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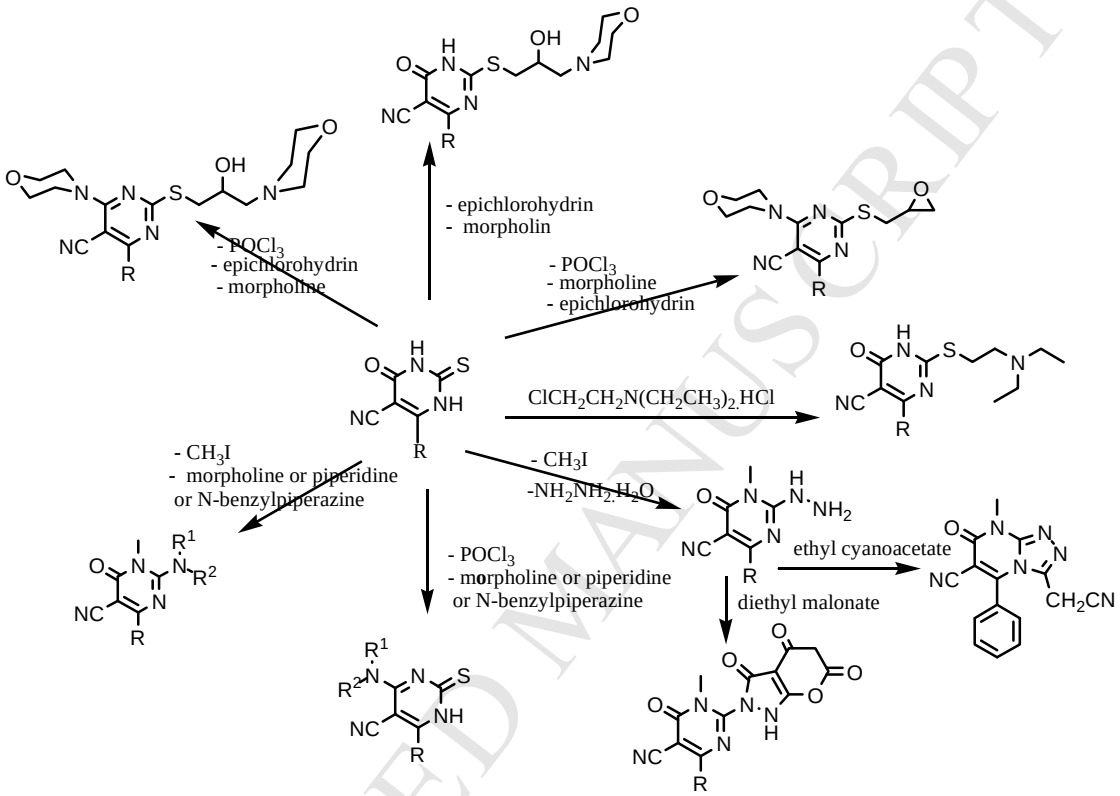
Graphical abstract

Synthesis of some pyrimidine analogues as a biologically active backbone with the hope to go a step forward in the field of anticancer and antimicrobial treatment.



Highlights

- The design and synthesis of novel pyrimidine derivatives.
- Compounds were investigated for anticancer and antimicrobial activities.
- Docking was performed for the five most active anticancer compounds.

Synthesis, biological evaluation and molecular docking studies of some pyrimidine derivatives

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A B S T R AC T

Some novel pyrimidine-5-carbonitrile derivatives bearing various substituent have been synthesized. The structures of target compounds were confirmed by elemental analysis and spectral data. Some selected members of the newly synthesized compounds were investigated for their cytotoxic potency against certain human tumor cell lines. Five representative active anticancer compounds **6a**, **6c**, **6d**, **17a** and **18a** were subjected to docking using MOE program on the 3D structure of two enzymes, namely; thymidylate synthase and dihydrofolate reductase. The antimicrobial activities of the synthesized compounds were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Candida albicans*. Compounds **2c**, **7a** and **9c** showed broad spectrum antimicrobial activity.

Keywords:

Pyrimidine Pyrimidine-5-carbonitrile Anticancer Antimicrobial Docking and Molecular modeling.

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1. Introduction

Pyrimidine ring is the building unit of DNA and RNA which explains the fact that pyrimidine derivatives exhibit diverse pharmacological activities. The most pronounced of which are anticancer [1-9], antiviral [10-12] especially anti-HIV [13-15], antimalarial [16-18] antimicrobial [19-20] and anti-inflammatory [21-22]. They also show activity against gonadotropin releasing hormone receptors [23-25] as well herbicidal activity targeting acetohydroxyacid synthase, which catalyze the first common step in branched-chain amino acid biosynthesis [26-28]. Furthermore, many pyrimidine-5-carbonitrile derivatives proved to exhibit potent anticancer [29,30] as well as antimicrobial activities [29,31,32]. Patients with neoplastic disorders are mostly subjected to microbial infections. The coadminstration of multiple drugs for treating patients suffering from cancer disease accompanied with microbial infections might inflect some added health problems especially in patients with impaired liver and/or kidney functions. Therefore, the concept of monotherapy by a single drug which possesses dual utility might be advantageous from both therapeutic as well as cost-effective stand points.

Consequently, our efforts were devoted to synthesize and investigate further innovative pyrimidine analogues with dual function; anticancer/ antimicrobial,. Aligned with this scope, some pyrimidine-5-carbonitriles, bearing biologically active functionalities were adopted to be synthesized (schemes 1, 2 and 3). Thioethers were found to show enhanced antimicrobial activities [33,34]. Utilizing the same goal, it was designed to synthesize various thioethers having different side chains with the hope to go a step forward in the field of anticancer and antimicrobial treatment. Synthesis of compounds **5a-c** were adopted on the rationale that some chloropyrimidines were found to possess anticancer activity [35]. Furthermore, our interest was extended to investigate anticancer and antimicrobial activities of chlorosubstituted pyrimidines bearing 2,3-epoxypropyl functionality **16a,c**. Phosphochloridate **4**, although obtained unexpectedly, yet its preparation was not beyond the scope of our interest due to its structural similarity to the anti-HIV cyclic phosphotriesters [36].

SAR study of the 2,4,6-trisubstituted pyrimidines revealed that 4-morpholino substitution appeared to exhibit important anti-proliferative activity leading to the most potent anticancer compounds[37]. However, replacing 4-morpholino with various linear or cyclic alkylamino substituents led to decrease in activity[37]. Encouraged by the foretasted findings and in a search for new and efficient agents, it was designed to synthesize 4-substituted amino-2-thioxopyrimidines **6a-i** and 2-substituted amino-6-oxopyrimidines **11a-f**. Moreover, it was desired to incorporate both morpholine functionality together with either 2,3-epoxypropyl or 2-hydroxypropyl substituents onto the substituted pyrimidine core **17a,b** and **18a,b** respectively, in an attempt to improve efficacy by increasing one more bioactive moiety. The fact that hydrazino derivatives are capable of exhibiting anticancer activity by alkylating DNA through a free radical intermediate [38] prompted us to synthesize 2-hydrazinopyrimidines **12a-c**. The latter was also considered as key intermediate to incorporate other biologically active ring systems fused or attached to pyrimidine core.

The integrity for the structures of the newly synthesized compounds would be substantiated by microanalyses, IR, ^{1}H-NMR, ^{13}C-NMR and MS data.

Furthermore, molecular modeling concept was adopted in an attempt to gain a better insight on the molecular interactions. Molecular docking studies were designed to help in understanding the mode of action of the active compounds through their interactions with the active sites of the proposed enzymes.

2. Results and discussion

2.1. Chemistry

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in schemes 1-3. In scheme 1, the starting compound 6-Substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles **1a-c** were prepared by adopting a previous reported literature procedure [29]. Alkylation of these pyrimidine thione derivatives **1a-c** following El-Meligie, S. et al. method[39], where an aqueous solution of the potassium salt of the pyrimidine thione **1a-c** was stirred at room temperature with an equimolar amount of epichlorohydrin to yield the desired compounds **2a-c. IR spectra** of compounds **2a-c** showed the characteristic absorption bands of the epoxy C–O–C function at 1239-1237, 1002-854, 772-755 cm^{-1} in addition to bands corresponding to C–S–C group at 1287-1281, 1078-1075 cm^{-1}. **^{1}H-NMR spectra** of compounds **2a-c** revealed a doublet of doublet integrated for one proton at 3.10-3.47 *ppm* corresponding to *cis* hydrogen of epoxy CH_2, a doublet of doublet integrated for one proton at 3.40 – 3.52 *ppm* assigned for *trans* hydrogen of epoxy CH_2, a multiplet at 3.80-4.48 *ppm* assigned for CH epoxy proton, two doublet of doublets at 2.84-4.31 *ppm* corresponding to the S–CH_2 protons. Opening of oxirane ring was achieved by adopting El-Meligie, S. et al. procedure [39], in which the epoxide derivative **2a,c** was refluxed with morpholine in absolute ethanol to produce 2-hydroxy-3-morpholinopropylthiopyrimidine derivative, **3a,b**. **IR spectra** of compounds **3a,b** showed the characteristic absorption bands corresponding to OH and NH functionalities at 3558-3413, 3456-3211, 3437-3140 cm^{-1} respectively. Besides, two bands at 1234-1180 and 1043-1030 cm^{-1} attributed to C–O–C function of the morpholine ring. They also showed bands at 1147-1137 cm^{-1} corresponding to the aliphatic C–N function, 1109-1108 cm^{-1} corresponding to secondary alcoholic group. **^{1}H-NMR spectrum** of compound **3b** showed a multiplet at 2.99-3.01 *ppm* attributed to S-CH_2 protons, two multiplets at 3.60-3.64 and 3.66-3.72 *ppm* corresponding to the morpholine $C_{3,5}$, $C_{2,6}$ protons respectively; a multiplet at 4.09-4.10 *ppm* attributed to $\underline{CH}$-$\underline{CH_2}$-N protons, two deuterium oxide exchangeable singlets at 6.78 and 8.09 *ppm* corresponding to NH and OH protons respectively; in addition to the signals due to furan protons. Oxiran-2-ylmethylthio **2a** was allowed to react with phosphorous oxychloride in order to yield the corresponding chloro derivative. However, the reagent not only introduced the chlorine atom in the 4-position of the reactant but also forced the oxirane ring to be opened, forming the cyclic phosphochloridate ring, furnishing compound **4**. **IR spectrum** of compound **4** showed the absorption bands corresponding to P=O function at 1250 cm^{-1}, C–Cl function at 766 cm^{-1} and P–Cl function at 696 cm^{-1} [40]and lacked the absorption bands due to C=O and C–O–C functions. **^{1}H-NMR spectrum** of compound **4** revealed two multiplets at 1.06-1.10, 4.40-4.51 *ppm* attributed to CH_2O and CHO protons of cyclic phosphochloridate moiety respectively [41]. Chlorination of pyrimidine thione derivatives **1a-c** were achieve by refluxing the precursors **1a-c** with excess

phosphorous oxychloride followed by cooling. The cold solution was poured on crushed ice with vigorous stirring, left in refrigerator overnight to obtain the chloro compounds **5a-c**. **IR spectra** of **5a-c** lacked the stretching absorption bands due to C=O group and displayed stretching absorption bands due to C–Cl functionality at 873-856 cm^{-1}. **^{1}H-NMR spectrum** of compound **5c** showed two doublets at 6.85 and 7.84 *ppm*, attributed to C_4 and C_3 protons of the furan ring respectively. It also revealed a singlet at 8.17 *ppm* corresponding to furan C_5 proton and a deuterium oxide exchangeable singlet at 13.07 *ppm* integrated for one proton, attributed to NH function. Replacement of the chloro function with secondary amines was reported to be achieved by heating the chloro compound together with the amine with or without catalyst [42-44]. The use of excess amine was also reported [45-47]. In the present work, the precursors **5a-c** were fused with two equivalents of the secondary amine on a sand bath at 160ºC, where the second mole of the amine is supposed to act as a proton abstractor. The residue was triturated with 10% acetic acid to get rid of the excess amine leaving the product in a pure form, which was then crystallized from the appropriate solvent. **IR spectra** of compounds **6a-i** revealed the absence of the absorption bands due to C–Cl function and showed the absorption bands due to the secondary amine moieties at their expected frequencies. **^{1}H-NMR spectrum** of compound **6a** revealed the presence of three triplets at 3.62, 3.67 and 3.78 *ppm* corresponding to C_5, C_3 and $C_{2,6}$ protons of the morpholine moiety. Furthermore, **^{1}H-NMR spectrum** of compound **6f** showed four multiplets attributed to the piperidine protons; at 1.45-1.51 *ppm* due to C_4 protons, 1.53-1.63 *ppm* due to $C_{3,5}$ protons, 2.60-2.62 *ppm* corresponding to C_6 protons and 2.84-2.86 *ppm* due to C_2 protons. **^{1}H-NMR spectrum** of compound **6g** revealed a multiplet at 3.70-3.90 *ppm* integrated for ten protons of the piperazine and CH_2 moieties and another multiplet at 7.20-7.80 *ppm* integrated for ten protons of the two phenyl groups. Compounds **7a,b** were prepared by stirring the pyrimidine thione derivatives **1a,b** with the hydrochloride salt of diethylaminoethyl chloride in aqueous potassium hydroxide solution. However, alkylation using anhydrous potassium carbonate in dry dimethylformamide was invalid. **IR spectra** of compounds **7a,b** lacked the absorption bands due to N–C=S functionality and displayed the stretching absorption bands due to C–S–C function at 1297, 1051-1046 $cm^{-1}$and C-N aliphatic at 1206-1181 and 1108-1103 cm^{-1}. **^{1}H-NMR spectrum** of compound **7b** showed two triplets at 0.87 and 0.92 *ppm* due to two methyl groups, a triplet at 1.15 *ppm* due to S–CH_2 protons, a triplet at 2.33 *ppm* corresponding to N–CH_2 protons and a quartet at 2.37 *ppm* due to the four protons of N($\underline{CH_2}$–CH_3)$_2$ moiety. In addition to a deuterium oxide exchangeable singlet at 4.07 *ppm* attributed to NH function. The scope of our investigation was extended to record the effect of S-alkylation with substituted acetanilide derivatives **8a,b** on the biological activity of the synthesized compounds **9a-d.** In scheme 2 compounds **9a-d** were achieved via the reaction of the pyrimidine-2-thione derivatives **1a,c** with 4-substituted chloroacetanilide derivatives **8a,b** and anhydrous potassium carbonate in dry dimethylformamide. It is to be noted down that 4-substituted chloroacetanilide derivatives **8a,b** , in their turn, were prepared by treating a solution of the appropriate aniline derivative in a mixture of dimethylformamide and tetrahydrofuran with chloroacetyl chloride according to reported procedure [48,49]. **IR spectra** of compounds **9a-d** lacked the absorption bands characteristic for N–C=S function and displayed the absorption bands characteristic for C–S–C function at 1296-1293, 1096 cm^{-1}. **^{1}H-NMR spectrum** of compound **9b** showed two doublets at 2.70 and 2.85 *ppm* each integrated for one proton assigned for S–CH_2 protons, a singlet at 3.67 *ppm* due to OCH_3 protons, two doublets at 6.84 and 7.42 *ppm* due to the p-methoxyphenyl $C_{2,6}$ and $C_{3,5}$ protons respectively. In addition to two deuterium oxide exchangeable singlets at 4.12 and 10.23 *ppm* attriubuted to pyrimidine NH and p-methoxyphenyl NH amide protons respectively. **^{1}H-NMR spectrum** of compound **9c** showed a singlet at 3.78 ppm assigned for the S–CH_2 protons, two doublets at 7.28 and 7.54 *ppm* attributed to the p-chlorophenyl $C_{2,6}$ and $C_{3,5}$ protons respectively. It also revealed two deuterium oxide exchangeable singlets at 4.07 and 10.96 *ppm* corresponding to the pyrimidine NH and p-chlorophenyl NH amide protons respectively. Methyl thio derivatives **10a-c** were prepared by stirring the thione derivatives **1a-c** with two equivalents of methyl iodide in presence of anhydrous potassium carbonate in dry dimethylformamide according to our previously reported method [29]. **IR spectra** of the title compounds **10a-c** lacked bands due to NH and N–C=S functions, whereas displayed absorption bands due to C–S–C function at 1283-1228 and 1094-1088 cm^{-1}. **^{1}H-NMR spectrum** of compound **10c** showed a singlet at 2.65 *ppm*

due to S-CH_3 protons, while N-CH_3 protons appeared at 3.38 *ppm* which overlapped with the signals of the solvent. Replacement of S-methyl function with an amine moiety ,was achieved by fusion of methylsulfide derivatives **10a-c** with two equivalents of the selected amine at 170 ºC, followed by treatment with ethanol to remove the excess amine leaving the desired compounds **11a-f**, in a pure form which was then crystallized. **IR spectra** of compounds **11a-f** lacked the absorption bands due to C–S–C function and displayed the absorption bands characteristic for the C–N aliphatic function. **^{1}H-NMR spectrum** of compound **11b** revealed the presence of multiplets at 2.32-2.33, 2.60-2.61 and 3.61-3.75 *ppm* due to morpholine protons, and a singlet at 4.05 *ppm* attributed to N-CH_3 protons. **^{1}H-NMR spectrum** of compound **11e** showed a singlet at 2.88 *ppm* due to N-CH_3 protons, a multiplet at 3.40-3.50 *ppm* attributed to piperazine protons, a singlet at 3.64 *ppm* corresponding to N-CH_2 protons and a multiplet at 7.30-7.44 *ppm* corresponding to phenyl and benzyl $C_{3,5}$ protons. Furthermore, two triplets at 7.50 and 7.54 *ppm* attributed to C_4 protons of both benzyl and phenyl respectively and two doublets at 7.51 and 7.80 *ppm* due to $C_{2,6}$ protons of benzyl and phenyl moieties respectively. 2-Hydrazino derivatives **12a-c** were prepared by hydrazinolysis of the 2-methylthio precursors **10a-c** in ethanol according to reported conditions [29]. **IR spectra** of compounds **12a-c** lacked the absorption bands due to C–S–C function and displayed the absorption bands corresponding to NH function at 3458-3114 cm^{-1}. **^{1}H-NMR spectrum** of compound **12c** lacked the characteristic singlet of methyl sulfide protons while revealed the presence of two deuterium oxide exchangeable singlets at 9.84 and 9.86 *ppm* each integrated for half proton due to NH indicating amino-imino tautomerism. It also showed a deuterium oxide exchangeable singlet at 10.33 *ppm* integrated for two protons corresponding to NH_2 function. Pyranopyrazole derivative **13a,b** was obtained upon refluxing hydrazino derivatives **12a,c** with excess diethyl malonate. **IR spectra** of compounds **13a,b** revealed the presence of absorption bands attributed to carbonyl functions at 1746-1744, 1662-1658 cm^{-1} and absorption bands due to C–O–C function at 1284-1222, 1082-1037 cm^{-1}. **^{1}H-NMR spectrum** of compound **13b** showed a singlet at 3.43 *ppm* attributed to pyranopyrazole CH_2 protons, a singlet at 3.46 *ppm* due to N-methyl protons and a deuterium oxide exchangeable singlet at 10.42 *ppm* due to NH function. **The mass spectrum** of compound **13b** revealed a molecular ion peak m/z at 367[M^+] absent, with a base peak m/z at 158 and 157(100). In the present investigation, fusion of the hydrazino compound **12a** with two equivalents of ethyl cyanoacetate gave rise to the desired cyanomethyltriazolo derivative **14**, in addition to another product which was found to be ethyl 3-iminopropanoate derivative **15**. Formation of **15** can be postulated on the fact that one molecule of ethyl cyanoacetate reacts with the substrate through its ester functionality, while the other molecule reacts via its nitrile group. **IR spectrum** of compound **14** lacked the absorption bands due to NH function and showed absorption bands corresponding to C≡N, C=O, C=N and C=C functions at their expected frequencies; **^{1}H-NMR spectrum** of compound **14** revealed the presence of two doublets at 3.15 and 3.53 *ppm* attributed to the cyanomethyl protons, a singlet at 3.81 *ppm* due to N-methyl protons. **The mass spectrum** of compound **14** revealed a molecular ion peak m/z at 291[M^++1](6), with a base peak m/z at 69(100). **IR spectrum** of compound **15** showed the absorption bands due to NH function at 3336 cm^{-1}, C≡N at 2224 cm^{-1} and an ester carbonyl function at 1727 cm^{-1}. It also showed C–O–C at 1292, 1017 cm^{-1}. On the other hand, **^{1}H-NMR spectrum** of compound **15** displayed a triplet at 1.18 *ppm* and a quartet at 4.11 *ppm* due to the ethyl ester protons. It also revealed two singlets at 3.37 and 3.56 *ppm* corresponding to CH_2CN and CH_2CO protons respectively. **The mass spectrum** of compound **15** revealed a molecular ion peak m/z at 421[M^+] absent, with a base peak m/z at 198(100). Scheme 3 shows the preparation of dimorpholino derivatives **18a,b**. This was achieved through two different precursors. In the **first method**, starting with 2-(oxiran-2-yl)methylthio-4-chloropyrimidine derivatives **16a,b**, which were prepared by treating a solution of chloropyrimidine thione derivative **5a,c** in DMF with two equivalents of aqueous sodium hydroxide solution followed by dropwise addition of an equivalent amount of epichlorohydrin. **IR spectra** of compounds **16a,b** lacked the absorption bands corresponding to NH and N–C=S functions, while revealed the absorption bands corresponding to C–S–C function at 1266-1255, 1085-1081 cm^{-1}, epoxy C–O–C function at 1266-1255, 1036-1030, 779-760 cm^{-1} and absorption band due to C–Cl function at 885-873 cm^{-1}. In addition to absorption bands due to CH_2, C≡N, C=N and C=C at their expected frequencies.**^{1}H-NMR spectrum** of compound **16a** showed two multiplets at 2.30-2.34 and 2.59-

2.63 *ppm* attributed to S-CH_2 protons. It also revealed the oxirane protons as two multiplets at 2.68-2.71 and 4.02-4.10 *ppm* corresponding to methine and methylene protons respectively. **16a,b**, were separately refluxed with four equivalents of morpholine in dry dioxane. It is assumed that one mole of morpholine is used to open the oxirane ring system and the other replaces the 4-chloro functionality. While the other two moles are supposed to act as catalysts, facilitating the reaction. The **second method,** involved refluxing 2-(oxiran-2-yl)methylthio-4-morpholino-pyrimidine derivatives **17a,b** with two equivalents of morpholine in dry dioxane. **17a,b** were fruitfully achieved by treating 4-morpholinopyrimidine thione derivatives **6a,c** with epichlorohydrin in alkaline medium. **IR spectra** of compounds **17a,b** lacked the absorption bands due to N–C=S function, while displayed absorption bands of C–S–C function at 1285-1257, 1069-1067 cm^{-1} and epoxy C–O–C function at 1255-1229, 1028-1022, 783-772 cm^{-1}. It also showed absorption bands due to C–N aliphatic function of morpholine at 1209-1206, 1115-1114 cm^{-1}. **^{1}H-NMR spectrum** of compound **17a** revealed the presence of signals corresponding to epoxypropyl side chain as two multiplets, at 2.59-2.60 *ppm* integrated for two protons due to OCH_2 moiety, and at 4.03-4.06 *ppm* integrated for one proton attributed to OCH proton. Signals corresponding to S–CH_2 protons were overlapped with the solvent signals. Compounds **18a,b** prepared by the aforementioned two methods were found to be identical, as revealed by TLC, m.p. and mixed m.p. **IR spectra** of compounds **18a,b** lacked absorption bands due to C–Cl and epoxy C–O–C functionalities, while revealed a broad band at 3438-3407 cm^{-1} due to alcoholic function.. **^{1}H-NMR spectrum** of compound **18a** revealed signals corresponding to the two morpholine moieties interpreted as two triplets at 4.47 and 4.50 *ppm* corresponding to $C_{3,5}$ protons of S-propylmorpholine and $C_{3,5}$ protons of 4-morpholino moieties respectively. A multiplet integrated for eight protons at 4.55-4.66 *ppm* attributed to $C_{2,6}$ protons of both morpholine moieties. It also showed a deuterium oxide exchangeable singlet at 8.75 *ppm* due to alcoholic proton.

2.2. Biological evaluation

2.2.1. *In-vitro* anticancer screening:

In the current protocol, 27 compounds were submitted to the NCI and were tested initially at a single high dose (10 μM) in the full NCI 60 cell panel. Results of the tested compounds were reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition caused by the test compounds (**table 1).** 4-Oxo-2-thioxopyrimidine derivative **1c** showed promising anticancer activity against many cell lines. Replacing the 4-oxo moiety with 4-morpholino group (**6a**, **6c, 17a & 18a)** resulted in marked increase in activity against several cell lines. Moreover, replacement of 4-oxo moiety with 1-piperidinyl group in compound **6d** resulted in slight increase in activity. On the other hand, replacement of 4-oxo with the bulky N-benzylpiperazine moiety **6g**, **6h** and **6i** diminished the anticancer activity. Furthermore, alkylation of the 2-thio group with an ethyl diethylamino function **(7a),** p-chloroacetanilides **(9c & 9d)**, methyl group **(10c)**, oxiranylmethyl group **(17a)** and hydroxypropylmorpholino moiety **(18a)** showed increase in activity against several cell lines. Replacement of 2-thioxo moiety with different amines did not increase the activity as much, this is contradictory to the marked increase in activity in case of 4-amino substitution, where compounds **11b**, **11c** and **11d** showed good activity against two Melanoma cell lines. Moreover, 2-hydrazino compound **12c** showed good activity against Colon cancer HCC-2998. Compound **17a** satisfied the threshold inhibition criteria and passed forwards for evaluation in the full panel 5-dose *in-vitro* anti-tumor screen. The three response parameters GI_{50}, TGI and LC_{50} values in μM/l of compound **17a** in the full panel, 5-dose assay are presented in **table 2**. The GI_{50}, TGI and LC_{50} values revealed that compound **17a** exhibited potential growth inhibition activity against most of the tested subpanel tumor cell lines. It showed promising activity toward several cell lines of Non-Small Cell Lung cancer especially EKVX, HOP-62, HOP-92, NCI-H23, and NCI-H322M. Concerning Colon cancer, it was most active against HCC-2998 and HCT-15 cell lines. It also showed promising activity toward CNS cancer SF-295 and SNB-75 cell lines. Moreover, it exhibited good activity against all Melanoma cell lines. However, it was most active against three Ovarian cancer cell lines; namely IGROV1, OVCAR-3 and OVCAR-5. It was most active against Renal cancer especially 786-0, A498 and CAKI-1 cell lines. Finally, it was highly active against all Breast cancer cell lines especially MCF7, HS 578T, BT-549 and T-47D cell lines. However, the compound was non-selective

toward almost all types of cancer but it exhibited moderate selectivity towards Renal cancer with selectivity ratio 3.22 (**table 3**).

2.2.2. Antimicrobial screening:

Antimicrobial activities of the newly synthesized compounds were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Candida albican.* using the cup diffusion technique [50]. Further evaluation was then carried out on compounds showing reasonable inhibition zones to determine their minimal inhibitory concentration (MIC) values using the two fold serial dilution method [51]. The results illustrated in **tables 4** and **5** revealed that from the tested compounds, non had superior antibacterial activity than the standard antibiotics. Moreover, compounds **2c**, **6i**, **7a**, **9c**, **15**, **18a** and **18b** displayed antifungal activity against *C.albicans*. However, most of them proved to possess nearly 50% of the activity of Clotrimazole, while compounds **6i** and **9c** were found to exhibit 75% of its activity. In a study of SAR, the 4-oxo-2-thioxopyrimidine derivative **1c** showed nearly 60% activity as that of Ofloxacin against the gram negative *Sh.flexneri*. The oxiranylmethylthio derivatives **2a-c** enhanced the activity against *Sh.flexneri*. However, the 4-(2-furyl) derivative **2c** exhibited additional activity toward *S.aureus*, *P.auregionsa* as well as *C.albicans.* On the other hand, ring opening of the oxiranylmethylthio derivatives to yield compounds **3a,b** or the treatment of **2a** with phosphorus oxychloride to yield the phosphochloridate derivative **4** diminished the antimicrobial activity. 4-Chloropyrimidines **5a,c** elicited activity against Gram negative bacteria, while their 4-substituted aminopyrimidines **6a-i** showed promising activity of which compounds **6c**, **6d**, **6e** and **6g** were the most active; nearly 60% that of Ofloxacin. Furthermore, compound **6i** exhibited both antibacterial and antifungal activities. Moreover, alkylation of the 2-thioxo function with diethylaminoethyl group, compound **7a** showed promising broad spectrum activity against the test organisms. The N-substituted-2-substituted thioacetamido derivatives **9a-d** showed variable antimicrobial activities depending on the substitution on the p-substitution on the phenyl ring. The 4-OCH_3 derivatives **9a** and **9b** showed 60% activity that of Ofloxacin against *Sh.flexneri* while the 4-chloro derivatives **9c** and **9d** were equipotent with Ampicillin and half the activity of Ofloxacin against *S.aureus* and *P.aureginosa* respectively. In addition, compound **9c** showed 75% activity that of Clotrimazole against the fungus *C.albicans*. Methylthio derivative **10c** exerted 50% the activity of Ampicillin against *S.aureus*. Replacement of S-alkyl by amino functionality; **11a-f** did not improve the activity. However, only compounds **11c** and **11e** showed activity against *Sh.flexneri* and *P.aeruginosa*. Moreover, the hydrazino compound **12c** exhibited antimicrobial activity against *Sh.flexneri* its potency reached 60% that of Ofloxacin. While introduction of pyranopyrazole moiety at the 2-position of the pyrimidine ring did not enhance the antimicrobial activity where only **13a** showed activity against *P. auerginosa*. Cyclization of hydrazinopyrimidine derivative **12a** into the corresponding cyanomethyltriazolopyrimidine **14**, did not improve the activity, while its open chain counter-part **15** exhibited activity reaching to 60% that of Clotrimazole against *C.albicans*. Introduction of an oxiranylmethylthio side chain to either chloropyrimidines **5a,c** or 4-morpholinopyrimidines **6a,c** furnishing compounds **16a,b** and **17a,b** respectively, resulted in diminished activity. Nevertheless, compound **16a** retained the activity of its precursor against *Sh.flexneri*. On the other hand, dimorpholinopyrimidine derivatives **18a,b** elicited activity about 60% that of Clotrimazole against *C.albicans*.

Computer aided drug design:

Five representative active anticancer compounds **6a**, **6c**, **6d**, **17a** and **18a** were subjected to docking using Molecular Operating Environment (MOE) program [52] on the 3D structure of two enzymes, namely; thymidylate synthase (TS)(1BID) and dihydrofolate reductase (DHFR). The study also shows the enzymes interactions with their substrates; 2'-deoxyuridine-5'-monophosphate (DUMP) and methotrexate(MTX), respectively.

Docking on the active site of thymidylate synthase:

MOE docking studies of the inhibitors was performed using thymidylate synthase co-crystallized with the substrate DUMP (PDB ID: 1BID) as a template.

Docking of DUMP into TS active site revealed that several interactions were considered to be responsible for the observed affinity of the compound. As it acts as a hydrogen bond acceptor with the backbone Asp 169 residue and the side chain residues; Arg 166, Ser 167, Asn 177, His 207 and Tyr 209 as well as hydrogen bond donor with the side chain residues; Asn 177, His 207

and Tyr 209. In addition to hydrophobic interactions with: Arg 21, Cys 146, His 147, Gln 165, Arg 166, Ser 167, Cys 168, Asp 169, Gly 173, Asn 177, His 207 and Tyr 209 as shown in **fig. (1)**.

Docking of compound 6a into TS active site revealed that several molecular interactions were considered to be responsible for the observed affinity, as the N of the cyano function acts as a hydrogen bond acceptor with the side chain residues; His 207 (3.47Aº) and Tyr 209 (3.20Aº) with a strength of 11.2% and 17.2%; respectively. In addition to many hydrophobic interactions between the phenyl moiety as well as the morpholine group and the sulphur atom with the following residues; Asp 20, Arg 21, Leu 143, Ala 144, Cys 146, Arg 166, Ser 167, His 207 and Tyr 209 as shown in **fig. (2).**

Docking of compound 6c into TS active site revealed the presence of hydrogen bond interaction between the N of the cyano group of the compound as it acts as hydrogen bond acceptor with the side chain residue Tyr 209 (3.22Aº) with a strength of 16.5%, in addition to hydrophobic interactions between the furan ring, morpholine moiety and sulphur atom with the following amino acid residues: Asp 20, Arg 21, Trp 83, Leu 143, Ala 144, Cys 146, Arg 166 and Try 209 as shown in **fig. (3).**

Docking of compound 6d into TS active site illustrated the interaction of the NH group of the compound as a hydrogen bond donor with the side chain residue Glu 58 (2.77 Aº) at a strength of 3% while the pyrimidine N-3 acted as a hydrogen bond acceptor with the side chain residue Tyr 94 (3.34Aº) at a strength of 6.8%. This beside many hydrophobic interactions concerning the piperidine moiety, phenyl ring and cyano function with the following amino acid residues: Glu58, Phe 62, Ile 79, Trp 80, Tyr 94, Leu 163, Ala 144, Cys 146, His 147 and Arg 166 as shown in **fig. (4).**

Docking of compound 17a into TS active site revealed the presence of hydrogen bond interaction between the N-1 of pyrimidine, as it acts as a hydrogen bond acceptor, and the side chain Asn 177 residue (2.92Aº) with a strength of 23.6%. There is also hydrophobic interactions between the morpholine moiety, CN function and benzene ring with many amino acid residues: Ser 54, Ile 55, Glu 58, Ile 79, Cys 146, Gln 165, Ser 167, Cys 168, Asp 169, Leu 172, Gly 173, Phe 176 and Asn 177as shown in **fig. (5)**.

Docking of compound 18a into TS active site revealed the presence of several molecular interactions which were considered to be responsible for the observed affinity of the compound; the hydrogen atom of the hydroxyl group acts as a hydrogen bond donor to the side chain residue Glu 58 (3.64 Aº) with a strength of 23.7%, also the oxygen atom of the hydroxyl group acts as a hydrogen bond donor and acceptor with His 147 residue (3.16Aº) with a strength 17.1%. Moreover the oxygen atom of morpholine moiety and N-1 of pyrimidine both acts as hydrogen bond acceptor to Trp 83 and Asn 177 residues at a strength of 13.9% and 11.3%; respectively. In addition to many hydrophobic interactions between pyrimidine C_4-morpholine, cyano function, phenyl group and the following amino acid residues: Glu 58, Ile 79, Trp 80, Trp 83, Cys 146, His 147, Gln 165, Ser 167, Cys 168, Asp 169, Leu 172, Gly 173, Phe 176 and Asn 177 as shown in **fig. (6).**

Docking on the active site of dihydrofolate reductase (DHFR):

Docking simulation study of the synthesized compounds 6a, 6c, 6d, 17a and 18a

MOE docking studies of the inhibitors were performed using dihydrofolate reductase co-crystallized with methotrexate (PDB ID: 4DFR) as a template.

Docking of MTX into DHFR active site revealed that hydrogen bond interactions beside hydrophobic interactions were considered to be responsible for the observed affinity as it acts as a hydrogen bond donor to the backbone Ile 5 and Ile 94 residues and the side chain Asp 27 residue. It also acts as a hydrogen bond accepter to Arg 52 and Arg 57 residues. This beside many hydrophobic interactions with various amino acid residues: ILe 5, Ala 6, Ala7, Asp 27, Leu 28, Phe 31, Lys 32, Ser 49, Ile 50, Arg 52, Leu 54, Arg 57, Ile 94, Tyr 100 and Thr 113 as shown in **fig. (7).**

Docking of compound 6a into DHFR active site revealed the presence of hydrogen bond interaction between the N of the cyano function as it acts as hydrogen bond acceptor and the backbone Ala 7 residue (2.66Aº) with a strength of 98.6% in addition to many hydrophobic interactions among morpholine moiety and the sulphur atom and the following amino acid

residues: Ile 5, Ala 6, Ala 7, Ile 14, Gly 15, Asp 27, Phe 31, Thr 46, Ile 50, Ile 94 and Tyr 100 as shown in **fig. (8).**

Docking of compound 6c into DHFR active site revealed the presence of strong hydrogen bond interactions with the N of the cyano moiety as it acts as a hydrogen bond acceptor with the backbone Ala 7 residue (2.68Aº) at a strength of 90.5% as well as with the side chain Tyr 100 residue (3.10 Aº) with a strength of 20.4%. Besides, many hydrophobic interactions between the morpholine moiety, thioxo function and the pyrimidine ring with: Ile 5, Ala 6, Ala 7, Ile 14, Gly 15, Asp 27, Phe 31, Thr 46, Gly 96 and Tyr 100 as shown in **fig. (9).**

Docking of compound 6d into DHFR active site revealed the presence of hydrogen bond interaction between the N of cyano function and side chain Tyr 100 residue (3.45 Aº) as it acts as a hydrogen bond acceptor with a strength of 7.1%. It also showed several hydrophobic interactions between the thioxo function, phenyl ring and pyrimidine carbons with: Ile 5, Ala 6, Ala7, Gly15, Met 16, Glu 17, Asn 18, Met 20, Trp 22, Asp 27, Phe 31, Ile 94 and Tyr 100 as show in **fig. (10).**

Docking of compound 17a into DHFR active site illustrated the presence of strong hydrogen bond interactions between N of cyano group as it acts as a hydrogen bond acceptor with the backbone Ala 7 residue (2.72Aº) with a strength of 70.5%. In addition to the oxygen of the morpholinyl group which acts as a hydrogen bond acceptor with the side chain Thr 46 residue (2.70Aº) at a strength of 45.4%. Besides, the hydrophobic interactions among the morpholine ring, oxiranylmethylthio function as well as the pyrimidine carbons and several amino acid residues namely; Ile 5, Ala 6, Ala7, Asp 27, Trp 30, Phe 31, Thr 46, Ile 50, Leu 54, Ile 94, Gly 95, Gly 96 and Tyr 100 as shown in **fig. (11).**

Docking of compound 18a into DHFR active site revealed the presence of strong hydrogen bond interaction between the N of the cyano moiety, as it act as a hydrogen bond acceptor, and the backbone Ala 7 residue (2.84Aº) with a strength of 77.9%. This besides, many hydrophobic interactions involving the S-propylmorpholine moiety, the 4-morpholino group, the phenyl ring as well as the cyano carbon and the following amino acid residues: Ala 7, Ile 14, Gly 15, Asp 27, Leu 28, Phe 31, Thr 46, Ser 49, Ile 50, Gly 96, Gly 97 and Tyr 100 **fig.(12).**

2. Conclusion

The design and synthesis of novel pyrimidine derivatives bearing various substituents or heterocyclic moieties joined to the pyrimidine ring system. Some selected members of the newly synthesized compounds were investigated for their cytotoxic potency against certain human tumor cell lines as well as their antimicrobial activities. Compound **17a** satisfied the threshold inhibition criteria and passed forwards for evaluation in the full panel, 5-dose *in-vitro* anti-tumor screen. Although most compounds were either devoid or of weak antimicrobial activity, compounds **2c** and **7a** were found to exhibit broad spectrum activity. Their potency against *S.aureus* was nearly half or equal to Ampicillin respectively, about 50% as potent as Ofloxacin against *P.aeruginosa* and 60% as potent against *Sh.flexneri*, beside exhibiting 62% the activity of Clotrimazole against *C.albicans*. Docking was performed for the five most active anticancer compounds **6a**, **6c**, **6d**, **17a** and **18a** on the two enzymes; thymidylate synthase and dihydrofolate reductase in a trial to predict their mode of action as anticancer agents. Compounds **17a** and **18a** exhibited strong interactions with dihydrofolate reductase enzyme.

4. Experimental

4.1. Chemistry

Melting points were determined in open glass capillaries on a Griffin-Kamp melting point apparatus and are uncorrected. IR spectra were recorded, for potassium bromide discs, on a Perkin-Elmer 1430 Infrared spectrophotometer. ^{1}H-NMR spectra were determined either on Joel spectrometer (500 *MHz)* at the central laboratory, Faculty of science, Alexandria University, or on Varian Mercury VX-300 NMR spectrometer, Faculty of science, Cairo University. Data are reported as δ values (*ppm*) relative to trimethylsilane(TMS) as an internal standard. The type of signal is indicated by one of the following letters: s = singlet,d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet. ^{13}C-NMR spectrum was recorded on Joel spectrometer at the central laboratory, Faculty of science, Alexandria University . Mass spectra were carried out

using either a Schimadzu GCMS-QP-1000EX mass spectrometer at 70 ev or HP5000988A mass spectrometer at Faculty of science, Cairo University. Elemental analyses were performed on Elementar Vario El III CHN analyzer (Germany) at the microanalytical unit, Faculty of science, Cairo University. Phosphorous was detected by using oxygen fust technique followed by measuring on Nanocolor 300D spectrophotometer at Faculty of science, Cairo University. Reactions were monitored by thin-layer chromatography (TLC) on silica gel (60 GF254, Merck), using glass plates. The spots were visualized by exposure to iodine vapour or UV-lamp at λ 254 nm for few seconds.

4.1.1. *6-Substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5- carbonitriles* **(1a-c).**

A mixture of thiourea (1.5 g, 20 mmole), ethyl cyanoacetate (2.6 g, 2.1 ml, 20 mmole), appropriate aldehyde (20 mmole) and K_2CO_3 (2.8 g, 20 mmole) in EtOH 95% (50 ml) was refluxed for 12h, then cooled. The obtained precipitate was filtered and washed with EtOH. The product was then dissolved in hot H_2O, filtered while hot and neutralized with gl.HOAc; where upon the desired thione was obtained. It was filtered, washed with H_2O, dried and crystallized from DMF/H_2O**.**

4.1.1.1. *4-Oxo-6-phenyl 2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile* **(1a)**

$C_{11}H_7N_3OS$ (229.26); yield: 90%; mp: 290-291 ºC [26]**.**

4.1.1.2. *6-(4-Nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5- carbonitrile* **(1b)**

$C_{11}H_6N_4O_3S$ (274.26); yield 76%; mp: 232-234 ºC [26].

4.1.1.3. *6-(2-Furyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile* **(1c)**

$C_9H_5N_3O_2S$ (219.22) ; yield 70%; mp: > 300 ºC. IR (KBr, cm^{-1}): 3121 (NH); 3087 (CH furan); 2232 (C≡N); 1674, 1661 (C=O); 1589, 1469 (C=N, δNH, C=C–Ar, C=C- furan); 1550, 1272, 1148, 1086 (N-C=S); 1410, 1326 (C–N lactam); 1224, 1039 (C–O–C); 767 (*oop* furan). ^{1}H-NMR of compound 1c (DMSO-d_6) δ*ppm*: 6.94 (dd, 1H, *J* = 3.8, 1.9*Hz*, furan C_4-H); 7.91 (d, 1H, *J* = 3.8*Hz*, furan C_3-H); 8.25 (s, 1H, furan C_5-H); 13.03 (s, 2H, 2NH, D_2O exchangeable). Anal. Calcd for $C_9H_5N_3O_2S$ (219.22): C, 49.31; H, 2.30. Found C, 50.24; H, 2.8.

4.1.2. *4-Substituted-2-(oxiran-2-ylmethylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(2a-c).**

The appropriate thione **1a-c** (2 mmole) was suspended in H_2O (30 ml) then treated with a solution of KOH (0.22 g, 4 mmole) in 5 ml H_2O. The suspension was stirred at R.T. for 30 min., where a clear solution was obtained. Epichlorohydrin (0.19 g, 0.16 ml , 2 mmole) was added and stirring was continued at R.T. for 6h, during which a precipitate was formed. It was filtered, washed with H_2O, dried and crystallized from the appropriate solvent

4.1.2.1 *2-(Oxiran-2-ylmethylthio)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile* **(2a).**

Crystallized from DMF/H_2O; yield: 76%; mp: 183ºC. IR (KBr, cm^{-1}): 3412, 3264 (NH); 2224 (C≡N); 1726, 1661 (C=O); 1599, 1516, 1500, 1489 (C=N, C=C), 1550 (δ NH); 1439, 1315 (C–N lactam); 1239, 1002, 770 (C–O–C epoxy); 1281, 1076 (C–S–C). ^{1}H-NMR of (DMSO-d_6) δ*ppm*: 3.47 (dd, 1H, *J* = 9.9, 4.2*Hz*, epoxy CH_2 *cis* hydrogen); 3.52 (dd, 1H, *J* = 9.9, 3.05*Hz*, epoxy CH_2 *trans* hydrogen); .80-3.82 (m, 1H, epoxy CH); 4.06 (2 overlapping dd, 2H, *J* = 14.15, 6.45*Hz*, S–CH_2);7.45 (t, 1H, *J* = 6.9*Hz*, phenyl C_4-H);7.64(dd, 1H, *J* = 11.5, 7.65*Hz*, phenylC_3-H);7.74 (dd, 1H, *J* = 12.2, 6.9*Hz*, phenyl C_5-H);7.88 (d, 1H, *J* = 7.65 *Hz*, phenyl C_2-H);8.27 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{14}H_{11}N_3O_2S$. H_2O(303.34);C, 55.44; H, 4.32; N,13.85. Found C, 55.21; H, 4.45; N,13.36.

4.1.2.2.*4-Nitrophenyl-2-(oxiran-2-ylmethylthio)-6-oxo-1,6-dihydropyrimidine-5carbonitrile***(2b).**

Crystallized from DMF/H_2O ; yield: 71%; mp: 150ºC. IR (KBr, cm^{-1}): 3374 (NH); 2204 (C≡N); 1713, 1660 (C=O); 1591 (C=N, C=C); 1524, 1348 (NO_2); 1239, 854, 755 (C–O–C epoxy); 1286, 1075 (C–S–C). H-NMR (DMSO-d_6) δ*ppm*: 2.68-2.71 (m, 2H, OCH_2 epoxy); 2.84-2.87 (m, 2H, S–CH_2);3.80-4.00 (m, 1H, CH epoxy); 6.69 (s, 1H, NH, D_2O exchangeable); 7.55-8.50 (m, 4H, 4-NO_2-C_6H_4). Anal. Calcd for $C_{14}H_{10}N_4O_4S$(330.32); C, 50.91; H, 3.05. Found C, 50.98; H, 2.85.

4.1.2.3.4-(2-Fury)l-2-(oxiran-2-ylmethylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitriles **(2c).**

Crystallized from DMF/EtOH ; yield: 37%; mp: 190ºC. IR (KBr, cm^{-1}): 3389 (NH); 3137 (CH furan); 2221 (C≡N); 1726, 1662 (C=O); 1586, 1518, 1500 (C=N, C=C); 1550 (δ NH); 1312 (C–N lactam); 1237, 989, 772 (C–O–C epoxy); 1287, 1078 (C–S–C); 1177, 1022 (C–O–C furan); 772 (*oop* furan). ^{1}H-NMR (DMSO-d_6) δ*ppm*: 3.10 (dd, 1H, *J* = 12.25, 3.83*Hz*, epoxy CH_2 *cis*

hydrogen); 3.40 (dd, 1H, *J* = 12.2, 2.25 *Hz*, epoxy CH_2 *trans* hydrogen); 4.28 (dd, 1H, *J* =14.15, 4.97 Hz, S–CH_2);4.31 (dd, 1H, *J* = 14.15, 4.97*Hz*, S–CH_2);4.44-4.48 (m, 1H, epoxy CH); 5.76 (s, 1H, NH, D_2O exchangeable); 6.75 (dd, 1H, *J* = 3.8, 1.9*Hz*, furan C_4–H); 7.42 (d, 1H, *J* = 3.8*Hz*, furan C_3–H); 7.99 (s, 1H, furan C_5–H). Anal. Calcd for $C_{12}H_9N_3O_3S$(276.29); C, 52.36; H, 3.30; N,15.26 Found C, 52.35; H, 2.95; N,15.60.

4.1.3. *4-Substituted-2-(2-hydroxy-3-morpholinopropylthio)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitriles* **(3a,b).**

The epoxide derivative **2a,c** (2 mmole) was suspended in absolute EtOH, whereafter morpholine (0.35 g, 0.35 ml, 4 mmole) was added. The reaction mixture was refluxed for 72h, allowed to cool, and filtered. The obtained product was triturated with pet. ether 40-60 to yield pale yellow solid which was crystallized from DMF/H_2O.

4.1.3.1. *2-(2-Hydroxy-3-morpholinopropylthio)-6-oxo-4-phenyl-1,6-dihydro-pyrimidine-5-carbonitrile* **(3a).**

Yield: 23%; mp: 210ºC. IR (KBr, cm^{-1}): 3558, 3456, 3437 (NH, OH); 2205 (C≡N); 1716, 1659 (C=O); 1586, 1501 (C=N, δNH, C=C); 1310 (C–N lactam); 1245, 1073 (C–S–C); 1180, 1043 (C–O–C); 1137 (C–N aliphatic); 1108 (2º OH). Anal. Calcd for $C_{18}H_{20}N_4O_3S$. 11/2H_2O (399.46) ;C, 54.12; H, 5.80; Found: C, 54.56; H, 5.35.

4.1.3.2. *2-(2-Hydroxy-3-morpholinopropylthio)-4-(2-furyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile* **(3b).**

Yield: 41%; mp: 250ºC. IR (KBr, cm^{-1}): 3413, 3211, 3140 (OH, NH); 3074 (CH furan); 2206 (C≡N); 1690, 1645 (C=O); 1587, 1515, 1485 (C=N, δNH, C=C); 1305 (C–N lactam); 1256, 1030 (C–S–C); 1234,1030 (C–O–C); 1147 (C–N aliphatic); 1109 (2º OH); 761 (*oop* furan). ^{1}H-NMR (DMSO-d_6) δ*ppm:* 2.99 – 3.01(m, 2H, S–CH_2, under DMSO); 3.60-3.64 (m, 4H, morpholine $C_{3,5}$–H);3.66-3.72 (m, 4H, morpholine $C_{2,6}$–H); 4.09–4.10 (m, 3H, CH, CH_2-N);6.56-6.69 (m, 1H, furan C_4-H);6.78 (br.s, 1H, NH, D_2O exchangeable)7.21 (d, 1H, *J* = 3.05*Hz*, furan C_3-H);7.84 (s, 1H, furan C_5-H);8.09 (s, 1H, OH, D_2O exchangeable). Anal. Calcd for $C_{16}H_{18}N_4O_4S$. 11/2H_2O(371.41);C, 51.17;H,5.16;Found: C,52.15; H,5.20.

4.1.4. *4-Chloro-2-[(2-chloro-2-oxo-1,3,2-dioxaphospholan-4-yl)thio]-6-phenyl-pyrimidine-5-carbonitrile***(4)**

A mixture of epichlorohydrin derivative **2a** (0.61 g, 2 mmole) and $POCl_3$ (10 ml) was heated under reflux in an oil bath for 12 h. The reaction mixture was left to cool; poured onto crushed ice and kept in refrigerator overnight. The obtained product was filtered washed with H_2O, left to dry and crystallized from EtOH/H_2O.

Yield: 56%; mp: 120ºC; IR (KBr, cm^{-1}): 2227 (C≡N); 1605, 1544, 1505 (C=N, C=C); 1250 (P=O); 1250, 1074 (C–S–C); 1027 (C–O–P); 766 (C-Cl); 696 (P-Cl). ^{1}H-NMR (DMSO-d_6) δ*ppm:* 1.06-1.10 (m, 2H, CH_2O); 3.91-4.30 (m, 2H, S–CH_2); 4.40 – 4.51 (m, 1H, CHO); 7.53-7.74 (m, 3H, Phenyl $C_{3,4,5}$-H); 7.84-7.92 (m, 2H, phenyl $C_{2,6}$-H). Anal. Calcd for $C_{14}H_{10}Cl_2N_3O_3PS$ (402.19): N,10.45;P,7.70; Found: 10.54; 7.22. EI-Mass spectrum of compound **4** m/z (relative abundance %):401($M^{+\cdot}$) (44); 400 (51); 326 (39); 325 (44); 324 (54); 303 (17); 302 (61); 289 (59); 288 (100); 270 (32); 269 (22); 268 (81); 255 (22); 252 (56); 209 (37); 200 (37); 186(24); 185 (66); 184 (37); 183(73); 182 (49); 180 (51); 141 (83); 140 (56); 127 (37); 116 (49); 115 (46); 114 (61); 113 (32); 111 (44); 110 (20); 109 (22); 105 (37); 104 (49); 103 (61); 101 (51); 100 (27); 99 (44); 95 (34); 89 (34); 88(27); 87 (49); 86 (32); 82 (76); 77 (88); 76 (95); 75 (63); 74 (27); 73 (46); 72 (56); 70 (27); 63 (54); 61(42); 60 (51); 59 (81); 58 (34); 56 (15); 54 (49); 52 (27); 51(49); 50 (90).

4.1.5. *6-Substituted-4-chloro-2-thioxo-1,2-dihydropyrimidine-5-carbonitriles* **(5a-c**)

A mixture of the appropriate thione derivative **1a-c** (2 mmole) and $POCl_3$ (5 ml) was heated under reflux in an oil bath for 12 h. The reaction mixture was left to cool and poured onto crushed ice. It was then left in refrigerator overnight. The obtained product was filtered, washed with H_2O and left to dry.

4.1.5.1. *4-Chloro-6-phenyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile(***5a)**

Yield: 84%; mp: 120ºC; IR (KBr, cm^{-1}): 3165 (NH); 2230 (C≡N); 1675, 1644 (C=N); 1591, 1505 (C=C); 1534, 1267, 1143, 1000 (δ-NH, N-C=S); 1445, 1336 (C–N thiolactam); 873 (C-Cl). Anal. Calcd for $C_{11}H_6ClN_3S$.(247.03) (402.19):N,16.97; Found: 16.67.

4.1.5.2. *4-Chloro- 6-(4-nitrophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(5b)**

Yield: 87%; mp: 300ºC; IR (KBr, cm^{-1}):
N); 1677, 1660 (C=N); 1629, 1598(C=C); 1525 (δNH); 1525, 1348 (NO_2); ≡3384, 3109 (NH); 2224 (C
1525, 1260, 1145, 1012 (N–C=S); 1413, 1348 (C–N thiolactam); 856 (C–Cl).
Anal. Calcd for $C_{11}H_5ClN_4O_2S.H_2O$(310.72):C,42.52; H,2.27; Found: 43.11;3.33.

4.1.5.3. *4-Chloro-6-(2-furyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(5c)**

Yield: 95%; mp: 160ºC; IR (KBr, cm^{-1}): 3136 (NH); 2921 (CH furan); 2230 (C≡N); 1662, 1645 (C=N); 1579, 1489 (C=C); 1533, 1265, 1146, 1016 (δNH, N–C=S); 1405, 1325 (C–N thiolactam); 1225, 1067 (C–O–C); 866 (C-Cl); 778 (*oop* furan).^{1}H-NMR (DMSO-d_6) δ*ppm:* 6.85 (d, 1H, J = 3.05 *Hz*, furan C_4–H); 7.84 (d, 1H, *J* = 3.85 *Hz*, furan C_3–H); 8.17 (s, 1H, furan C_5–H); 13.07 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_9H_4ClN_3OS.H_2O$: (255.68)N,16.43; Found: 16.71.

4.1.6. *6-Substituted-4-substitutedamino-2-thioxo-1,2-dihydropyrimidine-5-carbonitriles* **(6a-i).**

The appropriate chloropyrimidine derivative **5a-c** (2 mmole) together with the appropriate secondary amine (4 mmole) were fused on a sand bath at 160-170ºC for 6h. The reaction mixture was left to cool then triturated with 10% acetic acid. The obtained product was filtered, washed with EtOH, left to dry and crystallized from the appropriate solvent.

4.1.6.1. *4-Morpholinyl-6-phenyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6a).**

Crystallized from EtOH ; Yield: 80%; mp: 190ºC; IR (KBr, cm^{-1}): 3189 (NH); 2205 (C≡N); 1660, 1644 (C=N); 1607, 1518, 1491 (C=C); 1550 (δNH); 1585, 1284, 1117, 1003 (N-C=S); 1307 (C-N thiolactam); 1206 (C-N aliphatic); 1256, 1069 (C–O–C).^{1}H-NMR (DMSO-d_6) δ*ppm:* 3.62 (t, 2H, *J* = 4.6*Hz*, morpholine C_5–H); 3.67 (t, 2H, *J* = 4.6*Hz*, morpholine C_3–H); 3.78 (t, 4H, *J* = 4.6*Hz*, morpholine $C_{2,\ 6}$–H); 7.47 (t, 2H, *J* = 7.65*Hz*, phenyl $C_{3,\ 5}$–H); 7.52 (t, 1H, *J* = 7.65*Hz*, phenyl C_4–H); 7.77 (d, 2H, *J* = 7.65*Hz*, phenyl $C_{2,\ 6}$–H).. Anal. Calcd for $C_{15}H_{14}N_4OS$(298.36); N,18.78; found,18.58**.**

4.1.6.2. *4-Morpholinyl-6-(4-nitrophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6b).**

Crystallized from DMF/EtOH; Yield: 43%; mp: above 300 ºC; IR (KBr, cm^{-1}): 3374 (NH); 2201 (C≡N); 1643 (C=N); 1601, 1517 (C=C) 1551, 1369(NO_2); 1551, 1261, 1182, 1003 (N–C=S); 1412 (C–N thiolactam); 1261, 1076 (C–O–C); 1121 (C–N aliphatic). Anal. Calcd for $C_{15}H_{13}N_5O_3S$ (391.40) N,17.89; found,17.45**.**

4.1.6.3. *6-(2-Furyl)-4-morpholinyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6c).**

Crystallized from DMF/EtOH; Yield: 43%; mp: 180 ºC; IR (KBr, cm^{-1}): 3410 (NH); 3114 (CH furan); 2200 (C≡N); 1627 (C=N); 1585, 1550, 1500 (δNH, C=C); 1585, 1289, 1115, 1004 (N–C=S); 1438, 1309 (C–N thiolactam); 1260, 1068 (C–O–C); 1212 (C–N aliphatic); 757 (*opp* furan). Anal. Calcd for $C_{13}H_{12}N_4O_2S$(288.32) N,19.43; found,19.66.

4.1.6.4. *6-Phenyl-4-(1-piperidinyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6d).**

Crystallized from DMF/H_2O;Yield:50%; mp: 185ºC; IR (KBr, cm^{-1}): 3412 (NH); 2193 (C≡N); 1643 (C=N); 1586, 1506, 1492 (δNH, C=C); 1549, 1272, 1130, 1027(N–C=S); 1306 (C–N thiolactam);1212 (C–N aliphatic). Anal. Calcd for $C_{16}H_{16}N_4S$ $N2H_2O$(332.42) N,16.85; found,16.24.

4.1.6.5. *6-(4-Nitrophenyl)-4-piperidinyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6e).**

Crystallized from DMF/EtOH; Yield: 52%; mp: above 300ºC; IR (KBr, cm^{-1}): 3357, 3217 (NH); 2193 (C≡N); 1690 (C=N); 1602, 1517 (δNH, C=C); 1536, 1369 (NO_2); 1568, 1273, 1181, 1021 (N–C=S); 1311 (C–N thiolactam); 1214 (C–N aliphatic). Anal. Calcd for $C_{16}H_{15}N_5O_2S2H_2O$(377.42) N,18.56; found,18.00.

4.1.6.6. *6-(2-Furyl)-4-piperidinyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6f).**

Crystallized from DMF/H_2O; Yield: 93%; mp: 230 ºC; IR (KBr, cm^{-1}): 3135 (NH); 3108 (CH furan); 2194 (C≡N); 1643 (C=N); 1500 (C=C); 1550, 1272, 1138, 991 (δNH, N–C=S); 1307 (C–N thiolactam); 1232,1026 (C–O–C); 1213 (C–N aliphatic); 781 (*oop* furan).^{1}H-NMR (DMSO-d_6) δ*ppm:* 1.45-1.51 (m, 2H, piperidine C_4–H); 1.53-1.63 (m, 4H, piperidine $C_{3,\ 5}$–H); 2.60-2.62 (m, 2H, piperidine C_6–H); 2.84-2.86 (m, 2H, piperidine C_2–H); 3.80 (br.s, 1H, NH, D_2O exchangeable);6.68 (dd, 1H, *J* = 3.8, 2.3 *Hz*, furan C_4–H); 7.34 (d, 1H, *J* = 3.8 *Hz*, furan C_3–H); 7.93 (d, 1H, *J* = 1.8 *Hz*, furan C_5–H). Anal. Calcd for$C_{14}H_{14}N_4OS$(286.35)N, 19.57; found,19.88.

4.1.6.7. *4-N-Benzylpiperazinyl-6-phenyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6g).**

Crystallized from EtOH; Yield: 36 %; mp: 174 ºC; IR (KBr, cm^{-1}): 3396 (NH); 2201 (C≡N); 1659, 1643 (C=N); 1587, 1491 (δNH, C=C); 1537, 1261, 1181, 1027(N–C=S); 1209, 1101 (C–N, tertiary amine).^{1}H-NMR (DMSO-d_6) δ*ppm:* 3.70-3.90 (m, 10H, piperazine and N–CH_2); 4.49 (s, 1H, NH, D_2O exchangeable);7.20-7.80 (m, 10H, two phenyl protons).Anal. Calcd for $C_{22}H_{21}N_5S2H_2O$(435.54)N, 16.08; found, 16.00.

4.1.6.8. *2-N-Benzylpiperazinyl-6-(4-nitrophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6h).**

Crystallized from DMF/EtOH; Yield: 59 %; mp: above 300 ºC; IR (KBr, cm^{-1}): 3356, 3213 (NH); 2199 (C≡N); 1600, 1518 (C=N, C=C); 1530, 1345 (NO_2); 1313 (C–N thiolactam); 1263, 1180, 999 (N–C=S); 1209, 1109 (C–N, tertiary amine). Anal.Calcdfor $C_{22}H_{20}N_6O_2S2H_2O$(480.54)N, 17.49; found, 17.74. EI- Mass spectrum of compound **6h** m/z (relative abundance %):369(25); 368(59); 341(8); 313(19); 311(8); 285(14); 278(4); 264(16); 257(15); 256(21); 252(4); 250(7); 241(10); 239(17); 236(22); 227(11); 213(21); 201(5); 199(13); 194(8); 185(20); 171(17); 166(9); 157(14); 152(15); 149(29); 147(17); 139(19); 138(15); 137(23); 135(20); 133(17); 129(40); 127(15); 125(30); 124(17); 123(32); 121(23); 119(16); 115(18); 113(18); 112(20); 111(50); 110(26); 109(42); 107(21); 105(20); 101(15); 99(26); 98(39); 97(77); 96(33); 95(58); 93(23); 91(21); 87(20); 86(10); 85(62); 84(42); 83(78); 82(30); 81(59); 79(20); 73(40); 71(69); 70(35); 69(100); 68(21); 67(39); 60(35); 57(73); 56(23); 55(76).

4.1.6.9. *4-N-Benzylpiperazinyl-6-(2-furyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* **(6i).**

Crystallized from DMF/EtOH; Yield: 25 %; mp: above 300 ºC; IR (KBr, cm^{-1}): 3409 (NH), 3059 (CH furan); 2200 (C≡N); 1643 (C=N); 1586, 1550, 1500 (δNH, C=C); 1568, 1278, 1179, 1028 (N–C=S); 1278, 1028 (C–O–C); 1358 (C–N thiolactam); 1108 (C–N, tertiary amine); 750 (*oop* furan). Anal. Calcd for $C_{20}H_{19}N_5OS$. EtOH (423.53);C,62.39,H,5.95,N, 16.54; found: C,62.29, H,5.97, N,16.2.

4.1.7. *4-Substituted-2-[2-(diethylamino)ethylthio]-6-oxo-1,6-dihydropyrimidine-5-carbonitriles;* **(7a,b).**

To a suspension of the appropriate thione **1a,b** (2 mmole) in aqueous KOH solution (0.34 g, l ml) was added 2-(diethylamino)ethyl chloride.HCl (2 mmole, 0.34 g). The reaction mixture was stirred at R.T. for 4h during which a precipitate was formed. The product was then filtered, washed with H_2O, left to dry then crystallized from DMF/H_2O.

4.1.7.1 *2-[2-(Diethylamino)ethylthio]-4-phenyl-6-oxo-1,6-dihydropyrimid-ine-5-carbonitrile* **(7a).**

Yield: 67%; mp: 217ºC; IR (KBr, cm^{-1}): 3412 (NH); 2206 (C≡N); 1660 (C=O); 1644 (C=N); 1550 (δNH); 1585, 1525 (C=C); 1297, 1051 (C-S–C); 1435, 1316 (C–N lactam); 1206, 1103 (C–N aliphatic). Anal. Calcd for $C_{17}H_{20}N_4OS$. 1/2 H_2O(337.44) C 60.51,H 6.27,N 16.6; found,C,61.22, H,6.51, N,16.22.

4.1.7.2 *2-[2-(Diethylamino)ethylthio]-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimid-ine-5-carbonitrile* **(7b).**

Yield: 25%; mp: above 300ºC; IR (KBr, cm^{-1}): 3374 (NH); 2206 (C≡N); 1660 (C=O); 1643 (C=N); 1626, 1594, 1518 (δNH, C=C); 1551, 1346 (NO_2); 1452 (C–N lactam); 1181, 1108 (C–N aliphatic); 1046 (C–S–C).^{1}H-NMR (DMSO-d_6) δ*ppm:* 0.87 (t, 3H, *J* = 6.9 *Hz*, CH_2-$\underline{CH_3}$); 0.92 (t, 3H, *J* = 6.9 *Hz*, CH_2-$\underline{CH_3}$); 1.15 (t, 2H, *J* = 6.9 *Hz*, S-CH_2); 2.33 (t, 2H, *J* = 6.9 *Hz*, N-CH_2); 2.37 (q, 4H, *J* = 6.9 *Hz*, N-($\underline{CH_2}$-CH_3)$_2$); 4.07 (s, 1H, NH, D_2O exchangeable); 7.91 (d, 2H, *J* = 8.4 *Hz*, 4-NO_2- C_6H_4-$C_{2,\ 6}$-H); 8.28 (d, 2H, *J* = 8.4 *Hz*, 4-NO_2-C_6H_4-$C_{3,\ 5}$-H).Anal. Calcd for $C_{17}H_{19}N_5O_3S$.1/2 H_2O(382.44);C 53.39, H 5.27; found,C,53.43, H,5.13.

4.1.8 *N-(4-Substitutedphenyl)-2-[(4-substituted-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-yl)thio]acetamides***(9a-d)**

A mixture of the appropriate thione **1a,c** (2 mmole), anhydrous K_2CO_3 (0.28 g, 2 mmole) and the selected 4-substituted chloroacetanilide derivative **8a,b** (2 mmol) in dry DMF (10 ml) was stirred at R.T. for 6h. The reaction mixture was diluted with H_2O and the obtained product was filtered, washed with H_2O and crystallized from DMF/H_2O.

4.1.8.1 *N-(4-Methoxyphenyl)-2-[(4-phenyl-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-yl)thio]acetamides***(9a)**

Yield: 88%; mp: 230ºC; IR (KBr, *cm*$^{-1}$3269 (NH); 2220 (C≡N); 1654 (C=O); 1614 (C=N); 1595, 1511 (C=C); 1557, 1533 (δNH); 1415, 1369 (C–N lactam); 1293 (C–S–C); 1255, 1162, 1024(C–O–C). Anal. Calcd for $C_{20}H_{16}N_4O_3S$(392.43) C 61.21, H 4.11, N 14.28; found, C,60.87, H,3.57, N,13.92.

4.1.8.2 *N-(4-Methoxyphenyl)-2-[(4-(2-furyl)-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-yl)thio]acetamides***(9b)**

Yield: 85 %; mp: 230ºC; IR (KBr, *cm*$^{-1}$): 3267 (NH); 3130 (CH furan); 2223 (C≡N); 1691 1663 (C=O); 1588 (C=N); 1551 (δNH); 1411, 1330 (C–N lactam); 1294 (C–S–C); 1253, 1022(C–O–C); 779 (*oop* furan).^{1}H-NMR (DMSO-d_6) δ*ppm:* 2.70 (d, 1H, *J* = 1.55*Hz*, S–CH_2);2.85 (d, 1H, *J* = 1.5*Hz*, S–CH_2);3.67 (s, 3H, OCH_3);4.12 (s, 1H, pyrimidine NH, D_2O exchangeable); 6.74 (dd, 1H, *J* = 3.8, 1.5*Hz*, furan C_4–H); 6.84 (d, 2H, *J* = 9.2*Hz*, 4-OCH_3–C_6H_4–$C_{2,\ 6}$–H); 7.42 (d, 2H, *J* = 9.2*Hz*, 4-OCH_3–C_6H_4–$C_{3,\ 5}$–H); 7.54 (d, 1H, *J* = 3.05*Hz*, furan C_3–H); 8.05 (s, 1H, furan C_5–H); 10.23 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for$C_{18}H_{14}N_4O_4S$(382.39) C 56.54,H 3.69,N 14.68; found,C,55.81,H,2.77,N,14.25.

4.1.8.3 *N-(4-Chlorophenyl)-2-[(4-phenyl-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-yl)thio]acetamides***(9c)**

Yield: 88 %; mp: 240ºC; IR (KBr, *cm*$^{-1}$): 3379, 3272 (NH); 2195 (C≡N); 1655 (C=O); 1586, 1491 (C=N, C=C); 1554, 1535 (δNH); 1436, 1315 (C–N lactam); 1296, 1096 (C–S–C); 822 (C–Cl).^{1}H-NMR (DMSO-d_6) δ*ppm:* 3.78 (s, 2H, S–CH_2); 4.07 (s, 1H, pyrimidine NH, D_2O exchangeable); 7.28 (d, 2H, *J* = 8.4 *Hz*, 4-Cl-C_6H_4–$C_{2,\ 6}$–H); 7.35 (t, 1H, *J* = 7.65 *Hz*, C_6H_5–C_4–H); 7.43 (t, 2H, *J* = 7.65*Hz*, C_6H_5–$C_{3,\ 5}$–H); 7.54 (d, 2H, *J* = 8.4 *Hz*, 4-Cl-C_6H_4–$C_{3,\ 5}$–H); 7.70 (d, 2H, *J* = 7.65*Hz*, C_6H_5–$C_{2,\ 6}$–H); 10.96 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{19}H_{13}ClN_4O_2S.2H_2O$(432.88) N 12.94; found: 12.81.

4.1.8.4 *N-(4-Chloroxyphenyl)-2-[(4-(2-furyl)-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-yl)thio]acetamides***(9d)**

Yield: 68 %; mp: 245ºC; IR (KBr, *cm*$^{-1}$): 3436, 3318 (NH); 3126 (CH furan); 2226 (C≡N); 1689, 1657 (C=O); 1590, 1492 (C=N, C=C); 1538 (δNH); 1410, 1328 (C-N lactam); 1250, 1022 (C–O–C); 1096 (C–S–C), 823 (C–Cl); 762 (*oop* furan). Anal. Calcd $C_{17}H_{11}ClN_4O_3S$(386.81) C 52.79,H 2.87,N 14.48; found,C,53.24, H,2.83, N,14.75.

4.1.9 *4-Substituted-1-methyl-2-methylthio-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(10a-c)**

A mixture of the appropriate thione derivative **1a-c** (20 mmole), K_2CO_3 (4.1 g, 30 mmole) and CH_3I (5.68 g, 2.5 ml, 40 mmole) in dry DMF (10 ml) was stirred for 6 h at R.T. The reaction mixture was diluted with H_2O, filtered, washed with H_2O and crystallized from the appropriate solvent.

4.1.9.1 *1-Methyl-2-methylthio-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles* **(10a)[29]**

Crystallized from CH_3OH, Yield: 85 %; mp: 240ºC; IR (KBr, *cm*$^{-1}$): 2928 (CH_3); 2216 (C≡N); 1676 (C=O); 1647 (C=N); 1505 (C=C); 1373 (N–CH_3), 1308 (S–CH_3); 1228, 1088(C–S–C); 1415, 1344 (C–N lactam). $C_{13}H_{11}N_3OS$(257.32).

4.1.9.2 *1-Methyl-2-methylthio-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(10b)[29]**

Crystallized from DMF/H_2O; Yield: 82 %; mp: 242ºC; IR (KBr, *cm*$^{-1}$): 2929 (CH_3), 2218 (C≡N); 1665 (C=O); 1601, 1519 (C=N, C=C); 1568, 1346 (NO_2); 1412, 1316 (C-N lactam); 1369 (N–CH_3); 1316 (S–CH_3); 1283, 1089 (C–S–C). $C_{13}H_{10}N_4O_3S$(302.31)

4.1.9.3 *4-(2-Furyl)-1-methyl-2-methylthio-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(10c)**

Crystallized from DMF; Yield: 93 %; mp: 270ºC; IR (KBr, *cm*$^{-1}$): 3135 (CH furan); 2930 (CH_3); 2222 (C≡N); 1672 (C=O); 1628 (C=N); 1593; 1502 (C=C); 1412, 1340 (C–N lactam); 1365 (N–CH_3); 1309 (S-CH_3); 1234, 1094 (C–S–C); 1184, 1043 (C–O–C); 777 (*opp* furan). ^{1}H-NMR (DMSO-d_6) δ*ppm:* 2.65 (s, 3H, S–CH_3); 3.38 (s, 3H, N-CH_3, under DMSO); 6.81 (dd, 1H, *J* = 3.8, 1.5*Hz*, furan C_4–H); 7.55 (d, 1H, *J* = 3.8*Hz*, furan C_3–H); 8.12 (s, 1H, furan C_5–H).Anal. Calcd for $C_{11}H_9N_3O_2S$(247.27) C 53.43,H 3.64,N 16.99,S 12.97; found C,53.03, H,3.73, N,16.50, S,12.41.

4.1.10 *4-Substituted-2-substitutedamino-1-methyl-6-oxo-1,6-dihydro-pyrimidine-5-carbonitriles* **(11a-f).**

A mixture of the appropriate derivative of compound **10a-c** (2 mmole) and the selected secondary amine (4 mmole) was fused at 160-170ºC for 12 h. The reaction mixture was then

triturated with EtOH, filtered, washed with EtOH and left to dry. The product was crystallized from the appropriate solvent.

4.1.10.1. *2-(4-Mopholinyl)-1-methyl-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(11a**)

Crystallized from DMF/EtOH; Yield: 30 %; mp: 155ºC; IR (KBr, cm^{-1}): 2205 (C≡N); 1690 (C=O); 1604, 1569 (C=N, C=C); 1533, 1363 (NO_2); 1409, 1304 (C–N lactam); 1270, 1241, 1067 (C–O–C); 1112 (C-N aliphatic). Anal. Calcd for $C_{16}H_{15}N_5O_4.2H_2O$(377.36),N 18.55; found 18.37.

4.1.10.2. *4-(2-Furyl)-2-mopholinyl-1-methyl-6-oxo-1,6-dihydro-pyrimidine-5-carbonitriles* **(11b**)

Crystallized from DMF/H_2O; Yield: 35 %; mp: abouve 300ºC; IR (KBr, cm^{-1}): 2961 (CH furan); 2209 (C≡N); 1660 (C=O); 1644 (C=N); 1568, 1551, 1517 (C=C); 1447, 1306 (C–N lactam); 1263, 1067 (C–O–C); 1112 (C–N aliphatic); 786 (*oop* furan). ^{1}H-NMR (DMSO-d_6) δ*ppm:* 2.32-2.33, 2.60-2.61 (two m, 4H, morpholine $C_{3,5}$–H); 3.61-3.75(m,4H,morpholine $C_{2,6}$–H); 4.05 (s, 3H, N–CH_3); 6.71-6.74 (m, 1H,furan C_4–H);7.33 (d, 1H, *J* = 3.05*Hz*, furan C_3–H);7.99 (s, 1H, furan C_5-H). Anal. Calcd for$C_{14}H_{14}N_4O_3.H_2O$(304.31)C 55.26, H 5.29; found C,55.59, H,5.20. -EI- Mass spectrum of compound 11b m/z (relative abundance %): 285(M-1)(21); 284(14); 265(22); 264(39); 262(19); 256(17); 239(39); 237(25); 236(50); 227(13); 213(18); 211(15); 185(23); 152(23); 149(29); 137(22); 129(53); 125(26); 123(30); 121(24); 115(32); 112(25); 111(52); 110(26); 109(37); 100(17); 98(61); 97(87); 96(34); 95(67); 87(34); 86(26); 85(51); 84(48); 83(73); 82(27); 81(63); 79(23); 73(39); 71(64); 69(71); 67(39); 60(19); 57(100); 56(36); 55(77).

4.1.10.3. *1-Methyl-6-oxo-4-phenyl-2-(1-piperidinyl)-1,6-dihydropyrimidine-5-carbonitriles* **(11c**)

Crystallized from EtOH; Yield: 70%; mp: 230ºC; IR (KBr, cm^{-1}): 2207 (C≡N); 1663 (C=O); 1584, 1492 (C=N, C=C); 1447, 1307 (C–N lactam); 1212 (C– N aliphatic). Anal. Calcd for$C_{17}H_{18}N_4O$(294.35) C 69.37, H 6.16,N 19.03; found 68.63, 6.35, 18.47.

4.1.10.4. *4-(2-Furyl)-1-methyl-6-oxo-2-(1-piperidinyl)-1,6-dihydro-pyrimidine-5-carbonitriles* **(11d**)

Crystallized from DMF/H_2O;Yield:96%; mp: 220ºC; IR (KBr, cm^{-1}): 2931 (CH furan); 2198 (C≡N); 1660 (C=O); 1590, 1568, 1487 (C=N, C=C); 1446 (C-N lactam); 1256, 1022 (C–O–C); 1215 (C–N aliphatic); 782 (*oop* furan). Anal. Calcd for $C_{15}H_{16}N_4O_2$(284.31)C 63.57, H 5.67 ; found: C, 63.57, H,5.49.

4.1.10.5 *2-N-Benzylpiperazinyl-1-methyl-6-oxo-4-phenyl-1,6-dihydro-pyrimidine-5-carbonitriles* **(11e**)

Crystallized from EtOH; Yield: 57 %; mp: 105ºC; IR (KBr, cm^{-1}): 2213 (C≡N); 1665 (C=O); 1582, 1568, 1495 (C=N, C=C); 1447, 1313 (C-N lactam); 1210 (C–N aliphatic).^{1}H-NMR (DMSO-d_6) δ*ppm*; 2.88 (s, 3H, N–CH_3); 3.40-3.50 (m, 8H, piperazine protons);3.64 (s, 2H, N–CH_2); 7.30-7.44 (m, 4H, C_6H_5 and benzyl $C_{3,5}$-H);7.50 (t, 1H, *J* = 7.65*Hz*, benzyl C_4-H);7.51 (d, 2H, *J* = 7.65*Hz*, benzyl $C_{2,6}$-H);7.54 (t, 1H, *J* = 7.65*Hz*, C_6H_5-C_4-H);7.80 (d, 2H, *J* = 7.65*Hz*, C_6H_5-$C_{2,6}$-H).Anal. Calcd for $C_{23}H_{23}N_5O.2H_2O$(421.50): N, 16.61; found: 16.64.

4.1.10.6. *2-N-Benzylpiperazinyl-4-(2-furyl)-1-methyl-6-oxo-1,6-dihydro-pyrimidine-5-carbonitriles* **(11f**)

Crystallized from DMF/H_2O; Yield: 81 %; mp: 130ºC; IR (KBr, cm^{-1}): 3112 (CH furan); 2218 (C≡N); 1672 (C=O); 1595, 1568, 1490 (C=N, C=C); 1447, 1313 (C-N lactam); 1269, 1076 (C–O–C); 1206 (C–N aliphatic); 746 (*oop* furan). Anal. Calcd for $C_{21}H_{21}N_5O_2$(375.42) C, 67.18, H, 5.67, N, 18.65; found C,66.74, H,5.65, N,18.53.

4.1.11 *4-Substituted-2-hydrazino-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(12a-c)**

To a suspension of the appropriate S-CH_3 derivative **10a-c** (20 mmole) in EtOH (20 ml), hydrazine hydrate (1 ml, 0.1 mole) was added and the reaction mixture was heated under reflux for 6 h. The obtained product was filtered, washed with EtOH and crystallized from the appropriate solvent.

4.1.11.1 *2-Hydrazino-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles* **(12a)**[29]

Crystallized from DMF/CH_3Cl; Yield: 92 %; mp: 270ºC; IR (KBr, *cm*$^{-1}$): 3458, 3316 (NH); 2221 (C≡N); 1675 (C=O); 1643 (C=N); 1587, 1512 (C=C); 1542 (δNH). Anal. Calcd for $C_{12}H_{11}N_5O$ (241.25).

4.1.11.2 *2-Hydrazino-1-methyl-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(12b)[**29]

Crystallized from DMF/H_2O; Yield: 88 %; mp: 300ºC; IR (KBr, *cm*$^{-1}$): 3442, 3301, 3114 (NH); 2199 (C≡N); 1661 (C=O); 1646 (C=N); 1607, 1503 (C=C); 1552 (δNH); 1532, 1350 (NO_2). Anal. Calcd for $C_{12}H_{10}N_6O_3$(286.25).

4.1.11.3 *4-(2-furyl)-2-hydrazino-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitriles* **(12c)**

Crystallized from DMF/H_2O; Yield:70 %; mp: 264-265ºC; IR (KBr, *cm*$^{-1}$): 3316, 3141 (NH); 3121 (CH furan); 2213 (C≡N); 1659 (C=O); 1594, 1539 (C=N, δNH, C=C); 1411 (C–N lactam); 1467, 1380 (N–CH_3); 1260; 1020 (C–O–C); 775 (*opp* furan).^{1}H-NMR (DMSO-d_6) 3.32 (s, 3H, N–CH_3); 6.75 (dd, 1H, J = 3.8, 1.5*Hz*, furan C_4–H);7.38 (d, 1H, J = 3.8*Hz*, furan C_3–H);8.05 (s, 1H, furan C_5–H);9.84, 9.86 (two s, each 1/2 H, 2NH tautomers, D_2O exchangeable);10.33 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd for $C_{10}H_9N_5O_2$(231.21)C 51.95, H 3.92; found: C,51.50, H,3.25.

4.1.12 *4-Substituted-1-methyl-6-oxo-2-[3,4,6-trioxo-1,2,3,4,5,6-hexahydro-pyrano[2,3-c]pyrazol-2-yl]-1,6-dihydropyrimidine-5-carbonitriles* **(13a,b).**

The appropriate hydrazine derivative **12a,c** (2 mmole) was heated under reflux with diethyl malonate (3.18 g, 3 ml, 20 mmole) for 6h, whereupon needle crystals separated out. The reaction mixture was left to cool, filtered and the obtained product was washed with EtOH and crystallized from DMF/EtOH.

4.1.12.1 *1-Methyl-6-oxo-4-phenyl-2-[3,4,6-trioxo-1,2,3,4,5,6-hexahydro-pyrano[2,3-c]pyrazol-2-yl]-1,6-dihydropyrimidine-5-carbonitriles* **(13a).**

Yield: 60 %; mp: 230ºC; IR (KBr, *cm*$^{-1}$): 3278 (NH); 2218 (C≡N); 1746, 1658 (C=O); 1625 (C=N); 1595, 1492 (C=C); 1525 (δNH); 1253, 1222, 1037 (C–O–C). Anal. Calcd for $C_{18}H_{11}N_5O_5$(377.31) C 57.30, H 2.94, N 18.56; found 57.11, 3.07, 18.87.

4.1.12.2 *4-(2-furyl)-1-methyl-6-oxo-2-[3,4,6-trioxo-1,2,3,4,5,6-hexahydro-pyrano[2,3-c]pyrazol-2-yl]-1,6-dihydropyrimidine-5-carbonitriles* **(13b).**

Yield: 76 %; mp: 234ºC; IR (KBr, *cm*$^{-1}$): 3281 (NH); 2986 (CH furan); 2225 (C≡N); 1744, 1662 (C=O); 1592, 1520, 1467 (C=N, C=C); 1566 (δNH); 1284, 1263, 1224, 1082, 1040 (C–O–C); 776 (*opp* furan). ^{1}H-NMR (DMSO-d_6) 3.43 (s, 2H, pyranopyrazole C_5–H); 3.46 (s, 3H, N– CH_3); 6.75 (dd, 1H, *J* = 3.8, 1.5*Hz*, furan C_4–H); 7.41 (d, 1H, *J* = 3.05*Hz*, furan C_3–H); 8.02 (s, 1H, furan C_5–H);10.42 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ*ppm*:167.71 (pyranopyrazole C_6); 165.58 (pyranopyrazole C_4); 161.48 (pyranopyrazole C_3); 155.68 (pyrimidine C_6); 155.55 (pyranopyrazole C_{7a}); 151.0 (pyrimidine C_2); 150.21 (pyrimidine C_4); 147.93 (furan C_2); 117.92 (pyrimidine C_5); 117.83 (pyranopyrazole C_{3a}); 116.0 (C≡N);113.51 (furan C_5); 100(furan C_3); 82.27 (furanC_4);61.35 (pyranopyrazole C_5); 41.09 (N–CH_3). Anal. Calcd for$C_{16}H_9N_5O_6$.1/2H_2O(376.28) C 51.07, H 2.68; found 51.39, 2.18. EI- Mass spectrum m/z (relative abundance %):349(68); 347(59); 172(64); 158(100); 157(100); 113(68); 78(59).

4.1.13 *3-Cyanomethyl-8-methyl-7-oxo-5-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile* **(14)** *And ethyl 3-[2-cyano-N'-(5-cyano-1-methyl-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)acetohydrazido]-3-iminopropanoate***(15)**

The hydrazine derivative **12a** (0.48 g, 2 mmole) was heated with ethyl cyanoacetate (0.45 g, 0.42 ml, 4 mmole) in an oil bath at 160 for 5h. The solid mass was triturated with EtOH, filtered and washed with EtOH. The product was then crystallized from EtOH giving compound **14**, while the alcohol-insoluble part was found to be compound **15**.

3-Cyanomethyl-8-methyl-7-oxo-5-phenyl-7,8-dihydro[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile **(14)**

Yield: 26 %; mp: 185ºC; IR (KBr, *cm*$^{-1}$2978 (CH_2, CH_3); 2215 (C≡N); 1672 (C=O); 1626 (C=N); 1530, 1474 (C=C); 1343 (C–N lactam).^{1}H-NMR (DMSO-d_6) 3.51, 3.53 (two d, 2H, *J* = 3.05*Hz*, CH_2CN);3.81 (s, 3H, N-CH_3);7.46, 7.49 (two t, 2H, *J* = 8.4*Hz*, C_6H_5–$C_{3,5}$–H);7.61, 7.66 (two d, 2H, *J* = 7.65*Hz*, C_6H_5–$C_{2,6}$–H);7.73, 7.76 (two t, 1H, *J* = 6.1*Hz*, C_6H_5–C_4–H). Anal. Calcd for $C_{15}H_{10}N_6O$.1 1/2 H_2O C 56.78, H 4.13; found 56.97, 3.95.

EI- Mass spectrum m/z (relative abundance %): 291(M^++ 1) (6); 265(46); 264(38); 263(20) 239(9); 237(12); 236(20); 213(12); 211(11); 185(13); 163(10); 159(9), 149(19); 141(16); 137(21); 135(16); 133(16); 129(26); 127(22); 125(27); 123(30); 119(21); 111(48), 110(21); 109(40); 107(16); 105(20); 99(17); 98(24); 97(78); 96(30); 95(60); 93(19); 85(50); 84(29); 83(80); 82(26); 81(73), 79(21); 73(30); 71(67), 70(27); 69(100); 68(19); 67(40); 60(20); 57(96); 56(28); 55(92).

*Ethyl-3-[2-cyano-N'-(5-cyano-1-methyl-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)acetohydrazido]-3-iminopropanoate***(15)**

Yield: 40 %; mp: above 300ºC; IR (KBr, cm^{-1}): 3336 (NH); 2952 (CH_3, CH_2); 2224 (C≡N); 1727 (C=O ester); 1692 (C=O); 1617 (C=N); 1597, 1570, 1500 (C=C); 1534 (δNH); 1441, 1332 (C–N lactam) 1292, 1017 (C–O–C).^{1}H-NMR (DMSO-d_6) 1.18 (t, 3H, *J* = 7.2 *Hz*, CH_2-CH3);3.37 (s, 2H, CH_2–CN);3.56 (s, 2H, CH_2–CO);3.83 (s, 3H, N–CH_3);3.98 (s, 1H, NH, D_2O exchangeable); 4.11 (q, 2H, *J* = 7.2*Hz*, CH_2-CH_3);7.47-7.60 (m, 3H, C_6H_5-$C_{3,4,5}$–H);7.79 (dd, 2H, *J* = 7.9, 1*Hz*, C_6H_5-$C_{2,6}$–H).Anal. Calcd for $C_{20}H_{19}N_7O_4$(421.41) C 57.01, H 3.50, N 24.28; found 57.11, 3.24, 24.55. EI- Mass spectrum of compound 15 m/z (relative abundance %): 318(83); 317(61); 274(57); 273(57); 271(61); 213(65); 198(100); 159(57); 103(61); 96(87); 64(78); 63(65).

4.1.14 *6-Substituted-2-[(oxiran-2-yl)methylthio]-4-chloropyrimidine-5-carbonitriles* **(16a,b)**

To a solution of chloropyrimidine thione derivative **5a,c** (2 mmole) in DMF (5 ml) was added NaOH solution (0.16 g, 4 mmole) in 2 ml H_2O. The reaction mixture was stirred at R.T. and epichlorohydrin (0.19 g, 0.16 ml, 2 mmole) was added dropwise while stirring. Stirring was continued at R.T. for 48h. The reaction mixture was then diluted with H_2O and the obtained product was filtered, washed with H_2O and crystallized from the appropriate solvent.

4.1.14.1 *2-[(Oxiran-2-yl)methylthio]-6-phenyl-4-chloropyrimidine-5-carbonitriles* **(16a)**

Crystallized from EtOH; Yield: 48 %; mp: 100ºC; IR (KBr, cm^{-1}): 2912 (CH_2); 2226 (C≡N); 1597 (C=N); 1567 (C=C); 1266, 1085 (C–S–C); 1266, 1036, 760 (epoxy C–O–C); 873 (C–Cl).^{1}H-NMR (DMSO-d_6) 2.30-2.34 (m, 1H, S–CH_2);2.59-2.63 (m, 1H, S–CH_2);2.68-2.71 (m, 1H, O–CH);4.02-4.10 (m, 2H, O–CH_2);7.40-7.90 (m, 5H, C_6H_5).Anal. Calcd for $C_{14}H_{10}ClN_3OS$(303.77) C 55.36, H 4.32, N 13.83; found 56.84, 4.92, 14.06. EI- Mass spectrum of compound m/z (relative abundance %): 285(14); 284(45); 283(18); 281(53); 279(48) 270(22); 264(40); 257(45); 254(34); 253(23); 251(33); 238(33); 237(35), 227(36); 223(18); 222(37); 221(21); 215(31); 214(73); 213(28); 209(29); 196(28); 182(35); 179(18), 176(13); 169(25); 155(30); 154(22); 145(20); 141(23); 130(26); 127(25); 116(21); 105(26); 104(100); 103(23); 89(35); 82(22); 77(60), 76(24); 73(21); 70(29), 59(26); 57(13); 51(50); 50(37).

4.1.14.2 *6-(2-furyl)-2-[(oxiran-2-yl)methylthio]-4-chloropyrimidine-5-carbonitriles* **(16b)**

Crystallized from DMF/H_2O; Yield: 58 %; mp: above 300ºC; IR (KBr, cm^{-1}): 3137 (CH furan); 2919, 2849 (CH_2); 2224 (C≡N); 1643 (C=N); 1581, 1529, 1513 (C=C); 1255, 1081 (C–S–C); 1255; 1030, 779 (furan and epoxy C–O–C); 885 (C–Cl); 779 (*oop* furan). Anal. Calcd for $C_{12}H_8ClN_3O_2S$(293.73) C 49.07, H 2.75; found: C, 50.32, H,2.49.

4.1.15 *6-Substituted-2-[(oxiran-2-yl)methylthio]-4-morpholinopyrimidine-5-carbonitriles* (**17a,b).**

To solution of 4-morpholinopyrimidine derivative **6a** and **6c** separately (2 mmole) in DMF (5 ml) was added aqueous NaOH solution (0.16 g, 4 mmole). The reaction mixture was stirred at R.T. for 1h, then epichlorohydrin (0.19 g, 0.16 ml, 2 mmole) was added and stirring was continued for 48 h. The reaction mixture was then diluted with H_2O and the obtained product was filtered, washed with H_2O and crystallized from DMF/H_2O.

4.1.15.1. *2-[(Oxiran-2-yl)methylthio]-6-phenyl-4-morpholinopyrimidine-5-carbonitriles* (**17a).**

Yield: 70 %; mp: 198-200ºC; IR (KBr, cm^{-1}): 2920, 2850 (CH_2); 2207 (C≡N); 1638 (C=N); 1585, 1519, 1492 (C=C); 1285, 1069 (C–S–C); 1267, 1055 (C-O-C); 1255, 1028, 772 (epoxy C–O–C); 1206, 1115 (C–N aliphatic).^{1}H-NMR (DMSO-d_6) 2.59-2.60 (m, 2H, OCH_2 epoxy);3.20-3.40 (m, 2H, S-CH_2 under DMSO);3.62 (t, 2H, *J* = 4.6*Hz*, morpholine C_3–H);3.67 (t, 2H, *J* = 4.6*Hz*, morpholine C_5–H);3.78 (t, 4H, *J* = 4.6*Hz*, morpholine $C_{2,6}$–H);4.03-4.06 (m, 1H, CH, epoxy);7.46 (t, 2H, *J* = 7.6*Hz*, C_6H_5-$C_{3,5}$–H);7.50 (t, 1H, *J* = 7.6*Hz*, C_6H_5-C_4–H);7.73 (d, 2H, *J* = 7.6*Hz*, C_6H_5-$C_{2,6}$–H).Anal. Calcd for $C_{18}H_{18}N_4O_2S.H_2O$(372.45) N 15.04; found 14.86. EI- Mass spectrum m/z (relative abundance %):294(100); 95(44); 85(41); 73(9); 72(16) 71(3); 70(75); 55(47).

4.1.15.2. *6-(2-Furyl)-2-[(oxiran-2-yl)methylthio]-4-morpholinopyrimidine-5-carbonitriles*(**17b).** Yield: 62 %; mp: 190; IR (KBr, cm^{-1}): 3111 (CH furan); 2920, 2850 (CH_2); 2201 (C≡N); 1640 (C=N); 1587, 1561, 1530 (C=C); 1257, 1067 (C–S–C); 1229; 1022, 783 (morpholine, furan and epoxy C–O–C); 1209, 1114 (C–N aliphatic); 759 (*opp* furan).Anal. Calcd for $C_{16}H_{16}N_4O_3S$(344.40) N 16.27; found 16.51. EI- Mass spectrum m/z (relative abundance %):342(22); 341(87); 340(21); 312(27) 311(61); 310(51); 298(37); 296(75); 284(100); 283(28); 280(25); 252(16), 199(21); 156(21); 144(27); 143(34); 117(13); 115(14) 88(15); 86(21); 85(20); 83(12); 71(10); 58(15), 56(21); 55(30).

4.1.16 *6-Substituted-2-(2-hydroxy-3-morpholinopropylthio)-4-morpholino-pyrimidine-5-carbonitriles;* **(18a,b)**

Method A: To a suspension of 2-(oxiran-2-yl)methylthio-4-chloropyrimidine derivative **16a,b** (2 mmole) in dry dioxane (5 ml), morpholine (0.7 g, 0.7 ml, 8 mmole) was added and the reaction mixture was heated under reflux for 24 h. The reaction mixture was concentrated, allowed to cool, then diluted with H_2O. The obtained product was filtered and crystallized from the appropriate solvent.

Method B: The 2-(oxiran-2-yl)methylthio-4-morpholinopyrimidine derivatives **17a,b** (2 mmole) were allowed to react separately with morpholine (0.35 g, 0.35 ml, 4 mmole) in dry dioxane (5 ml) by heating under reflux for 12 h. The reaction mixture was concentrated, left to cool and diluted with H_2O. The obtained product was filtered, washed with H_2O, left to dry and crystallized from the appropriate solvent.

4.1.16.1 *2-(2-Hydroxy-3-morpholinopropylthio)-6-phenyl-4-morpholinopyrimidine-5-carbonitriles;* **(18a)**

Crystallized from EtOH/H_2O; Yield: 34 % (method A), 78% (method B); mp: 175ºC; IR (KBr, cm^{-1}): 3438 (OH br. band); 2920, 2850 (CH_2, CH); 2205 (C≡N); 1644 (C=N); 1585, 1520, 1492 (C=C); 1284, 1069 (C–S–C); 1256, 1023 (C–O–C); 1207, 1118 (C–N aliphatic).^{1}H-NMR (DMSO-d_6) 3.27-3.32 (m, 2H, S–CH_2);3.68-3.70 (m, 1H, CH); 4.08-4.12 (m, 2H, CH_2–N);4.47 (t, 4H, *J* = 4.43 *Hz*, $C_{3,5}$ morpholine of S-propyl side chain));4.50 (t, 4H, *J* = 4.43 *Hz*, morpholine $C_{3,5}$ of 4-morpholino);4.55-4.66 (m, 8H, morpholine $C_{2,6}$–H)8.26-8.38(m, 3H, C_6H_5-$C_{3,4,5}$–H);8.60 (d, *J* = 7.65 *Hz*, 2H, C_6H_5-$C_{2,6}$ –H);8.75 (s, 1H, OH, D_2O exchangeable).Anal. Calcd for $C_{22}H_{27}N_5O_3S.H_2O$(459.58)N 15.24; found 14.72. EI- Mass spectrum (relative abundance %):433(7); 429(36); 351(84); 350(100) 321(46); 320(92); 308(21); 306(53); 295(21); 294(78); 293(21); 292(20), 262(35); 249(13); 248(12); 236(24); 235(18); 224(10) 223(15); 219(15); 209(16); 208(27); 180(21); 154(24), 153(32); 152(15); 129(18); 127(14); 91(13); 86(26); 77(53); 56(26), 55(15); 51(18).

4.1.16.2 *6-(2-Furyl)-2-(2-hydroxy-3-morpholinopropylthio)-4-morpholino-pyrimidine-5-carbonitriles;* **(18b)**

Crystallized from DMF/H_2O; Yield: 86 % (method A), 90% (method B %; mp: above 300ºC; IR (KBr, cm^{-1}3407 (OH br. band); 2919 (CH furan); 2850 (CH_2, CH); 2206 (C≡N); 1658 (C=N); 1562, 1502 (C=C); 1264, 1066 (C–S–C); 1264, 1022 (morpholine and furan C–O–C); 1110 (C–N aliphatic); 754 (*opp* furan). Anal. Calcd for $C_{20}H_{25}N_5O_4S.2H_2O$(467.55) C 51.38,H 6.25; found: C, 50.84, H,6.28. EI- Mass spectrum (relative abundance %): 257(27); 112(7); 111(29); 109(9) 99(9); 98(14); 97(42); 95(30); 85(46); 83(52); 82(18); 81(19), 71(45); 70(15); 69(35); 68(10); 67(20); 58(17) 57(100); 56(29); 55(67).

4.2 **Antimicrobial activity**

All compounds were preliminarily evaluated for their *in-vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 6538P) as a representative of Gram-positive; *Pseudomonas aeruginosa* (ATCC 9027) and *Shigella flexneri* (ATCC 15391) as representatives of Gram-negative bacteria. The compounds were also evaluated for their *in-vitro* antifungal activity against *Candida albicans* (ATCC 2091). Their inhibition zones using the cup diffusion technique [50] were measured. Compounds showing reasonable inhibition zones were subjected to determine their minimal inhibitory concentration (MIC) values using the two fold serial dilution method[51]. Ampicillin and Ofloxacin (Tarivid $^{(R)}$, Sanofi-Aventis) were used as standard antibacterial agents, while Clotrimazole (Canesten $^{(R)}$, Bayer) was used as a standard antifungal agent. Dimethyl

formamide was used as a blank. Compounds were dissolved in dimethylformamide in a concentration of 1mg/ml. Each 100 ml of sterile molten agar (at 45ºC) received 1ml of 6 h. broth, then the seeded agar was poured into sterile Petri dishes. Cups (8mm in diameter) were cut in the agar. Each cup received 0.1 ml of the 1 mg/ml solution of the test compounds. Plates were then incubated at 37ºC for 24 h. for bacteria and 48 h. for fungi. The resulting inhibition zones were measured in mm diameter (**tables 4)**. The test organisms were allowed to grow in their suitable broth for 24 h. Two fold serial dilutions of the test compounds solution were prepared using the proper sterile nutrient broth to obtain concentrations 500, 250, 125, 62.5, 31.25, 15.63, 7.82 and 3.91µg/ml with the concentration of dimethylformamide not exceeding 2.5%. The tubes were then inoculated with the test organisms; each 5ml received 0.1ml of the inoculums and were incubated at 37ºC for 24 h. for bacteria and 48 h. for fungi. The tubes were observed for the presence or absence of microbial growth. The minimum inhibitory concentration was determined visually in which the non turbid tubes were observed and the lowest concentration giving no turbidity is the minimum inhibitory concentration.

4.3 **Computer aided drug design**

All the molecular studies were carried out on an Intel pentium 1.6 GHz processor, 512 MB memory with windows XP operating system using Molecular Operating Environment (MOE 2005.06; Chemical Computing Group, Montreal, Canada) as the computational software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol^{-1} A^{0-1} with MMFF94X forcefield and the partial charges were automatically calculated. The coordinates of the X-ray crystal structure of 2'-deoxyuridine-5'-monophosphate(DUMP) bound to thymidylate synthase enzyme were obtained from Protein Data Bank (PDB ID: 1BID) as well as the coordinates of the X-ray crystal structure of methotrexate (MTX) bound to dihydrofolate reductase (DHFR) enzyme (PDB ID: 4DFR). Enzyme structures were checked for missing atoms, bonds and contacts. Hydrogen atoms were added to the enzyme structure. Water molecules and bound ligands were manually deleted. The ligand molecules were constructed using the builder molecule and were energy minimized. The active site was generated using the MOE-Alpha site finder. Dummy atoms were created from the obtained alpha spheres. Ligands were docked within the thymidylate synthase and dihydrofolate reductase active sites using the MOE-Dock with simulated annealing used as the search protocol and MMFF94X molecular mechanics forcefield for 8000 interactions. The lowest energy conformation was selected and subjected to an energy minimization using MMFF94X force field.

References

[1] A. Esteban-Gamboa, J. Balzarini, R. Esnouf, E. De Clercq, M. J. Camarasa and M. J. Pérez-Pérez, *J Med Chem*, *43* **(2000)**, 971-983.

[2] R. C. Reynolds, A. Tiwari, J. E. Harwell, D. G. Gordon, B. D. Garrett, K. S. Gilbert, S. M. Schmid, W. R. Waud and R. F. Struck, *J Med Chem*, *43* **(2000**), 1484-1488.

[3] V. V. Iyer, G. W. Griesgraber, M. R. Radmer, E. J. McIntee and C. R. Wagner, *J Med Chem*, *43* **(2000**), 2266-2274.

[4] F. Focher, D. Ubiali, M. Pregnolato, C. Zhi, J. Gambino, G. E. Wright and S. Spadari, *J Med Chem*, *43* **(2000**), 2601-2607.

[5] S.A. Rostom ; H.M. Ashour; H.A. Abdel Razik; *Arch. Pharm*. *342(5)* (2009), 299-310.

[6] S. Raić-Malić, D. Svedružić, T. Gazivoda, A. Marunović, A. Hergold-Brundić, A. Nagl, J. Balzarini, E. De Clercq and M. Mintas, *J Med Chem*, *43* **(2000**), 4806-4811.

[7] N.R. Lomax and V.L. Narayanan, *Chemical structures of interest to the division of cancer treatment*, ***6*** (1988) 17.

[8] S. Budavari; , M.J. O'Neil; A. Smith; P.E. Heckelman and J.F. Kinneary; *The Merck Index*, twelfth edition; Merck Research laboratories ,division of Merck and Co.Inc.White house station, NJ. **(1996)** 708 .

[9] R. Griffith and T. Tracy; *Foye's Principles of Medicinal Chemistry*, D.A. Eds.Williams, and T.L. Lemke, Fifth edition, Lippincott Williams and Wilkins; **(2002).** p.900.

[10] H. Parveen, F. Hayat, A. Salahuddin and A. Azam, *Eur J Med Chem*, *45* **(2010**), 3497-3503.

[11] T.G. Kvaljevic; M. Klika; M. Kvalj; I.M. Kleimer; S. Jurmanovic; A. Milic; J. Padovan; S. Raic-Malic*; Bioorg. Med. Chem. Lett. 22* **(2012)**, 308-312.

[12] Y. Choi, L. Li, S. Grill, E. Gullen, C. S. Lee, G. Gumina, E. Tsujii, Y. C. Cheng and C. K. Chu, *J Med Chem*, *43* **(2000**), 2538-2546.

[13] C. McGuigan, H. Barucki, A. Carangio, S. Blewett, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini and J. T. Erichsen, *J Med Chem*, *43* **(2000**), 4993-4997.

[14] A. L. Hopkins, J. Ren, H. Tanaka, M. Baba, M. Okamato, D. I. Stuart and D. K. Stammers, *J Med Chem*, *42* **(1999**), 4500-4505.

[15] R. Pontikis, V. Dollé, J. Guillaumel, E. Dechaux, R. Note, C. H. Nguyen, M. Legraverend, E. Bisagni, A. M. Aubertin, D. S. Grierson and C. Monneret, *J Med Chem*, *43* **(2000**), 1927-1939.

[16] B. Tarnchompoo, C. Sirichaiwat, W. Phupong, C. Intaraudom, W. Sirawaraporn, S. Kamchonwongpaisan, J. Vanichtanankul, Y. Thebtaranonth and Y. Yuthavong, *J Med Chem*, *45* **(2000**), 1244-1252.

[17] C. Sirichaiwat, C. Intaraudom, S. Kamchonwongpaisan, J. Vanichtanankul, Y. Thebtaranonth and Y. Yuthavong, *J Med Chem*, *47* **(2004**), 345-354.

[18] S. Kamchonwongpaisan, R. Quarrell, N. Charoensetakul, R. Ponsinet, T. Vilaivan, J. Vanichtanankul, B. Tarnchompoo, W. Sirawaraporn, G. Lowe and Y. Yuthavong, *J Med Chem*, *47* **(2004)**, 673-680.

[19] D.W. Boykin; A. Kuma; M. Baji; G. Xiao; W.D. Wilson; B.C. Bender; D.R. McCurdy; J.E. Hall and R.R. Tidwell; *Eur.J.Med.Chem*., *32* **(2004)** 965.

[20] S. F. Chowdhury, V. B. Villamor, R. H. Guerrero, I. Leal, R. Brun, S. L. Croft, J. M. Goodman, L. Maes, L. M. Ruiz-Perez, D. G. Pacanowska and I. H. Gilbert, *J Med Chem*, *42* **(1999**), 4300-4312.

[21] M. S. Palanki, P. E. Erdman, L. M. Gayo-Fung, G. I. Shevlin, R. W. Sullivan, M. E. Goldman, L. J. Ransone, B. L. Bennett, A. M. Manning and M. J. Suto, *J Med Chem*, *43* **(2000**), 3995-4004.

[22] K. Ohmoto, T. Yamamoto, T. Horiuchi, H. Imanishi, Y. Odagaki, K. Kawabata, T. Sekioka, Y. Hirota, S. Matsuoka, H. Nakai, M. Toda, J. C. Cheronis, L. W. Spruce, A. Gyorkos and M. Wieczorek, *J Med Chem*, *43* **(2000**), 4927-4929.

[23] Z. Guo, Y. F. Zhu, T. D. Gross, F. C. Tucci, Y. Gao, M. Moorjani, P. J. Connors, M. W. Rowbottom, Y. Chen, R. S. Struthers, Q. Xie, J. Saunders, G. Reinhart, T. K. Chen, A. L. Bonneville and C. Chen, *J Med Chem*, *47* **(2004**), 1259-1271.

[24] F. C. Tucci, Y. F. Zhu, Z. Guo, T. D. Gross, P. J. Connors, Y. Gao, M. W. Rowbottom, R. S. Struthers, G. J. Reinhart, Q. Xie, T. K. Chen, H. Bozigian, A. L. Killam Bonneville, A. Fisher, L. Jin, J. Saunders and C. Chen, *J Med Chem*, *47* **(2004**), 3483-3486.

[25] F. C. Tucci, Y. F. Zhu, R. S. Struthers, Z. Guo, T. D. Gross, M. W. Rowbottom, O. Acevedo, Y. Gao, J. Saunders, Q. Xie, G. J. Reinhart, X. J. Liu, N. Ling, A. K. Bonneville, T. Chen, H. Bozigian and C. Chen, *J Med Chem*, *48* **(2005**), 1169-1178.

[26] YZ He, YX Li, XL Zhu, Z Xi, C Niu, J Wan, L Zhang, GF Yang, *J Chem Inf Model*.,*47* **(2007)**, 2335-44

[27] YX Li, YP Luo, Z Xi, C Niu, YZ He, GF Yang, *Agric Food Chem. 54(24)* **(2006)**, 9135-9.

[28] Z Xi, Z Yu, C Niu, S Ban, G Yang, *J Comput Chem. 27(13)* **(2006)**, 1571-6.

[29] N.S Habib, R. Soliman, K. Ismail, A.M. Hassan and , M.T. Sarg, *Boll.Chim.Farmac*., *142* **(2003)** 396.

[30] M. T. Cocco, C. Congiu, V. Lilliu and V. Onnis, *Bioorg Med Chem*, *14* **(2006**), 366-372.

[31] V.J. Ram, D.A.V. Berghe and A.J.Vlietinck, *J.Het.Chem*., *21* **(1984),** 1307.

[32] M. T. Cocco, C. Congiu, V. Onnis, M. L. Schivo and A. De Logu, *Farmaco*,*50* **(1995**), 73-76.

[33] M.K. Ibrahim, S.G. Abdel-Hamide and M.M. Khalifa, *Egypt.J.Pharm.Sci*., *40* **(1999),**1.

[34] A.M.E. Omar, O.M AboulWafa, A.A.B. Hazza, and S.A. El-Samahy, *Alex.J.Pharm.Sci*,*19* **(2005)** ,97.

[35] P. Reigan, P. N. Edwards, A. Gbaj, C. Cole, S. T. Barry, K. M. Page, S. E. Ashton, R. W. Luke, K. T. Douglas, I. J. Stratford, M. Jaffar, R. A. Bryce and S. Freeman, *J Med Chem*, *48* **(2005**), 392-402.

[36] C. Meier, C. Ducho, U. Görbig, R. Esnouf and J. Balzarini, *J Med Chem*, *47* **(2004**), 2839-2852.

[37] M. D. Thompson, T. L. Cupps, D. S. Wise, L. L. Wotring and L. B. Townsend, *J Med Chem*, *40* **(1997**), 766-770.

[38] W.A. Remers; *Wilson and Gisvold's Text book of Organic Medicinal and Pharmaceutical Chemistry*, J.H. Eds.Block and J.M. Beale, Eleventh edition, Lippincott Williams and Wilkins; **(2004)**, p.397.

[39] El-Meligie, S.; El-Ansary, A.K.; Said, M.M. and Hussien, M.M.M.; *Indian J. Chem., 40B* **(2001)**, 62.

[40] Pretsch; Clerc; Seibl and Simon; *Tabellen Zur Struktur aufKlarung Organischer Verbindungen*, second edition; Springer-Verlag Berlin Heidelberg New York, (**1981**), p.1245

[41] K. Misiura, *Pharmazie*, *59* **(2004**), 668-672.

[42] Y. J. Shaw, Y. T. Yang, J. B. Garrison, N. Kyprianou and C. S. Chen, *J Med Chem*, *47* **(2004)**, 4453-4462.

[43] S. W. Wright, A. A. Carlo, M. D. Carty, D. E. Danley, D. L. Hageman, G. A. Karam, C. B. Levy, M. N. Mansour, A. M. Mathiowetz, L. D. McClure, N. B. Nestor, R. K. McPherson, J. Pandit, L. R. Pustilnik, G. K. Schulte, W. C. Soeller, J. L. Treadway, I. K. Wang and P. H. Bauer, *J Med Chem*, *45***(2002**), 3865-3877.

[44] E. Mishani, G. Abourbeh, O. Jacobson, S. Dissoki, R. Ben Daniel, Y. Rozen, M. Shaul and A. Levitzki, *J Med Chem*, *48* **(2005**), 5337-5348.

[45] M. L. Richardson, K. A. Croughton, C. S. Matthews and M. F. Stevens, *J Med Chem. 47* **(2004)**, 4105-4108.

[46] A. Monge; J. A. Palop; T. Goni; F. Martinez-crespo and I. Recald; E. Fernandez-Alvarez; *J.Heterocyclic Chem*., *23* **(1986)**, 647.

[47] N. J. Curtin, H. C. Barlow, K. J. Bowman, A. H. Calvert, R. Davison, B. T. Golding, B. Huang, P. J. Loughlin, D. R. Newell, P. G. Smith and R. J. Griffin, *J Med Chem*, *47* **(2004**), 4905-4922.

[48] S. Hayao, H. J. Havera, W. G. Strycker, T. J. Leipzig and R. Rodriguez, *J Med Chem*, *10* **(1967**), 400-402.

[49] M.K. El-Sayed; A.S.V. El-Mogazy; F.A. Romel; F.F. Bovomaid and A.B. Hassan; *Egypt.J.Pharm.Sci*., *32* **(1991)**, 251.

[50] S. R. Jain and A. Kar ; *Planta Med*., *20* **(1971)**, 118.

[51] C.R. Manclark; M. J. Pickett and H. B. Moore; Laboratory manual for medical bacteriology, Fifth edition, Meredith corporation Mahony and Roese Inc., New York, **(1972)**, p22.

[52] Molecular Operating Enviroment (MOE), version 2005.06.Chemical Computing Group, Inc.Montreal, Canada. http://www.chemcomp.com

Table captions

Table 1: The mean growth percent, delta values and the percent growth inhibition against some subpanel cell lines of the selected compounds

Table 2: GI_{50}, TGI and LC_{50} of 5-Dose screening of compound 17a

Table 3: The MG-MID (GI_{50}) μM/l and the selectivity ratio of compound 17a

Table 4: Inhibition zones (IZ) in mm diameter

Table 5: MIC values in μg/ml for some selected compounds and for reference drugs

Scheme captions

Scheme 1: Synthetic pathways for compounds 2 - 6

Scheme 2: Synthetic pathways for compounds 9 - 15

Scheme 3: Synthetic pathways to the dimorpholino compounds 18a,b.

Figure captions

Fig (1) Docking of DUMP in the active site of TS

Fig (2)Docking of compound 6a in the active site of TS

Fig (3) Docking of compound 6c in the active site of TS

Fig (4) Docking of compound 6d in the active site of TS

Fig (5) Docking of compound 17a in the active site of TS

Fig (6) Docking of compound 18a in the active site of TS

Fig (7) Docking of MTX in the active site of DHFR

Fig (8) Docking of compound 6a in the active site of DHFR

Fig (9) Docking of compound 6c in the active site of DHFR

Fig (10) Docking of compound 6d in the active site of DHFR

Fig (11) Docking of compound 17a in the active site of DHFR

Fig (12) Docking of compound 18a in the active site of DHFR

Table 1: Mean growth percent, delta values and the percent growth inhibition against some subpanel cell lines of the selected compounds

Comp. No.	Mean growth percent	Delta	Panel	Subpanel cell lines (Growth inhibition percent)
1c	88.87	67.49	NonSmall Cell Lung Cancer	NCI-H23 (78.62)
			Breast Cancer	NCI/ADR-RES (74.23)
			Melanoma	MALME-3M (55.56)
			CNS Cancer	SNB-75 (42.8)
2a	104.84	46.33	NonSmall Cell Lung Cancer	HOP-92 (41.49)
3a	111.85	37.32	-	-
3b	113.60	43.66	-	-
5a	98.77	47.25	NonSmall Cell Lung Cancer	HOP-62 (47.36), HOP-92 (48.48)
5c	100.77	50.42	NonSmall Cell Lung Cancer	HOP-62 (49.65)
6a	65.97	73.38	NonSmall Cell Lung Cancer	EKVX (43.23), HOP-62 (48.67), HOP-92 (107.41), NCI-H522 (40.44)
			Breast Cancer	BT-549 (42.26), HS578T (73.25), MCF7 (43.42), T-47D (62.72)
			Ovarian Cancer	IGROV1 (70.9)
			Leukemia	SR (46.94)
			Renal Cancer	A498 (86.3), TK-10 (42.41)
			Melanoma	MALME-3M (103.74), SK-MEL-2 (58.19), UACC-62 (56.44)
			Prostate Cancer	PC-3 (52.98)
			CNS Cancer	SF-295 (45.47), SNB-75 (84.88)
6c	55.91	76.95	NonSmall Cell Lung Cancer	EKVX (51.2), HOP-62 (64.25), HOP-92 (111.63), NCI-H460 (49.93), NCI-H522 (50.41)
			Colon Cancer	HCC-2998 (121.04)
			Breast Cancer	MCF7 (73.27), NCI/ADR-RES (59.76), T-47D (81.09)
			Ovarian Cancer	IGROV1 (79.29), SK-OV-3 (45)
			Leukemia	CCRF-CEM (50.39), RPMI-8226 (56.98), SR (82.19)
			Renal Cancer	A498 (101.37), CAKI-1 (41.79), TK-10 (52.91)
			Melanoma	M14 (60.23), MALME-3M (97.83), SK-MEL-2 (62.95), UACC-62 (56.27)
			Prostate Cancer	PC-3 (57.37)
			CNS Cancer	SF-295 (52.6), SNB-75 (84.12)
6d	84.74	65.94	Non-SmallCell Lung Cancer	HOP-92 (44.53), NCI-H23 (81.2)
			Breast Cancer	NCI/ADR-RES (75.8)
			Leukemia	SR (44.56)
			Melanoma	MALME-3M (48.7)
6g	109.51	42.02	-	-
6h	104.68	76.50	Colon Cancer	HCC-2998 (71.82)
6i	105.38	30.33	-	-
7a	107.37	114.25	Colon Cancer	HCC-2998 (106.88)
			Breast Cancer	NCI/ADR-RES (42.03)
9c	108.90	93.33	Colon Cancer	HCC-2998 (84.43)
			Melanoma	M14 (44.95)
9d	99.24	69.50	NonSmall Cell Lung Cancer	HOP-62 (49.6)
			Breast Cancer	NCI/ADR-RES (70.26)
10c	96.40	94.78	Colon Cancer	HCC-2998 (98.38)
			Breast Cancer	NCI/ADR-RES (48.76)
11a	96.43	44.17	NonSmall Cell Lung Cancer	HOP-92 (44.28)
			Ovarian Cancer	IGROV1 (40.1)
			Leukemia	K-562 (41.8), RPMI-8226 (39.71)
			Renal Cancer	RXF393 (47.74)
11b	101.04	70.84	Ovarian Cancer	OVCAR-8 (47.1)
			Melanoma	UACC-257 (69.8)
11c	101.77	73.63	Ovarian Cancer	OVCAR-8 (42.4)

			Melanoma	SK-MEL-2 (69.76), UACC-257 (71.86)
11d	100.66	71.40	Melanoma	SK-MEL-2 (70.74)
11e	100.28	54.90	Ovarian Cancer	OVCAR-8 (54.62)
			Renal Cancer	UO-31 (49.09)
12c	99.46	83.83	NonSmall Cell Lung Cancer	NCI-H23 (54.69)
			Colon Cancer	HCC-2998 (84.37)
			Breast Cancer	NCI/ADR-RES (55.88)
16a	101.58	61.72	Ovarian Cancer	OVCAR-8(48.38)
			Melanoma	UACC-257 (60.14)
17a	61.03	76.27	NonSmall Cell Lung Cancer	HOP-62 (45.74), HOP-92 (81.74), NCI-H522 (42.1)
			Breast Cancer	BT-549(88.59), HS578T(49.97), MCF7(55.16), MDA-MB-468(44.08), T-47D (84.58)
			Ovarian Cancer	IGROV1 (61.86), SK-OV-3 (71.93)
			Leukemia	RPMI-8226 (52.1)
			Renal Cancer	A498 (115.24), ACHN (40.76), CAKI-1 (61.43), RXF393 (68.55), UO-31 (70.47)
			Melanoma	MALME-3M (74.29), UACC-62 (48.13)
			Prostate Cancer	PC-3 (63.91)
			CNS Cancer	SF-295 (90.86), SNB-75 (65.92)
18a	54.02	83.53	NonSmall Cell Lung Cancer	HOP-62 (52.02), HOP-92 (69.26), NCI-H322M (47.68), NCI-H460(66)
			Colon Cancer	HCT-116 (42.49)
			Breast Cancer	BT-549(70.53), HS578T(93.81), MCF7(95.01), MDA-MB-435(46.98), T-47D (74.01)
			Ovarian Cancer	IGROV1 (129.51), OVCAR-3 (64.62), OVCAR-8 (66.57), SK-OV-3 (58.89)
			Renal Cancer	786-O (61.91), A498 (103.34), ACHN (45.6), CAKI-1 (62.65), SN12C (42.18), TK-10 (59.19), UO-31 (58.27)
			Melanoma	M14 (40.12), MALME-3M (76.87), SK-MEL-2 (92.67), SK-MEL-28 (40.07), UACC-257 (82.93), UACC-62 (57.13)
			Prostate Cancer	PC-3 (43.49)
			CNS Cancer	SF-295 (56.39), SF-539 (43.39), SNB-75 (104.63)
18b	102.36	31.76	-	-

Table 2 GI_{50}, TGI and LC_{50} of 5-Dose screening of compound 17a

Panel-cell line	17a (μM/l) GI_{50}	TGI	LC_{50}
Leukemia :			
CCRF-CEM	> 100	> 100	> 100
HL-60 (TB)	34.1	> 100	> 100
K-562	> 100	> 100	> 100
MOLT-4	28.9	> 100	> 100
RPMI-8226	14.1	91.8	> 100
SR	33.3	> 100	> 100
Non-Small Cell Lung :			
A549/ATCC	27.9	> 100	> 100
EKVX	3.65	> 100	> 100
HOP-62	4.34	75.3	> 100
HOP-92	2.44	8.70	> 100
NCI-H226	10.1	> 100	> 100
NCI-H23	4.48	> 100	> 100
NCI-H322M	8.31	> 100	> 100

NCI-H460	10.3	> 100	> 100
NCI-H522	72.5	> 100	> 100
Colon cancer :			
HCC-2998	0.253	18.2	> 100
HCT-116	18.4	> 100	> 100
HCT-15	4.06	> 100	> 100
HT 29	36.8	> 100	> 100
KM 12	27.1	> 100	> 100
SW-620	25.0	> 100	> 100
CNS cancer :			
SF-268	13.0	> 100	> 100
SF-295	1.46	20.4	> 100
SF-539	19.8	> 100	> 100
SNB-19	84.5	> 100	> 100
SNB-75	2.15	68.2	> 100
U 251	22.1	> 100	> 100
Melanoma :			
LOX IMVI	6.48	> 100	> 100
MALME-3M	2.56	> 100	> 100
M 14	11.9	> 100	> 100
SK-MEL-28	5.45	98.3	> 100
SK-MEL-5	17.0	> 100	> 100
UACC-257	50.7	>100	> 100
UACC-62	3.12	75.5	> 100
Ovarian cancer :			
IGROV 1	1.79	23.3	> 100
OVCAR-3	4.19	47.8	> 100
OVCAR-4	85.1	> 100	> 100
OVCAR-5	1.70	> 100	> 100
SK-OV-3	14.1	75.2	> 100
Renal cancer :			
786-O	2.70	21.3	> 100
A498	1.06	5.28	> 100
ACHN	3.23	> 100	> 100
CAKI-1	0.635	31.4	> 100
SN12C	16.4	> 100	> 100
TK-10	4.63	> 100	> 100
UO-31	7.57	> 100	> 100
Prostate cancer :			
PC-3	22.1	> 100	> 100
DU-145	13.4	> 100	> 100
Breast cancer :			
MCF7	3.92	> 100	> 100
NCI/ADR-RES	11.8	> 100	> 100
MDA-MB-231/ATCC	10.0	> 100	> 100
HS 578T	1.15	6.09	> 100
MDA-MB-435	6.71	> 100	> 100
BT-549	3.22	> 100	> 100
T-47D	3.59	22.5	> 100
MDA-MB-468	4.82	> 100	> 100

Table 3: The MG-MID (GI_{50}) μM/l and the selectivity ratio of compound 17a

Fullpanel GI_{50} MG-MID (μM/l)	Subpanel tumor cell lines GI_{50} MG-MID (μM/l) (SI)								
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
16.65	27.60	16.00	18.60	23.84	13.89	21.38	5.18	17.75	5.65
	(0.60)	(1.04)	(0.90)	(0.70)	(1.20)	(0.78)	(3.22)	(0.94)	(2.95)

[I]Leukemia; [II]Non-Small Cell Lung cancer, [III]Colon cancer;
[IV]CNS cancer; [V] Melanoma; [VI]Ovarian cancer; [VII]Renal cancer;
[VIII]Prostate cancer; [IX]Breast cancer

Table 4: Inhibition zones (IZ) in mm diameter:

Comp. No.	S. aureus	P. aureginosa	Sh. Flexneri	C.albicans
1c	15	16	**21**	22
2a	15	16	**22**	22
2b	15	17	17	22
2c	**18**	**18**	**24**	25
3a	15	16	18	22
3b	15	16	18	22
4	15	17	19	23
5a	15	**18**	**22**	22
5b	15	16	17	22
5c	15	**18**	17	22
6a	15	**18**	17	22
6b	15	16	18	22
6c	15	16	**22**	22
6d	15	16	**20**	22
6e	15	17	**23**	22
6f	**18**	16	17	22
6g	15	**18**	**20**	22
6h	15	16	17	22
6i	**18**	**20**	17	**29**
7a	**18**	**18**	**21**	**25**
9a	15	16	**20**	22
9b	15	16	**20**	23
9c	**20**	**22**	17	**30**
9d	**18**	**18**	18	22
10c	**19**	17	17	22
11a	15	16	17	22
11b	15	17	17	22
11c	15	17	**24**	22
11d	15	16	19	22
11e	17	**18**	17	22
11f	15	17	17	22
12c	15	16	**20**	22
13a	15	**19**	17	22
13b	15	16	17	22
14	15	17	19	22
16a	15	16	**22**	22
16b	15	17	18	22
17a	15	17	18	22
17b	15	16	17	22
18a	15	16	18	**24**
18b	15	**18**	17	**25**

Ampicillin	41	16	22	-
Ofloxacin	43	43	32	-
Clotrimazole	-	-	-	40
Solvent	15	16	17	22

Table 5 : MIC values in µg/ml for some selected compounds and for reference drugs:

Comp. No.	S. aureus	P. aeruginosa	Sh. flexneri	C.albicans
1c	-	-	125	-
2a	-	-	125	-
2c	125	125	125	125
5a	-	125	125	-
5c	-	62.5	-	-
6a	-	125	-	--
6c	-	-	125	-
6d	-	-	125	-
6e	-	-	125	-
6f	125	-	-	-
6g	-	125	125	-
6i	3.91	125	-	125
7a	3.91	125	125	125
9a	-	-	125	-
9b	-	-	125	-
9c	3.91	125	-	125
9d	3.91	125	-	-
10c	125	-	-	-
11c	-	-	125	-
11e	-	125	-	-
12c	-	-	125	-
13a	-	125	-	-
15	-	125	-	125
16a	-	-	125	-
18a	-	-	-	125
18b	-	125	-	125
Ampicillin	3.91	-	-	-
Ofloxacin	-	1.95	7.81	-
Clotrimazole	-	-	-	12.5

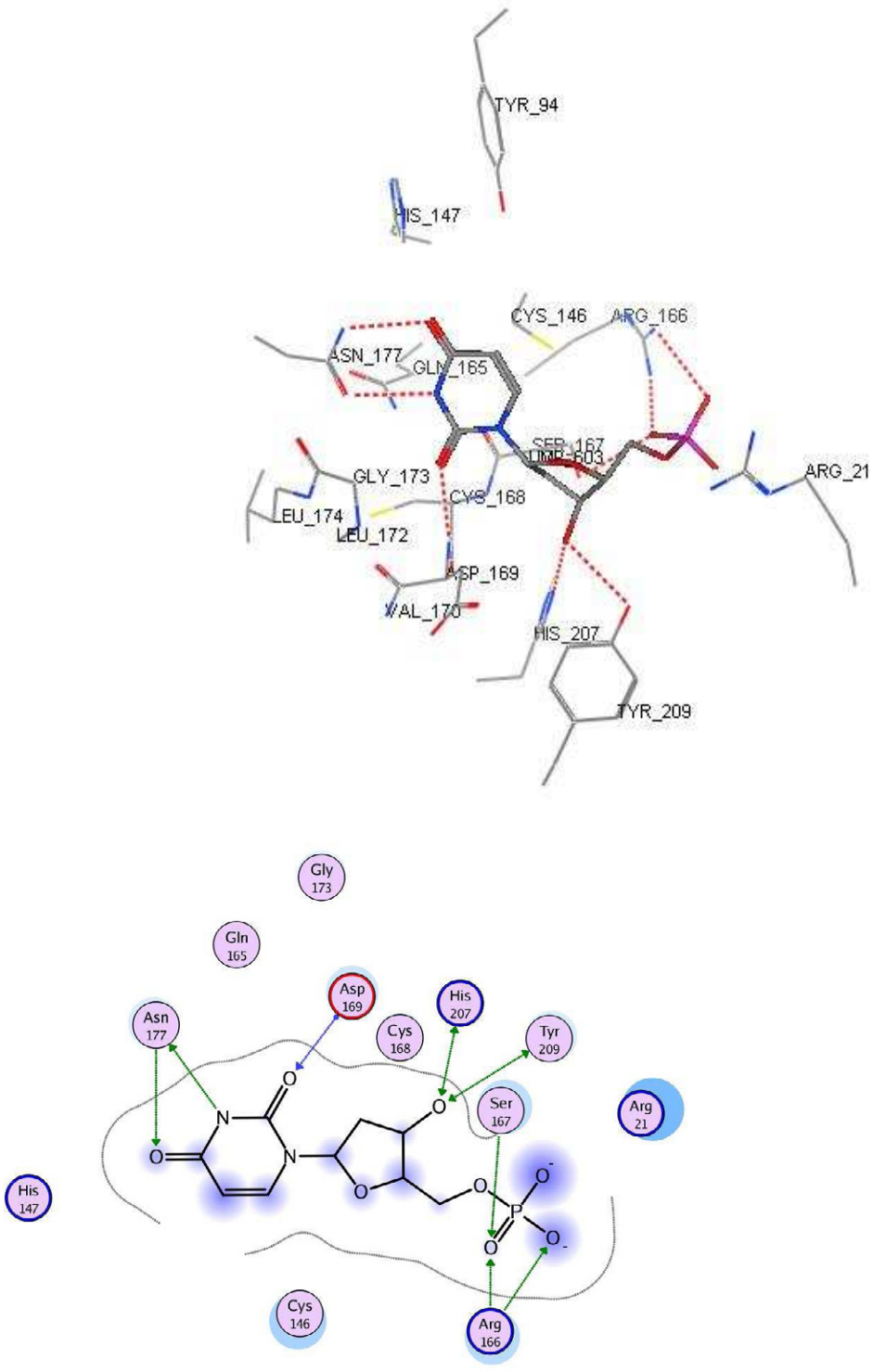


Fig (1) Docking of DUMP in the active site of TS

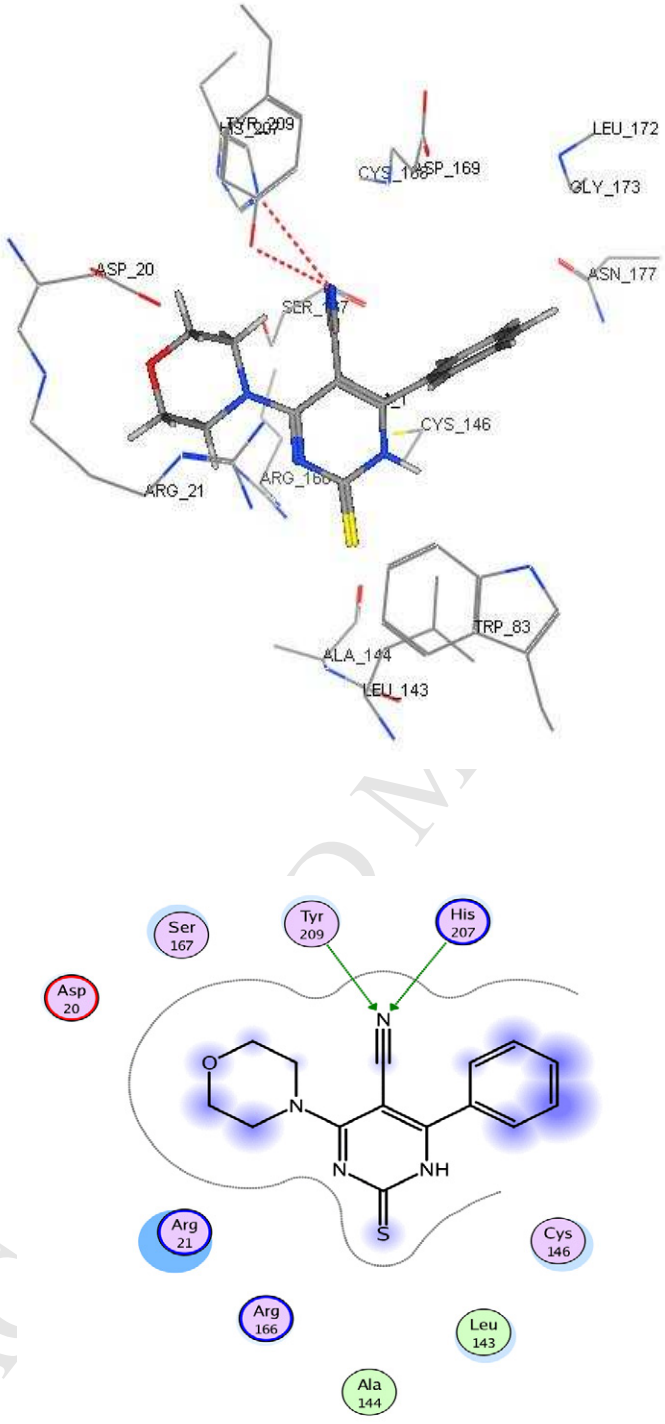


Fig (2)Docking of compound 6a in the active site of TS

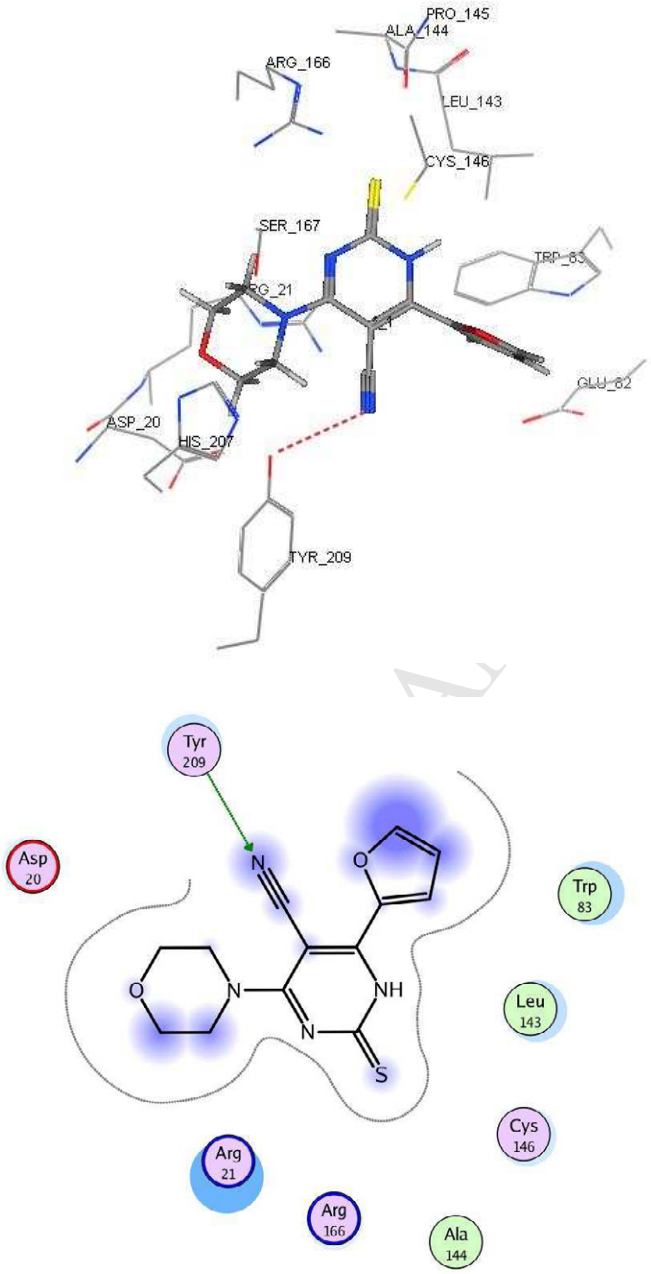


Fig (3) Docking of compound 6c in the active site of TS

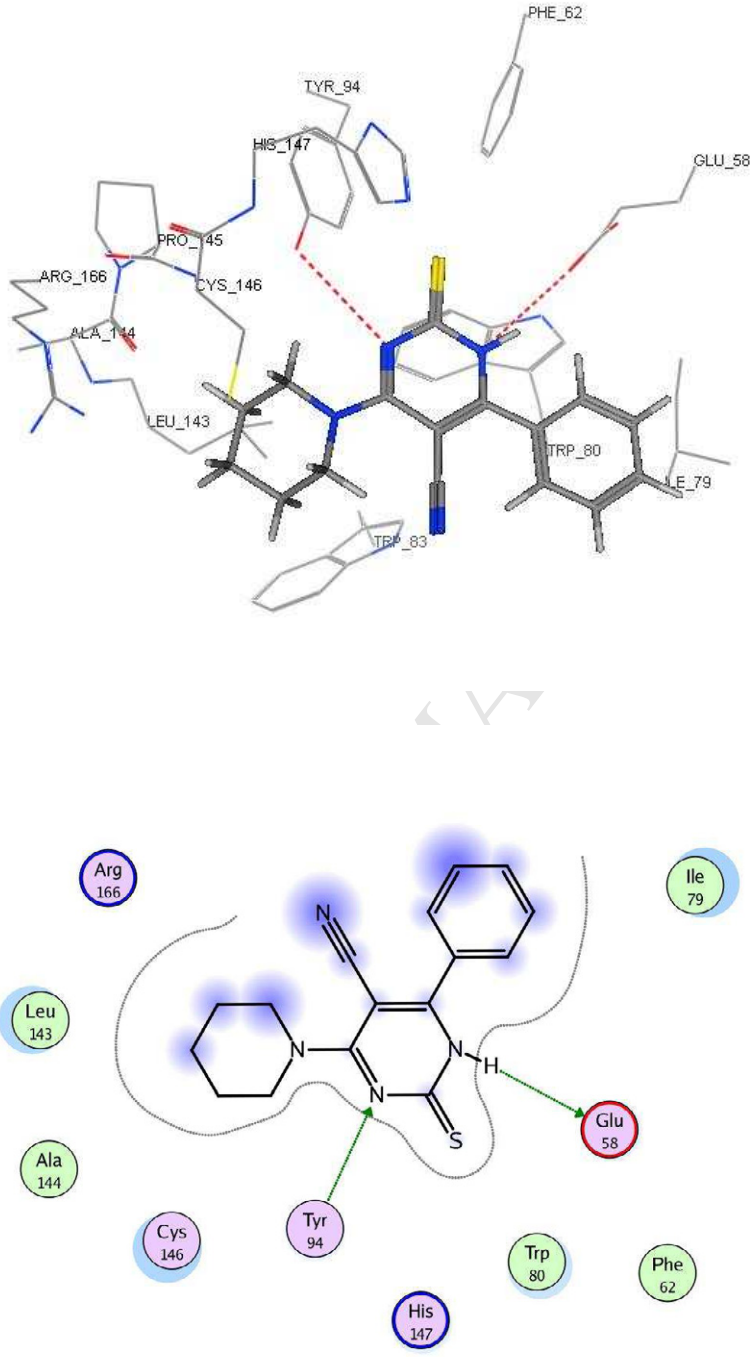


Fig (4) Docking of compound 6d in the active site of TS

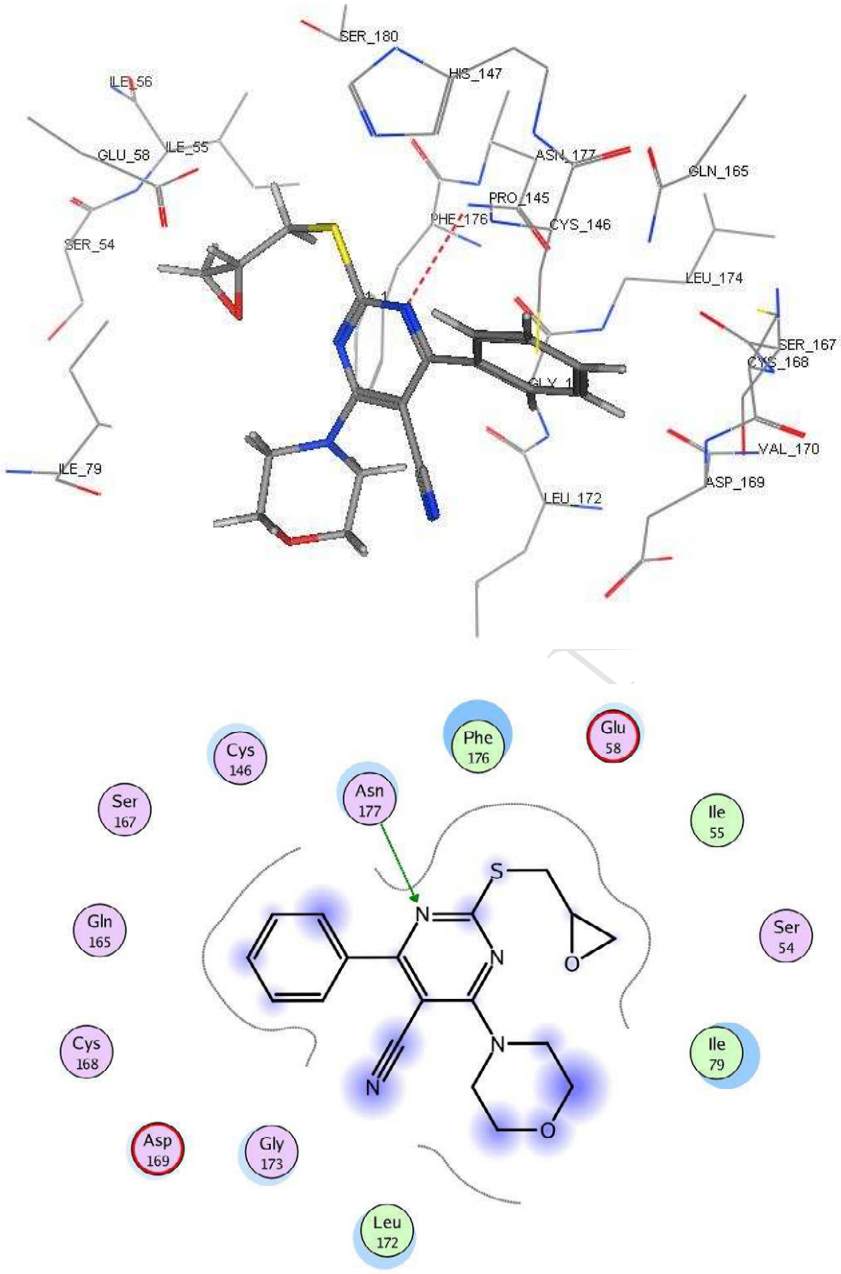


Fig (5) Docking of compound 17a in the active site of TS

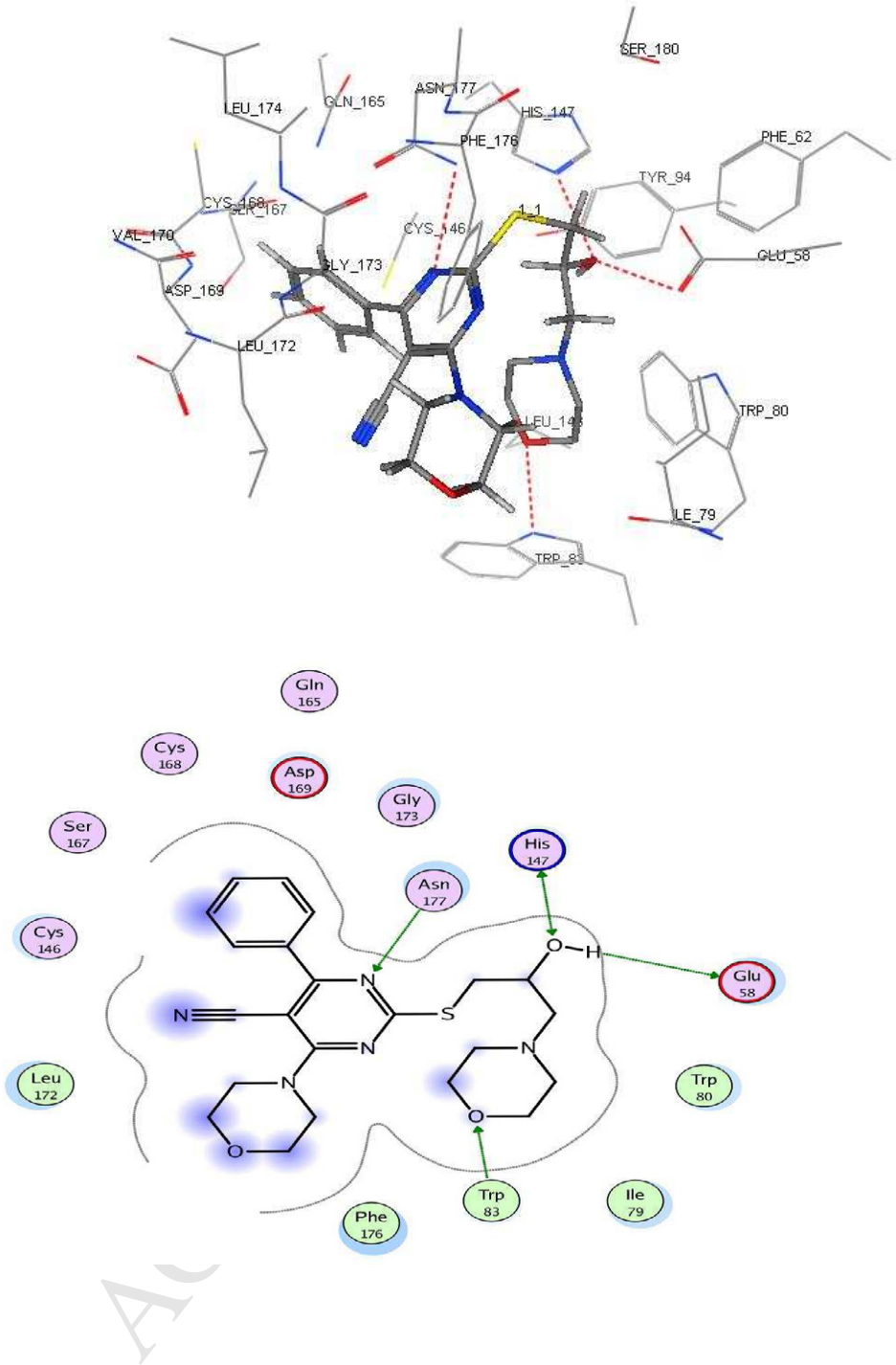


Fig (6) Docking of compound 18a in the active site of TS

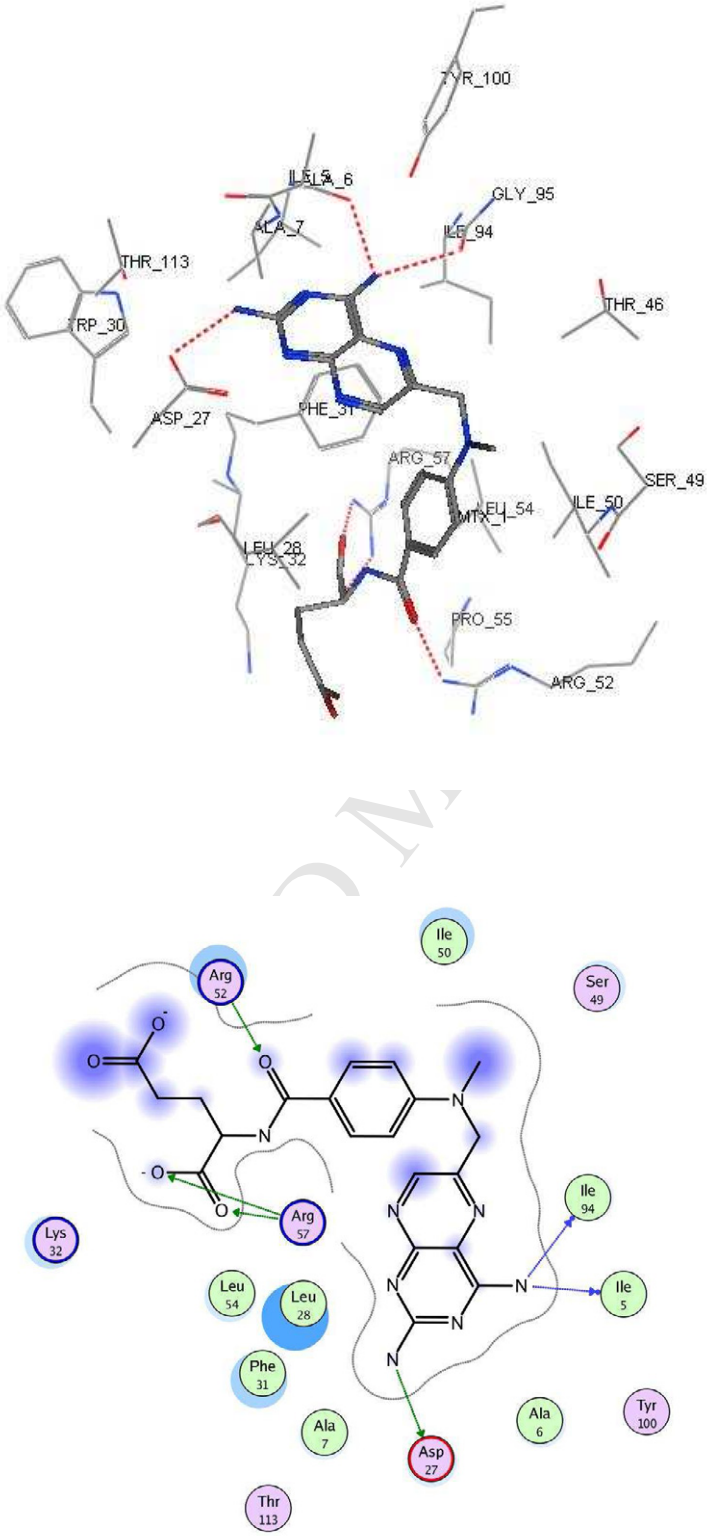


Fig (7) Docking of MTX in the active site of DHFR

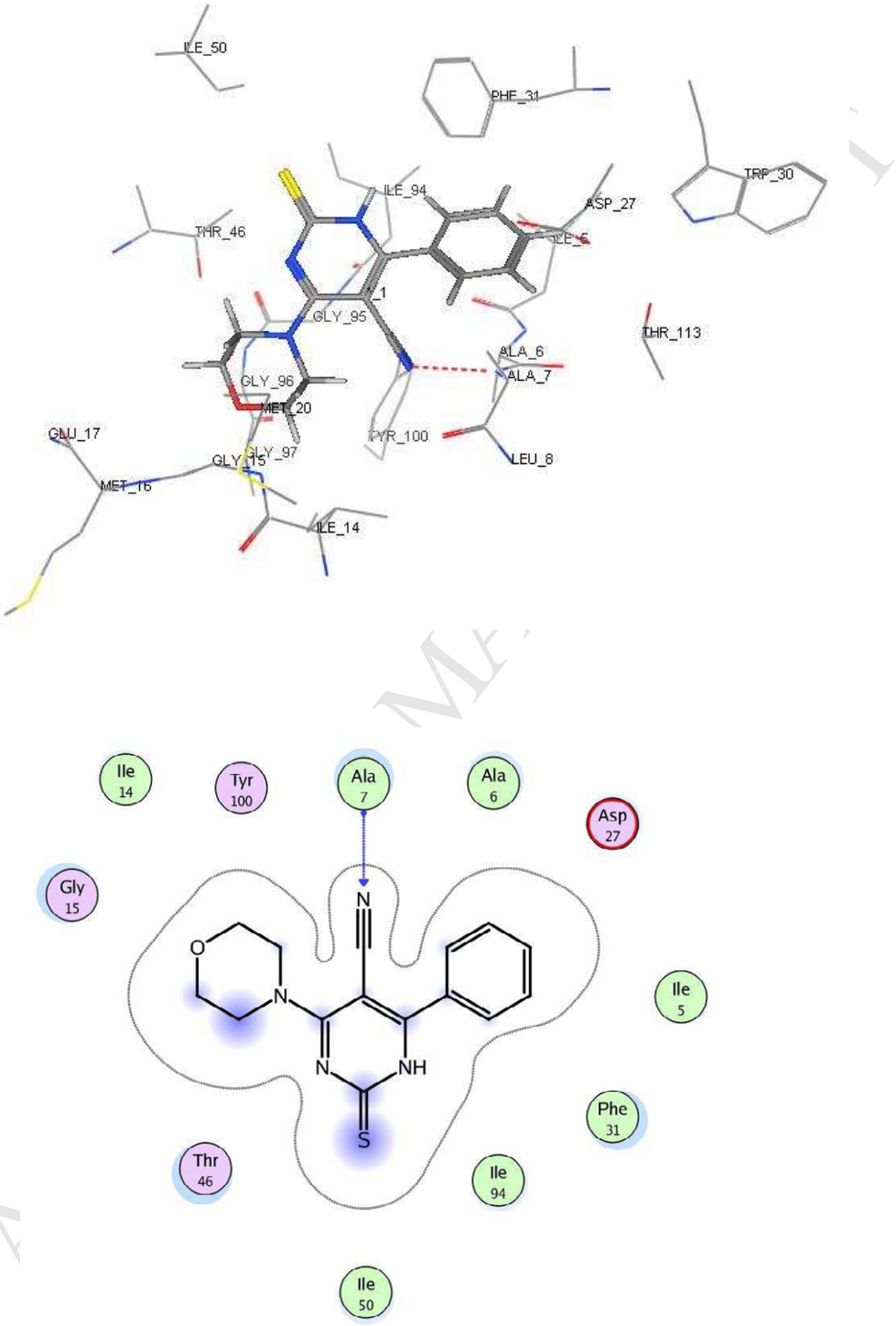


Fig (8) Docking of compound 6a in the active site of DHFR

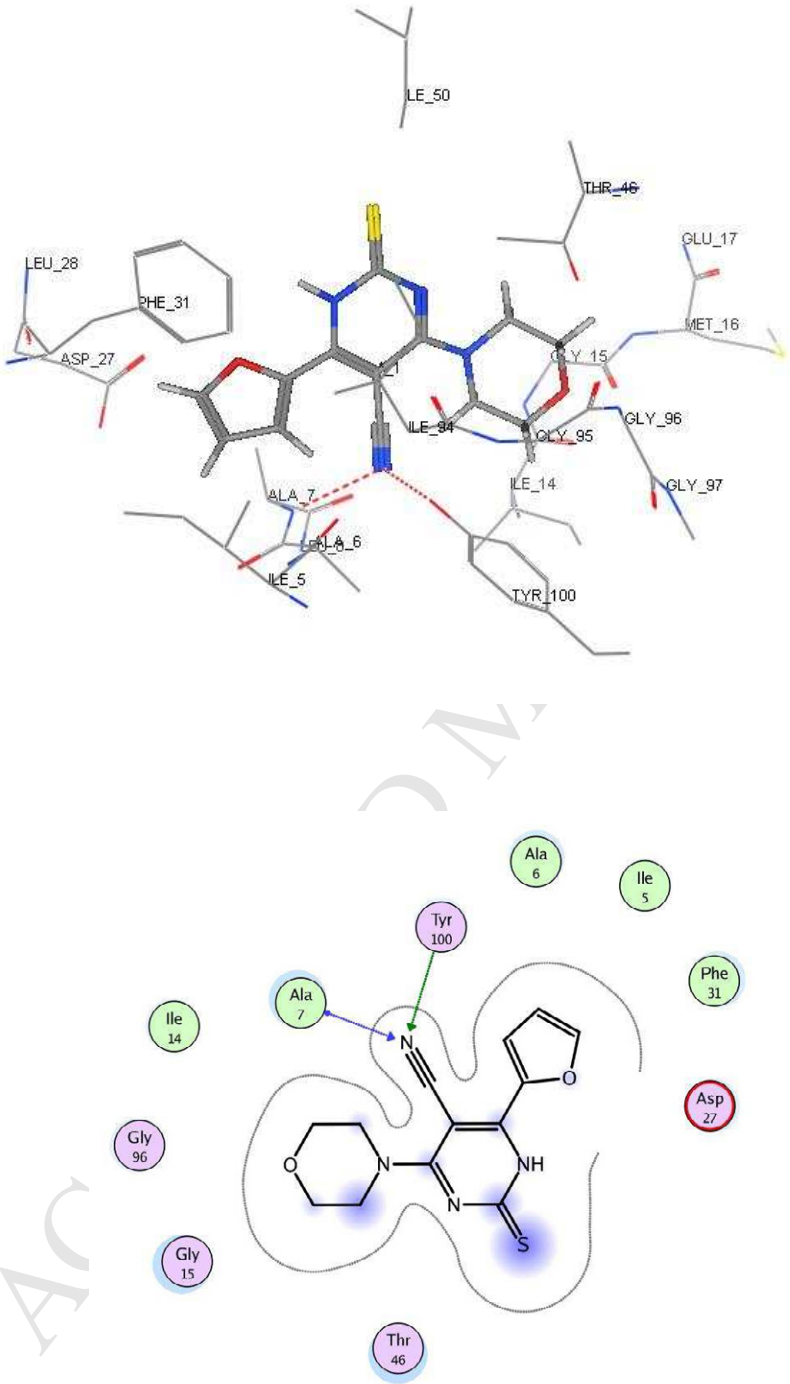


Fig (9) Docking of compound 6c in the active site of DHFR

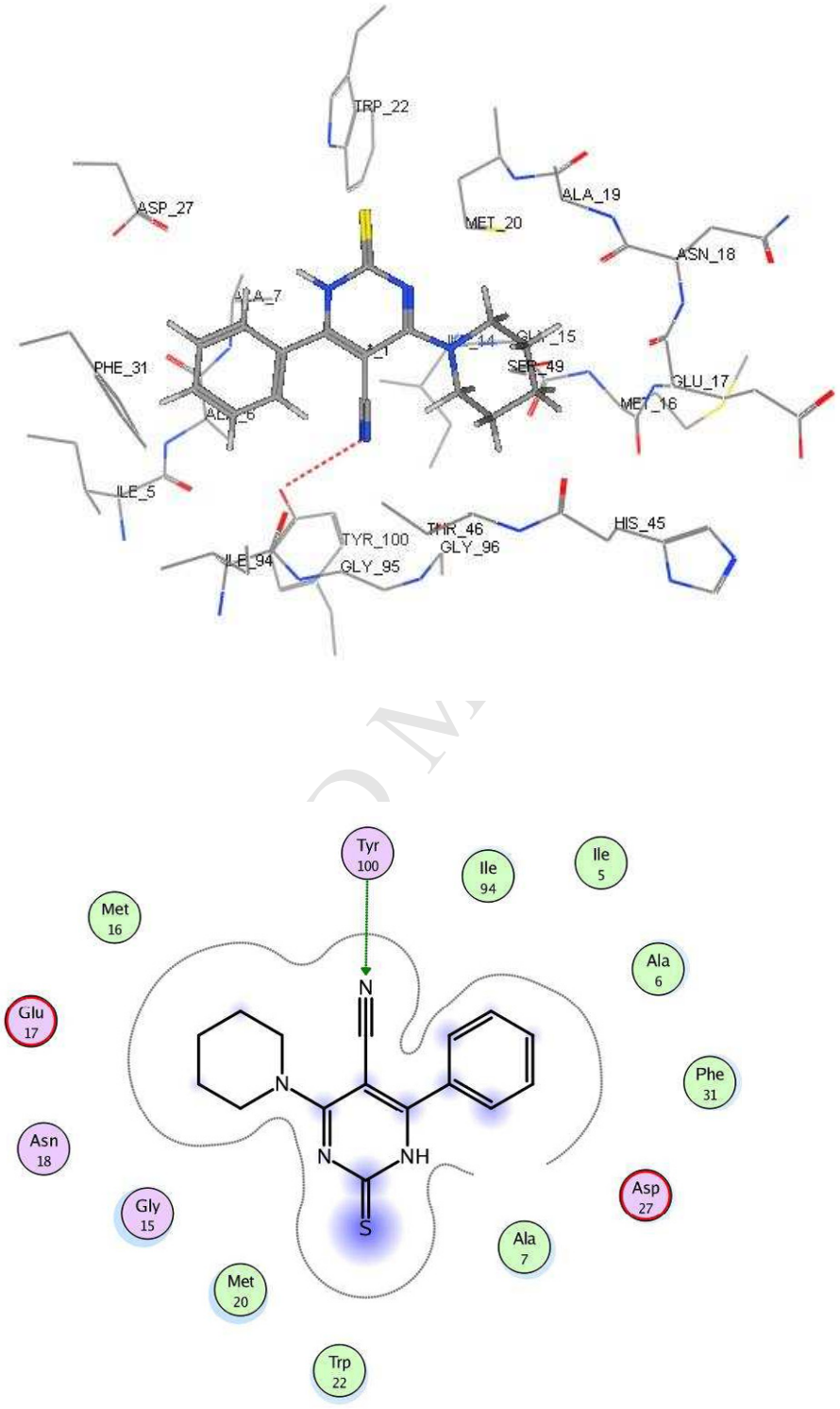


Fig (10) Docking of compound 6d in the active site of DHFR

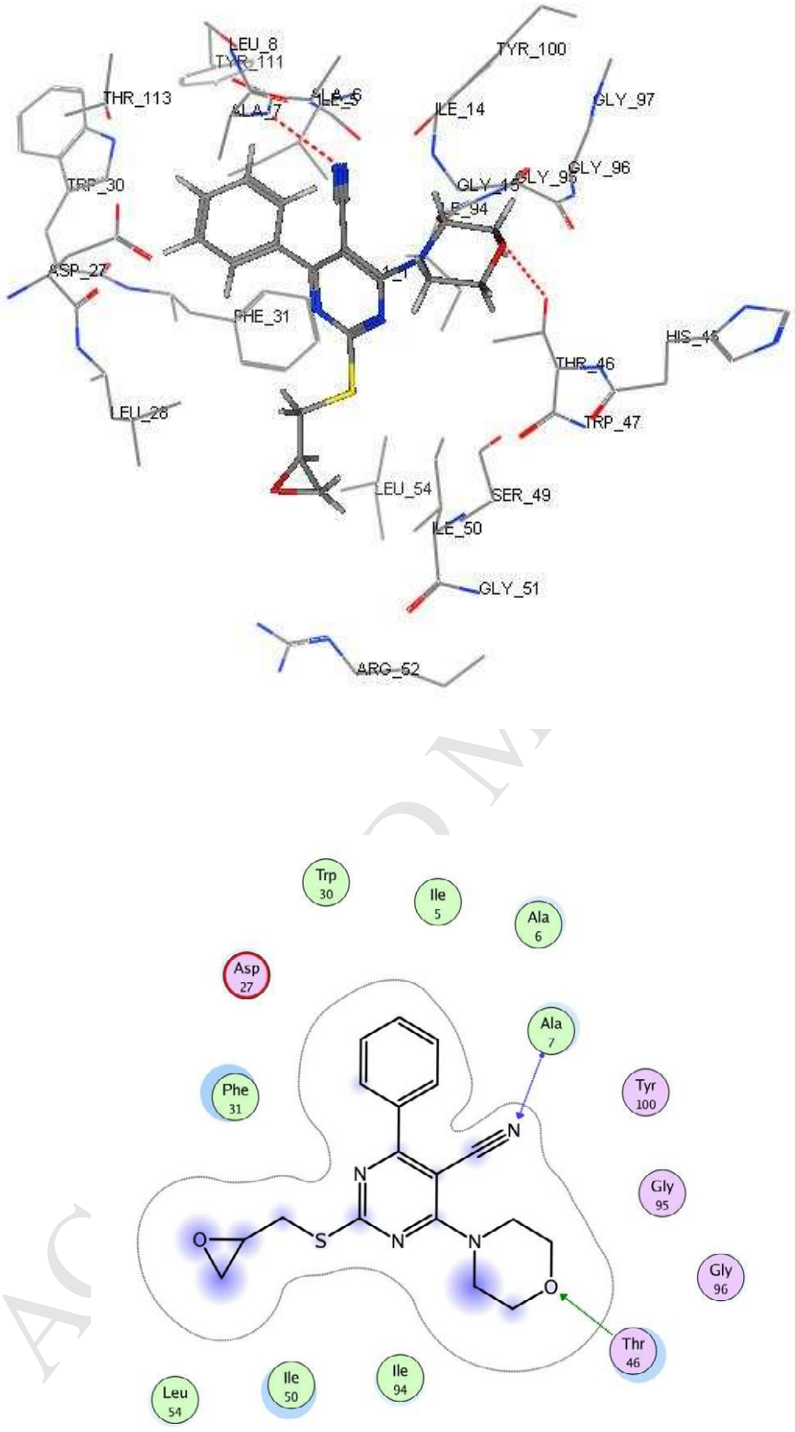


Fig (11) Docking of compound 17a in the active site of DHFR

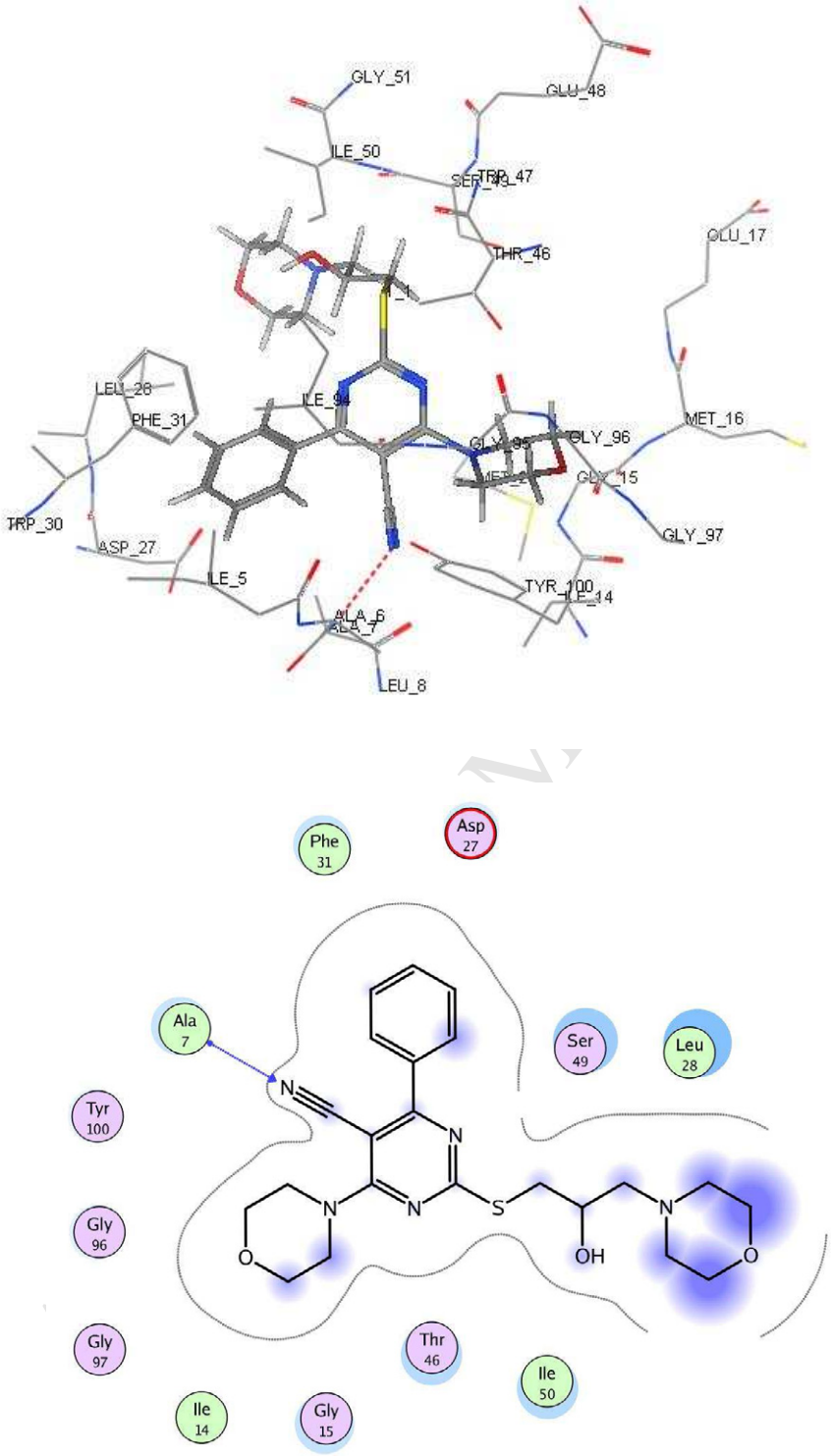
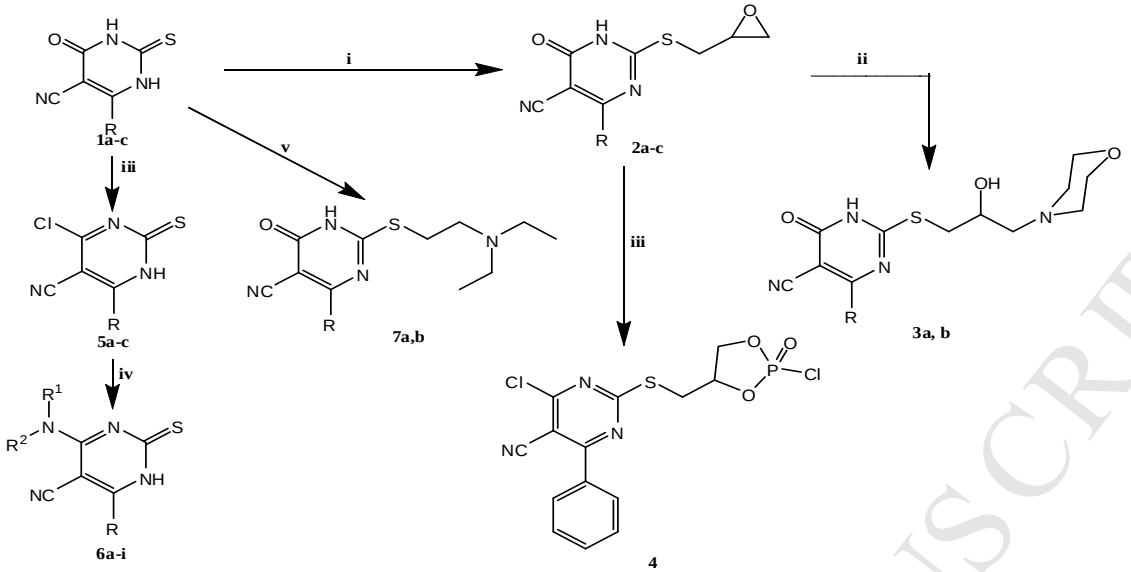


Fig (12) Docking of compound 18a in the active site of DHFR

Scheme 1: Synthetic pathways for compounds 2 - 6

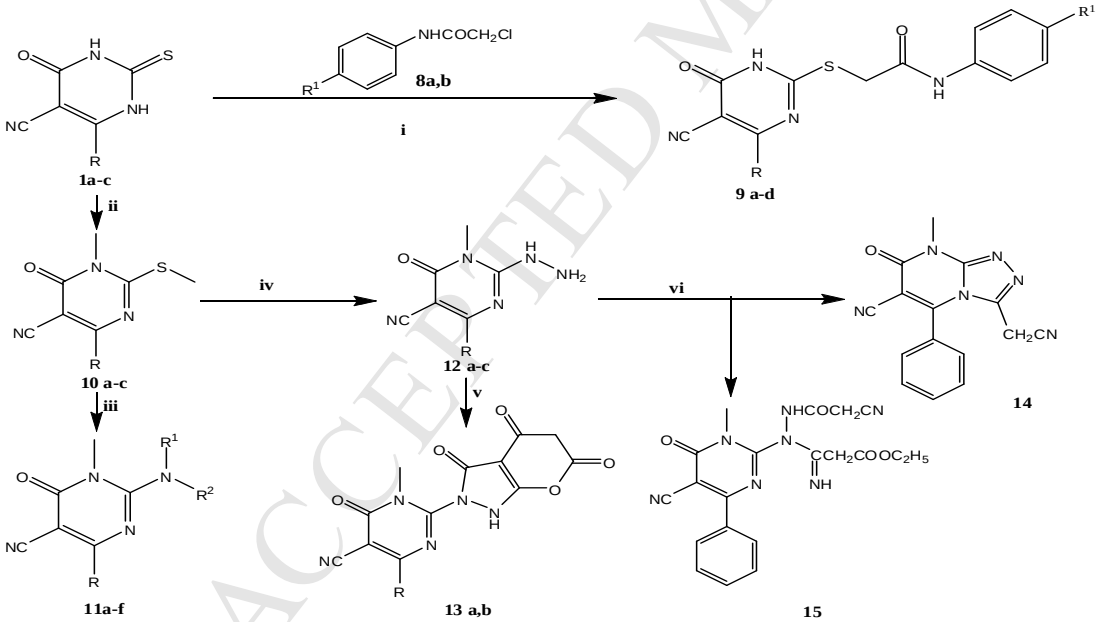


(i) epichlorohydrin, KOH/H_2O/R.T.; **(ii)** morpholine/Abs.EtOH/reflux; **(iii)** $POCl_3$/reflux; **(iv)** morpholine or piperidine or N-benzylpiperazine/fusion/160 ^oC ; **(v)** $ClCH_2CH_2N(CH_2CH_3)_2.HCl$, KOH/H_2O/R.T.

R = C_6H_5, 4-NO_2-C_6H_4, 2-furyl **NR^1R^2** = morpholino, piperidino, N-benzylpiperazinyl.

Scheme 1

Scheme 2: Synthetic pathways for compounds 9 – 15



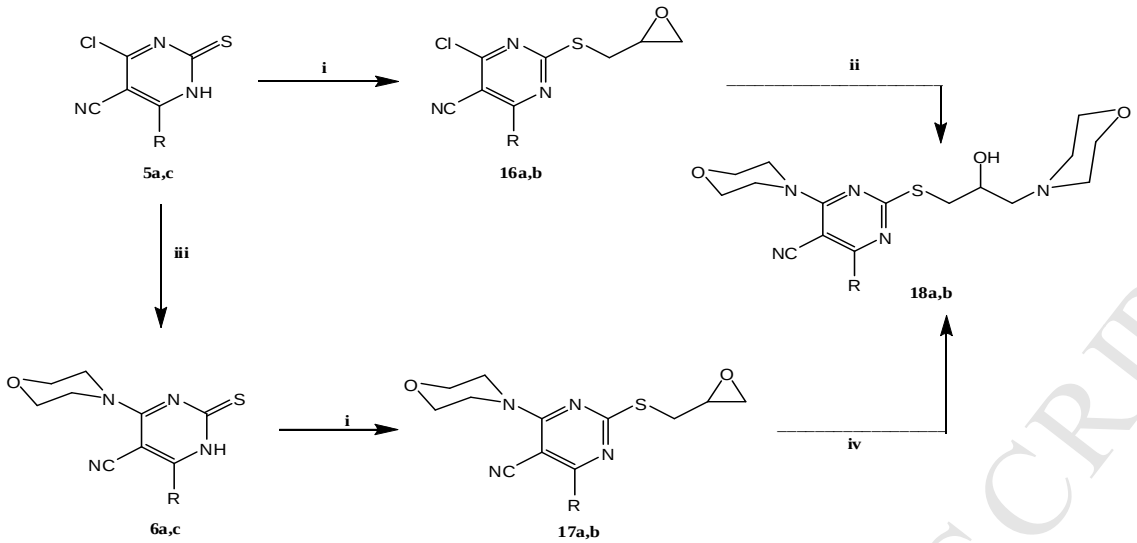
(i) K_2CO_3/DMF/R.T.; **(ii)** CH_3I, K_2CO_3/DMF/R.T.; **(iii)** morpholine or piperidine or N-benzylpiperazine/fusion/160; **(iv)** $NH_2NH_2.H_2O$/EtOH/reflux; **(v)** excess diethyl malonate/reflux; **(vi)** ethyl cyanoacetate/fusion.

1,10,12 R = C_6H_5, 4-NO_2-C_6H_4, 2-furyl. **8a,b R^1** = Cl, OCH_3. **9a-d R** = C_6H_5, 2-furyl ; **R^1** = Cl, OCH_3. **11a-f R** = C_6H_5, 4-NO_2-C_6H_4, 2-furyl ; **NR^1R^2** = morpholino, piperidino, N-benzyl piperazinyl.

13a,b R = C_6H_5, 2- furyl

Scheme 2

Scheme 3: Synthetic pathways to the dimorpholino compounds 18a,b.



(i) epichlorohydrin, NaOH/DMF/R.T.; **(ii)** 4 equivelants morpholine/dioxane/reflux; **(iii)** 2 equivelants morpholine/fusion; **(iv)** 2 equivelants morpholine/dioxane/reflux.
R = C_6H_5 , 2-furyl

Scheme 3

Highlights

- The design and synthesis of novel pyrimidine derivatives bearing various substituents or heterocyclic moieties joined to the pyrimidine ring system.
- Some selected compounds were investigated for their cytotoxic potency against certain human tumor cell lines as well as their antimicrobial activities.
- Two compounds; **17a** and **18a** were found to be promising and deserved further evaluation in the five dose sixty human tumor cell line assay.
- Compounds **2c** and **7a** showed broad spectrum antimicrobial activity, their potency against *S.aureus* nearly half or equal to ampicillin respectively.
- Docking was performed for the five most active anticancer compounds **6a**, **6c**, **6d**, **17a** and **18a**