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Cytotoxic and antimicrobial activities of some novel heterocycles employing 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile

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Abstract: A pyrimidinethione derivative namely, 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile, was readily synthesized and reacted with carbon electrophiles in an attempt to synthesize selected fused heterocycles. The reactivity of 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile was investigated towards selected nitrogen nucleophiles. Thiation and hydrolysis reactions of the tetrahydropyrimidine derivative were investigated. Antitumor and antimicrobial activity evaluation of some of the synthesized products exhibited promising results.

Keywords: Antitumor and antimicrobial activity, Carbon electrophiles, Tetrahydropyrimidine, Thiation, Thiazolopyrimidine.

Introduction

The chemistry of the pyrazole heterocycles has received great attention during the last few decades because of their outstanding pharmacological activities e.g. antiviral, antitumor and antimicrobial [1-7]. Considering all of these benefits and in continuation of our work to synthesize new heterocycles, starting from 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde [1-3], we introduce herein several new pharmacophores containing pyrimidine, pyrimidinethione, and thiazolopyrimidine moieties incorporated into the pyrazole nucleus, in an effort to obtain compounds with enhanced potency.

Results and discussion

A one-pot cyclo-condensation reaction of 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde **(1)** with ethyl cyanoacetate and thiourea yielded 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(2)** (Scheme 1) [7]. There are two possible tautomeric structures for pyrimidine **2** as depicted in Scheme 1.

The reactions of pyrimidine **2** with selected carbon electrophiles were studied (see schemes 2 and 3) in an attempt to design and synthesize *N*-heterocycles with pharmacological activity. The reactions of the thioamide and iminothiol tautomers of pyrimidinethione **2,** based on their thermodynamic and kinetic properties under experimental conditions are discussed below. The conjugate base of the iminothiol tautomer has been found to be thermodynamically more stable than that of the thioamide tautomer in the presence of a base (basicity is thermodynamically controlled). This is due to back donation involving the vacant d-orbital of the sulfur atom. Further, the sulfur anion is a stronger nucleophile than the nitrogen anion (nucleophilicity is kinetically controlled), making the iminothiol tautomer kinetically more reactive than the thioamide tautomer. Thus, under the experimental conditions used, the iminothiol tautomer is more thermodynamically and kinetically favored than the thioamide tautomer.

Chloroacetyl chloride was reacted with pyrimidinethione **2** in refluxing sodium ethoxide to yield thiazolopyrimidine derivative **3** as pale-yellow crystals (Scheme 2). The structure of compound **3** was deduced from its

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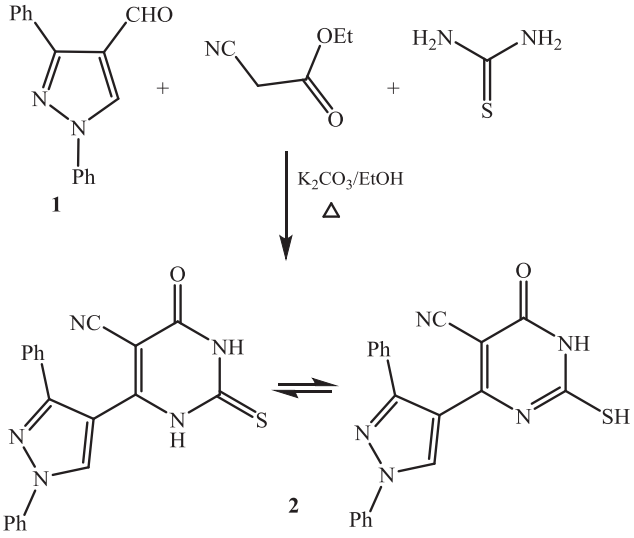
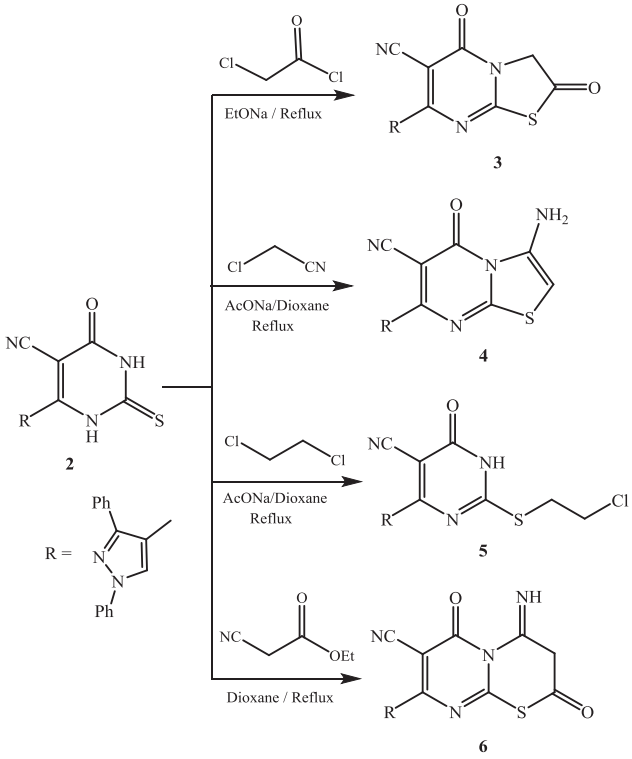
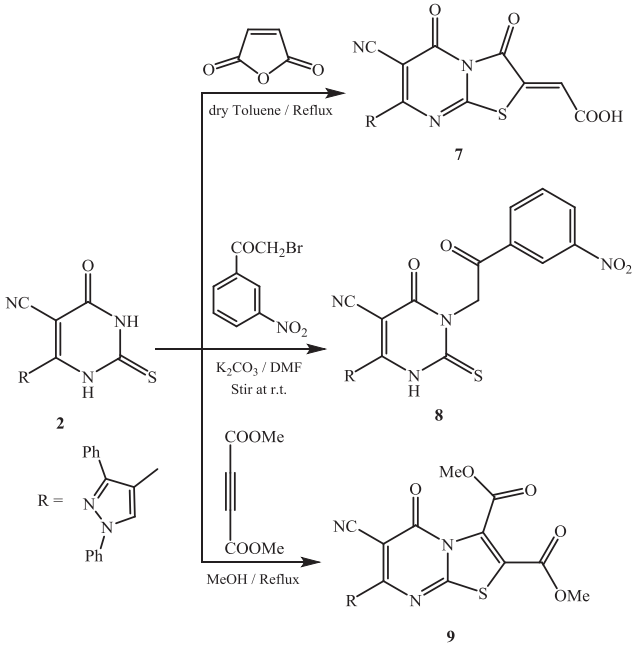


Scheme 1 Synthesis of pyrazolylpyrimidinethione **2**.



Scheme 2 Condensation of pyrimidinethione **2** with carbon electrophiles.



Scheme 3 Reaction of pyrimidinethione **2** with carbon electrophiles.

analytical and spectral data. The IR spectrum exhibited the stretching absorption bands of C=O groups of thiazolidinone and pyrimidinone at ν 1731 and 1690 cm^{-1}, respectively. The singlet in the ^{1}H-NMR spectrum at δ 3.58 ppm integrated to two protons, corresponding to methylene CH_2 protons. It is possible the reaction takes place *via* the tetrahedral mechanism (rather than the S_N^2 mechanism), by attack of the more nucleophilic sulfur atom on the carbonyl group of acid chloride followed by cyclization. In the tetrahedral mechanism, the S-C bond is formed before the C-Cl bond is broken and a lot of energy is accumulated in the reaction medium which offsets the activation energy and reactivity increase compared with the S_N^2 mechanism.

Chloroacetonitrile reacted with **2** to yield aminothiazolopyrimidine derivative **4** (Scheme 2). The spectral data of compound **4** agreed with the assigned structure. The *S*-alkylated product **5** was obtained from condensation of **2** with 1,2-dichloroethane in refluxing dioxane and anhydrous sodium acetate (Scheme 2). The IR spectrum showed the characteristic stretching absorption bands for NH, C≡N and C=O groups. The ^{1}H-NMR spectrum showed a singlet signal at δ 11.71 ppm, integrated to one exchangeable proton corresponding to a NH proton, as well as two triplet signals of CH_2-CH_2 protons (cf. Experimental). Ethyl cyanoacetate was treated with pyrimidinethione **2** in refluxing dioxane to yield pyrimidothiazine derivative **6** as yellow crystals (Scheme 2). The reaction could proceed *via* nucleophilic attack of the thiol group on the ester carbonyl group to eliminate ethanol molecule, followed by 1,6-exo-dig cyclization.

Treatment of pyrimidine **2** with maleic anhydride in refluxing dry toluene produced thiazolopyrimidine derivative **7** (Scheme 3). The spectral data of compound **7** was in accord with the proposed structure. The IR spectrum displayed the characteristic stretching absorption bands for OH, C≡N and C=O groups. The formation of

compound **7** could be interpreted *via* the ring opening of maleic anhydride due to nucleophilic attack by the NH of pyrimidine on the C=O of anhydride, followed by 1,5-exo-trig cyclization *via* intra thia-Michael addition, then finally dehydrogenation. The driving force for dehydrogenation of the desired product is to be more thermodynamically stable *via* conjugation with the carboxylic carbonyl group.

Pyrimidinethione **2** was treated with 3'-nitro-*w*-bromoacetophenone in *N,N*-dimethylformamide and anhydrous potassium carbonate and the *N*-alkylated product **8** was obtained (Scheme 3). The IR spectrum displayed two absorption stretching bands for carbonyl groups at ν 1735 and 1702 cm^{-1}, as well as the appearance of a CH_2 singlet in its ^{1}H-NMR spectrum. The higher values of ν C=O may be attributed to mutual induction between the two carbonyl groups.

Thiazolopyrimidine **9** was obtained when pyrimidinethione **2** reacted with dimethyl acetylenedicarboxylate in refluxing methanol (Scheme 3). The IR spectrum exhibited three absorption bands for C=O groups at ν 1736, 1719 and 1695 cm^{-1}. It was assumed that the higher values of ν C=O of the two ester groups may be due to mutual induction between the two carbonyl groups. The formation of compound **9** could be explained *via* thia-Michael addition on the C≡C followed by aza-Michael addition to give 1,5-endo-trig cyclization, then finally dehydrogenation.

This work was extended to investigate the reactivity of pyrimidinethione **2** towards 1,2-diaminoethane and thiourea (Scheme 4). The, pyrimidine dimer **10** was obtained from the reaction of compound **2** with 1,2-diaminoethane in refluxing ethanol. It seemed that the lactim form is more stable than the lactam form, due to its aromaticity. When thiourea was treated with pyrimidinethione **2** in refluxing ethanol/acetic acid, the pyrimidine derivative **11** was produced. The structures of compounds **10** and **11** were substantiated from their analytical and spectral data.

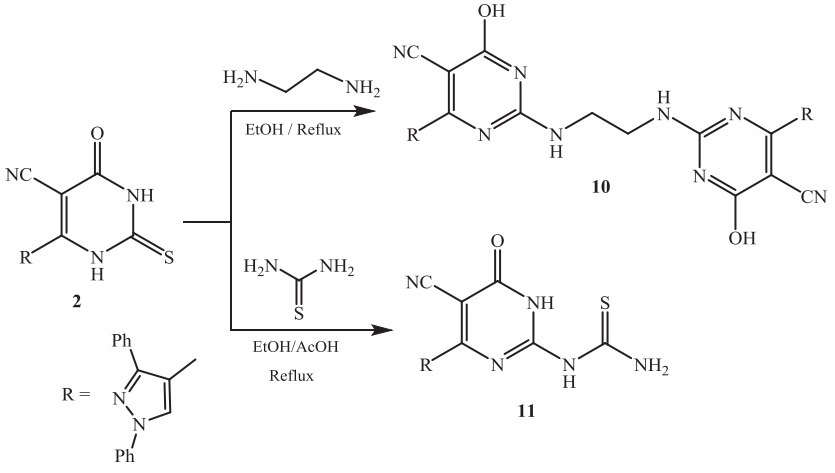
Thiation of compound **2** was undertaken using phosphorus pentasulfide in refluxing toluene to produce dithioxopyrimidine **12,** which was reacted with ethyl chloroacetate to yield the di-*S*-alkylated product **13** (Scheme 5). The IR spectrum of compound **12** showed the absence of the stretching absorption band of the carbonyl group and displayed the corresponding absorption band of C=S group. The IR spectrum of compound **13** lacked the NH absorption band and showed the absorption bands characteristic for the ester carbonyl group. The ^{1}H-NMR spectrum of compound **13** showed the absence of NH singlets (cf. Experimental). Compelling evidence for structure **13** was derived from the fact that, when the reaction was carried out in an equimolar ratio, the yield was 43%, however, by utilizing a double molar ratio of ethyl chloroacetate, the yield was increased to 75%.

Hydrolysis of 5-cyanopyrimidine **2** in 70% sulfuric acid yielded pyrimidine-5-carboxylic acid **14** (Scheme 5). The IR spectrum revealed the disappearance of the absorption band of the cyano group and the appearance of the submaxima for ν OH of the carboxylic group at 3408 cm^{-1}, as well as the stretching absorption band of carboxylic carbonyl group at ν 1709 cm^{-1}. The ^{1}H-NMR spectrum was in accord with the assigned structure.

Pharmacology

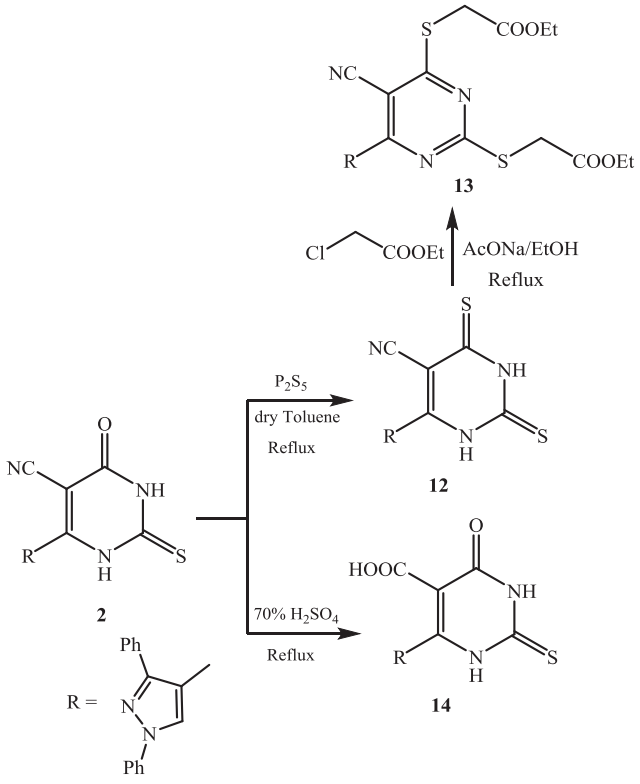
Antimicrobial activity evaluation

Six compounds were screened for their antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and



Scheme 4 Reaction of pyrimidine **2** with 1,2-diaminoethane and thiourea.

yeast-like pathogenic fungi (*Aspergillus Niger* and *Candida albicans*). The antimicrobial screening was carried out using a standard well agar diffusion assay according to Cheesbrough [8]. The broad-spectrum antibiotic Amoxicillin and the antifungal Fluconazole were used at a concentration of 100 µg/ml as positive controls. The results obtained (Table 1 and Figure 1) revealed that of the compounds tested **4, 8, 12** and **14** exhibited the highest antimicrobial activity. The rest of the tested compounds showed moderate to weak antimicrobial activity.



Scheme 5 Thiation and hydrolysis of compound **2**.

Structure-Activity Relationship for Antimicrobial Activity

Analysis of the previous results concerning the structural modifications that occurred only at positions-2,3 of the parent pyrimidine scaffold, revealed that Thiazolopyrimidine derivative **4**, with a free amino group showed strong inhibitory activity against all tested strains except *Aspergillus Niger*. Incorporation of 3-nitroacetophenone moiety at position-3, as in compound **8,** produced excellent activity. Replacement of an oxygen atom with sulfur as in compound **12** increased the activity [6]. Hydrolysis of the cyano group as in compound **14** achieved good activity.

In vitro anticancer activity

Six compounds were investigated for their *in vitro* cytotoxic activity against two human carcinoma cell lines; namely, colon cancer HCT-116 and mammary gland breast cancer MCF-7 cell lines, using doxorubicin as a standard anticancer drug. The anticancer activity was expressed as IC_{50} values (the concentration of the test compound required to kill 50% of the cell population). The results depicted in Table 2 and Figure 2 revealed that the most potent compounds are **8** and **12** against the tested cell lines. The rest of compounds exhibited moderate activity.

Structure-activity relationship for anticancer activity

The inhibitory activity of the tested derivatives could be correlated to structure variation and modifications. By investigating the variation in selectivity of the highly potent compounds, it was revealed that the existence of

Table 1 Antibacterial and antifungal activity (as inhibition zone in mm diameter).

Compound	Antibacterial Activity		Antifungal Activity	
	Staphylococcus aureus (G^+)	*Escherichia coli* (G^-)	*Candida albicans*	*Aspergillus Niger*
Amoxicillin	28	25	—	—
Fluconazole	—	—	20	22
3	6	8	11	0
4	24	22	17	0
6	14	11	12	9
8	20	21	17	0
12	23	19	16	0
14	15	13	17	0

G: Gram reaction 0.0: no activity (inhibition zone less than 7 mm).

7-10: weak activity 11-15: moderate activity. More than 15: strong activity.

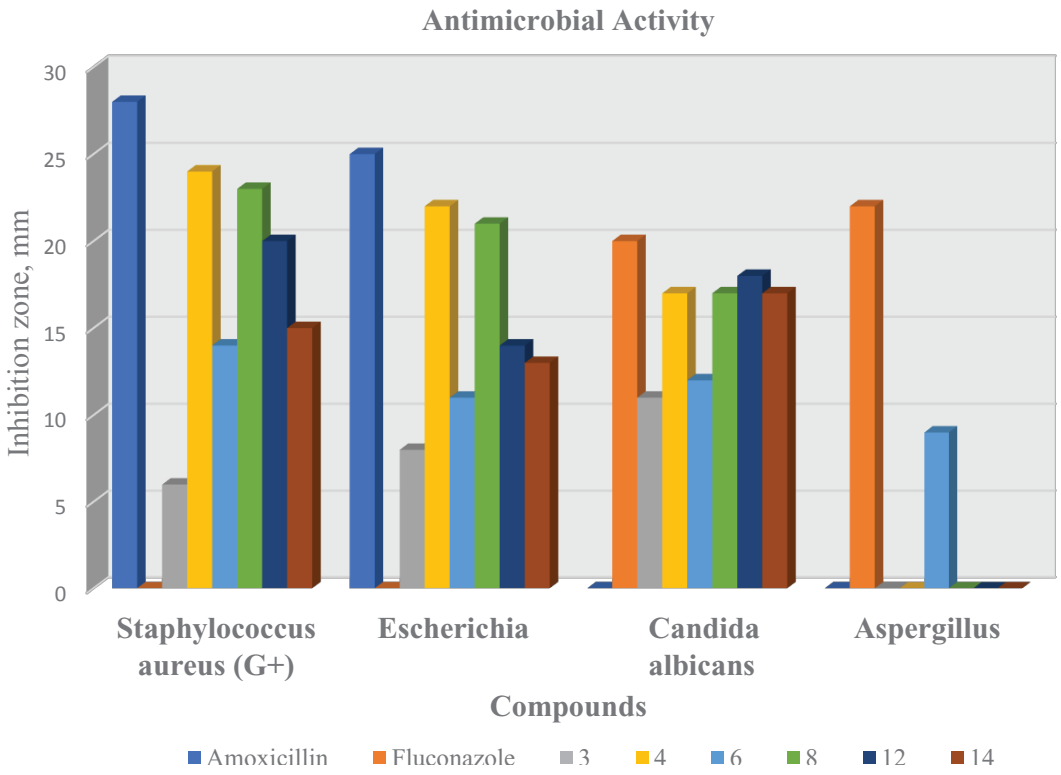


Figure 1 Antimicrobial activity of the tested compounds.

Table 2 *In vitro* cytotoxic activity of the tested compounds against different human cancer cell lines.

Compound	*In vitro* cytotoxicity IC_{50} (μM)	
	HCT-116	MCF-7
doxorubicin	5.23 ± 0.3	4.17 ± 0.2
3	30.48 ± 2.4	28.67 ± 2.7
4	39.55 ± 2.8	41.28 ± 3.6
6	35.07 ± 2.7	30.59 ± 2.8
8	13.72 ± 1.3	15.53 ± 1.4
12	17.79 ± 1.6	19.58 ± 1.8
14	43.06 ± 3.2	49.71 ± 3.9

IC_{50} (μM): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); 51-100 (weak), and > 100 (non-cytotoxic).

the free thioamide group of the parent pyrimidine scaffold led to enhanced activity. Also, the presence of the NO_2 group in compound **8** as a strong electron attracting group rendered the molecule positively charged, forming an electrostatic attraction with the DNA nucleobases [1].

Conclusion

Selected fused heterocycles bearing a pyrazole scaffold were synthesized *via* the reaction of 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile with carbon electrophiles and nitrogen nucleophiles. Thiation and hydrolysis of 6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile were also studied. Some of the synthesized compounds exhibited promising antimicrobial and antitumor activities.

Experimental

Chemistry

All melting points were measured on a Gallenkamp electric melting point apparatus and are uncorrected. The FTIR spectra (ν, cm^{-1}) were recorded using potassium bromide disks on a Fourier Transform Infrared Thermo Electron Nicolet iS10 Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at the Central Laboratory of Faculty of Science, Ain Shams University. The ^{1}H-NMR spectra (δ_H, ppm) were run at 400 *MHz* on a BRUKER 400 BB NMR Spectrometer (BRUKER, Manufacturing & Engineering Inc., Anaheim, CA, USA) using tetramethyl silane (TMS) as an internal standard in deuterated dimethylsulfoxide (DMSO-d_6) at the Faculty of Pharmacy, Ain Shams University. The mass spectra (MS) were recorded

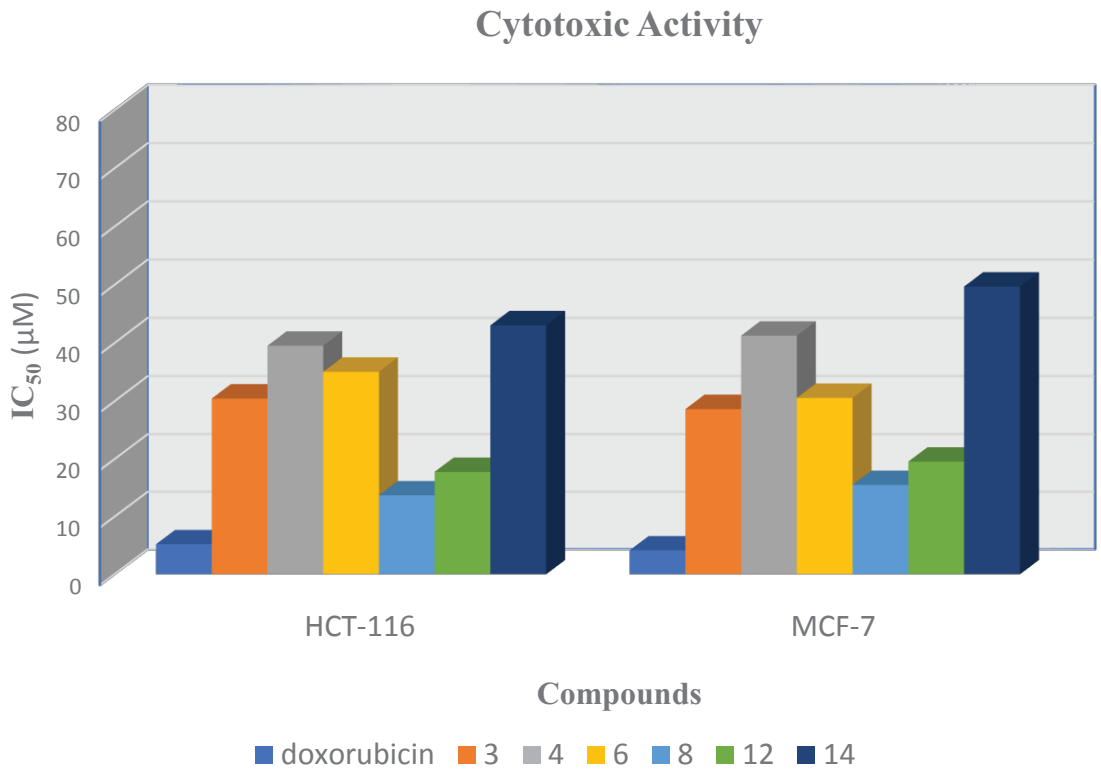


Figure 2 Cytotoxic activity of the tested compounds on HCT-116 and MCF-7 cell lines.

on a 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 of Al-Azhar University, Nasr City, Cairo, Egypt. Elemental analyses were carried out at the Microanalytical Unit, 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 F_{254} analytical sheets obtained from Fluka. The pharmacological activity assays were carried out at the Pharmacology Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

6-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-5-cyano-4-oxo-1,2,3,4-tetrahydropyrimidine-2-thione (2)

A mixture of pyrazole aldehyde **1** (2.48 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol), thiourea (0.76 g, 0.01 mol) and potassium carbonate (4.14 g, 0.03 mol) in absolute ethanol (60 ml) was heated under reflux for 12 h, cooled, and neutralized with glacial acetic acid. The product was crystallized from methanol to give the pyrimidine **2** as yellowish orange, mp. 196-198°C (Lit. [7] 195-197°C).

Synthesis of 7-(1,3-diphenyl-1H-pyrazol-4-yl)-2,5-dioxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (3)

A solution of pyrimidinethione **2** (0.74 g, 2 mmol), chloroacetyl chloride (0.22 g, 2 mmol) and sodium ethoxide (0.05 g/10 ml ethanol) in ethanol (20 ml) was heated under reflux for 10 h. The reaction mixture was poured onto ice/HCl (10%). The precipitated solid was filtered off, washed with water, dried, and then recrystallized from dioxane to yield thiazolopyrimidine derivative **3** as pale-yellow crystals, mp. 270-272°C, yield 68%. FTIR (KBr, ν, cm^{-1}): 2216 (C≡N), 1731 (C=O thiazolidine), 1690 (C=O pyrimidine). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 9.12 (s, 1H, C_5-H pyrazole), 7.98-7.43 (m, 10H, Ar-H), 3.58 (s, 2H, CH_2). MS (*m/z*, %): 411 ($M^{\cdot+}$, 15). Anal. Calcd. for $C_{22}H_{13}N_5O_2S$ (411.08): C, 64.22; H, 3.18; N, 17.02. Found: C, 64.01; H, 3.03; N, 17.05.

Synthesis of 3-amino-7-(1,3-diphenyl-1H-pyrazol-4-yl)-5-oxo-5H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (4)

A solution of compound **2** (0.74 g, 2 mmol), chloroacetonitrile (0.15 g, 2 mmol) and freshly prepared anhydrous sodium acetate (0.16 g, 2 mmol) in dioxane (10 ml)

was heated under reflux for 8 h. After concentration the reaction mixture was then cooled and the solid product that formed was filtered off and recrystallized from an ethanol/dioxane mixture (2:1) to give compound **4** as brown crystals, mp >360°C, yield 62%. FTIR (KBr, *v*, cm^{-1}): 3425, 3351 (NH_2), 2216 (C≡N), 1668 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 9.10 (s, 1H, C_5-H pyrazole), 7.99-7.42 (m, 11H, Ar-H + CH), 6.55 (s, 2H, NH_2, D_2O-exchangeable). MS (*m/z*, %): 410 (M^{+}, 12). Anal. Calcd. for $C_{22}H_{14}N_6OS$ (410.09): C, 64.38; H, 3.44; N, 20.48. Found: C, 64.09; H, 3.15; N, 20.46.

Synthesis of 2-((2-chloroethyl)thio)-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (5)

A mixture of compound **2** (0.74 g, 2 mmol), 1,2-dichloroethane (0.20 g, 2 mmol) and freshly prepared sodium acetate (0.16 g, 2 mmol) in dioxane (20 ml) was heated under reflux for 10 h. The excess solvent was evaporated under vacuum. The residue was triturated with water. The solid was filtered off and recrystallized from benzene to give compound **5** as yellow crystals, mp. 150-152°C, yield 54%. FTIR (KBr, *v*, cm^{-1}): 3435 (NH), 2211 (C≡N), 1660 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 11.71 (s, 1H, NH, D_2O-exchangeable), 9.07 (s, 1H, C_5-H pyrazole), 7.98-7.30 (m, 10H, Phenyl), 3.25-3.20 (t, 2H, CH_2Cl, *J*= 7.2 *Hz*), 3.07-3.01 (t, 2H, SCH_2, *J*= 7.2 *Hz*). Anal. Calcd. for $C_{22}H_{16}ClN_5OS$ (433.08): C, 60.90; H, 3.72; N, 16.14. Found: C, 60.72; H, 3.57; N, 16.17.

Synthesis of 8-(1,3-diphenyl-1H-pyrazol-4-yl)-4-imino-2,6-dioxo-3,4-dihydro-2H,6H-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile (6)

To a solution of compound **2** (0.74 g, 2 mmol) in dioxane (20 ml), ethyl cyanoacetate (0.22 g, 2 mmol) was added and the reaction mixture was heated under reflux for 6 h. The reaction mixture was concentrated and then cooled to room temperature. The precipitated solid was collected and recrystallized from ethanol to give compound **6** as yellow crystals, mp. 274-276°C, yield 66%. FTIR (KBr, *v*, cm^{-1}): 3448 (NH), 2220 (C≡N), 1700 (C=O thiazine), 1670 (C=O pyrimidine). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 13.15 (s, 1H, NH, D_2O-exchangeable), 9.20 (s, 1H, C_5-H pyrazole), 8.10-7.47 (m, 10H, Phenyl), 3.46 (s, 2H, CH_2). MS (*m/z*, %): 438 (M^{+}, 28). Anal. Calcd. for $C_{23}H_{14}N_6O_2S$ (438.09): C, 63.00; H, 3.22; N, 19.17. Found: C, 62.87; H, 3.11; N, 19.20.

Synthesis of 2-(6-cyano-7-(1,3-diphenyl-1H-pyrazol-4-yl)-3,5-dioxo-5H-thiazolo[3,2-a]pyrimidin-2(3H)-ylidene) acetic acid (7)

A mixture of **2** (0.74 g, 2 mmol) and maleic anhydride (0.19 g, 2 mmol) in dry toluene (20 ml) was heated under reflux for 6 h. The precipitated solid was cooled, collected and recrystallized from ethanol to give thiazolopyrimidine derivative **7** as yellow crystals, mp. 252-254°C, yield 69%. FTIR (KBr, *v*, cm^{-1}): 3424 (*br*. OH), 2227 (C≡N), 1670 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 13.19 (*br*.s, 1H, COOH, D_2O-exchangeable), 9.21 (s, 1H, C_5-H pyrazole), 8.07-7.43 (m, 10H, Phenyl), 6.66 (s, 1H, CH=). MS (*m/z*, %): 467 (M^{+}, 11). Anal. Calcd. for $C_{24}H_{13}N_5O_4S$ (467.07): C, 61.67; H, 2.80; N, 14.98. Found: C, 61.54; H, 2.11; N, 14.91.

Synthesis of 6-(1,3-diphenyl-1H-pyrazol-4-yl)-3-(2-(3-nitrophenyl)-2-oxoethyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (8)

A solution of pyrimidinethione **2** (0.74 g, 2 mmol) and 3'-nitro-*w*-bromoacetophenone (0.48 g, 2 mmol) in *N,N*-dimethylformamide (20 ml) containing anhydrous potassium carbonate (0.27 g, 2 mmol) was stirred at room temperature for 8 h. The reaction mixture was poured onto ice and acidified with dilute HCl (10%). The separated solid was collected and recrystallized from an ethanol/dioxane mixture (1:1) to give compound **8** as beige crystals, mp. 264-266°C, yield 68%. FTIR (KBr, *v*, cm^{-1}): 3143 (NH), 2224 (C≡N), 1735 (C=O acetophenone), 1702 (C=O pyrimidine), 1531, 1354 (NO_2), 1234 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 13.77 (s, 1H, NH, D_2O-exchangeable), 9.19 (s, 1H, C_5-H pyrazole), 8.09-7.36 (m, 14H, Ar-H), 4.50 (s, 2H, CH_2). Anal. Calcd. for $C_{28}H_{18}N_6O_4S$ (534.11): C, 62.91; H, 3.39; N, 15.72. Found: C, 62.70; H, 3.11; N, 15.75.

Synthesis of dimethyl 6-cyano-7-(1,3-diphenyl-1H-pyrazol-4-yl)-5-oxo-5H-thiazolo[3,2-a]pyrimidine-2,3-dicarboxylate (9)

An equimolar mixture of pyrimidine **2** (0.74 g, 2 mmol) and dimethyl acetylenedicarboxylate (0.28 g, 2 mmol) in methanol (20 ml) was heated under reflux for 4 h. The precipitated solid was filtered off, while hot, and recrystallized from methanol to give compound **9** as yellow crystals, mp. 284-286°C, yield 83%. FTIR (KBr, *v*, cm^{-1}): 2239 (C≡N), 1736, 1719 (C=O two ester), 1695 (C=O pyrimidine). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 9.19 (s, 1H,

C_5-H pyrazole), 8.09-7.37 (m, 10H, Ar-H), 3.73 (s, 6H, 2 CH_3). Anal. Calcd. for $C_{26}H_{17}N_5O_5S$ (511.10): C, 61.05; H, 3.35; N, 13.69. Found: C, 60.42; H, 3.09; N, 13.76.

Synthesis of 2,2'-(ethane-1,2-diylbis(azanediyl))bis(4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-hydroxypyrimidine-5-carbonitrile) (10)

To a solution of pyrimidine **2** (0.74 g, 2 mmol) in absolute ethanol (20 ml), 1,2-diaminoethane (0.12 g, 2 mmol) was added and the reaction mixture was heated under reflux for 4 h. The obtained precipitate was collected, while hot, and recrystallized from dioxane to give compound **10** as white crystals, mp. >360°C, yield 61%. FTIR (KBr, *v*, cm^{-1}): 3422 (*br*. OH, NH), 2211 (C≡N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 11.30 (s, 2H, OH, D_2O-exchangeable), 9.04 (s, 2H, C_5-H pyrazole), 8.41 (s, 2H, NH, D_2O-exchangeable), 7.91-7.44 (m, 20H, Ar-H), 3.11 (s, 4H, CH_2). Anal. Calcd. for $C_{42}H_{30}N_{12}O_2$ (734.26): C, 68.65; H, 4.12; N, 22.88. Found: C, 68.32; H, 3.87; N, 22.84.

Synthesis of 1-(5-Cyano-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-oxo-1,6-dihydropyrimidin-2-yl)thiourea (11)

A solution of compound **2** (0.74 g, 2 mmol) and thiourea (0.15 g, 2 mmol) in absolute ethanol (20 ml) was heated under reflux for 5 h in the presence of 3-drops of glacial acetic acid. The reaction mixture was concentrated and the separated solid was filtered off, after cooling and recrystallized from a benzene/ethanol mixture (1:1) to give compound **11** as yellow crystals, mp. 258-260°C, yield 49%. FTIR (KBr, *v*, cm^{-1}): 3473, 3422 (NH, NH_2), 2214 (C≡N), 1696 (C=O), 1233 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 11.01 (s, 2H, 2 NH, D_2O-exchangeable), 9.14 (s, 1H, C_5-H pyrazole), 7.08 (s, 2H, NH_2, D_2O-exchangeable), 8.01-7.46 (m, 10H, Ar-H). MS (*m/z*, %): 413 (M^{+}, 17). Anal. Calcd. for $C_{21}H_{15}N_7OS$ (413.11): C, 61.01; H, 3.66; N, 23.71. Found: C, 60.74; H, 3.19; N, 23.76.

Synthesis of 6-(1,3-diphenyl-1H-pyrazol-4-yl)-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (12)

A suspension of compound **2** (0.74 g, 2 mmol) and phosphorus pentasulfide (0.22 g, 1 mmol) in dry toluene (20 ml) was heated under reflux for 2 h. The precipitated solid was collected while hot and recrystallized from ethanol to yield the corresponding dithioxopyrimidine derivative **12** as brown crystals, mp. 160-162°C, yield 74%. FTIR (KBr, *v*, cm^{-1}): 3418 (NH), 2211 (C≡N), 1222 (C=S). MS (*m/z*, %): 387 (M^{+}, 24). Anal. Calcd. for $C_{20}H_{13}N_5S_2$ (387.06): C, 62.00; H, 3.38; N, 18.07. Found: C, 61.76; H, 3.02; N, 18.00.

Synthesis of diethyl 2,2'-((5-cyano-6-(1,3-diphenyl-1H-pyrazol-4-yl)pyrimidine-2,4-diyl)bis(sulfanediyl)) diacetate (13)

A solution of dithioxopyrimidine derivative **12** (0.77 g, 2 mmol), ethyl chloroacetate (0.24 g, 2 mmol or 0.49 g, 4 mmol) and anhydrous sodium acetate (0.16 g, 2 mmol or 0.33 g, 4 mmol) in absolute ethanol (20 ml) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature. The precipitated solid was collected and recrystallized from ethanol to give compound **13** as brown crystals, mp. 102-104°C, yield 43% [1:1 mol], 75% [1:2 mol]. FTIR (KBr, *v*, cm^{-1}): 2210 (C≡N), 1733 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 9.10 (s, 1H, C_5-H pyrazole), 7.98-7.41 (m, 10H, Ar-H), 4.40 (s, 4H, 2 S-CH_2), 4.21-3.92 (q, 4H, 2 $\underline{CH_2}CH_3$, *J*= 6.8 *Hz*), 1.21-1.42 (t, 6H, 2 $CH_2\underline{CH_3}$, *J*= 6.8 *Hz*). Anal. Calcd. for $C_{24}H_{19}N_5O_2S_2$ (473.10): C, 60.87; H, 4.04; N, 14.79. Found: C, 60.45; H, 3.72; N, 14.76.

Synthesis of 6-(1,3-diphenyl-1H-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid (14)

A solution of pyrimidine **2** (0.74 g, 2 mmol) in sulfuric acid 70% (10 ml) was heated under reflux for 3 h. The reaction mixture was allowed to cool and poured onto ice, then neutralized by NH_4OH. The precipitate was then filtered off, dried and recrystallized from ethanol to give the corresponding acid derivative **14** as grey powder, mp. >360°C, yield 57%. FTIR (KBr, *v*, cm^{-1}): 3408 (*br*. OH), 1709 (C=O acid), 1655 (C=O pyrimidine), 1225 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H, ppm: 13.02 (s, 2H, COOH and NHCS, D_2O-exchangeable), 11.20 (s, 1H, NHCO, D_2O-exchangeable), 9.11 (s, 1H, C_5-H pyrazole), 7.99-7.42 (m, 10H, Ar-H). MS (*m/z*, %): 392 (M^{+}, 32). Anal. Calcd. for $C_{20}H_{16}N_4O_3S$ (392.09): C, 61.21; H, 4.11; N, 14.28. Found: C, 60.92; H, 3.89; N, 14.31.

Biological evaluation

Antimicrobial assay

The antimicrobial activities of the synthesized compounds were examined against two bacterial strains,

Staphylococcus aureus and *Escherichia coli*, and two fungal strains, *Aspergillus Niger* and *Candida albicans*, using a standard well agar diffusion assay [8]. Plates containing nutrient agar medium and sabouraud dextrose agar medium (for bacteria and fungi, respectively) were surface inoculated with 106 CFU/ml of freshly prepared microorganisms. Using a 6-mm sterile cork borer wells were punched in the agar and filled separately with 100 µl of the tested compounds (100 µg/ml in DMSO). The plates were left in a refrigerator for 2 h to allow diffusion of the tested compounds. After that, the plates were incubated for 24 h at 37°C for bacteria and for 72 h at 28°C for fungi Following incubation, the inhibition zones surrounding the wells were measured in millimeters. Amoxicillin and Fluconazole were used as the standard against bacteria and fungi, respectively, at the same concentration (100 µg/ml).

Cytotoxicity Assay

Materials and methods

Two cell lines were utilized for this work: colorectal carcinoma (colon) HCT-116 and mammary gland breast cancer MCF-7. The cell lines were obtained from ATCC *via* a holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison, according to a previously reported MTT method [9]. Chemical reagents: The reagents RPMI-1640 medium, MTT, and DMSO (Sigma Co., St. Louis, USA), fetal bovine serum (GIBCO, UK).

References

[1] Ramadan SK, Sallam HA. Synthesis, Spectral Characterization, Cytotoxic, and Antimicrobial Activities of Some Novel Heterocycles Utilizing 1,3-Diphenylpyrazole-4-carboxaldehyde Thiosemicarbazone. J Heterocycl Chem. 2018;55:1942.

[2] Ramadan SK, Abou-Elmagd WSI. Synthesis and anti H_5N_1 activities of some novel fused heterocycles bearing pyrazolyl moiety. Synth Commun. 2018;48(18):2409.

[3] Abou-Elmagd WSI, El-Ziaty AK, Ramadan SK, Elzahar MI, Hashem AI. Synthesis and antitumor activity evaluation of some *N*-heterocycles derived from pyrazolyl-substituted 2(3*H*)-furanone. Synth Commun. 2016;46(14):1197.

[4] (a) El-Ziaty AK, Abou-Elmagd WSI, Ramadan SK, Hashem AI. Synthesis and biological screening of some chromonyl-substituted heterocycles derived from 2(3*H*)-furanone derivative. Synth Commun. 2017;47(5):471. (b) Ramadan SK, El-Helw EAE. Synthesis and antimicrobial activity evaluation of some novel heterocycles derived from chromonyl-2(3*H*)-furanone. J. Chem. Res. 2018;42:332.

[5] Hashem AI, Ramadan SK, Abou-Elmagd WSI, El-Ziaty AK. Ring transformation of a 2(3*H*)-furanone derivative into oxazinone and pyrimidinone heterocycles. J Heterocycl Chem. 2017;54:3711.

[6] Youssef AM, El-Ziaty AK, Abou-Elmagd WSI, Ramadan SK. Novel Synthesis of Some Imidazolyl-, Benzoxazinyl-, and Quinazolinyl-2,4-dioxothiazolidine Derivatives. J Heterocycl Chem. 2015;52:278.

[7] El-Emary TI, Abdel-Mohsen ShA. Synthesis and Antimicrobial Activity of Some New 1,3-Diphenylpyrazoles Bearing Pyrimidine, Pyrimidinethione, Thiazolopyrimidine, Triazolopyrimidine, Thio- and Alkylthiotriazolopyrimidinone Moieties at the 4-Position. Phosphorus Sulfur Silicon. 2006;181:2459.

[8] Cheesbrough M. District Laboratory Practice in Tropical Countries; Cambridge University Press: Cambridge, UK; 2000; Part 2.

[9] Amr AE, Abo-Ghalia MH, Moustafa G, Al-Omar MA, Nossier ES, Elsayed EA. Design, Synthesis and Docking Studies of Novel Macrocyclic Pentapeptides as Anticancer Multi-Targeted Kinase Inhibitors. Molecules 2018;23(10):2416.