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ARTICLE

Synthesis and antitumor activity of some nitrogen heterocycles bearing pyrimidine moiety

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Abstract

Synthesis of novel pyrimidine derivatives **4-16** was accomplished by heterocyclization of polarized system, for example, Chalcone. Claisen-Schmidt condensation of 2-acetyl naphthalene with 4-(N, N-dimethylaminoben zaldehyde) afforded chalcone **3**, which was utilized for synthesis various pyrimidine derivatives by treatment with urea, thiourea, and guandine hydrochloride in ethanolic sodium hydroxide solution. The reactivity of the synthesized pyrimidine derivatives towards different nucleophilic and electrophilic reagent were examined. The constructions of the newly synthesized pyrimidine derivatives were elucidated from their spectral and elemental analysis. All the synthesized compounds were tested in vitro for their anticancer activities against HePG-2 and MCF-7 cell lines. Some of them posses a wide range of pharmacological activity. Finally, a molecular docking study was conducted to reveal the probable interaction with the dihydrofolate reductase (DHFR) active site.

1 | INTRODUCTION

Chalcones have a considerable interest to medicinal chemists and are widely used as antimicrobial,[1–3] anticancer,[4,5] antitumor,[6] antitubercular,[7] antioxidant, antidiabetic,[8,9] anti-inflammatory,[10] antimalarial,[11] antileishmanial,[12] and antiviral activity.[13]

They are well known intermediates for synthesizing various heterocyclic compounds. Cyclization of chalcones, leading to thiazines, pyrimidines, pyrazoline has been a developing field within the realm of heterocyclic chemistry for the past several years because of their ready accessibility and the broad spectrum of biological activity of the products as antibacterial, antifungal, antiprotozoal, antiinflammatory substances.

A survey of literature in the recent past reveals that pyrimidine heterocyclic core has a capital value in pharmacologically active skeletons since it contains the base for uracil, thiamine, and cytosine nitrogen bases, which are the building blocks of the nucleic acids.[14,15] Furthermore, pyrimidine derivatives have an extensive scope of healing actions such as: anti-microbial,[16–19] anti-viral, anti-HIV,[20,21] anti-tubercular,[22,23] anti-malarial,[24,25] analgesic, anti-inflammatory,[26,27] diuretic,[28] cardiovascular,[29,30] hypnotic for the nervous system,[31,32] and antioxidant,[33,34] anticancer.[35]

On the other hand, many pyrimidine-5-carbo nitriles,[2,36–39] and pyrimidinethione derivatives[40–44] have been reported to possess a significant anticancer and antimicrobial actions. Moreover, the presence of either methoxy and/or benzyloxy substituents resulted in a significant increase of several biological potency due to the improved in lipophilicity of compounds.[45–48] Consequently, we designed and synthesized compounds enclosing the pyrimidinethione ring system decorated with a cyano group and alkoxylated aryl moiety. The strategy focused on the thione functional group that was linked to a range of active substituents through —CH_2— or —CH_2CO— groups or directly attached to the sulfur atom providing thioethers of high antimicrobial and antitumor activities.[49–51]

In view of these observations and in continuation of our previous work to discover new biologically active heterocyclic compounds,[52–55] we aimed in this context to synthesized new hybrid compounds having the pyrimidine moiety conjugated with different aromatic/ heterocylic/ side chains of documented cytotoxic potency against different cancer cell lines such as: benzothioate, naphthalene, thiazole, tetrahydropyrimidin, pyrimidine, and chloroethanethiolate. The antiproliferative activity of all synthesized compounds was measured against human liver (HePG-2) and human breast (MCF-7) carcinoma cell lines using doxorubicin as a reference drug.

2 | RATIONALE OF MOLECULAR DESIGN

Depending on ligand-based drug design approach,[56–61] it was determined to select compounds, some reported as DHFR inhibitors (I, II, III, IV, V, and VI), as lead compounds. I, III, IV, V, and VI), as lead compounds.

A survey of the structure-activity relationships (SAR) and common pharmacophoric features of various DHFR antagonists revealed that they have four main features as indicated in Figure 1. These features are (a) a hetero aromatic ring system containing at least one H-bond acceptor and one hydrogen bond donor, (b) a linker moiety of two or three carbon bridge, (c) a central aryl ring (spacer), (d) terminal pharmacophore comprises at least two hydrogen bond acceptors.[62,63]

The target of our work is to synthesize new agents with the same essential pharmacophoric features of the reported and clinically used DHFR inhibitors.

The main core of our molecular design rationale comprised bio-isosteric modification strategies of DHFR inhibitors at four different positions (Figure 2).

The first position was the heterocyclic aromatic ring; 4,6-diaryl pyrimidine moiety was used. The second position was the linker region, where thioacetamide group was utilized as a linker. The third position was the central aryl ring, where the phenyl group was incorporated.

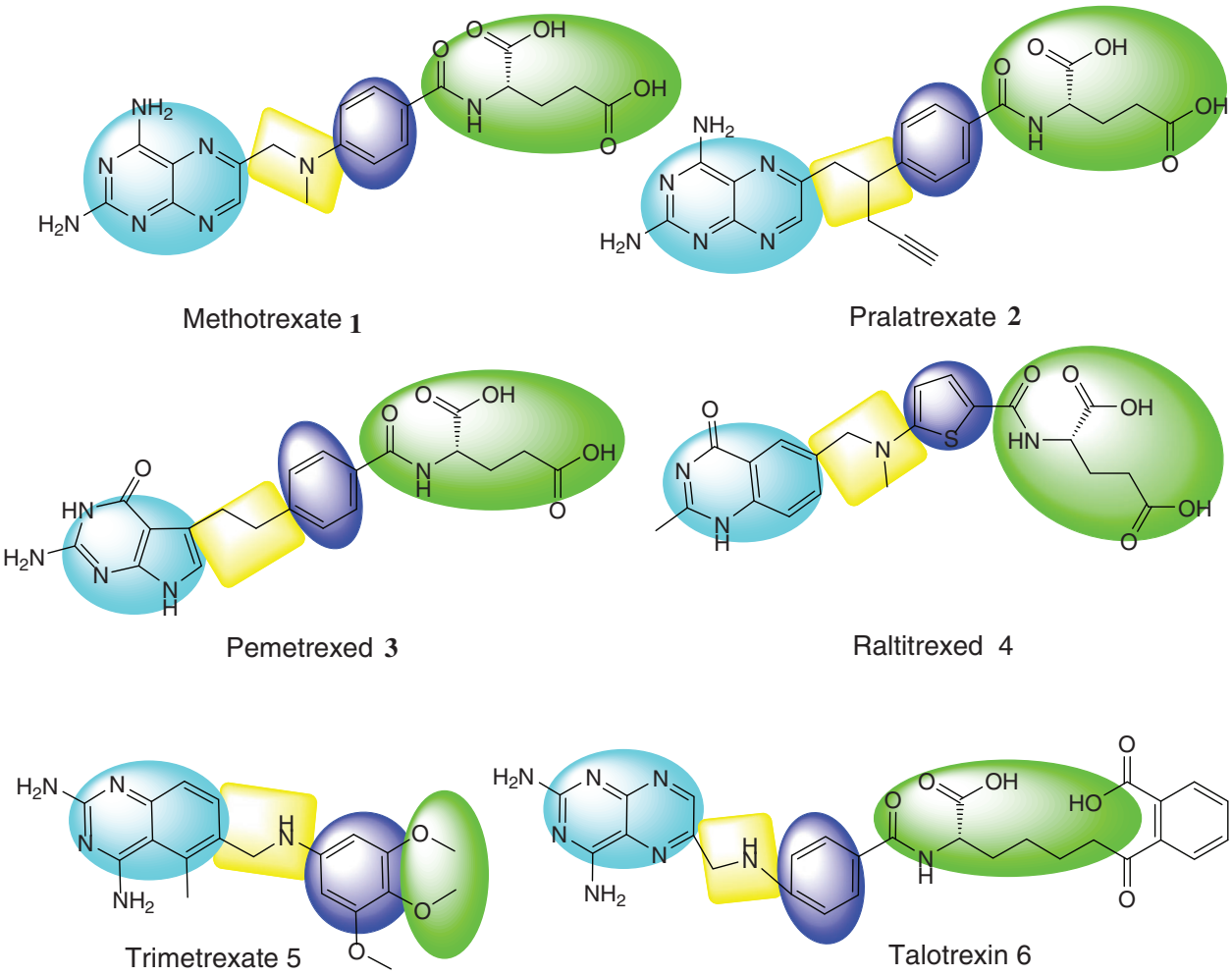


FIGURE 1 Basic pharmacophoric features of DHFR inhibitors

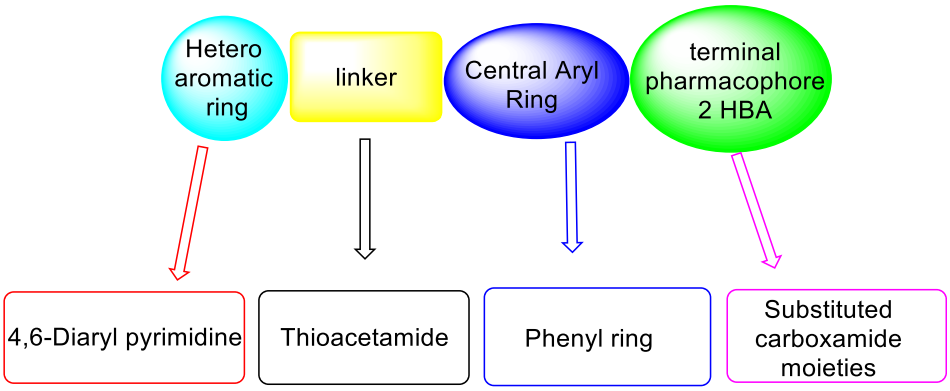


FIGURE 2 Summary of the possible modifications of DHFR inhibitors

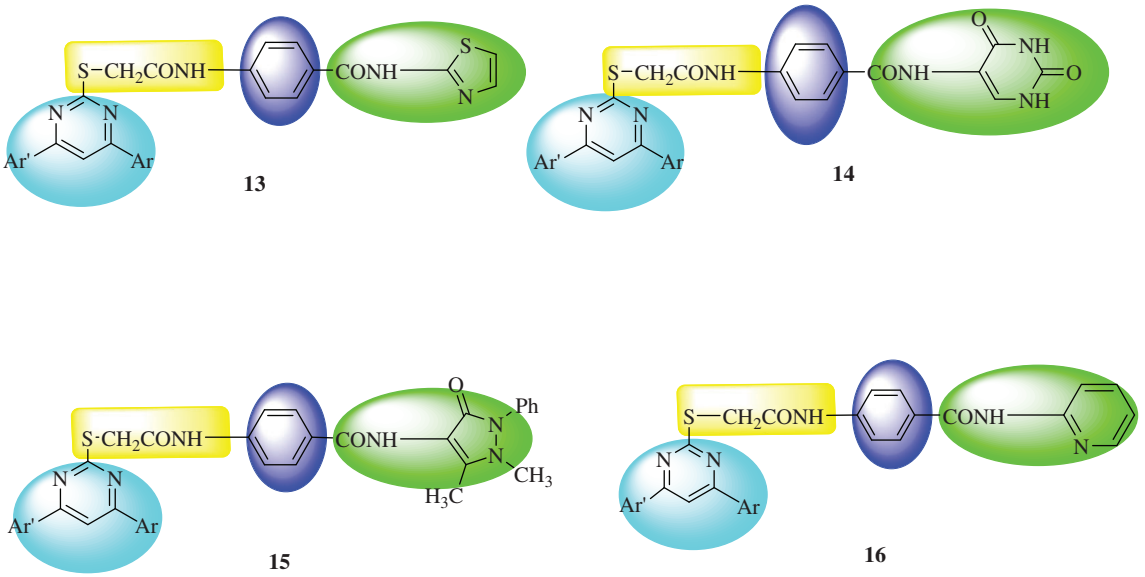


FIGURE 3 The rationale of molecular design of the newly synthesized compounds having the same pharmacophoric features of DHFR inhibitors

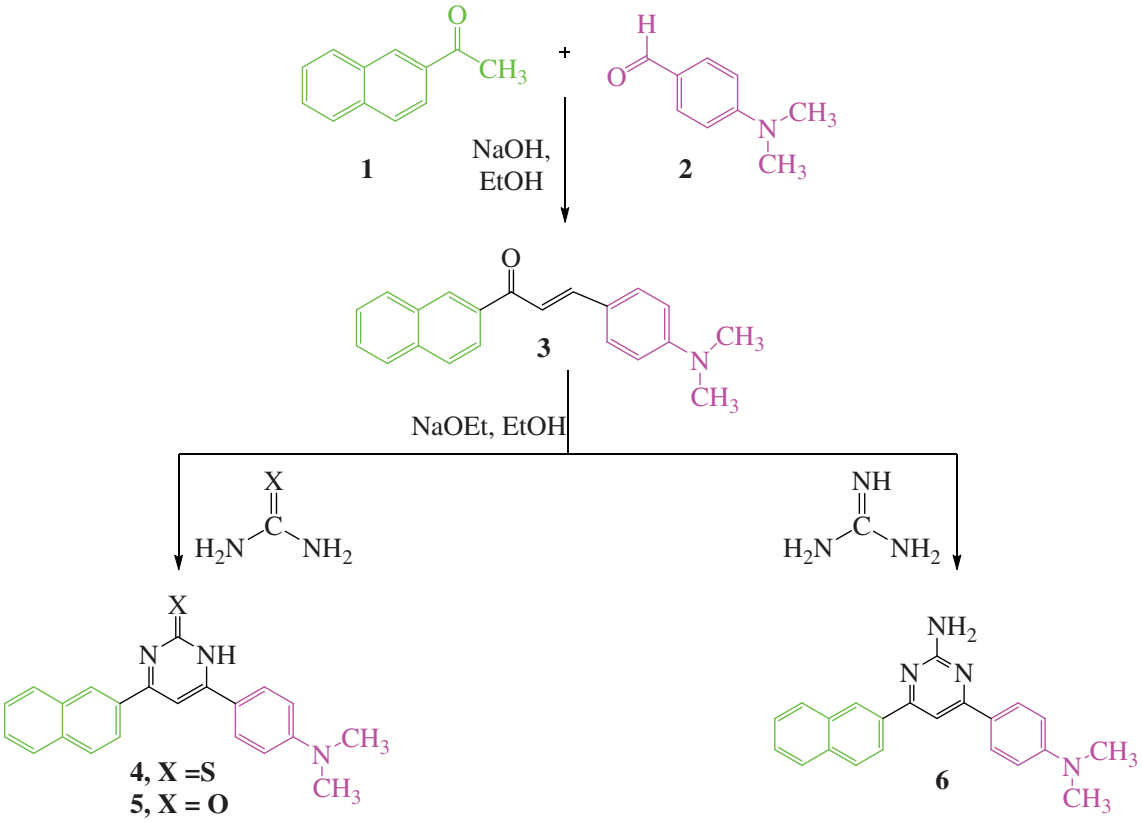
Finally, the terminal pharmacophore where different substituted carboxamide moieties were used. (Figure 3).

In addition, molecular docking studies were conducted to understand the expected binding interactions of the target compounds with DHFR active sites and explore and emphasize the mechanism of action of the synthesized compounds.

3 | RESULTS AND DISCUSSION

In the present study 3-(4-[dimethylamino]phenyl)-1-(naphthalen-2-yl)prop-2-en-1-one **3** was synthesized by the base-catalyzed Claisen-Schmidt condensation[64,65] of 2-acetyl naphthalene with 4-(N,N-dimethylamino benzaldehyde (Scheme 1).

The structure of compound **3** was confirmed by its elemental analysis as well as spectroscopic data. The IR spectrum of chalcone **3** displayed a weak band at 3059 cm^{-1} due to a stretching vibration of (C-H) group. The two bands at 1649 cm^{-1} and 1623 cm^{-1} were due to C═O and C═C stretching vibrations, respectively. Condensation of chalcone **3** with thiourea, urea and guanidine hydrochloride under basic conditions (EtONa) produced 6-(4-[dimethylamino] phenyl)-4-(naphthalen-2-yl) pyrimidin-2(1H)thione (**4**), 6-(4-[dimethylamino] phenyl)-4-(naphthalen-2-yl) pyrimidin-2(1H)-one (**5**) and 4-(4-[dimethylamino] phenyl)-6-(naphthalen-2-yl) pyrimidin-2-amine (**6**), respectively (Scheme 1). The purity of the compounds was determined by TLC and elemental analyses. Spectral data (I.R, ^{1}H-NMR and Mass) revealed that these compounds were in full agreement with the proposed structures. The IR spectra of compounds **4** and **5** showed absorption bands at 3394 and 3450 cm^{-1} attributed to NH group, respectively. Compound **5** showed absorption band at 1490 cm^{-1} attributed



SCHEME 1 Synthesis of chalcone **3** and compounds **4-6**

to C═S group. The ^{1}HNMR spectra of compounds **4** and **5** exhibited signals at δ = 9.25 and 11.74 ppm due to NH group. The IR spectrum of compound **6** showed a characteristic absorption band at 3318 cm^{-1} for NH_2. ^{1}H-NMR spectrum of compound **6** exhibited a signals at δ = 6.55 ppm attributed to NH_2. Mass spectra of compounds **4**, **5**, and **6** exhibited their molecular ion peaks that confirmed their molecular formula. The reactivity of compounds **4** and **5** towards different carbon electrophiles was examined. So, Treatment of compounds **4** and/or **5** with benzoyl chloride in pyridine afforded pyrimidine derivatives **7a** and **8a**.

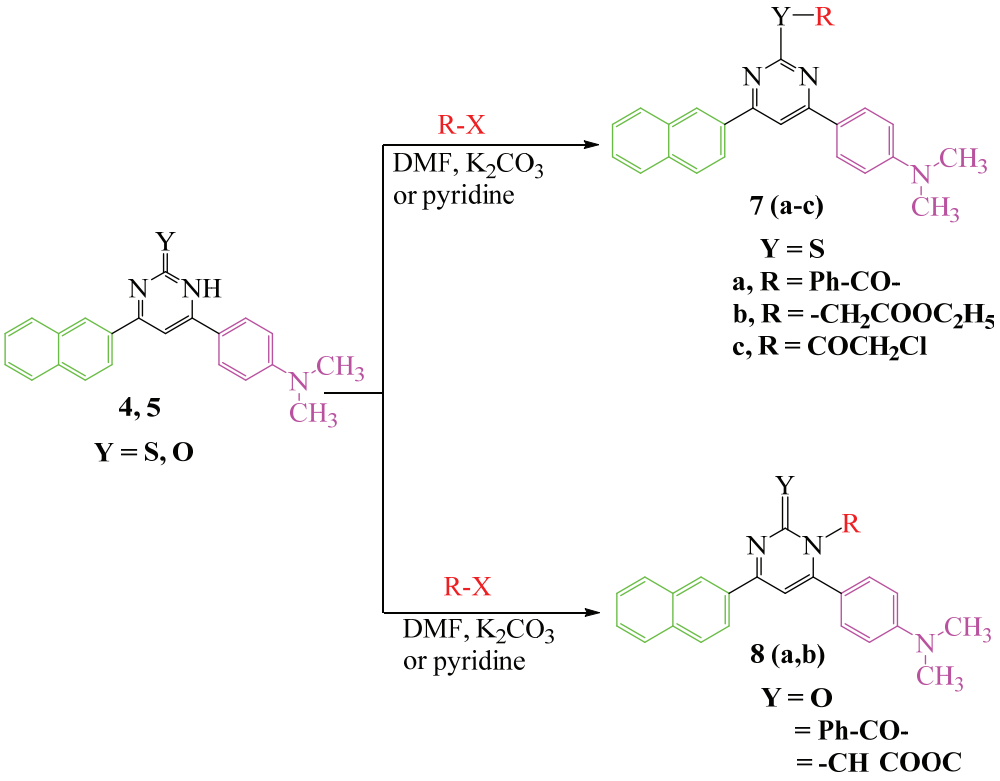
The IR spectra of compounds **7a** and **8a** revealed the disappearance of the NH band and the appearance of a characteristic band of C═O group at 1783 and 1638 cm^{-1}, respectively. The ^{1}H-NMR spectra showed that the signal of NH group was disappeared for both compounds **7a** and **8a (**Scheme 2).

Also, treatment of pyrimidine derivative **4** and pyrimidinethione derivative **5** with ethyl bromoacetate in DMF in presence of anhydrous K_2CO_3 gave pyrimidine derivatives **7b** and **8b**, respectively. The IR spectrum of compound **7a** showed a characteristic band at 1728 cm^{-1} corresponding to C═O ester, however compound **8b** showed two bands at 1743 and 1652 corresponding to 2 C═O groups. ^{1}H-NMR spectra of compounds **7b** and **8b** showed beside the expected methyl and aromatic signals, other two signals in the range at 1.03 to 1.18 ppm for $\underline{CH_2}CH_3$ and at 4.03 to 4.47 ppm for $CH_2\underline{CH_3}$.

The activities of the thioamide and iminothiol tautomer based on their thermodynamic and kinetic control under experimental condition have been explained. The conjugate base of the iminothiol tautomer has been found to be thermodynamically more stable than the conjugate base of the thioamide tautomer (basicity is thermodynamic control). In the presence of anhydrous K_2CO_3 or pyridine and DMF due to back donation involving the vacant *d*-orbital of the sulfur atom. Further, the sulfur anion is strong nucleophile thane the nitrogen anion (nucleophilicity is kinetic control), thus making the iminothiol tautomer to be kinetically more stable than the thioamide tautomer. Thus, under the experimental conditions used the iminothiol tautomer is more thermodynamically and kinetically favored than the thioamide tautomer, which practically spell out the reactivity of the iminothiol tautomer. However, in amide tautomer the nucluphilicity of nitrogen atom is stronger than the nucleophilicity of the oxygen atom in immediate tautomer.

Refluxing a mixture of **4** with chloroacetylchloride in DMF in the presence of anhydrous potassium

SCHEME 2 Synthesis of compounds **7(a-c)** and **(8a, b)**



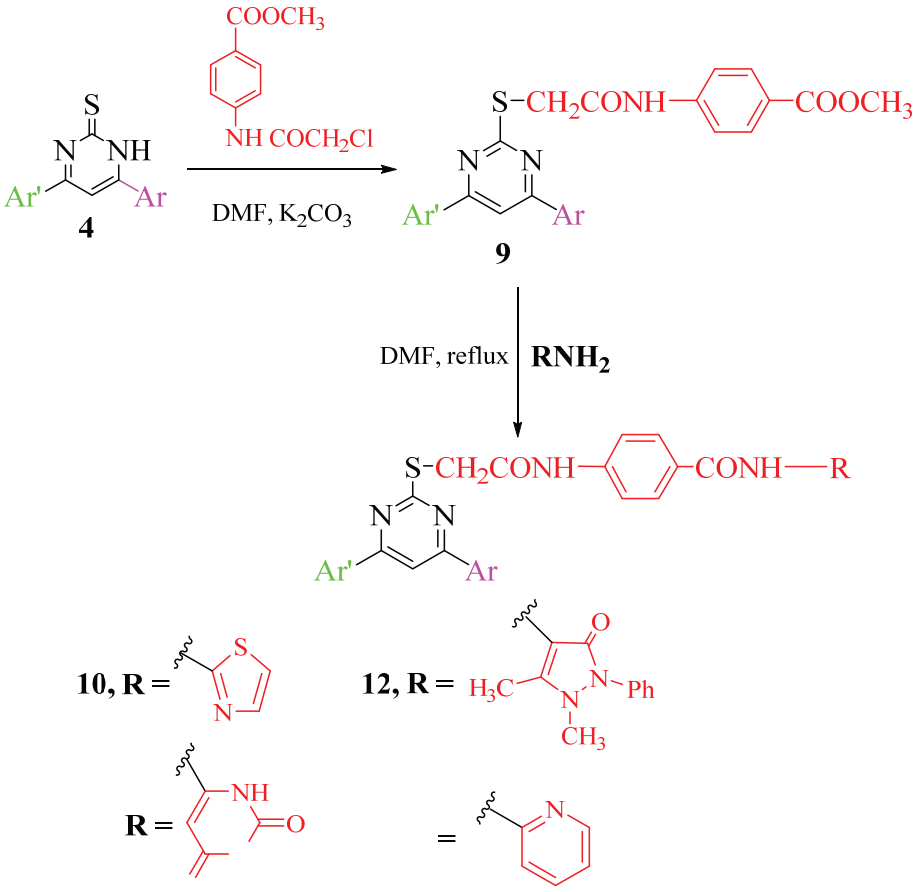
carbonate afforded the pyrimidine derivative **7c**. The IR spectrum of **7c** showed absorption bands at 1722 cm^{-1} due to the C=O group. ^{1}H-NMR spectrum of compound **7c** exhibited two singlet signals at 4.32 and 3.02 ppm attributed to CH_2 and two CH_3 groups, respectively.

Compound **4** was submitted to react with methyl 4-(2-chloroacetamido) benzoate furnished compound **9** via reaction of sulfur atom (Scheme 3). The IR spectrum showed two bands for C=O amide and C=O ester. The ^{1}H-NMR spectrum exhibited signals at 3.79 and 4.22 ppm due to $COOCH_3$ and CH_2, respectively. Compound **9** was reacted with different amines namely, 2-aminothiazole, 4-amino-2,6-dihydroxypyrimidine, 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one and 2-aminopyri dine in DMF to afford compounds **10-13**, respectively (Scheme 3). For the synthesis of compounds **9-13**, we use DMF as solvent due to its high dielectric constant and its ability to soluble the reactants of the reaction. The structures of compounds **10-13** were confirmed by elemental analyses as well as spectroscopic data. The ^{1}H-NMR spectra of compounds **10-13** showed two singlet signals in the range 10.00-10.75 ppm attributed to two NH groups.

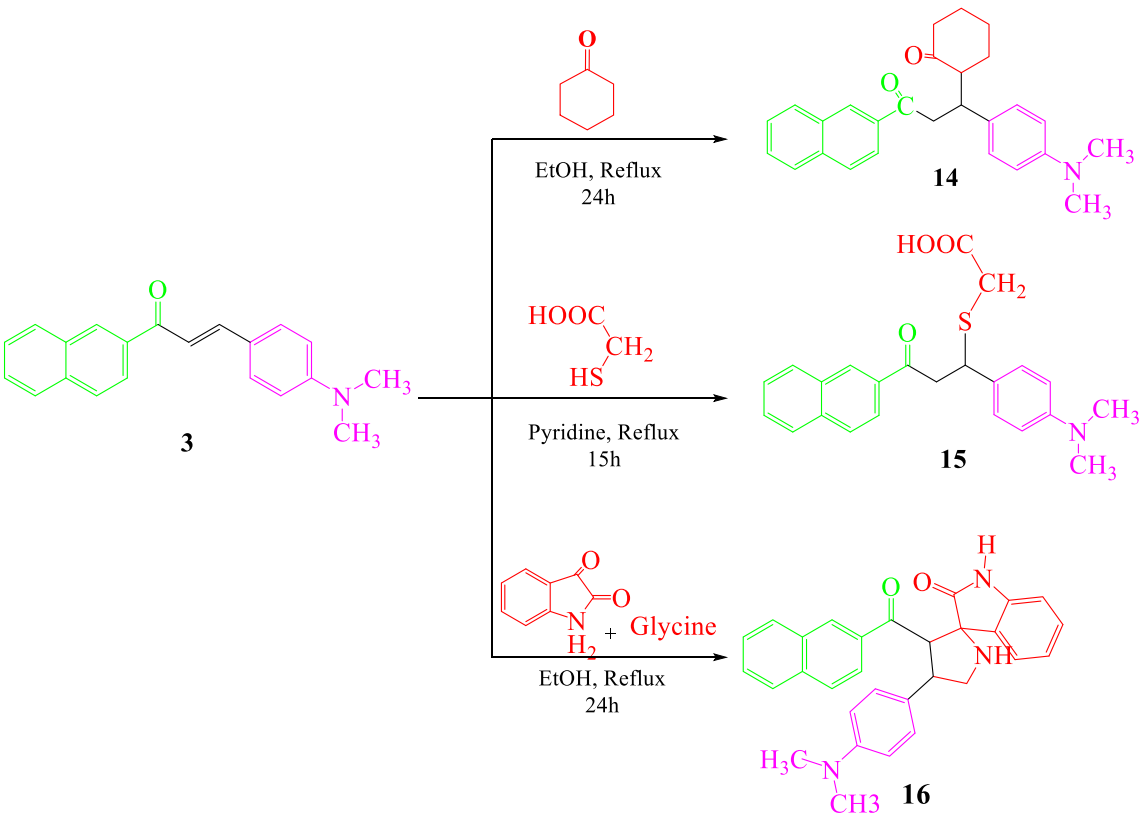
The reaction of Chalcone **3** with cyclohexanone in boiling ethanol afforded the corresponding addition product **14**. The IR spectrum of compound **14** exhibited two absorption bands at 1701, 1676 cm^{-1} attributed to two (C=O) groups, which have high frequency value due to mutual induction between the two carbonyl groups (Scheme 4).

Treatment of a mixture of compound **3** with thioglycolic acid in pyridine afforded 2-((1-(4-[dimethyla mino] phenyl)-3-(naphthalen-2-yl)-3-oxopropyl)thio)acetic acid **(15)**. The IR spectrum of compound **15** showed the following characteristic absorption bands at 3439, 1648 and 1669 due to OH and 2C=O, respectively. Moreover the ^{1}H-NMR spectrum of compound **15** exhibited signals at δ = 5.19 and 9.62 ppm corresponding to CH_2 and OH groups, respectively. One -pot three component reaction of chalcone **3** with isatine and glycine in methanol[66] afforded 4′-(2-naphthoyl)-3′-(4-[dimethylamino] phenyl) spiro[indoline-2,2′-pyrrolidin]-3-one (**16**). The reaction takes place via the following route. Glycine attack the more reactive carbonyl group in istain and yielded the addition product followed by dehydrogenation to give the corresponding lactam moiety, which loss carbon dioxide and yielded the reactive 1, 3-dipolar system. The reaction of the later one with chalcone **3** afforded the desired product **16**.

The structure of the spiropyrrolidin derivative **16** was established from the elemental analysis and spectral data. The IR spectrum of compound **16** showed characteristic absorption bands at 3407, 1712, and 1670 corresponding to NH and CO, respectively. ^{1}H-NMR spectrum of compound **16** exhibited signals at δ = 2.86, 3.55, 3.56, 3.91,



SCHEME 3 Synthesis of compounds **9-13**



SCHEME 4 Synthesis of compounds **14-16**

and 10.40 ppm corresponding to CH, NH, CH_2, CH, and NH, respectively.

4 | ANTICANCER ACTIVITY

A literature survey[40,41] as well as our previous work on pyrimidine moiety.[55,67,68] Revealed the cytotoxicity of pyrimidine derivatives as potential anticancer agents against the two cell lines MCF7 and HePG2. So, the in vitro antiproliferative activity of the newly Pyrimidine derivatives (**3-16**) were assessed against human liver (HepG2) and breast (MCF7) cancer cell lines in addition to the normal fibroblast (WI-38) and compared to the activity of doxorubicin. The results were expressed as growth inhibitory concentration (IC50) values, which represent the compound concentrations required to produce a 50% inhibition of cell growth after 72 hours of incubation, compared with the untreated controls (Table 1), Figure 4. The data showed that doxorubicin had an IC50 at ∼4-7 μM against all cells investigated with no differentiation between cancer and normal cells. The novel Pyrimidine derivatives (**7c**, **12**, **14**, **15** and **16**) showed a promising antiproliferative activity against cancer cell lines with IC50 at 7.36-21.7 μM. Only three derivatives (**7c**, **12**, and **16**) were safe to the normal fibroblasts with IC50 at ∼58-61 μM. The other 2 derivatives (**14** and **15**) showed some toxicity against the fibroblasts at ∼22 and 45 μM, respectively; a concentration that is 4 to 6 times that of doxorubicin This indicates that derivatives **7c**, **12**, and **16** have selectivity against cancer cell lines and therefore, these derivatives deserve more focused mechanistic studies.

4.1 | Structure-activity relationship

By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure activity relationships (SAR) were postulated.

- The linker region with sulfur atoms was found to be more effective in cytotoxic activity as shown in compound **7b** (IC_{50} = 42.50 ± 2.8 μM, 58.39 ± 3.5 μM and 44.6 ± 4.2 μM) when compared with compound **8b** (IC_{50} = 82.74 ± 3.9 μM, 77.74 ± 4.0 μM and 35.4 ± 2.7 μM).

TABLE 1 Anti-proliferative activity toward HePG-2, MCF-7cell lines

	In vitro cytotoxicity IC_{50} (μM)[a]		
Compounds	**HePG2**	**MCF-7**	**WI-38**
3	53.14 ± 3.1	40.28 ± 2.9	33.9 ± 1.49
4	13.81 ± 1.4	28.81 ± 2.3	39.1 ± 2.1
5	72.92 ± 4.0	81.47 ± 4.4	42.2 ± 3.2
6	24.65 ± 2.1	37.58 ± 2.8	23.5 ± 1.9
7a	>100	94.81 ± 5.3	29.5 ± 2.2
8a	92.74 ± 4.9	89.32 ± 4.9	72.4 ± 4.8
7b	42.50 ± 2.8	58.39 ± 3.5	44.6 ± 4.2
8b	82.74 ± 3.9	77.74 ± 4.0	35.4 ± 2.7
7c	9.17 ± 1.0	8.03 ± 0.7	58.9 ± 2.5
9	71.64 ± 3.9	78.25 ± 4.1	47.0 ± 4.7
10	31.26 ± 2.5	49.16 ± 3.2	68.7 ± 2.5
11	63.97 ± 3.7	67.51 ± 3.8	27.8 ± 2.4
12	11.08 ± 1.2	21.17 ± 1.6	51.4 ± 4.1
13	84.66 ± 4.2	82.45 ± 4.6	23.2 ± 1.2
14	16.29 ± 1.7	12.90 ± 1.1	44.6 ± 2.1
15	19.42 ± 1.9	15.83 ± 1.4	21.6 ± 1.8
16	7.36 ± 0.8	10.16 ± 1.0	61.1 ± 4.3
DOX[b]	4.50 ± 0.2	4.17 ± 0.2	6.7 ± 0.5

Note: IC50 (μM): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); 51-100 (weak); and above 100 (non-cytotoxic).
[a]IC50: values are the mean ± SD of three separate experiments.
[b]Doxorubicin.

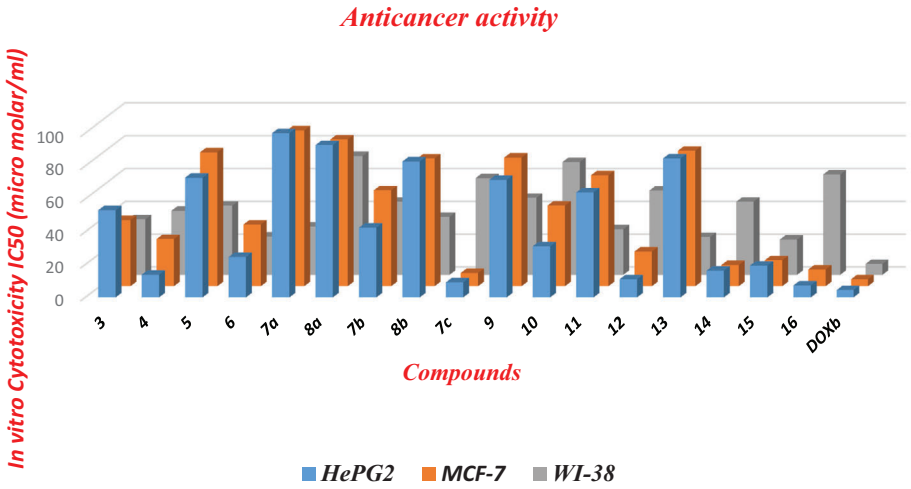


FIGURE 4 Anticancer activity of the synthesized compounds

Comp. No	CDocker interaction energy (CDocker energy) kcal/mol	Number of hydrogen bonding	Number of cationic-π interactions
A	**−7.90**	4 (ILE 10, ILE123, GLU32)	1 (PHE 36)
7c	−7.92	—	2 (ILE 65)
12	−10.163	2 (LYS 37, LYS 73)	1 (ALA12)
16	−8.362	—	(PHE 36)

TABLE 2 The docking binding free energies of the synthesized compounds **7c**, **12**, and **16** with dihydrofolate reductase (DHFR) forming hydrogen bonding and π interactions

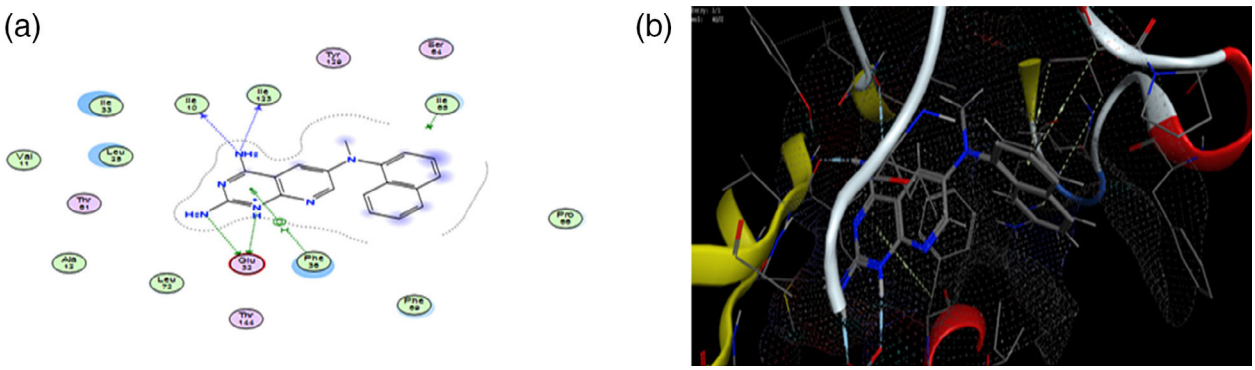


FIGURE 5 Interaction diagram of the ligand-binding domain of dihydrofolate reductase (DHFR) with **N ∼ 6 ∼ −methyl-N ∼ 6 ∼ −(naphthalen-1-yl)pyrido[2,3-d]pyrimidine-2,4,6-triamine**, **(A)**

- Introducing the $COCH_2Cl$ to the pyrimidine moiety with the sulfur atom increases the cytotoxic activity against the two cell lines as in compound **7c** (IC_{50} = 9.17 ± 1.0 μM, 8.03 ± 0.7 μM and 58.9 ± 2.5 μM) when compared with compound **8b** (IC_{50} = 82.74 ± 3.9 μM, 77.74 ± 4.0 μM and 35.4 ± 2.7 μM).
- Incorporation of thioacetic acid moiety in the linker region increases the cytotoxic activity as shown in compound **15** (IC_{50} = 19.42 ± 1.9 μM, 15.83 ± 1.4 μM and 21.6 ± 1.8 μM).
- The Spiro derivative **16** (IC_{50} = 7.36 ± 0.8 μM, 10.16 ± 1.0 μM and 61.1 ± 4.3 μM) has higher cytotoxic effect than that of the corresponding derivatives.

4.2 | Molecular docking

Molecular docking of pyrimidine derivatives (**3**-**16**) to the active site of dihydrofolate reductase (DHFR) enzyme was performed to investigate their binding interactions

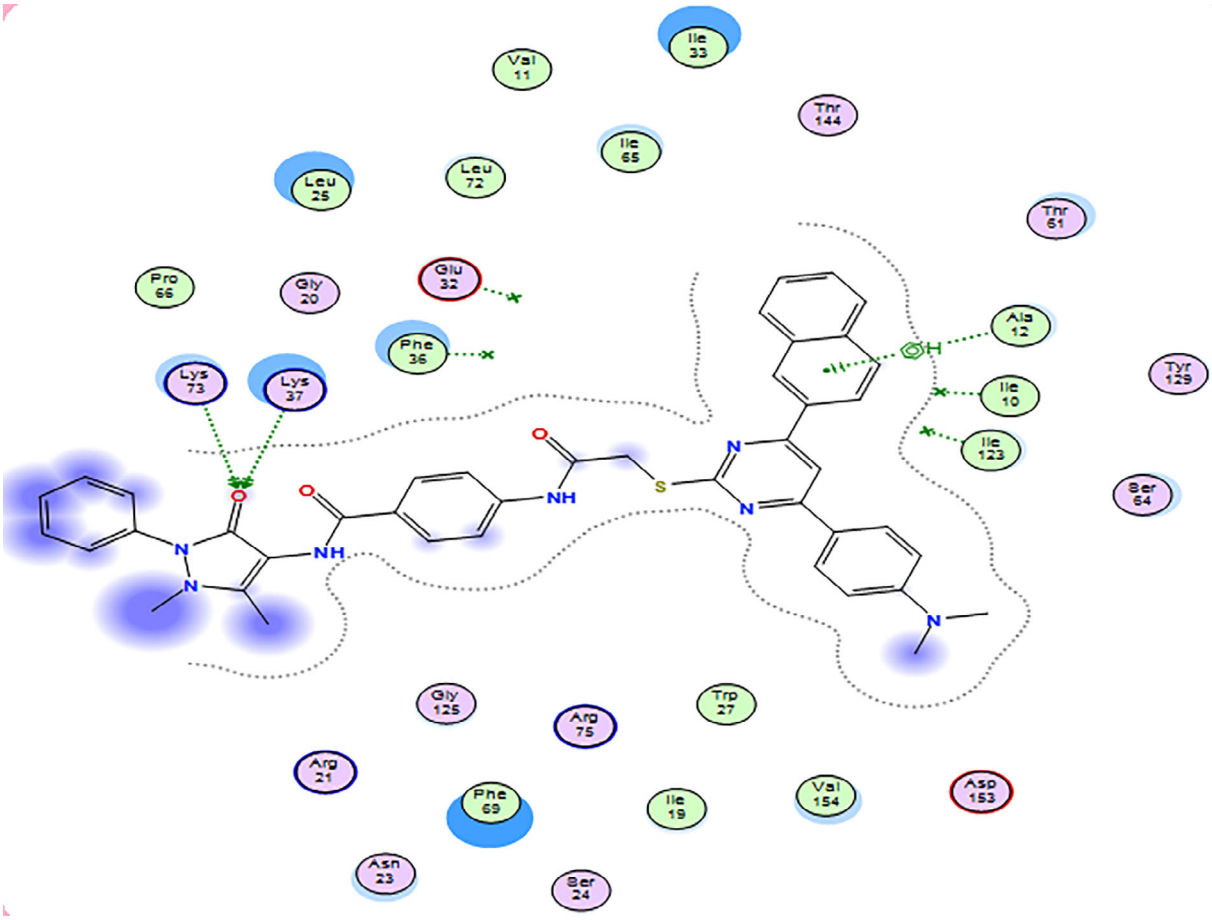


FIGURE 6 (2D) diagrams of binding interactions of dihydrofolate reductase (DHFR) with compound **12**

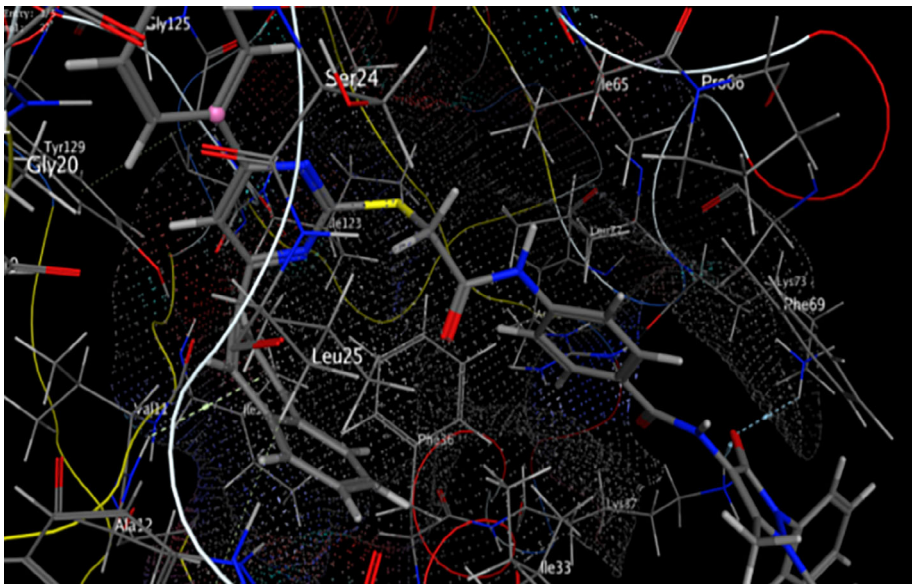


FIGURE 7 Docking conformations of compound **12** in the active site and corresponding Connolly surface

and to explore their binding modes Table 2. Moreover, our lead compound **A** was also docked in order to investigate its binding pattern to the (DHFR) active site (Figure 5). The interaction of compound **A** with (DHFR), illustrated the formation of four hydrogen bonding with the amino acids ILE 10. ILE 123, GLU 32 and one pi bond with the amino acid PHE 36. Additionally, it showed a good interaction CDOCKER score **− 7.90** kcal/mol)

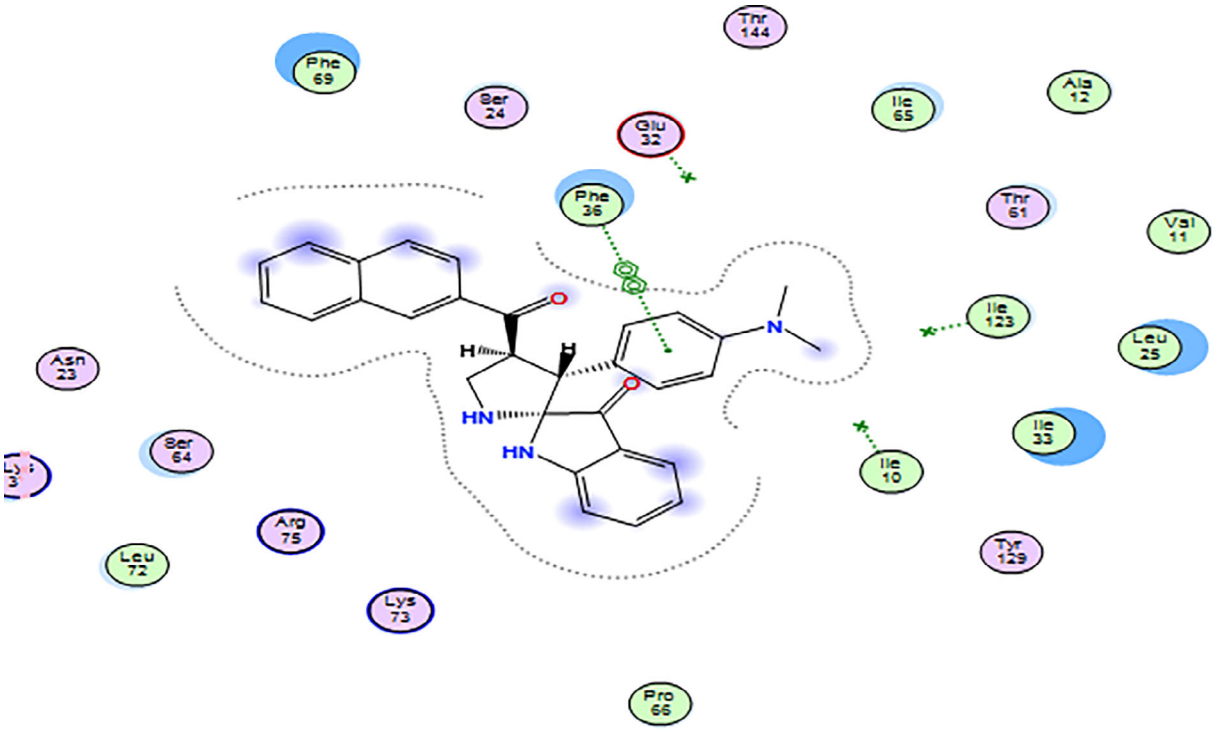


FIGURE 8 (2D) diagrams of binding interactions of dihydrofolate reductase (DHFR) with compound **16**

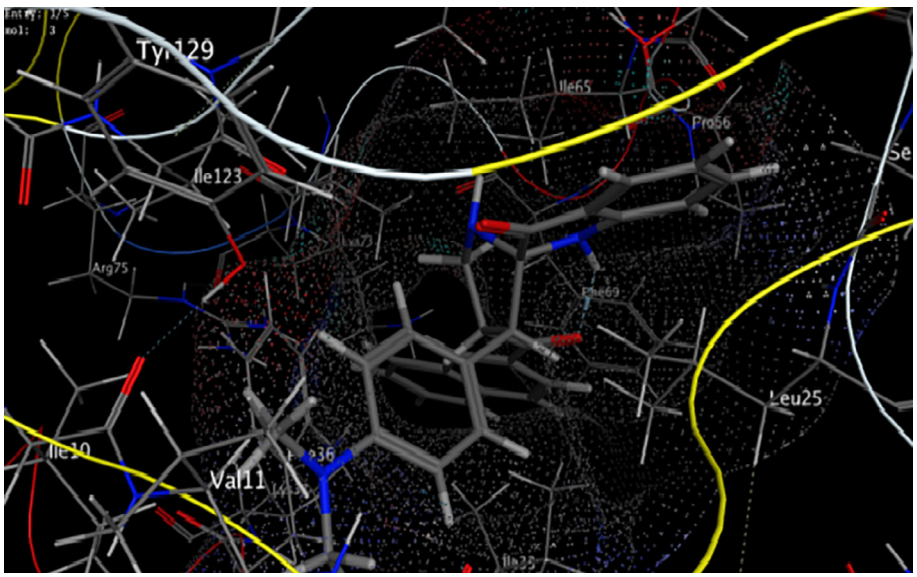


FIGURE 9 Docking conformations of compound **16** in the active site and corresponding Connolly surface

(See supplementary data). Scrutinizing the binding pattern between the most active compound **7c** and the (DHFR) enzyme (Figure 10) (See supplementary data) and exploring the interaction energy, unravels that it was able to form two pi- bonds between the naphthayl moiety and the key amino acid ILE 65 with a good CDOCKER interaction energy score (−7.92 kcal/mol) that is comparable to the lead compound CDOCKER score (**−7.90** kcal/mol) (See supplementary data). Compound **12** formed pi- bonds between Carbonyl group of pyrazolone moiety with the two amino acid LYS 37 and LYS 73 and Pi- bond between the aromatic ring of naphthalene moiety with the amino acid ALA 12 with good CDOCKER score more than the ref (−10.163) (Figures 6 & 7). Finally, Compound **16** showed the binding mode with (DHFR) involved in aromatic stacking interactions with PHE 36 with a good CDOCKER score (−8.3623 kcal/mol) (Figures 8 & 9).

5 | CONCLUSIONS

This study reveals successful synthesis of new pyrimidine and thiopyrimidine derivatives **4-16** with different heteroaryl ring systems. This was achieved by treatment of chalcone **3** with urea, thiourea, and guandine hydrochloride. The reactivity of the synthesized pyrimidine derivatives towards different nucleophilic and electrophilic reagent was examined. The structures of the newly synthesized pyrimidine derivatives were elucidated by using IR, ^{1}H NMR, ^{13}C NMR, EI-MS, and elemental analysis. Anticancer activities of all the synthesized derivatives were screened against HepG2, MCF-7, and WI-38 cell lines. Compounds **7c**, **12**, and **16** appeared to be the most active cytotoxic compounds against the two cell lines. They produced growth inhibition of the tested cancer cells slightly less than that of the standard drug doxorubicin.

6 | EXPERIMENTAL PART

6.1 | Chemistry

All melting points were measured with a Gallenkamp melting point apparatus and were uncorrected. The IR spectra were recorded on a Pye-Unicam SP-3-300 infrared spectrophotometer (KBr dicks) and expressed in wave number (cm^{-1}). ^{1}H-NMR spectra were run at 400 MHz, on a Varian Mercury VX-300, and BrukerAvance III NMR spectrometer, respectively. TMS was used as an internal standard in deuterated dimethylsulphoxide (DMSO-*d6*). While ^{13}C NMR spectra were run at 100 MHz. TMS was used as an internal standard in deuterated dimethylsulphoxide (DMSO-*d6*). Chemical shifts (δ) are quoted in ppm. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. All coupling constant (J) values are given in hertz. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were performed on CHN analyzer and all compounds were within $\pm$0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. All reagents and solvents were purified and dried by standard techniques.

Synthesis of 3-(3-[dimethylamino]phenyl)-1-(naphthalen-2-yl)prop-2-en-1-one (3).

To a solution of 2-acteylnaphathalene (1.7 g, 0.01 mol) in ethanol (20 mL) 50% sodium hydroxide solution (20 mL) was added dropwise with stirring in an ice bath for 15 minutes; 4-(N, N-dimethylaminobenzaldehyde) (1.49 g, 0.01 mol), was added. The reaction mixture was stirred in ice bath for another 30 minutes; left over two nights in the refrigerator. The obtained precipitate was filtered off, washed with H_2O, dried; and recrystallized from ethanol to give **3**.

Yield 95%; orange crystals; mp. 220-222°C (DMF/EtOH); IR (cm^{-1}) ν: 3059 (CH aromatic), 2802 (CH aliphatic), 1623 (C═C), 1649 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.06 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 6.09-6.70 (d, 2H, Ar-H; J = 8.8 Hz), 7.47-7.51 (d, 1H, H-C═C, J = 15.2 Hz), 7.53-7.58 (m, 1H, Ar-H), 7.59 (d, 2H, Ar-H, J = 8.8 Hz), 7.84-8.05 (m, 3H, Ar-H), 7.85 (d, 1H, CH═CH, J = 15.2); 8.10 (d, 2H, Ar-H, J = 8.8 Hz), 8.52 (s, 1H, Ar-H of naphthalene ring); MS (m/z %): 301 (M^+, 7), 302 (9), 303 (23); Anal. Calcd for: $C_{21}H_{19}NO$ (301.1): C, 83.69; H, 6.35; N, 4.65%; Found C, 83.45; H, 6.29; N, 4.55%.

General method for the synthesis of compounds (4, 5 and 6).

a) Method I.

A mixture of **3** (3.01 g, 0.01 mol) thiourea (0.76 g, 0.01 mol), urea (0.6 g, 0.01 mol), and /or guanidine (0.6 g, 0.01 mol) in the presence of sodium ethoxide (5 g, 25 mL) in absolute ethanol was refluxed for 24 hours. The reaction mixture was cooled, acidified with dilute hydrochloric acid and the solid obtained was filtered off, dried, and then recrystallized to give **4**, **5**, and **6**.

b) Method II (one-pot multicomponent reaction).

A mixture of 2-acteylnaphathalene (1.7 g, 0.01 mol), 4-N,N-aminodimethyl benzaldhyde (1.49 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) and/or urea (0.6 g, 0.01 mol) in case of 5 and/or guanidine (0.6 g, 0.01 mol) in case of 6 in 20 mL ethanol and drops of piperdine was refluxed for 4 hours. The formed precipitate was filtered, off, dried, and then recrystallized from a proper solvent to give 4, 5 and 6.

6-(4-[Dimethylamino] phenyl)-4-(naphthalen-2-yl) pyrimidine-2(1H)-thione (4).

Yield 75%; yellow crystals; mp. 242-244°C (DMF/EtOH)); IR (cm^{-1}) ν: 3394 (NH), 3051 (CH aromatic), 2850 (CH aliphatic), 1490 (C═S); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.02 (s, 6H, $2CH_3$), 6.80 (d, 2H, Ar-H, J = 8.4 Hz), 7.46 (s, 1H of pyrimidin ring), 7.48-8.73 (m, 8H, Ar-H); 8.77 (s, 1H, Ar-H of naphthalene ring), 9.24 (1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-*d6*): 42.3 (2), 112.5 (2), 118.1 (2), 125.7, 126.2 (2), 127.3, 128.1 (2), 128.4 (2), 129.0, 130.1 (2), 154.8, 164.1, 165.9, 176.2, 180.5; MS (m/z %): 357 (7), 295 (60), 289 (69) and 287 (100); Anal. Calcd for: $C_{22}H_{19}N_3S$ (357.48): C, 73.92; H, 5.89; N, 11.75; S, 8.97; Found: C, 73.54; H, 5.80; N, 11.69; S, 8.80%.

6-(4-[Dimethylamino] phenyl)-4-(naphthalen-2-yl) pyrimidin-2(1H)-one (5).

Yield 65%; yellow crystals; mp. 300-302°C (DMF/EtOH)); IR (cm^{-1}) ν: 3450 (NH), 3100 (CH aromatic),

2920 (CH aliphatic), 1620 (C═N), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.02 (s, 6H, $2CH_3$), 6.80 (d, 2H, Ar-H, J = 8.8 Hz), 7.51 (s, 1H of pyrimidine ring), 7.60 (d, 2H, Ar-H, J = 8.8 Hz), 7.97-8.21 (m, 6H, Ar-H), 8.77 (s, 1H, Ar-H of naphthalene ring), 11.74 (s, 1H, NH); MS (m/z) (%): 341.2 (2), 285 (100), 57 (100); Anal. Calcd for: $C_{22}H_{19}N_3O$ (341.43): C, 77.40; H, 5.61; N, 12.31; Found: C, 77.25; H, 5.52; N, 12.29%.

4-(4-[Dimethylamino] phenyl)-6-(naphthalen-2-yl) pyrimidin-2-amine (6).

Yield 75%; yellow crystals; mp. 208-211°C (DMF/EtOH)); IR (cm^{-1}) ν: 3318 (NH), 3194 (CH aromatic), 2852 (CH aliphatic), 1641 (C=N), 1607 (C=C), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 2.99 (s, 6H, $2CH_3$), 6.55 (s, 2H, NH_2, D_2O exchangeable), 6.62 (d, 2H, Ar-H, J = 8.4 Hz), 6.65-7.58 (m, 3H, Ar-H), 7.73 (s, 1H of pyrimidine ring), 7.89-8.63 (m, 5H, Ar-H), 8.78 (s, 1H, Ar-H of napthalen ring); ^{13}C NMR (100 MHz, DMSO-*d6*): 40.3 (2), 103.0, 111.7 (2), 125.7, 126.2 (2), 127.3 (2), 128.1 (2), 128.4 (2), 129.7, 130.1 (2), 132.5, 136.8, 164.6, 165.9, 168.2; MS (m/z %): 340.2 (2.5), 331 (52), 329 (50.5) and 57 (100); Anal. Calcd for: $C_{22}H_{20}N_4$ (340.2): C, 77.16; H, 6.84; N, 16.36; Found: C, 77.35; H, 5.92; N, 16.46%.

General procedure for the Synthesis of compounds 7a and 8a.

A mixture of **4** (2.77 g, 0.01 mol) and/or **5** (2.16 g, 0.01 mol) and benzoyl chloride (1.4 mL; 0.01 mol) in pyridine (20 mL) was refluxed on water bath for 3 hours. The solid obtained was filtered off, dried, and then recrystallized from the suitable solvent to give compounds **7a** and **8a**, respectively.

S-(4-(4-[Dimethylamino]phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl) benzothioate (7a).

Yield 55%; brown crystals; mp. 138-140°C (EtOH); IR (cm^{-1}) ν: 3445 (NH), 3057 (CH aromatic), 2850 (CH aliphatic), 1607 (C=N), 1783 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 2.92 (s, 6H, $2CH_3$), 6.67-8.26 (m, 17H, Ar-H); MS (m/z %): 461.2 (M^+, 2), 331 (100) and 325 (27); Anal. Calcd for: $C_{29}H_{23}N_3OS$ (461.2): C, 75.46; H, 5.02; N, 9.10; S, 6.95; Found: C, 75.55; H, 5.11; N, 9.00; S, 6.81%.

1-Benzoyl-6-(4-[dimethylamino]phenyl)-4-(naphthalen-2-yl)pyrimidin-2(1H)-one (8a).

pi tlsb -0.01wYield 55%; orange crystals; mp. 207-209°C (EtOH); IR (cm^{-1}) ν: 3059 (CH aromatic), 2922 (CH aliphatic), 1638 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.02 (s, 6H, $2CH_3$), 6.80 (d, 2H, Ar-H, J = 8.4 Hz), 7.46 (s, 1H of pyrimidine ring), 7.48-8.73 (m, 13H, Ar-H); 8.77 (s, 1H, Ar-H of naphthalene ring); ^{13}C NMR (100 MHz, DMSO-*d6*): 42.3 (2), 51.1, 107.0, 116.7 (2), 123.1, 127.7 (2), 126.2 (2), 126.9, 127.5 (2), 127.6, 127.9, 128.1 (2), 128.4 (2), 128.9 (2), 132.0, 135.7, 136.5, 142.8, 152.6, 154.9, 168.2; MS (m/z) (%): 445.2 (M^+, 15), 311 (28.8), 122 (78.3), 57 (100); Anal. Calcd for: $C_{29}H_{23}N_3O_2$ (445.2): C, 78.18; H, 5.20; N, 9.43; Found: C, 78.35; H, 5.11; N, 9.54%.

General procedure for the Synthesis of compounds 7b and 8b.

A solution of compound **4** (2.77 g, 0.01 mol) or **5** (2.16 g, 0.01 mol) in (10 mL) DMF was refluxed with ethyl bromoacetate (1.65 mL; 0.01 mol) and anhydrous potassium carbonate (5.52 g, 0.004 mol) for 24 hours on water bath, cool, and pour into water, the obtained red solid was filtered off, dried, and recrystallized from the suitable solvent to afford compounds **7b** and **8b**, respectively.

Ethyl – 2-((4-(4-[dimethylamino] phenyl)-6-(naphthalen-2-yl) pyrimidin-2-yl)thio)acetate (7b).

Yield 23%; reddish brown crystals mp.103-105°C; (EtOH); IR (cm^{-1}) ν: 3053 (CH aromatic), 2836 (CH aliphatic), 1728 (CO ester), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 1.03 (t, 3H, $CH_2\underline{CH_3}$), 3.02 (s, 6H, $2CH_3$), 4.03-4.47 (q, 2H, $\underline{CH_2}CH_3$), 4.95 (s, 2H, SCH_2), 6.81-8.93 (m, 12H, Ar-H); MS (*m/z*) (%): 313 (100), 311 (37), 297 (7.3) and 77 (2.4); Anal. Calcd for: $C_{26}H_{25}N_3O_2S$ (443.57): C, 70.40; H, 5.68; N, 9.47; Found: C, 70.23; H, 5.59; N, 9.39%.

Ethyl 2-(6-(4-[dimethylamino]phenyl)-4-(naphthalen-2-yl)-2-oxopyrimidin-1(2H)-yl)acetate. (8b).

Yield 33%; red crystals; mp.158-160°C, (EtOH); IR (cm^{-1}) ν: 3053 (CH aromatic), 2854 (CH aliphatic), 1743 (CO), 1652 (CO), 1611 (C═N), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 1.18 (t, 3H, CH_3), 3.02 (s, 6H, $2CH_3$), 4.15-4.18 (q, 2H, OCH_2CH_3), 5.01 (s, 2H, OCH_2), 6.81 (d, 2H, Ar-H, J = 6.4 Hz), 6.97 (d, 2H, Ar-H, J = 6.8 Hz), 7.08 (d, 2H, Ar-H, J = 8.0 Hz), 7.45-8.37 (m, 5H, Ar-H), 8.9 (s, 1H, Ar-H); MS (m/z) (%): 429 (M^{+2}, 1), 361 (31.6) and 286 (100); Anal. Calcd for; $C_{26}H_{25}N_3O_3$ (427.5): C, 73.05; H, 5.89; N, 9.83; Found: C, 72.88; H, 5.94; N, 9.79%.

Synthesis of S-(4-(4-[dimethylamino]phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl) 2-chloroethanethioate (7c).

A mixture of compound **4** (2.77 g, 0.01 mol), chloroacetyl chloride (1.12 mL; 0.01 mol), and anhydrous potassium carbonate (5.52 g, 0.004 mol) in (10 mL) DMF was refluxed for 8 hours. The obtained red solid was filtered off, dried, and recrystallized from ethanol to give compound **7c**.

Yield 56%; red crystals; mp.112-114°C, (EtOH); IR (cm^{-1}) ν: 3054 (CH aromatic), 2851 (CH aliphatic), 1722 (CO), 1643 (C═N), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm):), 3.02 (s, 6H, $2CH_3$), 4.32 (s, 2H, CH_2), 6.81 (d, 2H, Ar-H, J = 8 Hz), 7.51-8.22 (m, 9H, Ar-H, J = 8 Hz), 8.74 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-*d6*): 42.3 (2), 49.3, 109.0, 112.6 (2), 126.2 (2), 126.9, 127.3 (2), 127.6,

128.3 (2), 129.4(2), 130.4, 130.9, 132.6, 133.8, 157.8, 164.6, 165.9, 190.5; MS (m/z %): 433.19 (M^{-1}, 1), 3.88 (6.4), 311 (100); Anal. Calcd for; $C_{24}H_{20}ClN_3OS$ (434.0): C, 66.43; H, 4.65; N, 9.68; Found: C, 66.23; H, 4.59; N, 9.56%.

Synthesis of methyl 4-(2-((4-(4-[dimethylamino] phenyl)-6-(naphthalen-2-yl) pyrimidin-2-yl)thio)acetamido)benzoate (9).

A mixture of compound **4** (2.77 g, 0.01 mol), methyl 4-(2-chloroacetamido) benzoate (2.27 g, 0.01 mol) and anhydrous potassium carbonate (5.52 g, 0.004 mol) in (10 mL) DMF was refluxed for 24 hours. The obtained red solid was filtered off, dried, and recrystallized from ethanol to give compound **9**.

Yield 62%, orange crystals; mp.173-175°C (EtOH); IR (cm^{-1}) ν: 3420 (NH), 3054 (CH aromatic), 2851 (CH aliphatic), 1714 (CO ester), 1666 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.01 (s, 6H, $2CH_3$), 3.79 (s, 3H of $COO\underline{CH_3}$), 4.22 (s, 2H, CH_2), 6.74 (d, 2H, Ar-H), 7.09 (t, 1H, Ar-H), 7.17 (d, 2H, Ar-H), 7.45-8.37 (m, 9H, Ar-H), 8.87 (s, 2H, Ar-H), 10.81 (s, 1H, NH); MS (m/z) (%): 548.2 (M^+, 1), 325 (74) and 105 (100); Anal. Calcd for: $C_{32}H_{28}N_4O_3S$ (548.3): C, 70.05; H, 5.14; N, 10.21; Found: C, 69.87; H, 5.21; N, 10.15%.

General method for the synthesis of compounds 10-13.

A mixture of **9** (4.68 g, 0.01 mol) and different amines namely 2-amino thiazole (0.1 g, 0.01 mol), 4- amin ouracile (1.27 g, 0.01 mol), 4-aminoantipyrine (2.03 g, 0.01 mol), and 2-aminopyridine (0.94 g, 0.01 mol) in DMF (20 mL) was refluxed for 12-15 hours. The solids that obtained were filtered off, dried, and then recrystallized from the suitable solvent to give compounds **10-13**.

4-(2-((4-(4-[Dimethylamino] phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl)thio)acetamido)-N-(thiazol-2-yl) benzamide (10).

Yield 70%; redish brown crystals; mp.140-142°C. (EtOH); IR (cm^{-1}) ν: 3445 (NH), 3083 (CH aromatic), 2851 (CH aliphatic), 1632 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.02 (s, 6H, $2CH_3$), 3.82 (s, 2H, SCH_2), 6.79-8.26 (m, 16H, Ar-H + 2H thiazole); 10.0 (s, 1H, NH, exchangeable D2O), 10.76 (s, 1H, NH, D_2O exchangeable); MS (m/z) (%): 311 (14.7), 265 (44) and 238 (100); Anal. Calcd for: $C_{34}H_{28}N_6O_2S_2$ (616.76): C, 66.21; H, 4.58; N, 13.63; Found: C, 66.01; H, 4.50; N, 13.56%.

4-(2-((4-(4-[Dimethylamino]phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl)thio)acetamido)-N-(2 ,4-dioxo-1,2,3, 4-tetrahydropyrimidin-5-yl)benzamide (11).

Yield 70%; brown crystals; mp. 216-218°C. (DMF/EtOH)); IR (cm^{-1}) ν: 3173 (NH), 3389 (NH), 3173 (CH aromatic), 2850 (CH aliphatic), 1740 (CO), 1709 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 3.02 (s, 6H, $2CH_3$), 4.39 (s, 2H, SCH_2), 6.15 (s, 1H, NH, D_2O exchangeable), 7.59-8.93 (m, 17H, Ar-H); 10.2 (s, 1H, NH, D_2O exchangeable), 10.05 (s, 2H, 2NH, D_2O exchangeable); MS (m/z) (%): 643.2 (M^+, 2), 339 (100) and 57 (67.8); Anal. Calcd for: $C_{35}H_{29}N_7O_4S$ (643.72): C, 65.31; H, 4.54; N, 15.23; Found: C, 65.12; H, 4.42; N, 15.13%.

N-(1 ,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-(2-((4-(4-(dimethylamino)phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl)thio)acetamido)benzamide (12).

Yield 70%; brown crystals; mp.109-112°C (EtOH); IR (cm^{-1}) ν: 3439 (NH), 3055 (CH aromatic), 2850 (CH aliphatic), 1652 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 2.71, 2.86 (2 seconds, 6H, $2CH_3$) 3.02 (s, 6H, $2CH_3$), 4.21 (s, 2H, SCH_2), 6.81(s, 1H, CH), 7.95-8.9 (m, 21H, Ar-H); 10.05 (s, 1H, NH exchangeable), 10.75 (s, 1H, NH exchangeable); Anal. Calcd for: $C_{43}H_{38}N_6O_3S$ (718.88): C, 71.84; H, 5.33; N, 11.69; Found: C, 71.66; H, 5.20; N, 11.58%.

4-(2-((4-(4-[Dimethylamino] phenyl)-6-(naphthalen-2-yl)pyrimidin-2-yl)thio)acetamido)-N-(pyridin-4-yl) benzamide (13).

Yield 61%; red crystals; mp. 110-113 C (EtOH); IR (cm^{-1}) ν: 3446 (NH), 2919 (CH aromatic), 2849 (CH aliphatic),1635 (CO); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 3.02 (s, 6H, $2CH_3$), 3.82 (s, 2H, SCH_2), 6.81-8.92 (m, 20H, Ar-H); 10.0 (s, 1H, NH), 10.05 (s, 1H, NH); MS (m/z) (%): 610.2 (M^+, 7), 339 (66.4), 340 (100) and 148 (46.3); Anal. Calcd for: $C_{36}H_{30}N_6O_2S$ (610.2): C, 70.80; H, 4.95; N, 13.76; Found: C, 70.65; H, 5.04; N, 13.69%.

Synthesis of 2-(1-(4-(dimethylamino)phenyl)-3-(naphthalen-2-yl)-3-oxopropyl)cyclohexan-1-one (14).

A mixture of **3** (3.01 g, 0.01 mol) and cyclohexanone (0.98 mL, 0.01 mol) was refluxed in ethanol (25 mL) for 24 hours. The reaction mixture was cooled, acidified with dilute hydrochloric acid and the solid obtained was filtered off, dried and then recrystallized form ethanol to give compound **17**.

Yield 75%; red crystals; mp.116-118°C (MeOH); IR (cm^{-1}) ν: 3054 (CH aromatic), 2855 (CH aliphatic), 1701, 1676 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 1.50-2.5 (m, 9H of cyclohexane ring), 3.02 (s, 6H, $2CH_3$), 3.41 (m, 1H, CH), 3.51 (m, 2H, CH_2), 6.67 (d. d, 2H, Ar-H); 7.10 (d, d, 2H, Ar-H), 7.23-8.76 (m, 7H, Ar-H); ^{13}C NMR (400 MHz, DMSO-*d6*): 24.6, 26.4, 27.4, 43.1 (2), 45.9, 46.3, 112.7 (2), 125.7, 123.9, 125.3, 126.2 (2), 127.3 (2), 128.1 (2), 128.3, 128.5, 129.2, 129.7, 130.7, 132.5, 135.8, 150.7, 211.5; MS (*m/z*) (%): 250 (10.7), 228 (100), 215 (87), 200 (96.8); Anal. Calcd for: $C_{27}H_{29}NO_2$ (399.2): C, 81.17; H, 7.32; N, 3.51; Found: C, 80.88; H, 7.23; N, 3.43%.

Synthesis of 2-((1-(4-(dimethylamino) phenyl)-3-(naphthalen-2-yl)-3-oxopropyl)thio) acetic acid (15).

A mixture of **3** (3.01 g, 0.01 mol) and thioglycolic acid (0.92 g, 0.01 mol) was refluxed in pyridine (25 mL) for 15 hours. The reaction mixture was cooled, acidified with dilute hydrochloric acid and the solid obtained was filtered off, dried and then recrystallized form ethanol to give **15**.

Yield 75%; brown crystals; mp. 88-90°C (MeOH); IR (cm^{-1})ν: 3439 (OH), 3053, 3014 (CH aromatic), 2850 (CH aliphatic), 1648, 1669 (CO), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 2.99 (s, 6H, $2CH_3$), 3.56 (m, 2H, CH_2 +1H, CH), 5.19 (s, 2H, CH_2COOH), 6.65-8.84 (m, 11 H, Ar-H), 9.62 (s, 1H, OH exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-*d6*): 40.3 (2), 38.3, 51.1, 110.0, 112.7 (2), 118.1 (3), 125.7, 126.2 (2), 127.3 (2), 127.6, 128.1 (2), 128.4 (2), 129.0, 129.7, 130.1 (2), 132.5, 133.8, 142.8, 164.1, 164.6, 165.9, 168.2, 172.3; MS (m/z) (%): 393.1 (2.1), 369 (100), 340 (54.2) and 57 (100); Anal. Calcd for: $C_{23}H_{23}NO_3S$ (393.1): C, 70.20; H, 5.89; N, 3.56; Found: C, 70.00; H, 5.96; N, 3.48%.

Synthesis of 3′-(2-naphthoyl)-4′-(4-(dimethylamino) phenyl)spiro[indoline-3 ,2′-pyrrolidin]-2-one (16).

A mixture of **3** (3.01 g, 0.01 mol), glycine (1 g, 0.013 mol) and isatine (1.47 g, 0.01 mol), was refluxed in ethanol (25 mL) for 24 hours. The reaction mixture was cooled, acidified with dilute hydrochloric acid and the solid obtained was filtered off, dried and then recrystallized form ethanol to give compound (**16**).

Yield 65%; brown crystals; mp. 92-94°C (MeOH); IR (cm^{-1}) ν: 3407 (NH), 3192 (CH aromatic), 2852 (CH aliphatic), 1712, 1670 (CO), 1613 (C=C), ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 2.86 (m, 1H, CH), 2.99 (s, 6H, $2CH_3$), 3.55 (s, 1H, NH, D_2O exchangeable), 3.56 (d, 2H, CH_2), 3.91 (t, 1H, CH), 6.56-8.84 (m, 15H, Ar-H), 10.40 (s, 1H, NH); Anal. Calcd for: $C_{30}H_{27}N_3O_2$ (461.2): C, 78.07; H, 5.90; N, 9.10; Found: C, 77.82; H, 5.81; N, 9.01%.

6.2 | Cytotoxicity assay

Cell line

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

Chemical reagents

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

6.3 | MTT assay

The different cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay.[69–71] This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0 × 104 cells/well[66] at 37°C for 48 hours under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 hours. After 24 hours of drug treatment, 20 μL of MTT solution at 5 mg/mL was added and incubated for 4 hours. Dimethyl sulfoxide (DMSO) in volume of 100 μL is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT). The relative cell viability in percentage was calculated as (A570 of treated samples/A_{570} of untreated sample) × 100.

6.4 | Molecular docking

Molecular docking studies were carried out using Molecular Operating Environment (MOE, version 2015) of Chemical Computing Group (https://www.chemcomp.com/ MOE-Molecular_Operating_Environment.htm). Docking simulations were performed on the crystal structure of dihydrofolate reductase (DHFR) complexed with **N ∼ 6 ∼ −methyl-N ∼ 6 ∼ −(naphthalen-1-yl)pyrido [2,3-days]pyrimidine-2,4,6-triamine**, extracted from Protein Data Bank (http://www.rcsb.org/, PDB ID: 4QJZ). During the docking process, water molecules were removed while the co-factor NAD was kept. "Protonate 3D" tool of MOE was applied to pose the missing hydrogen atoms in order of the correct ionization states to be assigned to the protein structure. "Docking" module in MOE was run to perform the molecular docking. Docking procedure has been implemented with default settings. The top 30 poses as ranked by London dG were kept and minimized using MMFF94x within a rigid receptor. The GBVI/WSA dG (Generalized-Born Volume Integral/Weighted Surface area) scoring function was then applied to score the resulting poses and 5 best poses were recorded. "Ligand Interactions" MOE tool was further used to analyze the molecular docking results by a visualization of the protein-ligand interactions in the active site of the complex. The tool presents in a diagram

form an identification and visualization of the interactions between the ligand and the receptor interacting entities, solvent molecules, and ions in the active site of the protein. Among the main interactions included are hydrogen bonds, salt bridges, hydrophobic cation-π interactions; and solvent exposure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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