56.08; H, 3.49; N, 13.00 %.

Formation Of 2-(2-(4-Methoxybenzylidene)Hydrazinyl)-4-(4-Methoxyphenyl)-6-Oxo-1,6-Dihydro Pyrimidine-5-Carbonitrile (8).

A mixture of **7** (10 mmol) and 2-(4-methoxybenzylidene)malononitrile (10 mmol) was refluxed in pyridine (20 mL)for 8 hr. The reaction mixture was cooled, acidified with dilute acetic acid and the solid obtained was filtered off, dried and then recrystallized form ethanol to give **8**.

Yield 75 %; brown crystal; m.p. 296-297 ^{o}C (EtOH); IR (cm^{-1}) *v*: 3223 (NH), 3168 (CH aromatic), 2835 (CH aliphatic), 2208 (CN), 1658 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.79 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.96 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.06 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.87 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.94 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.10 (s, 1H, CH), 12.19 (s, 1H, NH); 12.37 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 57.2 (2), 95.0, 114.3 (2), 116.4 (2), 118.8, 128.1, 129.5 (2), 132.8, 135.5,

136.1, 145.4, 160.8, 162.9, 164.7, 171.2, 174.4; **MS (*m/z*):** 375 (M^+, 5 %), 226 (38 %), 107 (100 %); Anal. Calcd for: $C_{20}H_{17}N_5O_3$ (375.38): C, 63.99; H, 4.56; N, 18.66; Found: C, 63.65; H, 4.62; N, 18.59 %.

Formation Of 5-(4-Methoxyphenyl)-7-Oxo-3,7-Dihydrotetrazolo[1,5-A]Pyrimidine-6-Carbonitril (12).

Compound **7** (10 mmol) was dissolved in (10 mL) concentrated hydrochloric acid and then (7 mL) of sodium nitrite (10 %) was added dropwise with stirring over a period of 2 hours at 0 ^oC. The obtained yellow solid was filtered off, dried and recrystallized from ethanol to give **12** as yellow crystals.

Yield 77 %; yellow crystal; m.p. 237-238 ^oC (EtOH); IR (cm^{-1}) *v*: 3223 (NH), 3076 (CH aromatic), 2836 (CH aliphatic), 2222 (CN), 1651 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.83 (s, 3H, OCH_3), 5.96 (s, 1H, NH), 7.08 (d, 2H, Ar-H, J = 8.0 Hz), 7.78 (d, 2H, Ar-H, J = 8.0 Hz). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 55.2, 94.4, 117.1 (2), 118.9, 128.4 (2), 130.3, 132.5, 164.1, 167.5, 171.6; **MS (*m/z*):** 268 (M^+, 20 %), 225 (100 %); Anal. Calcd for: $C_{12}H_8N_6O_2$ (268.23): C, 53.73; H, 3.01; N, 31.33; Found: C, 53.61; H, 2.92; N, 31.25 %.

Formation Of 7-(4-Methoxyphenyl)-5-Oxo-1,5-Dihydro-[1,2,4]Triazolo[4,3-A]Pyrimidine-6-Ca-Rbonitrile (13).

A mixture of **7** (10 mmol) and formic acid (20 mL) was refluxed for 8-10 h. the solid was filtered off, dried and then recrystallized from ethanol to give **13**. Yield 80 %; yellow

crystal; m.p. 250-252 ^{o}C (EtOH); IR (cm^{-1}) *v*: 3408 (NH), 3111 (CH aromatic), 2848 (CH aliphatic), 2219 (CN), 1701 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.84 (s, 3H, OCH_3), 7.07 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.09 (s, 1H, NH), 7.87 (d, 2H, Ar-H, *J* = 8.0 Hz); 9.29 (s, 1H, CH); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ (ppm): 55.4, 82.3, 113.4, 113.7, 117.2, 128.3, 130.2, 130.4, 133.1, 150, 155.5, 161.7, 168,7; Anal. Calcd for: $C_{13}H_9N_5O_2$ (267.24): C, 58.43; H, 3.39; N, 26.21; Found: C, 58.29; H, 3.29; N, 26.12 %.

Biological Assay

Antimicrobial Activity

Materials And Methods

Chemical compounds were individually tested against a panel of two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) using Muller Hinton agar medium (Oxoid). The anti-fungal activities of the compounds were tested against two fungi (*Candida albicans* and *Aspergillus flavus*) using Sabouraud dextrose agar medium (Oxoid). Each of the compounds was dissolved in DMSO and solution of the concentration 1 mg /mL was prepared. Separately paper discs of Whatman filter paper were prepared with standard size (5cm), were cut and sterilized in an autoclave [26]. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petri dishes containing Muller Hinton agar medium (Oxoid) seeded with *Staphylococcus aureus , Bacillus subtilis , E. coli , Pseudomonas aeuroginosa* and *Candida albicans , Aspergillus flavus*. using Sabouraud dextrose agar medium (Oxoid). The petri dishes were incubated at 36 c and the inhibition zones were recorded after 24 h

of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic *ampicillin* and antifungal *Colitrimazole* was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under :

$$\%\,Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ \ diametre}{Zone\ of\ inhibition\ by\ standard\ \ diametre} \times 100$$

Cytotoxicity Assay

Cell Line

The cytotoxic activity of fourteen compounds was tested against two human tumor cell lines namely hepatocellular carcinoma HePG2 and mammary gland breast cancer MCF-7. The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil was used as a standard anticancer drug for comparison.

Chemical Reagents

The reagents used were RPMI-1640 medium, MTT, DMSO, 5-fluorouracil (sigma co., St. Louis, USA), and Fetal Bovine serum (GIBCO, Paisley, UK).

MTT Assay

The different cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay.[27-29] This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in

RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0x104 cells/well[66] at 37 °C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech,Winoosky, VT,USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A_{570} of untreated sample) X 100.

Molecular Docking

All docking studies were performed using 'Internal Coordinate Mechanics [Molsoft ICM Discovery Studio]. A set novel thiopyrimidine derivatives, were compiled by us using ChemDraw. 3D structures were constructed using Chem 3D ultra12.0 software [Molecular Modeling and Analysis, Cambridge Soft Corporation, USA (2010)]. The selected compounds were energetically minimized by using MOPAC (semi-empirical quantum mechanics), ynternational coordinate 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.

CONCLUSION

A series of novel cyanothiouracil derivatives (**2-15**) was synthesized from 6-aryl-cyano thiouracils and 6-aryl-4-hydrazino-2-thioxo-1, 2-dihydropyrimidine-5-carbonitriles derivatives. Antimicrobi al and anticancer studies were performed. Potent activities were observed for some of the synthesized compounds.

REFERENCES

1. Kreutzberger, A.; Schimmelpfenning, H. Tumour inhibitory agents. Part-12: 2-Ureido-4-(3, H)-pyrimidinones. *Arch. Pharm.* **1981**, *314*, 34–41.

2. Ueda, T.; Sakkakibara, J.; Nakagami, J. Synthesis of 5-Amino-4 (3H)-pyrimidinones. II. Synthesis of 5-Acyl- and 5-Alkylamino-3-phenyl-6-methyl-4 (3H)-pyrimidinones and Determination of Their Analgesic and Antiinflammatory Activities**.** *Chem. Pharm. Bull***. 1983,** *31***,** 4263 **-** 4269**.**

3. Kotwa, R.; Krepelka, J.; Melka, M. Czech CS 254, 620 (Cl C 07 D 239/47) **1988**, *907*, 1986-3.

4. Atwal, K. US Patent US 4,769,371 (Cl514-275, C 07 D 239/42) 1988, Appl. 45956 01, **1987**, 14.

5. Ozeki, K.; Ichikawa, T.; Hiroyuki, T.; Tanimury, K.; Sato, M.; Yaginuna, H. Studies on antiallergy agents. III. Synthesis of 2-anilino-1,6-dihydro-6-oxo-5-pyrimidinecarboxylic acids and related compounds. *Chem. Pharm. Bull*. **1989**, *37*, 1780–1787.

6. Youssif, S.; Mohamed, S. F. 6-Amino-2-thio- and 6-Aminouracils as Precursors for the Synthesis of Antiviral and Antimicrobial Methylenebis (2-

thiouracils), Tricyclic Pyrimidines, and 6-Alkylthiopurine-2-ones. *Chem*. **2008**, *139*, 161-168.

7. Mondal, P.; Jana, S.; Kanthal, L. K. Synthesis of novel mercapto-pyrimidine and amino-pyrimidine derivatives of indoline-2-one as potential antioxidant & antibacterial agent. *T. Pharm. Res*. **2010**, *3*, 17-26.

8. Agbaje, O. C.; Fadeyi, O.O.; Fadeyi, S.A.; Myles, L.S.E.; Okoro, C.O. Synthesis and in vitro cytotoxicity evaluation of some fluorinatedhexahydropyrimidine derivatives. *Med. Chem. Lett*. **2011**, *21*, 989-992.

9. Prachayasittikul, S.; Sornsongkhram, N.; Pingaew, R. Techatanachai, S.; Ruchirawat, S.; Prachayasittikul,V. Synthesis and novel bioactivities of substituted 6-propylthiouracils. *Eur. J. Sci. Res*. **2009**, *36*, 236-245.

10. Ram, V. J.; Vanden, B. D. A.; Vlietinck, A. Chemotherapeutical Agents V. Syntheses and Activities of Novel Pyrimidines Derived from
5- Cyano- 6- aryl- 2- thiouracil. *Liebigs Ann. Chem.* **1987**, *9*, 797-801.

11. Wyrzykiewicz, E.; Bartkowiak, G.; Nowakowska, Z.; Kedzia, B. Synthesis and antimicro- bial properties of S-substituted derivatives of 2- thiouracil. *Farmaco.* **1993**, *48 (7)*, 979-988.

12. Kamalakannan P.; Venkappayya, D. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. *J. Inorg. Biochem.* **2002**, *90 (1)*, 22-37.

13. Ibrahim, D. A.; El-Metwally, A. M. synthesis, and biological evaluation of novel pyrimidine derivatives as CDK2 inhibitors. *Eur J Med Chem*. **2010**; *45 (3)*, 1158-1166.

14. Ding, Y.; Girardet, L.; Smith, K. L.; Larson, G.; Prigaro, B.; Wu J. Z.; Yao, N. Parallel synthesis of 5-cyano-6-aryl-2-thiouracil derivatives as inhibitors for hepatitis C viral NS5B RNA-dependent RNA polymerase. *Bioorg. Chem*. **2006**, *34*, 26–38.

15. Chan Katie, K. L.; Zhang, Q.; Dianov, G. L. Base excision repair fidelity in normal and cancer cells. *Mutagenesis*. **2006**, *21(3)*, 173-178.

16. MacKenzie, R. E. Biogenesis and interconversion of substituted tetrahydrofolates. *Folates and Pterins*, **1984**, *1*, 255-306.

17. Eissa, I. H.; El-Naggar, A. M.; El-Hashash, M. A.; Design, synthesis, molecular modeling and biological evaluation of novel 1H-pyrazolo[3,4-b]pyridine derivatives as potential anticancer agents, *Bioorg. Chem*. **2016**, 67, 43–56.

18. El-Hashash, M. A.; El-Naggar, A. M.; El-Bordany1, E.A.; Marzouk, M.I.; S. Nawar, T. M. Regioselectivity and regiospecificity of benzoxazinone (2-isopropyl-4H-3,1-benzoxazi- none) derivatives toward nitrogen nucleophiles and evaluation of antimicrobial activity. *Synth Commun*. **2016**, *46 (14)*, 1230–1241.

19. El-Hashash, M. A.; El-Naggar1, A. M.; El-Bordany1, E.A.; Marzouk1, M.I.; S. Nawar, T. M. 6-Iodo-2-Isopropyl-4*H*-3,1-Benzoxazin-4-one as Building Block in Heterocyclic Synthesis. *Synth Commun*. **2016**, *46 (24)*, 1230–1241.

20. Hamouda, A. M. Synthesis of novel pyrimidines thiazolopyrimidines, triazolopyrimidines and pyrimidotriazines as potent antimicrobial agent. *Der Pharma Chemica*, 2014, *6(6)*, 346-357.

21. Edwin, S. G. Mechanism and Structure in Organic Chemistry; Holt, Rinchart and Winston: New York, 1995, pp 377-381.

22. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; New colorimetric cytotoxicity assay for anticancer-drug screening, *J. Natl. Cancer Inst*. **1990**, *82 (13)*, 1107–1112.

23. Longley, D. B.; Harkin, D. P.; Johnston, P. G. 5- fluorouracil: mechanisms of action and clinical strategies. *Nat. Rev. Cancer*. **2003**, *3 (5)*, 330-338.

24. Ghorab, M. M.; Abou El Ella, D. A.; Heiba, H. I.; Soliman, A. M. Synthesis of Certain New Thiazole Derivatives Bearing a Sulfonamide Moiety with Expected Anticancer and Radiosensitizing Activities. *Mater Sci Eng A Struct Mater*. **2011**, *A1*, 684-691.

25. Rami, C.; Patel1, L.; Patel, C. N.; Parmar, J. P. Synthesis, antifungal activity, and QSAR studies of 1,6‑dihydropyrimidine derivatives *J Pharm Bioall Sci*. **2013**, 5, 277-89.

26. Stylianakis, A.; Kolocouris, N.; Kolocouris, G.; Fytas, G.B.; Foscolos, E.; Padalko, J.; Neyts, D.; Clerq, E. Spiro[pyrrolidine-2,2'-adamantanes]: synthesis, anti-influenza virus activity and conformational properties. *Bioorg, Med. Chem. Lett*. **2003,** *13*, 1699 -1703.

27. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* **1983**, *65*, 55-6

28. Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods.* **1986**, *22*, 271-277

29. Mauceri, H. J.; Hanna, N. N.; Beckett,M. A.; Gorski, D. H.; Staba, M. J.; Stellato, K. A.; BigelowK.; Heimann, R.; Gately, S.; Dhanabal, M.; Soff, G. A.; Sukhatme, V. P.;

Kufe D.; Weichselbaum, R. R. Combined effects of agiostatin and ionizing radiation in antitumour therapy. *Nature.* **1998**, *394*, 287-291.

Table 1. Preliminary antibacterial activity for some of the synthesized compounds.

Compound	Diameter of inhibition zone (mm). % Activity index					
	E. coli	P.aeuroginosa	S. aureus	B. subtilis	C. Albicans	A. flavus
1	7 (30.4)	13 (54.2)	8 (36.4)	12 (50.0)	6 (26.1)	9 (36.0)
2a	NA	2 (8.3)	NA	2 (8.3)	NA	5 (20.0)
2b	7 (30.4)	11 (45.8)	8 (36.4)	10 (41.7)	5 (21.7)	9 (36.0)
2c	11 (47.8)	17 (70.8)	12 (54.5)	16 (66.7)	15 (65.2)	18 (72.0)
2d	14 (60.9)	20 (83.3)	16 (72.7)	20 (83.3)	17 (73.9)	19 (76.0)
2e	13 (56.5)	18 (75.0)	15 (68.2)	18 (75.0)	12 (52.2)	17 (68.0)
2f	8 (34.8)	13 (54.2)	11 (50.0)	15 (62.5)	7 (30.4)	10 (40.0)
2g	6 (26.1)	10 (41.7)	7 (31.8)	8 (33.3)	NA	4 (16.0)
2h	10 (43.5)	14 (58.3)	12 (54.5)	15 (62.5)	11 (47.8)	16 (64.0)
4a	NA	2 (8.3)	2 (9.1)	4 (16.7)	NA	3 (12.0)
4c	2 (8.7)	5 (20.8)	4 (18.2)	6 (25.0)	3 (13.0)	7 (28.0)
4b	NA	4 (16.7)	2 (9.1)	5 (20.8)	NA	2 (8.0)
5	10	15(62.5)	10	15 (62.5)	9 (39.1)	14

	(43.5)		(45.4)			(56.0)
7	17 (73.9)	21 (87.5)	17 (77.3)	22 (91.7)	18 (78.3)	23 (92.0)
9	18 (78.3)	22 (91.7)	19 (86.4)	23 (95.8)	17 (73.9)	21 (84.0)
13	NA	NA	NA	NA	NA	NA
Ampicillin	23 (100)	24 (100)	22 (100)	24 (100)	NA	NA
Colitrimazole	NA	NA	NA	NA	23 (100)	25 (100)

*NA: No activity.

Table 2. Anti-proliferative activity toward HePG-2, MCF-7cell lines.

Compound	In vitro Cytotoxicity IC_{50} (µ molar/ml)[a]	
	HePG2	MCF-7
1	89.90±4.20	91.83±4.43
2a	84.12±4.17	NA [b]
2b	86.14±3.92	NA [b]
3c	67.92± 3.35	58.13 ± 2.99
2d	24.16±1.89	27.24± 2.7
2e	40.98±2.76	22.46± 2.95
2f	56.28±3.24	49.35± 2.21
2g	23.40± 2.94	52.3±3.24
2h	60.93±3.71	36.90 ±2.14
4a	NA [b]	NA [b]
4c	93.31±4.82	98.08±4.90
4b	NA [b]	NA [b]
5	31.55 ± 3.00	56.46± 2.11
6	NA [b]	NA
7	25.52±1.6	23.91±1.23
8	NA	NA
9	25.73 ±2.2	27.71± 2.7
10	NA [b]	NA [b]
11	NA [b]	NA [b]
12	NA [b]	NA [b]

13	NA [b]	NA [b]
14	NA [b]	NA [b]
15	34.92± 3.6	87.30±4.82
5- Fu [c]	38.44±2.14	41.53±2.30

[a] IC_{50}: values are the mean ± S.D. of three separate experiments.

[b] NA: Compounds having IC_{50} value >100 µM.

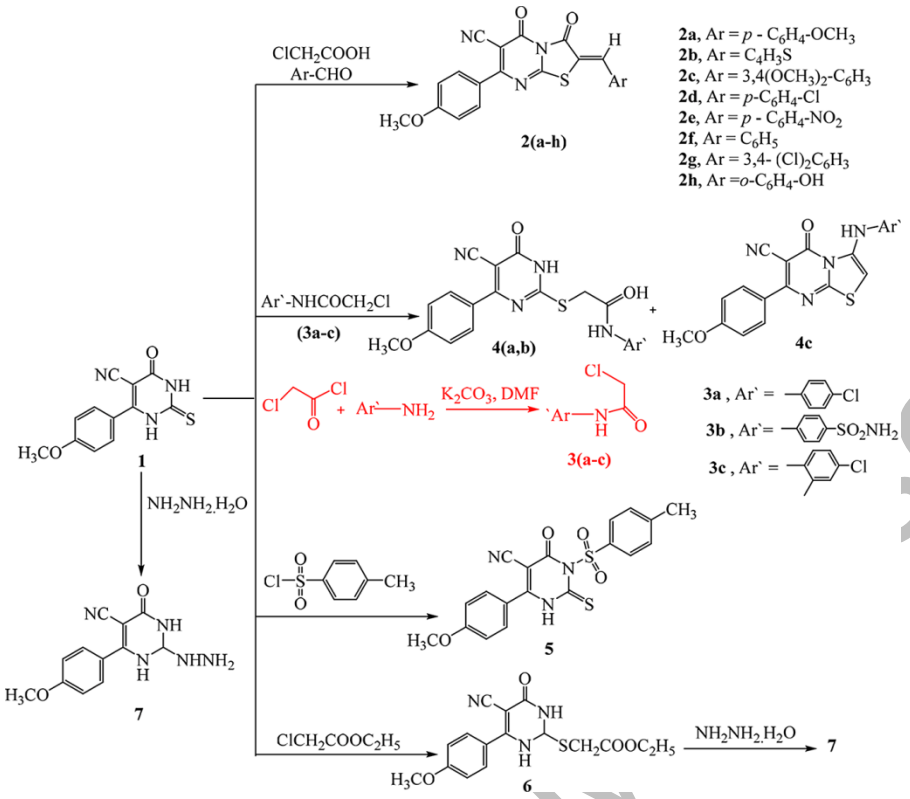
[c] 5- Flourouracil

Table 3. The docking energy scores of compounds **5-FU**, **7**, **9** and **2d** with the amino acid residues of the target enzyme thymidilate synthase forming hydrogen bonds.

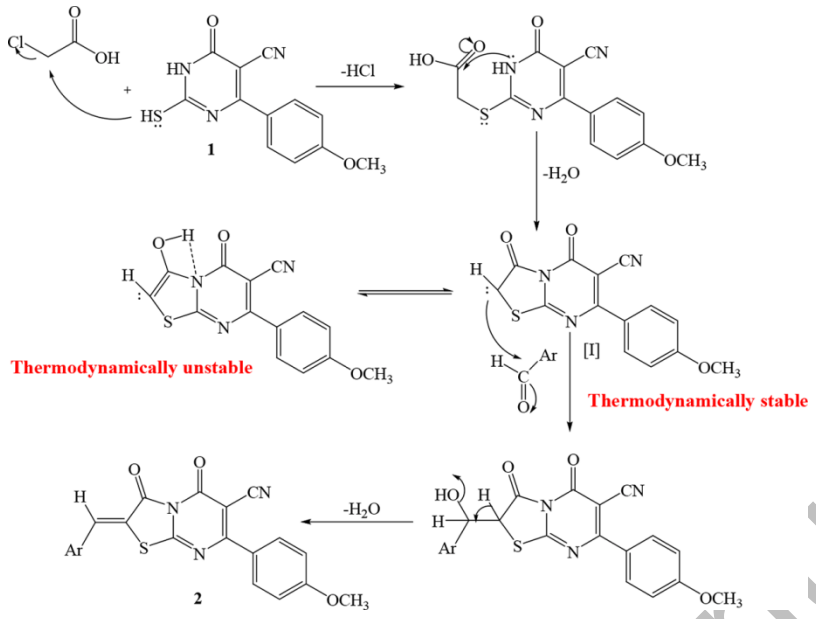
Cpd. No.	Docking score (Kcal/mol)	No. of Hydrogen bonds	Amino acid residues – Atom of ligand involved H-bond distance (A^0)
5-FU	- 18.87	4	Asp218: HN -- O8 2.1
			His256: HE2 -- O9 2.2
			Ser216: OG -- H10 2.3
			Try258: OH --H11 2.4
7	-42.23	6	Try258: OH-- H27 2
			Ala312: O--H29 2.2
			Val313: OCT2-- H29 2.5
			Arg50: HH21-- N19 2
			Asp218: HN -- N16 2.3
			His256: HE2-- O17 2
9	-39.36	5	Asn226: OD1--H30 2
			Arg50: HH21--O7 2.2
			Asn112: HD22--O7 2
			Gln214: HE21--O17 2.2
			Asp218: HN -- O17 2.2
2d	-39.19	4	Lys47: HZ1-- O21 2.5
			Arg50: HH11-- O21 1.8
			Ser216: HG -- S18 2.4

			Asn226: HD21-- O7 1.9

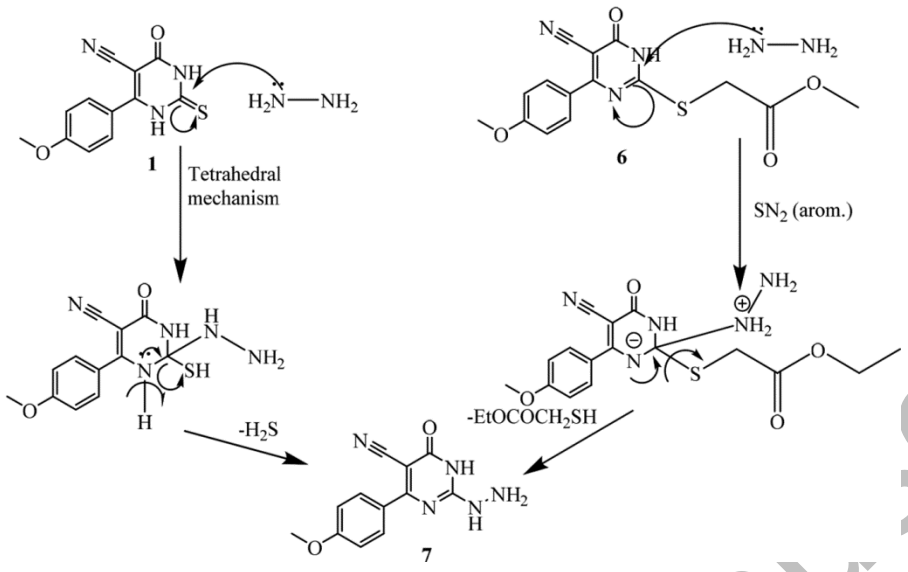
Scheme 1. Synthetic pathway of compounds **2-7.**



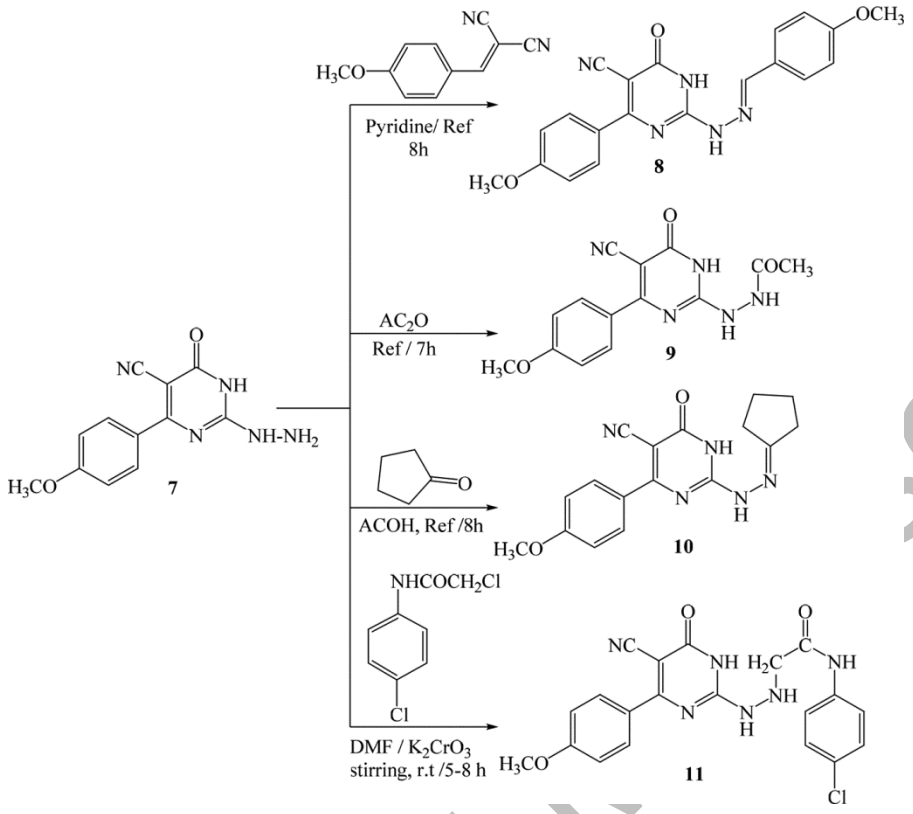
Scheme 2. The proposed mechanism for synthesis of compound **2**.



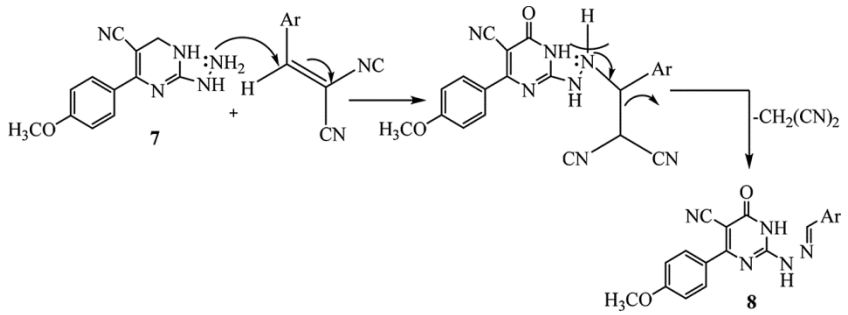
Scheme 3. The proposed mechanism for formation of compound **7**.



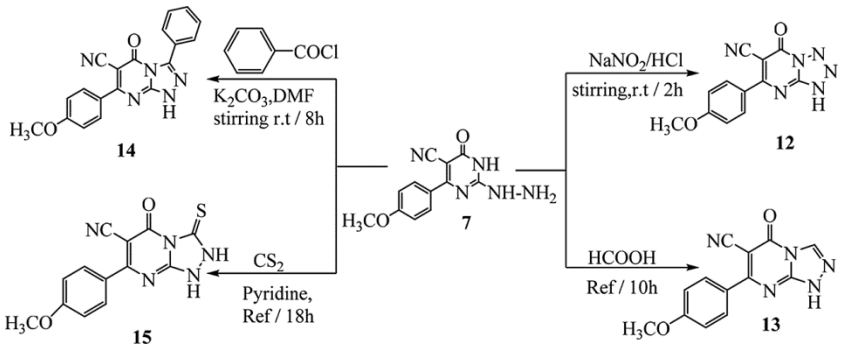
Scheme 4. Conversion of **7** to pyrimidinone derivatives **8-11**.



Scheme 5. The proposed mechanism for synthesis of compound **8**.



Scheme 6. Synthesis of triazolo and tetrazolopyrimidinone derivatives **12-14**.



Scheme 7. The proposed mechanism for synthesis of compound **15**.

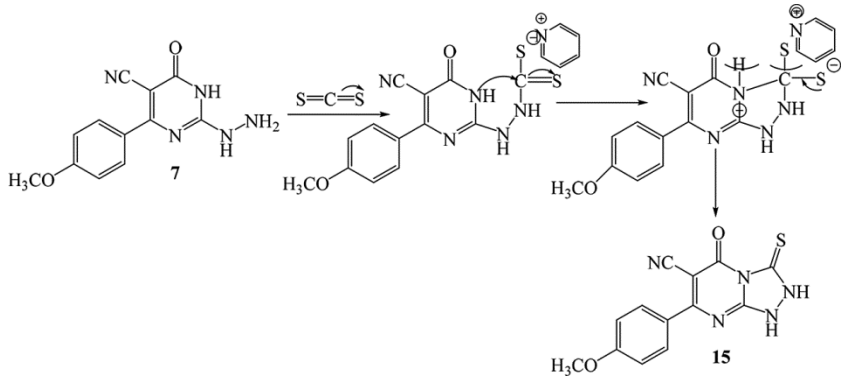


Figure 1. Structure of some potent 5-cyano-2-thiouracils.

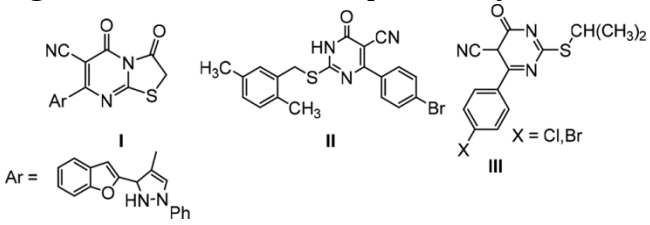


Figure 2. Antibacterial activity of synthesized compounds.

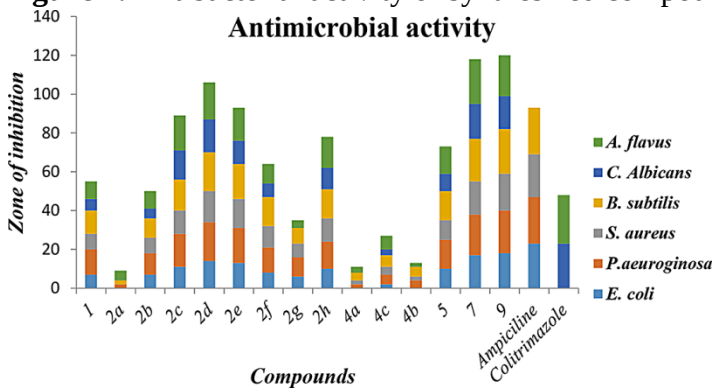


Figure 3. The proposed binding mode of FU inside the active site of TS. It formed four hydrogen bonds with Asp218, His256, Ser216 and Tyr258. (H-bonds Green dotted lines). Hydrogen (white), nitrogen (blue), and oxygen (red)

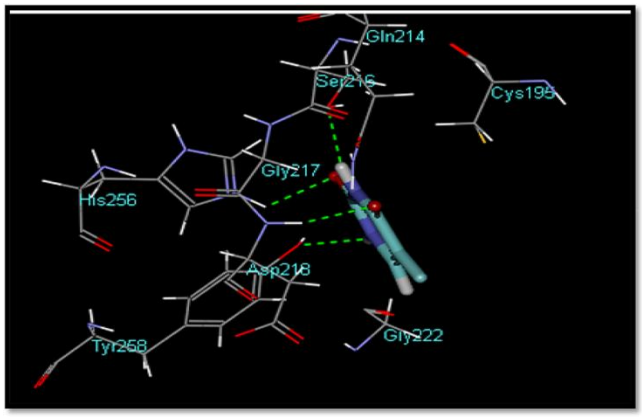


Figure 4. 3D diagram of the interaction of compound **7** in the active site of the thymidylate synthase (TS) showing five hydrogen bonds with Try258, Ala312, Val313, Arg50, Asp218, His256. Atoms are coloured by atom type and hydrogen bonds are represented by Green dotted lines.

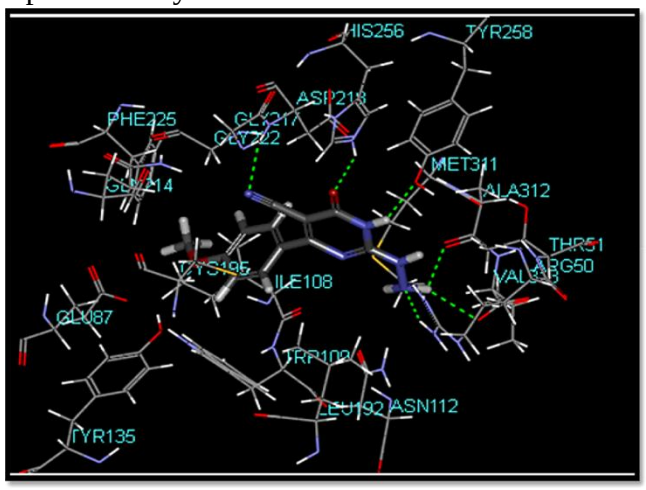


Figure 5. 3D diagram of the interaction of compound **9** in the active site of the thymidylate synthase TS showing five hydrogen bonds with Asn226, Arg50, Asn112, Gln214, Asp218. Atoms are coloured by atom type and hydrogen bonds are represented by Green dotted lines.

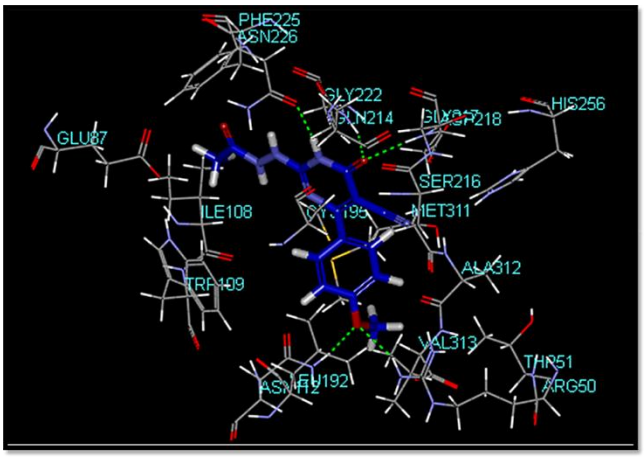


Figure 6. 3D diagram of the interaction of compound **2d** in the active site of the thymidylate synthase TS showing four hydrogen bonds with Lys47, Arg50, Ser216, Asn226. Atoms are colored by atom type and hydrogen bonds are represented by Green dotted lines.