



Cytotoxic activity and density functional theory studies of some 1,3-diphenylpyrazolyltetrahydropyrimidine derivatives

Sayed K. Ramadan¹ · Karema N. M. Halim¹ · Sameh A. Rizk¹ · Maher A. El-Hashash¹

Received: 29 November 2019 / Accepted: 17 February 2020 / Published online: 21 February 2020
© Iranian Chemical Society 2020

Abstract

Proclivity of tetrahydropyrimidinethione derivative encompassing 1,3-diphenylpyrazole moiety **1** was studied toward some carbon electrophiles in an attempt to synthesize some heteroannulated compounds. Also, pyrimidine dimer and pyrimidinotriazepine derivative were obtained through reactions with 1,2-diaminoethane and 2-cyanoacetohydrazide, respectively. Oxidation of the starting pyrimidinethione **2** was mainly dependent on the oxidizing agent used. The newly synthesized compounds were characterized by their analytical and spectral data including IR, ¹H NMR, ¹³C NMR and mass spectral data. The synthesized compounds were examined as antitumor agents against four human different cancer cell lines including hepatocellular carcinoma (HePG2), breast adenocarcinoma (MCF7), prostate cancer (PC3) and colon cancer (HCT-116). The results obtained revealed that some of them exhibited satisfactory activities.

Keywords Cytotoxic · Pyrimidines · Thiazolopyrimidine · *S*-Alkylation · *N*-Alkylation

Introduction

Pyrimidines (component of nucleic acids) and pyrazoles constitute influential classes of natural and non-natural products due to their pharmacological properties such as antiviral, anticancer, antioxidant, antimicrobial, anti-inflammatory, antihypertension and insecticidal activities [1–19]. The natural products with these heterocyclic moieties have been studied as new leads for AIDS therapies [20] and in potent HIV gb-120-CD4 inhibitors [21]. Enthused by the aforesaid findings and in continuation of our research group [22–30], we aimed to develop and synthesize some new pyrazolyltetrahydropyrimidine derivatives in an attempt to enhance their antitumor potency. The DFT calculations were discussed.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13738-020-01880-8>) contains supplementary material, which is available to authorized users.

✉ Sayed K. Ramadan
sayed.karam2008@sci.asu.edu.eg

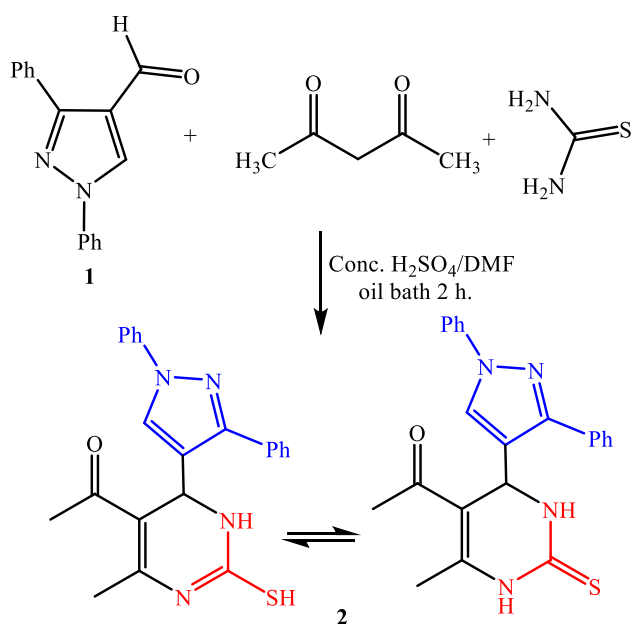
¹ Chemistry Department, Faculty of Science, Ain Shams University, Abassia, Cairo 11566, Egypt

Results and discussion

Chemistry

The key starting material in this work was pyrazolyltetrahydropyrimidinethione derivative **2** which was previously prepared by our research group via Biginelli cyclocondensation reaction of pyrazole aldehyde **1**, acetylacetone and thiourea in dimethylformamide containing few drops of concentrated sulfuric acid [30] (Scheme 1).

Thus, the proclivity of the former pyrimidine **2** toward some carbon electrophiles and nitrogen nucleophiles was studied *vide infra* (Schemes 2, 3). Thus, treatment of pyrimidine **2** with ethyl iodide in refluxing ethanol containing anhydrous sodium acetate afforded the corresponding *S*-alkylated product **3** in a good yield. The chemical structure of compound **3** was substantiated from its spectral data. The IR spectrum revealed the absence of both NH and C=S absorption bands. The ¹H NMR spectrum lacked to NH signals and displayed the triplet and quartet signal for ethyl group. The absence of singlet for the methine proton confirmed the dehydrogenation step. The mass spectrum supported the assigned structure as it provided the correct molecular ion peak at *m/z* 414 (17%) corresponding to the molecular formula C₂₄H₂₂N₄OS and some abundant peaks



Scheme 1 Synthesis of the target tetrahydropyrimidinethione **2**

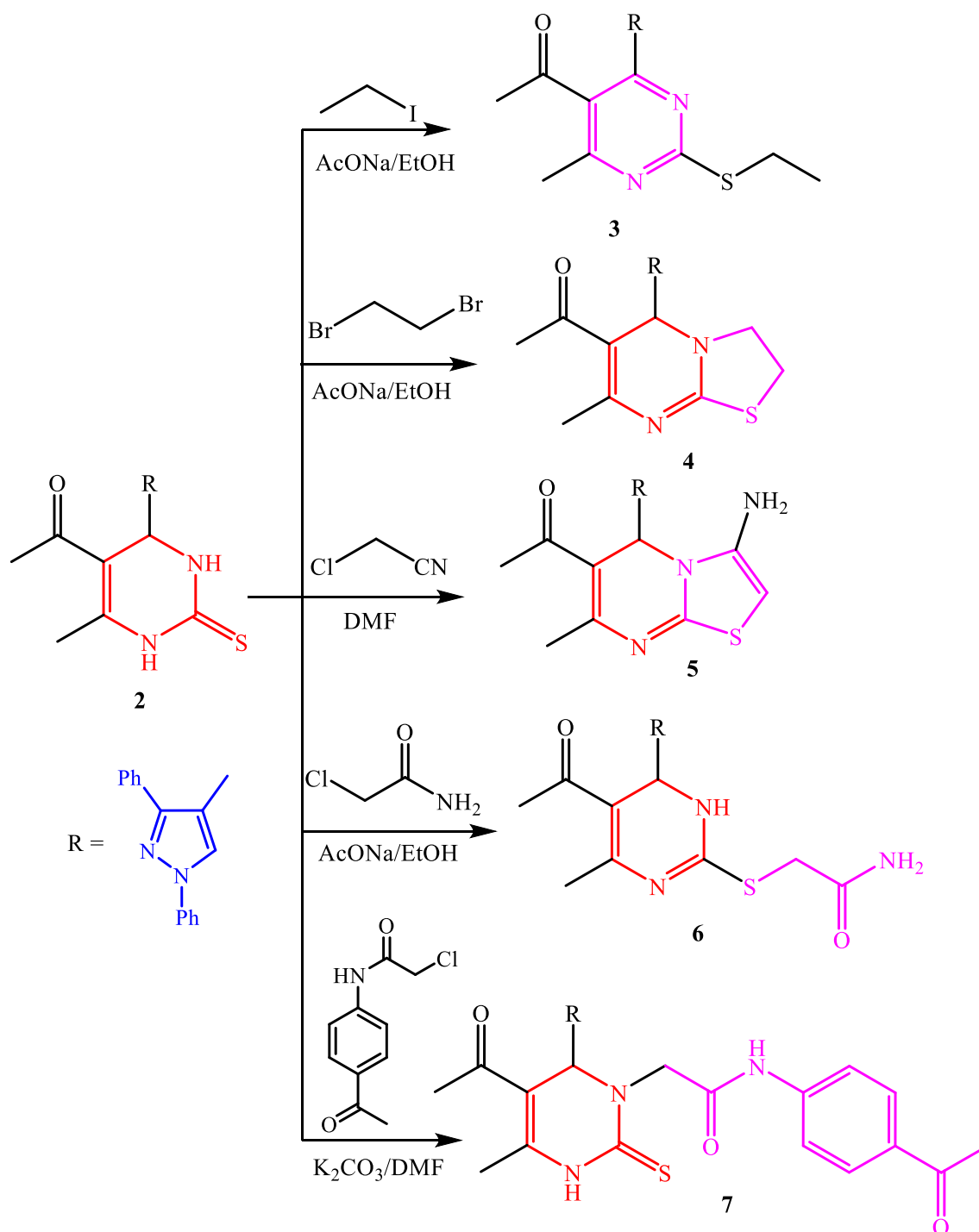
(cf. Experimental). Noteworthy, reaction of pyrimidine **2** with 1,2-dibromoethane, as an attempt to synthesize fused heterocycles, led to the construction of thiazolopyrimidine **4**. Its IR spectrum lacked to NH and C=S absorption bands. The ^1H NMR spectrum exhibited two triplets at the upfield region corresponding to $\text{CH}_2\text{-CH}_2$ group and devoid from NH signals. The mass spectrum showed the correct molecular ion peak at m/z 414 (19%) and the base peak at m/z 327 (100%) corresponding to loss of thiazole ring, as well as some of the important abundant peaks. A speculated mechanistic pathway for the formation of compound **4** could be explicated via elimination of two HBr molecules with consequent annulation of thiazole ring. In turn, refluxing pyrimidine **2** with chloroacetonitrile in *N,N*-dimethylformamide solution furnished aminothiazolopyrimidine **5** as brown crystals. The IR spectrum exhibited the absorption band for NH_2 group. The ^1H NMR spectrum showed exchangeable singlet for NH_2 group as well as singlet for methine proton. Further, the mass spectrum confirmed the proposed chemical structure. Formation of compound **5** could be interpreted via elimination of HCl molecule by S_N^2 mechanism followed by cyclization. Treating 2-chloroacetamide with pyrimidine **2** in the presence of anhydrous sodium acetate acquired the corresponding *S*-alkylated derivative **6**. The IR spectrum displayed the absorption bands for amide C=O and NH_2 groups. The ^1H NMR and mass spectra were in complete agreement with the assigned chemical structure. It was fortunate that stirring pyrimidine **2** with *N*-(4-acetylphenyl)-2-chloroacetamide in DMF solution containing anhydrous potassium carbonate at room temperature afforded the corresponding *N*-alkylated product **7** as brown crystals. The IR

spectrum retained the absorption band of C=S and showed the appearance of amide C=O group. The ^1H NMR spectrum provided two exchangeable singlets in the downfield region for NHCS and NHCO groups in addition to two singlets in the upfield region for CH_2CO and CH_3CO groups as well as retaining the signals for the two methyl groups. Furthermore, the mass spectrum displayed the correct molecular ion peak at m/z 563 (21%) as well as some important abundant peaks. All the foregoing reactions are illustrated in Scheme 2.

On the other hand, behavior of the starting pyrimidine **2** toward some carbonyl reagents for example acetic anhydride, oxalyl chloride and formaldehyde was investigated (Scheme 3). Thus, acetylation of pyrimidine **2** with acetic anhydride prompted the corresponding *N*-acetyl derivative **8** as gray crystals. The IR spectrum showed a new absorption band for amide carbonyl group. The ^1H NMR spectrum retained one exchangeable singlet signal for NH group and displayed a new singlet signal in the upfield region for methyl protons of the acetyl group. A chemical evidence for the structure of compound **8** was forthcoming from its reaction with ethyl chloroacetate under basic conditions to produce the corresponding ethyl ester derivative **9** as yellow crystals. The ^1H NMR spectrum revealed the existence of new triplet and quartet signals for ethyl ester group as well as a singlet signal for the methylene protons. The mass spectra confirmed all structures by showing their molecular ion peaks. A successful heterocyclic transformation of pyrimidine **2** into dioxothiazolopyrimidine **10** has been accomplished by treating with oxalyl chloride under basic conditions (Scheme 3). The IR spectrum displayed three singlets for three carbonyl groups. The lack of NH absorption bands in its IR spectrum and singlets for NH in its ^1H NMR spectrum evidenced the proposed structure.

Furthermore, reaction of pyrimidine **2** with formaldehyde in methanol containing few drops of piperidine as a base afforded the *S*-hydroxymethyl derivative **11** as pale-brown crystals (cf. Scheme 3). The IR spectrum displayed the absence of C=S absorption band and the appearance of the absorption bands for OH, NH, C=O groups. The ^1H NMR spectrum provided singlet for methylene protons and two exchangeable singlet signals for NH and OH groups. A further evidence for its structure was gained from its mass spectrum (cf. Experimental).

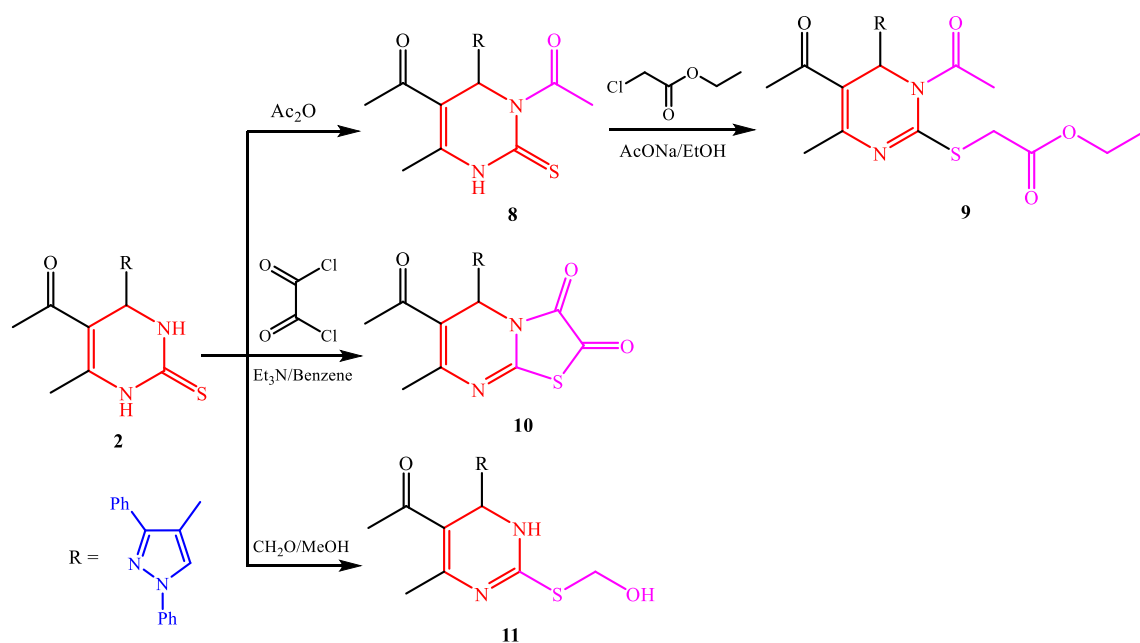
In view of the usefulness of 2-thio functionality in pyrimidine **2**, its treatment with 1,2-diaminoethane and 2-cyanoacetohydrazide in boiling ethanol or dioxane acquired the pyrimidine dimer **12** and pyrimidotriazepine **13**, respectively (Scheme 4). The spectral data were completely consistent with the assigned structures. The IR spectrum of compound **12** exhibited absorption bands for NH and C=O groups. Its ^1H NMR spectrum revealed the absence of singlet of methine proton and the presence of three singlet signals for protons of CH_2 , CH_3 and CH_3CO



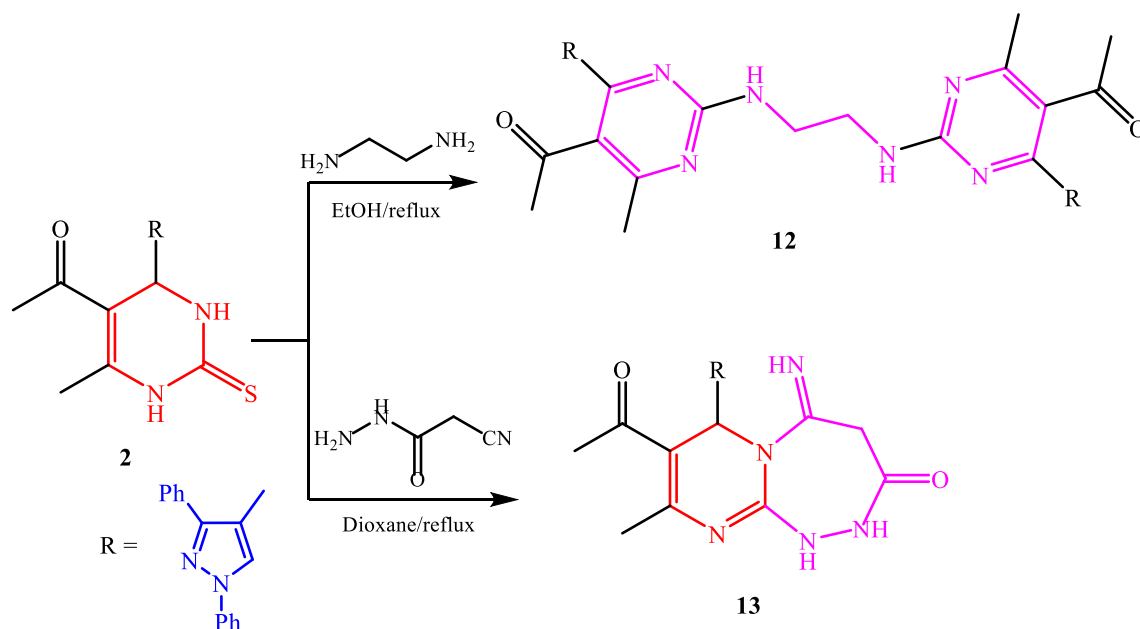
Scheme 2 Reactions of pyrimidine **2** with some halogenated reagents

groups, as well as one exchangeable singlet signal for NH proton. Furthermore, its mass spectrum provided the correct molecular ion peak besides some abundant peaks (cf. Experimental). Formation of compound **12** could be exploited via elimination of two H_2S molecules followed by dehydrogenation. The ^1H NMR spectrum of triazepine

13 displayed three exchangeable singlet signals for three NH protons as well as four singlets for methine (CH), methylene (CH_2), acetyl (CH_3CO) and methyl (CH_3). Its mass spectrum supported the proposed structure. The reaction pathway could be interpreted via elimination of H_2S molecule pursued by cyclization (Scheme 5).



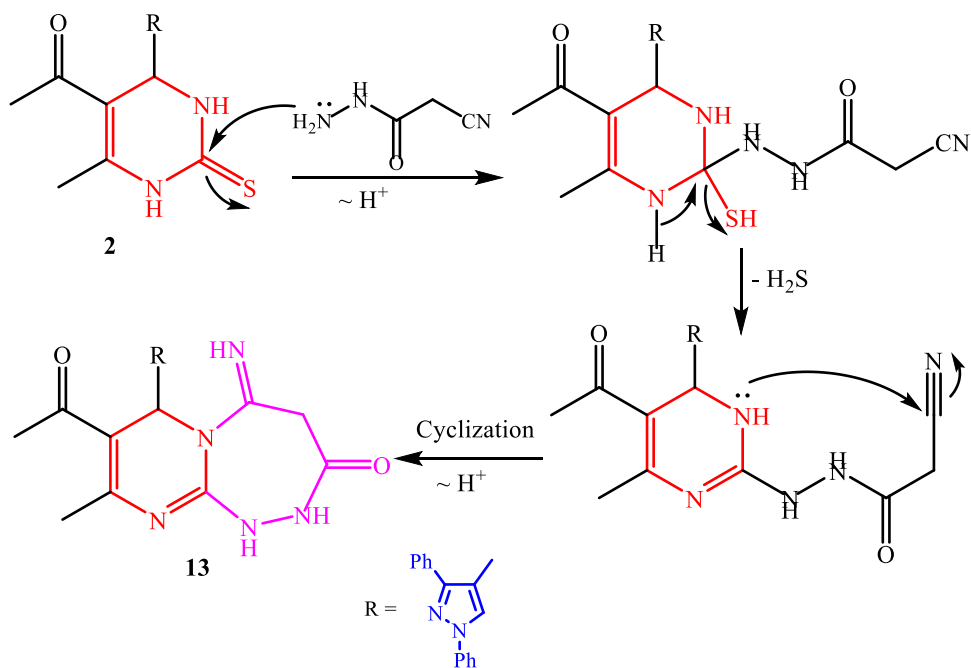
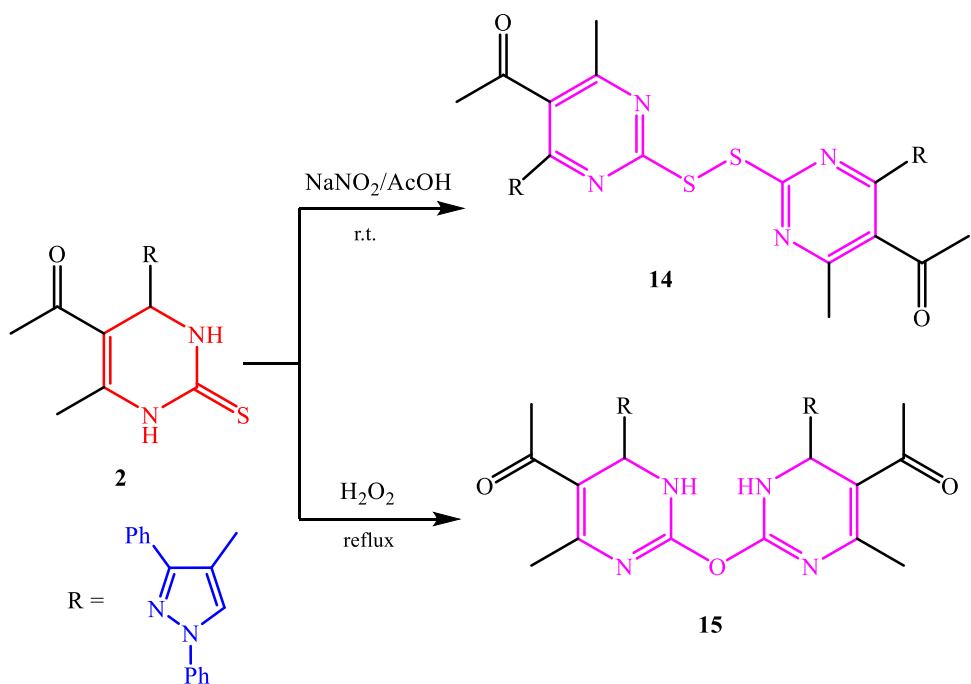
Scheme 3 Reactions of pyrimidine **2** with some carbonyl electrophiles



Scheme 4 Reaction of pyrimidine **2** with ethylenediamine and 2-cyanoacetohydrazide

The present investigation was extended to investigate the oxidation of the former pyrimidine **2** which was mainly dependent on the oxidizing agent used. Indeed, mild oxidizing agent such as sodium nitrite in the presence of acetic acid converted the pyrimidinethione derivative **2** to dithiopyrimidine derivatives **14** as yellow crystals. In

turn, hydrogen peroxide oxidized the pyrimidinethione **2** to pyrimidinyloxy pyrimidine derivative **15** as brown crystals (Scheme 6). The structures of these compounds were established on the basis of their spectral data (cf. Experimental). Presumably, the oxidation with hydrogen peroxide could be visualized via Scheme 7 [31].

Scheme 5 Plausible pathway for the formation of compound 13**Scheme 6** Oxidation of pyrimidine 2

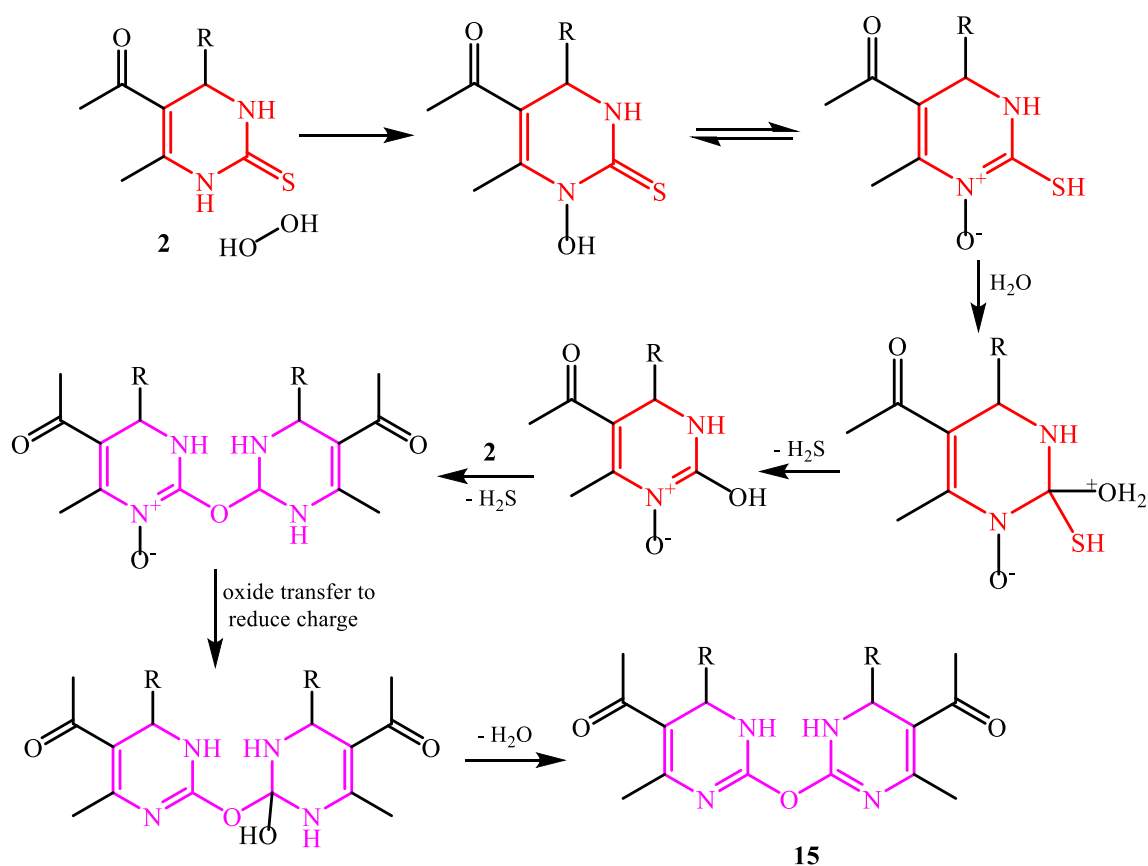
Pharmacology

In vitro antiproliferative activity

Cytotoxic effect on human cell lines (HepG2, MCF7, PC3 and HCT-116)

Fourteen compounds were tested against four human carcinoma cell lines, namely hepatocellular carcinoma

(HepG2), breast adenocarcinoma (MCF7), prostate cancer (PC3) and colon cancer (HCT-116) cell lines using doxorubicin as a standard anticancer drug. In vitro cytotoxic activity was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT assay) [32–35]. The anticancer activity was expressed as IC_{50} values. The results are displayed in Table 1 and Fig. 1 and revealed that the tested compounds exhibited variable degrees of inhibitory activity toward the four tested human tumor cells. It



Scheme 7 Suggested pathway for oxidation of pyrimidinethione **2** with hydrogen peroxide

Table 1 Cytotoxic activity of some compounds against human tumor cells

Compound	In vitro cytotoxicity IC ₅₀ (μM) ^a			
	HePG2	MCF7	PC3	HCT-116
Doxorubicin	4.50 ± 0.2	4.17 ± 0.2	8.87 ± 0.6	5.23 ± 0.3
2	7.19 ± 0.5	5.63 ± 0.3	12.46 ± 1.0	7.32 ± 0.5
3	14.08 ± 1.2	21.12 ± 1.6	27.52 ± 1.9	9.74 ± 0.8
4	91.21 ± 4.9	83.48 ± 4.7	> 100	76.35 ± 4.2
5	64.35 ± 3.8	75.15 ± 4.1	90.45 ± 5.1	61.06 ± 3.3
6	41.93 ± 3.1	63.02 ± 3.7	51.11 ± 3.0	38.21 ± 2.5
7	24.27 ± 1.9	29.56 ± 1.8	45.29 ± 2.6	17.87 ± 1.4
8	53.81 ± 3.3	58.41 ± 3.5	65.38 ± 3.4	44.19 ± 2.8
9	30.14 ± 2.3	48.43 ± 3.4	70.93 ± 3.8	28.67 ± 1.9
10	72.07 ± 4.0	79.29 ± 4.3	86.07 ± 4.5	68.08 ± 3.7
11	18.20 ± 1.4	15.29 ± 1.3	31.84 ± 2.2	13.28 ± 1.2
12	36.64 ± 2.7	42.30 ± 2.9	32.12 ± 2.3	29.83 ± 2.1
13	6.48 ± 0.4	3.87 ± 0.2	8.93 ± 0.7	4.39 ± 0.3
14	9.52 ± 0.8	10.14 ± 0.9	16.18 ± 1.4	8.16 ± 0.7
15	59.17 ± 3.5	67.04 ± 3.9	78.27 ± 4.1	52.14 ± 3.0

^aIC₅₀ (μM): 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic)

was noted that the most potent cytotoxic activities against the four tumors were displayed by three compounds **2**, **14** and **15**. Compound **3** showed strong potency against HePG2 and HCT-116 cell lines, while it exhibited moderate activity against MCF7 and PC3 tumors. On the other hand, compound **11** showed strong activity against three tumors HePG2, MCF7 and HCT-116 while it was moderate in cytotoxic activity against prostate tumor (PC3). The rest of compounds displayed weak cytotoxic activities against the four tumor cell lines.

Structure activity relationships (SAR)

DNA is made up of chemical building blocks called nucleotides. The four types of nitrogen bases found in nucleotides are as follows: adenine (A), thymine (T), guanine (G) and cytosine (C). The base adenine always pairs with thymine, while guanine always pairs with cytosine through hydrogen bond. The cytotoxic activity of the tested compounds toward different cell lines depends on two factors: (i) the formation of intermolecular hydrogen bond with DNA bases and (ii) the positive charge on the tested compounds attracted to the negative

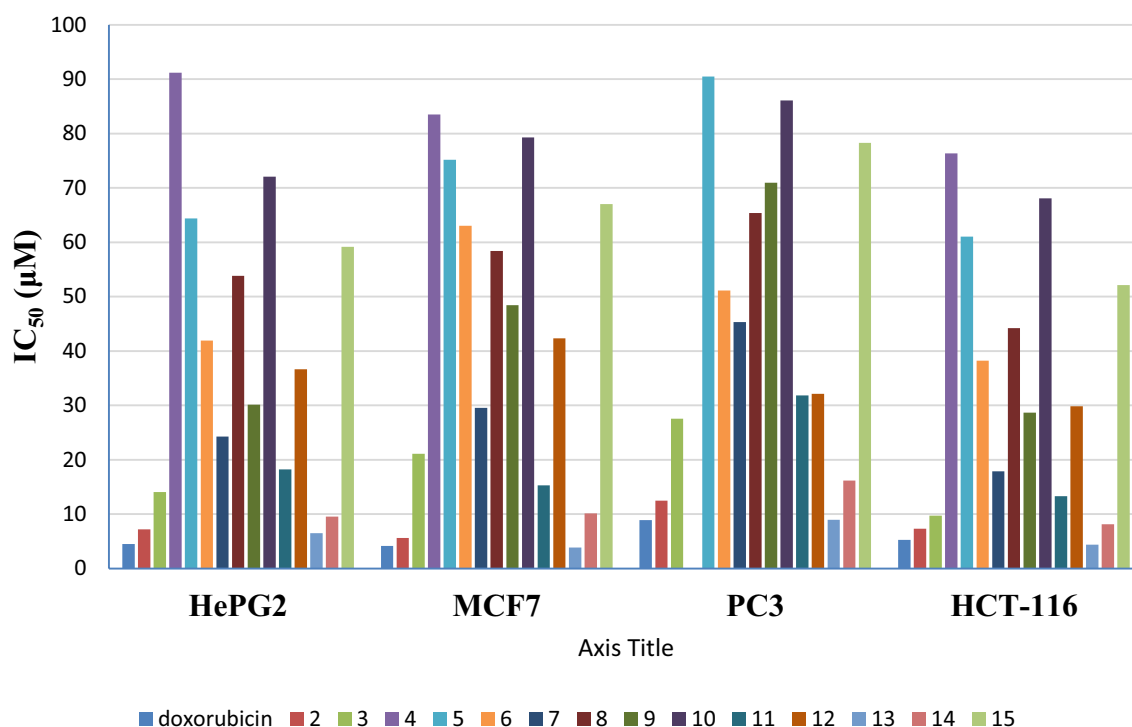


Fig. 1 Cytotoxic activity of some compounds against human tumor cells

charge on the cell wall. By comparing the experimental cytotoxic activity of the synthesized compounds in this study to their structures, the following structure–activity relationship was postulated (Fig. 2). 2'-C-Cyano-2'-deoxy-1-β-D-arabinopentofuranosylcytosine (CNDAC) [36] is a nucleoside analog with a mechanism of action that is being evaluated in clinical trials. Incorporation of CNDAC triphosphate into DNA and extension during replication lead to single-strand breaks directly caused by β-elimination. These breaks, or the lesions that arise from further processing, cause cells to arrest in G2 (Scheme 8). The proposed mechanism of DNA interaction with compound **13** as compared with CNDAC is displayed in Scheme 9. More than one active sites in the formimide and hydrazide functional groups of the triazepinopyrimidine derivative **13** increase its flexibility and enhance the anticancer activity in the four carcinoma cell lines (see more in DFT section that outlined in Table 1; Fig. 3). Herein, the tautomerism plays an important role in the anticancer reagents, which increases the functionality and flexibility. So, the anticancer activity as in compounds **2** and **14** is including the functionality thioamide-thioimidine and disulfane, respectively, that improve the blocked cell cycle in G2 phase. No tautomeric structure in the compounds **3** and **11** decreases the flexibility (softness) of compounds as anticancer activity.

Computational studies (DFT methods)

Density functional theory (DFT) studies were carried out for pyrimidine and thiazolopyrimidine compounds using Materials Studio 6.0 (MS 6.0) software from Accelrys, Inc. DMol3 module was used to perform the DFT calculations utilizing Perdew and Wang LDA exchange–correlation functional and DND basis set. The calculated parameters included the electron density, dipole moment and Frontier molecular orbitals and the molecular surface area. Frontier molecular orbitals include the highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMOs) [37, 38].

The molecular structures of the synthesized compounds are superior to be formed to a stable geometry which is known as optimization. Such the energy of the structure is transported to a stationary point, the geometry of the compounds as an example, compounds of pyrimidine is gradually optimized, and its energy is continuously decreased until the fluctuations in the molecule energy are minimized. Frontier molecular orbitals possess the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) (Fig. 3, see all compounds in the supplemental file). The regions of highest electron density (HOMO) characterize the electrophilic-attacking sites, while the LUMO imitates the nucleophilic-attacked sites [39, 40]. The quantum chemical computation

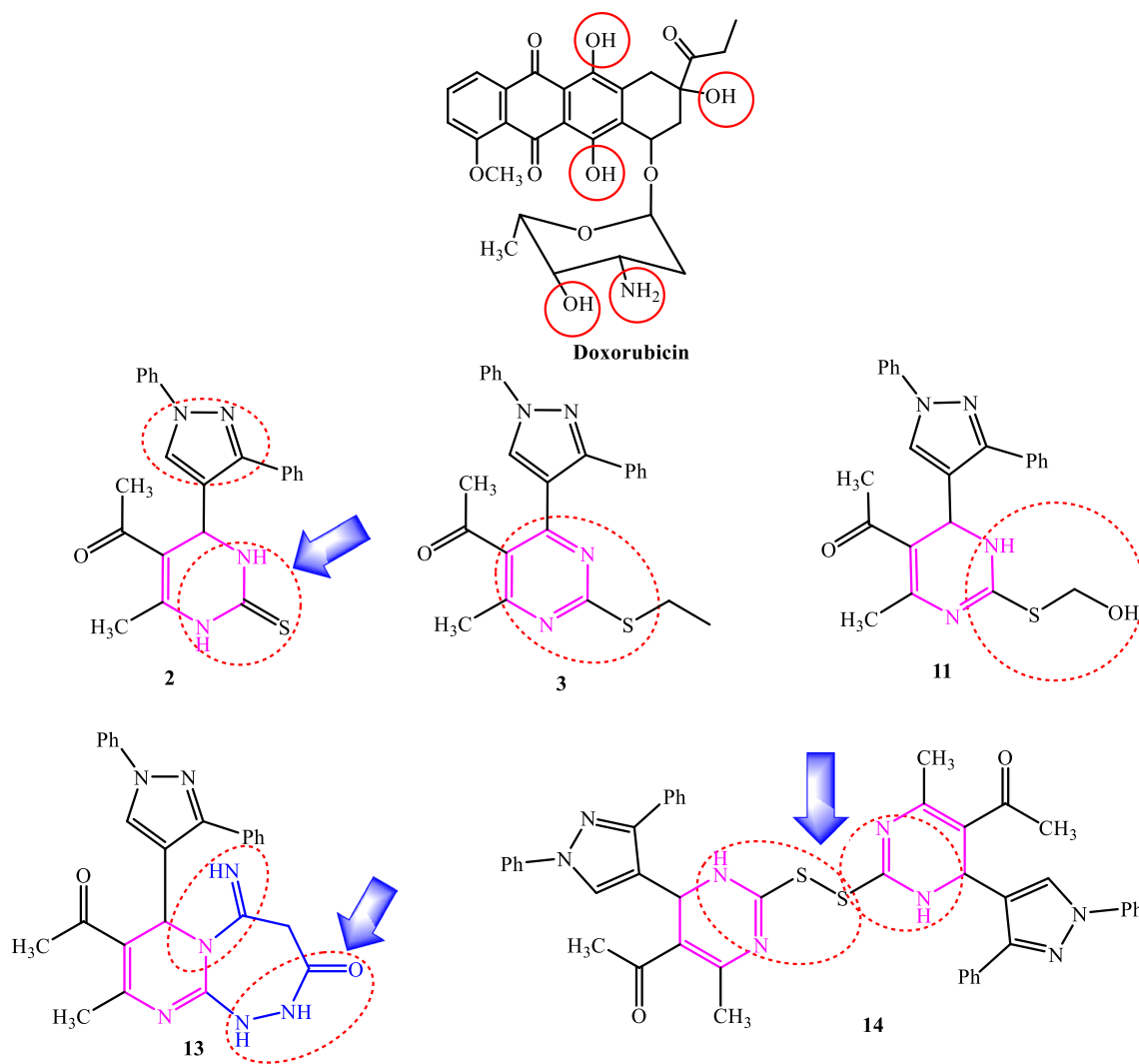


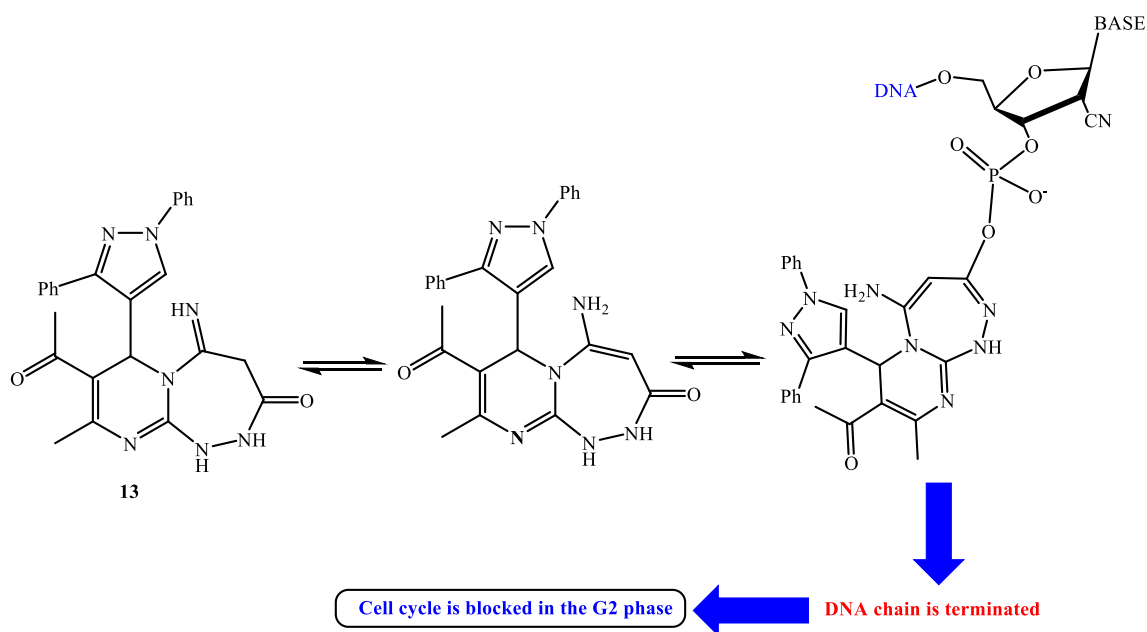
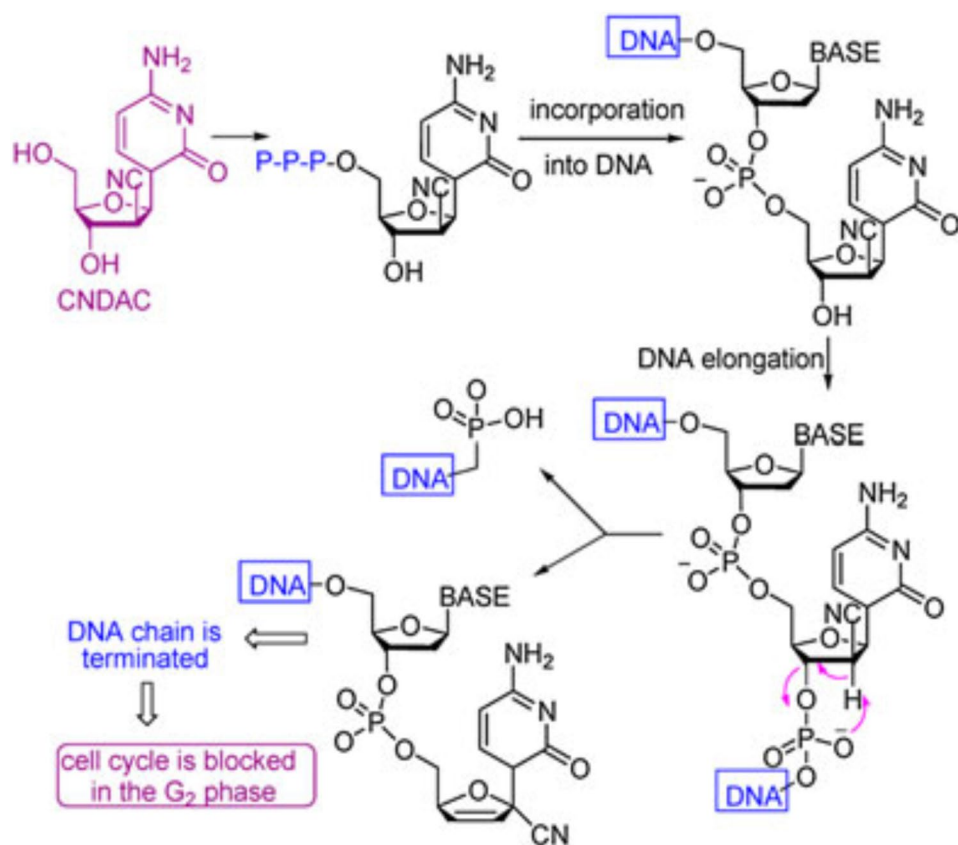
Fig. 2 Structure activity relationships of the potent compounds

could explain this reaction at the different conditions achieving different products **3–10**.

Functionality and their electron densities of the compounds **2**, **13** and **14** can be confirmed the relationship between their activities. Softness of these compounds can be determined from this equation $\sigma = 2/E_{LU} - E_{HO}$. Table 2 outlines that the softness of the compound **13** is larger than **2** than **14** that are good agreement with the experimental activity. Nucleophilicity index, dipole moment and surface area of the active compounds are not involved in the cytotoxic activity. Although compound **14** has higher surface area than compounds **2** and **13**, it has the lower activity.

Conclusion

Tetrahydropyrimidinethione bearing a pyrazole moiety was synthesized and utilized for design and synthesis of some fused heterocycles through treating with some carbon electrophilic reagents. Alkylation and oxidation of the target pyrimidinethione were studied. Also, pyrimidine dimer and pyrimidinotriazepine derivative were obtained through reactions with 1,2-diaminoethane and 2-cyanoaceto-hydrazide, respectively. The anticancer activity evaluation of the obtained products against four human tumors revealed that some compounds were interesting antitumor agents.

Scheme 8 Mechanism of the antitumor action of CNDAC**Scheme 9** Inhibition of the cancer cell lines

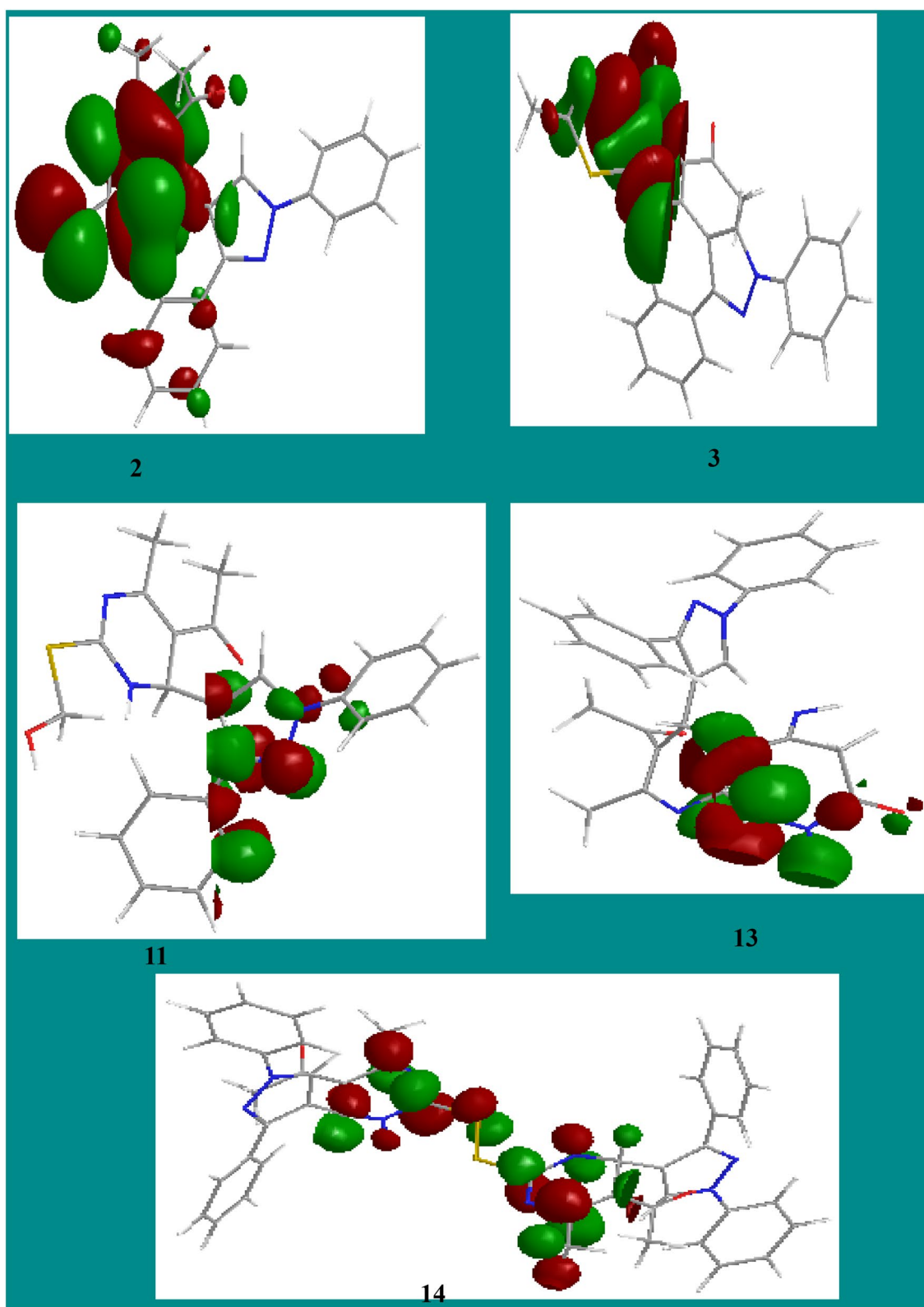


Fig. 3 Optimized structures (left), highest occupied molecular orbital (middle) and lowest unoccupied molecular orbital (right) for the most potent antitumor compounds. Color index: white H, gray C, blue N,

red O and yellow S. (the rest of compounds can be found in the supplementary file)

Table 2 Parameters of the quantum chemical computation for the synthesized pyrimidines

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	I (eV)	A (eV)	χ (eV)	η (eV)	σ eV^{-1}	ΔN	μ (Debye)	A_{molec} (nm^2)
2	-2.572	1.524	3.48	2.49	1.48	2.74	1.05	1.9048	0.26	2.190	297.443
3	-4.130	-0.525	3.60	4.28	0.60	2.36	1.93	1.0360	0.34	4.558	542.608
11	-5.237	-1.207	4.03	5.16	1.12	3.18	2.04	0.9801	0.38	2.139	590.401
13	-3.475	-2.724	0.751	3.535	2.569	3.09	0.45	4.3281	0.31	3.395	519.866
14	-3.644	0.504	3.41	3.432	0.723	2.16	1.71	1.1690	0.27	1.065	872.768

I Ionization potential, A electron affinity, χ electronegativity, η hardness, σ softness, ΔN nucleophilicity index, μ dipole moment, A_{molec} surface area

Experimental

General

All melting points were measured on a GALLENKAMP electric melting point apparatus (USA) and are uncorrected. The IR spectra (ν , cm^{-1}) were recorded using potassium bromide disks on Fourier Transform Infrared Thermo Electron Nicolet iS10 Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at Chemistry Department, Faculty of Science, Ain Shams University. The ^1H NMR spectra (δ_{H} , ppm) were run at 400 MHz on BRUKER NMR Spectrometer (BRUKER, Manufacturing & Engineering Inc., Anaheim, CA, USA) using tetramethyl silane (TMS) as internal standard in deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) as a solvent at Faculty of Pharmacy, Ain Shams University. The ^{13}C NMR spectra (δ_{H} , ppm) were recorded using the same solvent and spectrometer at 100 MHz. The mass spectra (MS) were recorded on Shimadzu GC-MS-QP-1000 EX mass spectrometer (Shimadzu Scientific Instruments, Inc., USA) operating at 70 eV at the regional center for Mycology and Biotechnology (RCMB) of Al-Azhar University, Nasr City, Cairo, Egypt. Elemental analyses were carried out at the Microanalytical Laboratory, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer, and satisfactory analytical data (± 0.4) were obtained for all compounds. The reactions were monitored by thin layer chromatography (TLC) using Merck Kiesel gel 60 F₂₅₄ analytical sheets obtained from Fluka, Switzerland. The pharmacological activity assays were carried out at Pharmacology Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. All chemicals and solvents used were obtained from commercial sources and used as received or dried by standard procedures. The starting material, 1-(4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethan-1-one (**2**) was previously prepared [30].

1-(4-(1,3-Diphenyl-1H-pyrazol-4-yl)-2-(ethylthio)-6-methylpyrimidin-5-yl)ethan-1-one (**3**)

A solution of pyrimidinethione **2** (0.7 g, 1.8 mmol), ethyl iodide (0.14 mL, 1.8 mmol) and freshly prepared anhydrous sodium acetate (0.18 g, 1.8 mmol) in absolute ethanol (20 mL) was heated under reflux for 4 h. The precipitated solid after cooling was collected and recrystallized from ethanol to furnish ethyl thiopyrimidine **3** as brown crystals, mp. 208–210 °C, yield 67%. IR (KBr, ν , cm^{-1}): 3053 (CH-aromatic), 2958, 2922, 2852 (CH-aliphatic), 1678 (C=O), 1628 (C=N). ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ , ppm): 7.99 (s, 1H, C₅-H pyrazole), 7.97–7.07 (m, 10H, Ar-H), 3.01–2.97 (q, 2H, CH_2CH_3 , $J=5.2$ Hz), 2.23 (s, 3H, CH_3), 2.11 (s, 3H, CH_3CO), 1.36–1.33 (t, 3H, CH_2CH_3 , $J=5.2$ Hz). EIMS (70 eV, m/z , %): 414.08 (M^+ , 17), 401.15 (18), 380.85 (32), 369.02 (32), 342.79 (40), 333.98 (54), 318.07 (25), 300.92 (36), 282.86 (46), 260.99 (50), 248.21 (59), 222.41 (32), 206.99 (100), 191.64 (14), 173.59 (29), 146.63 (19), 112.51 (26), 91.89 (25). Anal. Calcd. for $\text{C}_{24}\text{H}_{22}\text{N}_4\text{OS}$ (414.53): C, 69.54; H, 5.35; N, 13.52. Found: C, 69.30; H, 5.01; N, 13.49%.

1-(5-(1,3-Diphenyl-1H-pyrazol-4-yl)-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidin-6-yl)ethan-1-one (**4**)

A mixture of pyrimidinethione **2** (0.7 g, 1.8 mmol), 1,2-dibromoethane (0.15 mL, 1.8 mmol) and freshly prepared anhydrous sodium acetate (0.15 g, 1.8 mmol) in absolute ethanol (20 mL) was heated under reflux for 6 h. The precipitated solid after cooling was collected and recrystallized from ethanol to furnish thiazolopyrimidine **4** as brown crystals, mp. 222–224 °C, yield 71%. IR (KBr, ν , cm^{-1}): 3056 (CH-aromatic), 2922, 2860 (CH-aliphatic), 1679 (C=O), 1625 (C=N). ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ , ppm): 8.40 (s, 1H, C₅-H pyrazole), 8.01–7.21 (m, 10H, Ar-H), 5.36 (s, 1H, C₅-H thiazolopyrimidine), 3.91–3.72 (t, 2H, N- CH_2 , $J=6.8$ Hz), 3.11–2.92 (t, 2H, S- CH_2 ,

$J=6.9$ Hz), 2.25 (s, 3H, CH₃), 2.03 (s, 3H, CH₃CO). ¹³C NMR (100 MHz, DMSO, δ , ppm): 190.1, 181.3, 150.7, 148.9, 1.39.5, 132.9, 131.2, 129.3, 129.2, 128.6, 127.4, 126.1, 122.8, 118.9, 117.1, 65.5, 52.3, 27.1, 24.3, 21.1. EIMS (70 eV, m/z , %): 414.12 (M⁺, 19), 403.60 (27), 395.28 (58), 384.77 (55), 377.05 (39), 338.23 (27), 327.54 (100), 307.74 (35), 296.48 (76), 274.29 (59), 266.23 (54), 242.06 (28), 227.81 (36), 210.57 (76), 204.68 (36), 182.17 (57), 177.36 (48), 156.21 (57), 145.46 (66), 140.35 (32), 86.02 (32). Anal. Calcd. for C₂₄H₂₂N₄OS (414.53): C, 69.54; H, 5.35; N, 13.52. Found: C, 69.33; H, 5.08; N, 13.47%.

1-(3-Amino-5-(1,3-diphenyl-1H-pyrazol-4-yl)-7-methyl-5H-thiazolo[3,2-a]pyrimidin-6-yl)ethan-1-one (5)

A solution of pyrimidine **2** (0.7 g, 1.8 mmol) and chloroacetonitrile (0.19 mL, 1.8 mmol) in DMF (10 mL) was heated under reflux for 4 h. The reaction mixture was then cooled to room temperature and poured onto ice-cooled water. The precipitated solid was collected and recrystallized from ethanol to afford aminothiazolopyrimidine **5** as brown crystals, mp. 215–217 °C, yield 62%. IR (KBr, ν , cm⁻¹): 3320, 3310 (NH₂), 3057 (CH-aromatic), 2924, 2854 (CH-aliphatic), 1665 (C=O), 1628 (C=N). ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 8.55 (s, 1H, C₅-H pyrazole), 8.01–7.21 (m, 10H, Ar-H), 7.13 (s, 1H, C₂-H thiazolo), 5.02 (s, 1H, C₅-H thiazolopyrimidine), 4.22 (br.s, 2H, NH₂, exchangeable), 2.89 (s, 3H, CH₃), 2.74 (s, 3H, CH₃CO). EIMS (70 eV, m/z , %): 427.09 (M⁺, 24), 412.96 (9), 401.73 (21), 384.76 (15), 373.20 (100), 365.21 (17), 323.54 (16), 305.99 (9), 279.00 (17), 251.30 (24), 222.93 (16), 180.75 (20), 170.87 (70), 148.48 (11), 122.18 (21), 84.53 (18). Anal. Calcd. for C₂₄H₂₁N₅OS (427.53): C, 67.43; H, 4.95; N, 16.38. Found: C, 67.22; H, 4.71; N, 16.31%.

2-((5-Acetyl-6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-methyl-1,6-dihydropyrimidin-2-yl)thio)acetamide (6)

A solution of pyrimidine **2** (0.7 g, 1.8 mmol), 2-chloroacetamide (0.16 g, 1.8 mmol) and anhydrous sodium acetate (0.15 g, 1.8 mmol) in absolute ethanol (20 mL) was heated under reflux for 2 h. Upon cooling, the reaction mixture was poured onto ice-cold water. The precipitated solid was collected and recrystallized from ethanol to afford compound **6** as brown crystals, mp. 200–202 °C, yield 68%. IR (KBr, ν , cm⁻¹): 3320, 3250, 3115 (NH, NH₂), 3050 (CH-aromatic), 2958, 2921, 2853 (CH-aliphatic), 1680 (C=O), 1623 (C=N). ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 9.17 (br.s, 1H, NH, exchangeable), 8.73 (br.s, 2H, NH₂, exchangeable), 8.30 (s, 1H, C₅-H pyrazole), 8.02–7.15 (m, 10H, Ar-H), 5.47 (s, 1H, C₆-H pyrimidine), 3.57 (s, 2H, CH₂), 2.29 (s, 3H, CH₃), 2.02 (s, 3H, CH₃CO). EIMS (70 eV, m/z , %): 445.43

(M⁺, 49), 431.23 (60), 408.95 (32), 384.21 (34), 369.51 (99), 356.60 (73), 331.04 (51), 320.35 (82), 312.40 (100), 302.77 (41), 265.08 (52), 244.54 (47), 217.62 (33), 196.09 (49), 184.97 (58), 170.72 (52), 165.80 (43). Anal. Calcd. for C₂₄H₂₃N₅O₂S (445.54): C, 64.70; H, 5.20; N, 15.72. Found: C, 64.59; H, 4.97; N, 15.75%.

2-(5-Acetyl-6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-methyl-2-thioxo-3,6-dihydropyrimidin-1(2H)-yl)-N-(4-acetylphenyl)acetamide (7)

To a stirred solution of pyrimidine **2** (0.7 g, 1.8 mmol) and anhydrous potassium carbonate (0.25 g, 1.8 mmol) in DMF (10 mL), *N*-(4-acetylphenyl)-2-chloroacetamide (0.38 g, 1.8 mmol) was added and the reaction mixture was further stirred for 8 h. The reaction mixture was poured onto ice-cooled water. The precipitated solid was collected and recrystallized from ethanol to afford compound **7** as brown crystals, mp. 136–138 °C, yield 58%. IR (KBr, ν , cm⁻¹): 3220, 3113 (NH), 3052 (CH-aromatic), 2922, 2853 (CH-aliphatic), 1675 (C=O), 1626 (C=N), 1222 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 10.54 (br.s, 1H, NHCO, exchangeable), 9.08 (br.s, 1H, NHCS, exchangeable), 8.01 (s, 1H, C₅-H pyrazole), 7.99–7.06 (m, 14H, Ar-H), 5.66 (s, 1H, C₆-H pyrimidine), 4.16 (s, 2H, CH₂), 2.44 (s, 3H, CH₃CO), 2.37 (s, 3H, CH₃), 2.02 (s, 3H, CH₃CO pyrimidine). EIMS (70 eV, m/z , %): 563.21 (M⁺, 21), 526.12 (23), 512.39 (26), 491.32 (32), 453.94 (34), 437.43 (54), 414.33 (53), 387.94 (35), 372.61 (29), 327.41 (23), 299.85 (60), 271.21 (38), 203.29 (28), 158.24 (53), 120.56 (34), 102.89 (32), 59.06 (100). Anal. Calcd. for C₃₂H₂₉N₅O₃S (563.68): C, 68.19; H, 5.19; N, 12.42. Found: C, 67.99; H, 5.04; N, 12.29%.

1,1'-(6-(1,3-Diphenyl-1H-pyrazol-4-yl)-4-methyl-2-thioxo-3,6-dihydropyrimidine-1,5(2H)-diyl)bis(ethan-1-one) (8)

A solution of pyrimidine **2** (0.7 g, 1.8 mmol) in freshly distilled acetic anhydride (5 mL) was heated under reflux for 4 h. The reaction mixture was poured onto ice-cooled water. The precipitated solid was collected and recrystallized from ethanol to afford *N*-acetyl derivative **8** as gray crystals, mp. 144–146 °C, yield 73%. IR (KBr, ν , cm⁻¹): 3124 (NH), 3055 (CH-aromatic), 2923, 2864 (CH-aliphatic), 1703 (C=O ketone), 1673 (C=O amide), 1615 (C=N), 1225 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 11.96 (br.s, 1H, NH, exchangeable), 8.33 (s, 1H, C₅-H pyrazole), 8.02–7.35 (m, 10H, Ar-H), 6.80 (s, 1H, C₆-H pyrimidine), 2.39 (s, 3H, CH₃), 2.33 (s, 3H, COCH₃), 1.92 (s, 3H, N-COCH₃). EIMS (70 eV, m/z , %): 430.52 (M⁺, 13), 409.06 (16), 395.21 (32), 368.23 (40), 329.85 (49), 316.55 (47), 277.28 (39), 268.13 (100), 232.15 (21), 183.01 (23), 133.72 (37), 114.52

(50), 89.24 (56). Anal. Calcd. for $C_{24}H_{22}N_4O_2S$ (430.53): C, 66.96; H, 5.15; N, 13.01. Found: C, 66.81; H, 4.89; N, 12.97%.

Ethyl 2-((1,5-diacetyl-6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-methyl-1,6-dihydropyrimidin-2-yl)thio)acetate (9)

A mixture of the *N*-acetyl derivative **8** (0.7 g, 1.6 mmol), ethyl chloroacetate (0.23 mL, 1.6 mmol) and anhydrous sodium acetate (0.15 g, 1.8 mmol) in absolute ethanol (20 mL) was heated under reflux for 6 h. The separated solid after cooling was collected and recrystallized from ethanol to furnish compound **9** as yellow crystals, mp. 160–162 °C, yield 67%. IR (KBr, ν , cm^{-1}): 3053 (CH-aromatic), 2959, 2921, 2852 (CH-aliphatic), 1736 (C=O ester), 1708 (C=O ketone), 1674 (C=O amide). 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 8.57 (s, 1H, C₅-H pyrazole), 8.04–7.15 (m, 10H, Ar-H), 6.38 (s, 1H, C₆-H pyrimidine), 3.88–3.85 (q, 2H, CH₂CH₃, $J=7.1$ Hz), 3.65 (s, 2H, SCH₂), 2.37 (s, 3H, CH₃), 2.20 (s, 3H, CH₃CO), 2.15 (s, 3H, N-COCH₃), 1.05–0.91 (t, 3H, CH₂CH₃, $J=7.2$ Hz). EIMS (70 eV, m/z , %): 516.65 (M^+ , 5), 490.59 (4), 457.01 (5), 364.60 (28), 344.24 (46), 312.29 (15), 253.40 (13), 167.41 (9), 104.17 (81), 85.07 (100), 77.26 (63). Anal. Calcd. for $C_{28}H_{28}N_4O_4S$ (516.62): C, 65.10; H, 5.46; N, 10.85. Found: C, 64.92; H, 5.29; N, 10.73%.

6-Acetyl-5-(1,3-diphenyl-1H-pyrazol-4-yl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-2,3-dione (10)

A solution of pyrimidine **2** (0.7 g, 1.8 mmol) and oxalyl chloride (0.22 mL, 1.8 mmol) in dry benzene (20 mL) was heated under reflux for 6 h in the presence of triethylamine (0.5 mL). The obtained solid after cooling was collected and recrystallized from benzene to produce thiazolopyrimidine **10** as pale-brown crystals, mp. 144–146 °C, yield 69%. IR (KBr, ν , cm^{-1}): 3061 (CH-aromatic), 2972, 2936 (CH-aliphatic), 1741, 1682 (C=O), 1625 (C=N). 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 8.31 (s, 1H, C₅-H pyrazole), 8.02–7.30 (m, 10H, Ar-H), 5.49 (s, 1H, C₅-H thiazolopyrimidine), 2.38 (s, 3H, CH₃), 2.02 (s, 3H, CH₃CO). ^{13}C NMR (100 MHz, DMSO, δ , ppm): 195.3, 188.1, 174.2, 158.1, 151.8, 149.7, 139.5, 133.1, 131.8, 129.3, 129.1, 128.8, 127.2, 126.1, 123.0, 119.7, 117.0, 66.8, 27.1, 21.5. EIMS (70 eV, m/z , %): 442.53 (M^+ , 14), 427.46 (15), 397.21 (22), 380.34 (17), 334.33 (32), 269.44 (28), 253.10 (100), 225.54 (56), 206.06 (30), 136.00 (22), 102.16 (50), 90.83 (31). Anal. Calcd. for $C_{24}H_{18}N_4O_3S$ (442.49): C, 65.15; H, 4.10; N, 12.66. Found: C, 64.09; H, 3.91; N, 12.59%.

1-(6-(1,3-Diphenyl-1H-pyrazol-4-yl)-2-((hydroxymethyl)thio)-4-methyl-1,6-dihydropyrimidin-5-yl)ethan-1-one (11)

To a stirred solution of pyrimidine **2** (0.7 g, 1.8 mmol) and formaldehyde (0.05 mL, 1.8 mmol) in methanol (20 mL), piperidine (0.35 mL, 3.6 mmol) was added. The reaction mixture was further stirred for 8 h. The resulting precipitate was collected and recrystallized from ethanol to afford compound **11** as pale-brown crystals, mp. 190–192 °C, yield 42%. IR (KBr, ν , cm^{-1}): 3424 (OH), 3235 (NH), 3067 (CH-aromatic), 2993, 2923 (CH-aliphatic), 1655 (C=O), 1621 (C=N). 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 9.72 (br.s, 1H, NH, exchangeable), 8.34 (s, 1H, C₅-H pyrazole), 8.01–7.31 (m, 10H, Ar-H), 7.10 (br.s, 1H, OH, exchangeable), 5.48 (s, 1H, C₆-H pyrimidine), 2.38 (s, 2H, CH₂), 2.29 (s, 3H, CH₃), 2.02 (s, 3H, CH₃CO). EIMS (70 eV, m/z , %): 418.43 (M^+ , 21), 413.55 (35), 388.90 (72), 359.35 (100), 345.88 (99), 335.73 (71), 310.23 (72), 290.87 (55), 282.26 (46), 246.09 (47), 211.77 (50), 190.41 (85), 164.52 (55), 129.36 (43), 114.82 (37), 86.06 (50). Anal. Calcd. for $C_{23}H_{22}N_4O_2S$ (418.52): C, 66.01; H, 5.30; N, 13.39. Found: C, 65.87; H, 5.14; N, 13.29%.

1,1'-((Ethane-1,2-diylbis(azanediyl))bis(4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-methylpyrimidine-2,5-diyl))bis(ethan-1-one) (12)

1,2-diaminoethane (0.13 mL, 1.8 mmol) was added to a solution of pyrimidine **2** (0.7 g, 1.8 mmol) in absolute ethanol (20 mL) was heated under reflux for 5 h. The solid obtained after cooling was collected and recrystallized from ethanol to furnish pyrimidine dimer **12** as brown crystals, mp. 242–244 °C, yield 57%. IR (KBr, ν , cm^{-1}): 3200 (NH), 3055 (CH-aromatic), 2968, 2923 (CH-aliphatic), 1674 (C=O), 1626 (C=N). 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 8.74 (br.s, 2H, 2 NH, exchangeable), 8.31 (s, 2H, 2 C₅-H pyrazole), 8.01–7.15 (m, 20H, Ar-H), 3.39 (s, 4H, 2 CH₂), 2.30 (s, 6H, 2 CH₃CO), 2.03 (s, 6H, 2 CH₃). EIMS (70 eV, m/z , %): 764.84 (M^+ , 7), 701.19 (16), 639.80 (45), 558.00 (11), 418.80 (13), 310.95 (19), 220.37 (33), 141.94 (39), 105.32 (55), 77.27 (95), 51.13 (100). Anal. Calcd. for $C_{46}H_{40}N_{10}O_2$ (764.89): C, 72.23; H, 5.27; N, 18.31. Found: C, 72.06; H, 5.04; N, 18.26%.

8-Acetyl-7-(1,3-diphenyl-1H-pyrazol-4-yl)-5-imino-9-methyl-1,4,5,7-tetrahydropyrimido[2,1-c][1,2,4] triazepin-3(2H)-one (13)

A solution of pyrimidine **2** (0.7 g, 1.8 mmol) and 2-cyanoacetohydrazide (0.28 g, 1.8 mmol) in dioxane (20 mL) was heated under reflux for 6 h. The reaction mixture was concentrated and then allowed to stand at room

temperature. The deposited solid was collected and recrystallized from ethanol/dioxane mixture (2:1) to furnish compound **13** as white crystals, mp. 230–232 °C, yield 61%. IR (KBr, ν , cm^{-1}): 3150, 3200 (NH), 3060 (CH-aromatic), 2960, 2922, 2854 (CH-aliphatic), 1682 (C=O), 1628 (C=N). ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 10.24 (br.s, 1H, =NH, exchangeable), 9.70 (br.s, 1H, NHCO, exchangeable), 9.00 (br.s, 1H, NHNHCO , exchangeable), 8.30 (s, 1H, $\text{C}_5\text{-H}$ pyrazole), 8.12–7.33 (m, 10H, Ar-H), 5.49 (s, 1H, $\text{C}_7\text{-H}$ pyrimidotriazepine), 3.67 (s, 2H, CH_2), 2.29 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3). EIMS (70 eV, m/z , %): 453.08 (M^+ , 16), 443.73 (44), 430.70 (50), 423.68 (98), 403.01 (69), 394.67 (100), 390.93 (27), 357.80 (52), 332.09 (28), 296.01 (27), 262.37 (33), 217.89 (44), 200.29 (40), 198.70 (32), 137.30 (34), 77.13 (42). Anal. Calcd. for $\text{C}_{25}\text{H}_{23}\text{N}_7\text{O}_2$ (453.51): C, 66.21; H, 5.11; N, 21.62. Found: C, 66.02; H, 4.98; N, 21.57%.

1,1'-(Disulfanediy)bis(6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-methyl-1,6-dihydropyrimidine-2,5-diy) bis(ethan-1-one) (14)

A mixture of pyrimidine **2** (1.94 g, 5 mmol) and sodium nitrite (1.72 g, 25 mmol) in acetic acid (10 mL) was stirred at room temperature for 2 h. The reaction mixture was poured onto ice. The separated solid that formed was filtered off, washed with water, dried and then recrystallized from ethanol to produce the disulfide derivative **14** as yellow crystals, mp. 230–232 °C, yield 44%. IR (KBr, ν , cm^{-1}): 3390 (NH), 3060 (CH-aromatic), 2968 (CH-aliphatic), 1655 (C=O), 1621 (C=N). ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 9.74 (br.s, 2H, 2 NH, exchangeable), 8.73 (s, 2H, 2 $\text{C}_5\text{-H}$ pyrazole), 8.33–7.12 (m, 20H, Ar-H), 5.48 (s, 2H, 2 $\text{C}_6\text{-H}$ pyrimidine), 2.37 (s, 6H, 2 CH_3CO), 2.29 (s, 6H, 2 CH_3). EIMS (70 eV, m/z , %): 770.07 ($[\text{M}^+ - 2\text{H}_2]$, 8), 755.84 (16), 739.53 (20), 676.39 (28), 600.66 (36), 507.21 (33), 495.08 (61), 478.64 (100), 454.79 (23), 404.25 (72), 319.28 (26), 268.54 (21), 224.70 (39), 149.15 (33), 144.01 (44), 105.18 (25). Anal. Calcd. for $\text{C}_{44}\text{H}_{38}\text{N}_8\text{O}_2\text{S}_2$ (774.96): C, 68.19; H, 4.94; N, 14.46. Found: C, 68.02; H, 4.80; N, 14.42%.

1-(2-((5-Acetyl-6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-methyl-1,6-dihydropyrimidin-2-yl)oxy)-6-(1,3-diphenyl-1H-pyrazol-5-yl)-4-methyl-1,6-dihydropyrimidin-5-yl)ethan-1-one (15)

A mixture of pyrimidinethione **2** (1.94 g, 5 mmol) and hydrogen peroxide (1.1 mL, 10 mmol, 30%) was stirred under reflux for 6 h. Most of the solvent was evaporated under vacuum and the reaction mixture was then poured onto ice. The separated solid was collected, washed with water, dried and recrystallized from ethanol to produce compound **15** as brown crystals, mp. 150–152 °C, yield

33%. IR (KBr, ν , cm^{-1}): 3234 (NH), 3063 (CH-aromatic), 2962, 2912 (CH-aliphatic), 1685 (C=O), 1622 (C=N). ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 9.72 (br.s, 2H, 2 NH, exchangeable), 8.31 (s, 2H, 2 $\text{C}_5\text{-H}$ pyrazole), 8.02–7.30 (m, 20H, Ar-H), 5.47 (s, 2H, 2 $\text{C}_6\text{-H}$ pyrimidine), 2.36 (s, 6H, 2 CH_3), 2.02 (s, 6H, 2 CH_3CO). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 195.02 (C=O), 152.15, 151.23, 144.41, 139.71, 133.40, 129.97, 128.96, 128.89, 128.54, 128.47, 126.95, 124.94, 118.90, 111.70, 46.14, 30.67, 18.83. EIMS (70 eV, m/z , %): 726.20 (M^+ , 16), 694.25 (47), 685.58 (45), 641.34 (35), 577.41 (100), 550.66 (24), 517.43 (48), 482.78 (31), 436.78 (72), 320.15 (44), 303.36 (99), 283.42 (50), 236.36 (60), 183.62 (72), 158.29 (22), 112.02 (28). Anal. Calcd. for $\text{C}_{44}\text{H}_{38}\text{N}_8\text{O}_3$ (726.84): C, 72.71; H, 5.27; N, 15.42. Found: C, 72.49; H, 5.01; N, 15.29%.

Cytotoxicity assay

Materials and methods

Cell line

Four human tumor cell lines, namely hepatocellular carcinoma (HePG2), mammary gland breast cancer (MCF7), human prostate cancer (PC3) and colorectal carcinoma (HCT-116). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical reagents

The reagents used were RPMI-1640 medium, MTT, DMSO, doxorubicin (Sigma Co., St. Louis, USA), and Fetal Bovine serum (GIBCO, Paisley, UK). Doxorubicin was used as a standard anticancer drug for comparison.

MTT assay

The different cell lines mentioned above were utilized to measure the inhibitory effects of compounds on cell growth using the MTT assay [32–35]. 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 $\mu\text{g}/\text{mL}$ streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 . After incubation, the cells treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 μL of MTT solution at 5 mg/mL was added and

then incubated for 4 h. DMSO in volume of 100 μ L 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, Bio-Tech, Winosky, VT, USA). The relative cell viability in percentage was calculated as (A_{570} of treated samples/ A_{570} of untreated sample) \times 100.

Acknowledgements Technical support from Chemistry Department, Faculty of Science, Ain Shams University is gratefully acknowledged.

Funding The authors received no specific funding for this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies involving animals or human participants performed by any of the authors.

References

- G. Maga, M. Radi, M.A. Gerard, M. Botta, E. Ennifar, *Viruses* **2**, 880 (2010)
- M.B. Deshmukh, S.M. Salunkhe, D.R. Patil, P.V. Anbhule, *Eur. J. Med. Chem.* **2009**(44), 2651 (2009)
- B.R. Prashantha Kumar, G. Sankar, R.B. Nasir Baig, S. Chandrashekar, *Eur. J. Med. Chem.* **44**, 4192 (2009)
- K.V. Sujith, J.N. Rao, P. Shetty, B. Kalluraya, *Eur. J. Med. Chem.* **44**, 3697 (2009)
- A.M. Isloor, B. Kalluraya, P. Shetty, *Eur. J. Med. Chem.* **44**, 3784 (2009)
- M. Larhed, A. Hallberg, *Drug Discov. Today* **6**(8), 406 (2001)
- T.P. Selby, G.P. Lahm, T.M. Stevenson, K.A. Hughes, D. Cordova, I.B. Annan, *Bioorg. Med. Chem. Lett.* **23**, 6341 (2013)
- H. Yu, M. Xu, Y. Cheng, H. Wu, Y. Luo, B. Li, *Arkivoc* **6**, 26 (2012)
- P. Lidström, J. Tierney, B. Wathey, J. Westman, *Tetrahedron* **57**(51), 9225 (2001)
- P. Callery, P. Gannett, *Cancer and cancer chemotherapy*, in *Foye's Principles of Medicinal Chemistry*, 5th edn., ed. by D.A. Williams, T.L. Lemke (Lippincot Williams and Wilkins, Philadelphia, 2002), p. 934
- M.S. Masoud, A.A. Ibrahim, A.E. Khalil, A. El-Marghany, *Spectrochim. Acta Mol. Biomol. Spectrosc.* **67**, 662 (2007)
- O.A. Fathalla, S.M. Awad, M.S. Mohamed, *Arch. Pharm. Res.* **28**, 1205 (2005)
- E.A. El-Helw, H.A. Derbala, M.M. El-Shahawy, M.S. Salem, M.M. Ali, *Russ. J. Bioorg. Chem.* **45**(1), 42 (2019)
- A. Odani, H. Kozlowski, J. Swiatek-Kozlowska, J. Brasun, B.P. Operschall, H.J. Sigel, *J. Inorg. Biochem.* **101**, 727 (2007)
- S.K. Ramadan, W.S.I. Abou-Elmagd, *Synth. Commun.* **48**(18), 2409 (2018)
- S.K. Ramadan, S.S. Shaban, A.I. Hashem, *Synth. Commun.* **50**(2), 185 (2020)
- W.S.I. Abou-Elmagd, A.K. El-Ziaty, M.I. Elzahar, S.K. Ramadan, A.I. Hashem, *Synth. Commun.* **46**(14), 1197 (2016)
- S.K. Ramadan, H.A. Sallam, *J. Heterocycl. Chem.* **55**, 1942 (2018)
- S.K. Ramadan, E.A.E. El-Helw, H.A. Sallam, *Heterocycl. Commun.* **25**(1), 107 (2019)
- C.O. Kappe, *Eur. J. Med. Chem.* **35**, 1043 (2000)
- A. Shaabani, A. Bazgir, F. Teimouri, *Tetrahedron Lett.* **44**, 857 (2003)
- S.A. Rizk, S.S. Abdelwahab, H.A. Sallam, *J. Heterocycl. Chem.* **55**, 1604 (2018)
- S.A. Rizk, S.S. Abdelwahab, A.A. El-Badawy, *J. Heterocycl. Chem.* **56**, 2347 (2019)
- M.A. Hussein, O.H. Zyaan, A.H. Abdel Monsef, S.A. Rizk, S.M. Farag, S.E. Hafez, A.S. Khaled, O.M. Helmy, *Int. J. Mosq. Res.* **5**, 22 (2018)
- S.A. Rizk, A.M. El-Naggar, A.A.J. El-Badawy, *Mol. Str.* **1155**, 720 (2018)
- A.I. Hashem, W.S.I. Abou-Elmagd, A.K. El-Ziaty, S.K. Ramadan, *J. Heterocycl. Chem.* **54**, 3711 (2017)
- S.K. Ramadan, E.A.E. El-Helw, M.E. Azab, *Russ. J. Org. Chem.* **55**(12), 1940 (2019)
- A.K. El-Ziaty, W.S.I. Abou-Elmagd, S.K. Ramadan, A.I. Hashem, *Synth. Commun.* **47**(5), 471 (2017)
- S.K. Ramadan, E.A.E. El-Helw, *J. Chem. Res.* **42**, 332 (2018)
- K.N.M. Halim, S.K. Ramadan, S.A. Rizk, M.A. El-Hashash, *Synthesis, DFT study, molecular docking and insecticidal evaluation of some pyrazole-based tetrahydropyrimidine derivatives*. *Synth. Commun.* (2020). <https://doi.org/10.1080/00397911.2020.1720739>
- B.Y. Bhong, P.B. Thorat, N.N. Karade, *Tetrahedron Lett.* **54**(14), 1862 (2013)
- T.J. Mosmann, *Immunol. Methods* **65**, 55 (1983)
- P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, *J. Natl. Cancer Inst.* **82**(13), 1107 (1990)
- H.J. Mauceri, N.N. Hanna, M.A. Beckett, D.H. Gorski, M.J. Staba, K.A. Stellato, K. Bigelow, R. Heimann, S. Gately, M. Dhanabal, G.A. Soff, V.P. Sukhatme, D. Kufe, R.R. Weichselbaum, *Nature* **394**, 287 (1998)
- ShM Abu-Bakr, KhM Abouzid, M. Youns, A. Hashim, H.I. El-Diwan, *Res. J. Pharm. Biol. Chem. Sci.* **4**(4), 1350 (2013)
- X. Liu, Y. Guo, Y. Li, Y. Jiang, S. Chubb, A. Azuma, P. Huang, A. Matsuda, W. Hittelman, W. Plunkett, *Cancer Res.* **65**, 6874 (2005)
- X.L. Yu, Y.X. Liu, Y.Q. Li, Q.M. Wang, *J. Agric. Food Chem.* **63**, 9690 (2015)
- R.B. Xu, R. Xia, M. Luo, X.Y. Xu, J.G. Cheng, X.S. Shao, Z. Li, *J. Agric. Food Chem.* **62**, 381 (2014)
- S.A. Abdel-Latef, A.S. Darwish, S.A. Rizk, S.K. Atya, M.E. Helal, *J. Mol. Liq.* **288**, 111006 (2019)
- S.K. Attia, A.T. Elgendy, S.A.J. Rizk, *Mol. Str.* **1184**, 583 (2019)