Synthesis of Novel Substituted Tetrahydropyrimidine Derivatives and Evaluation of Their Pharmacological and Antimicrobial Activities

Naglaa F. H. Mahmoud* and Eman A. Ghareeb

Chemistry Department, Faculty of Science, Ain Shams University, Abbassia, Cairo 11566, Egypt
*E-mail: naglaa.fawzy@yahoo.com

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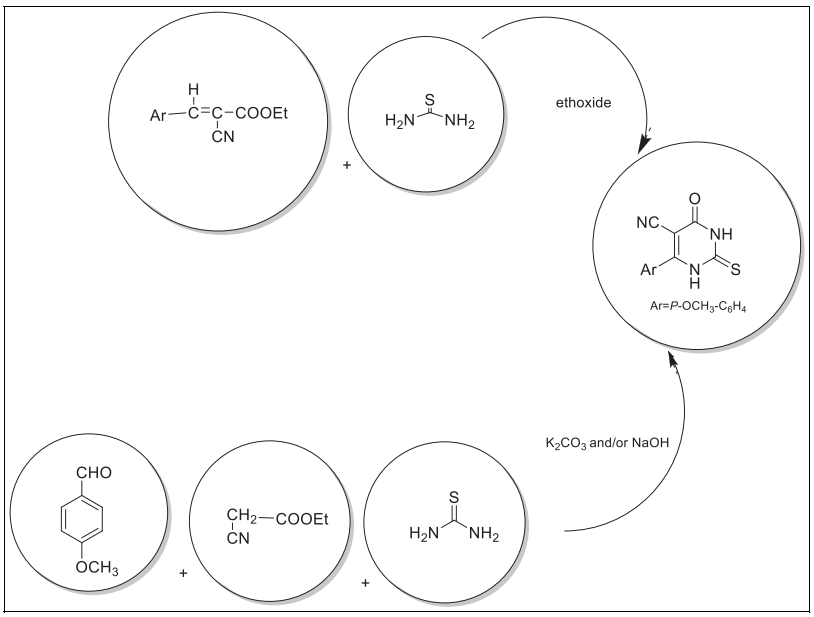

Tetrahydropyrimidine derivative **1** was employed as intermediate compound, which in turn was allowed to react with different electrophilic and nucleophilic reagents to synthesize new polyfunctionalized series of substituted pyrimidine-2-thione derivatives. Structures of the newly synthesized compounds have been elucidated by spectroscopic data and elemental analyses. The pharmacological and antimicrobial activities of synthesized products have been evaluated as drug candidates.

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INTRODUCTION

Heterocyclic compounds have been widely utilized in synthesis of pharmacologically active-compounds [1–4]. In particular, pyrimidines and fused pyrimidines as key building units of DNA and RNA revealed the fact that their derivatives exhibit diverse pharmacological activities such as anticancer [5], antiviral [6], anti-inflammatory [7], antioxidant [8], antibacterial,-and antifungal-agents [9]. Recently, it was reported that some series of 5-cyano-2-thiouracil derivatives showed antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* besides their remarkable antifungal activity against *Candida albicans* and *Aspergillus niger* [10,11]. Furthermore, thiouracils exhibited potential therapeutics as antiviral, anticancer, and antimicrobial agents [12–14]. For example, *S*-alkylation and *N*-alkylation products of pyrimidine-2-thione have been recently reported as novel antibacterial, cytotoxic agents [15,16], and unique HIV reverse transcriptase inhibitors-[17]. Moreover, 6-aryl-5-cyano-2-thiouracil derivatives and their condensed heterocycles exerted promising chemotherapeutic activities as antimicrobial and anticancer agents [18–21]. Because of all aforementioned facts, pyrimidine and thiouracil derivatives served as promising starting material to synthesize polyfunctionally pyrimidine derivatives. The antimicrobial, antioxidant, and cyctotoxic activities of newly synthesized compounds were evaluated.

RESULTS AND DISCUSSION

Chemistry. As stated vide supra and in continuation of our previous work on the synthesis of the biologically active compounds [22,23], herein, we investigated 6-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile **1** as a key starting material to design-and synthesize a library of biologically active series of polyfunctionalized pyrimidine-2-thione

derivatives. Therefore, compound **1** upon alkylation with different reagents such as ethyl iodide in the presence of sodium acetate using (1:1) molar ratio afforded *S*-alkylated product **2**. ^{1}H NMR of compound **2** showed signals at δ = 3.23 and 1.34 ppm due to CH_2 and CH_3 protons that ascertained its proposed feature.

Next, 4-aryl-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **3** was afforded either *via* the treatment of **1** with an excess amount of hydrazine hydrate and refluxed in ethanolic solution or treatment of *S*-alkylated product **2** with hydrazine hydrate. The IR spectrum exhibited strong absorption bands at υ = 3326, 3276 cm^{-1} (NH_2), 3186, 3111 cm^{-1} (NH), 2208 cm^{-1} (C═N), 1686 cm^{-1} (C═O). On the other hand, reaction of **1** with ethyl chloroacetate in the presence of anhydrous potassium carbonate afforded the ethyl ester derivative **4**, which in turn was subjected to react with hydrazine hydrate to give **3**.

Alkaline hydrolysis of tetrahydropyrimidine **1** using hydrogen peroxide in ammonium hydroxide gave dioxo tetrahydropyrimidine derivative **5**. Thus, IR spectrum of **5** showed two carbonyl groups signals at υ = 1684 and 1659 cm^{-1}.

Meanwhile, acid hydrolyses of **1** using concentrated sulfuric acid (70%) afforded the carboxylic acid derivative **6**. The structure of **6** was confirmed by the disappearance of a characteristic absorption band for cyano group in IR spectrum as well as appearance of an absorption band of a carbonyl of acidic group at υ = 1709 cm^{-1}.

Dimerization of compound **1** through disulphide linkage was achieved upon by oxidation when it was treated with iodine in an alkaline medium to give the corresponding disulphide derivative **7**. Mass spectrum of compound **7** showed its molecular ion peak at *m/z* 516.

Chlorination of **1** with a mixture of phosphorus pentachloride and phosphorus oxychloride as a chlorinating reagent gave chloropyrimidine derivative **8**. The structure of **8** was confirmed by the spectral data such as the IR that lacked a band corresponding to carbonyl group **(**Scheme 1**)**.

The chloropyrimidine **8** was used as a building block to synthesize new heterocyclic compounds. Thus, it underwent variant substitution and cyclization reactions when it was treated with primary amines such as anthranilic acid and/or *p*-aminoacetophenone to afford the corresponding pyrimidoquinazoline derivative **9** and aniline pyrimidine-2-thione derivative **10**, respectively (Scheme 2).

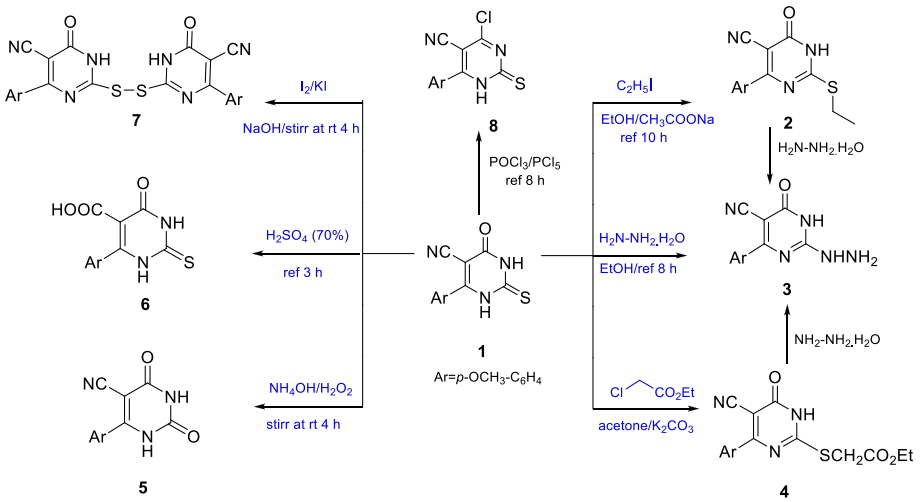
The structure of **9** and **10** were elucidated from their spectral data, where the IR spectrum of compound **9** exhibited a strong absorption band at υ = 1673 cm^{-1} corresponding to (C═O) group, while the mass spectrum revealed the molecular ion peak at *m/z* 360. On the other hand, the IR of compound **10** showed absorption band of carbonyl group (C═O) at υ = 1677 cm^{-1}, and mass spectrum exhibited the molecular ion peak at *m/z* 376.

Furthermore, when chloropyrimidine derivative **8** underwent thiation using thiourea and/or phosphorus pentasulfide, it afforded the dithioxo-1,2,3,4-tetrahydropyrimidine derivative **11**. The IR spectrum of compound **11** showed bands characteristic for NH and C═S groups at υ = 3127, 1256, and 1232 cm^{-1}. While the ^{1}H NMR exhibits two exchangeable broad singlet signals at δ = 14.13 and 8.05 ppm due to NH protons. Further evidence was gained from mass spectrum as it revealed the molecular ion peak at *m/z* 275.

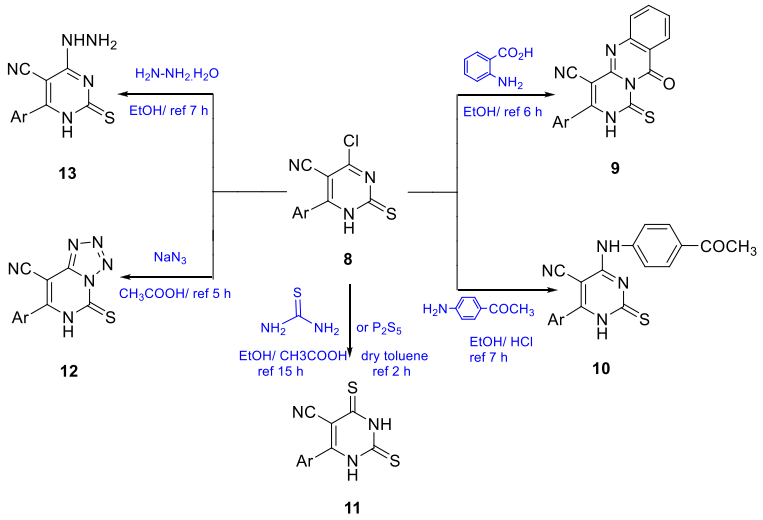
Treatment of chloropyrimidine derivative **8** with sodium azide and hydrazine hydrate gave tetrazolopyrimidine derivative **12** and 4-hydrazinyl pyrimidine derivative **13**, respectively (Scheme 2).

The mass spectrum of derivative **12** showed the molecular ion peak at *m/z* 285. Meanwhile, IR of 4-hydrazinyl pyrimidine **13** showed absorption bands at

Scheme 1 [Color figure can be viewed at wileyonlinelibrary.com]



Scheme 2 [Color figure can be viewed at wileyonlinelibrary.com]



υ = 3333, 3314, 3289, and 3170 cm^{-1} corresponding to NH_2 and NH. While ^1H NMR of compound **13** showed D_2O-exchangeable singlet signals at δ = 13.15, 8.29, and 4.74 ppm due to NH and NH_2 protons, hydrazinyl-6-oxo-1,6-dihydropyrimidine derivative **3** has been utilized as a versatile material for the synthesis of numbers of fused heterocyclic compounds. So the hydrazine derivative **3** was reacted with electrophilic reagents such as ethyl chloroacetate or chloroacetyl chloride, ethyl chloroformate, and/or acetylacetone to give the corresponding pyramido triazine derivative **14**, ethyl pyrimidine hydrazine carboxylate derivative **15**, and dimethyl pyrazol pyrimidine derivative **16**, respectively. The structure of compound **14** was confirmed by the IR spectrum that exhibited absorption bands at υ = 3261, 3087 cm^{-1} (NH), 1695, 1665 cm^{-1} (C═O). In addition, ^1H NMR of compound **14** showed D_2O-exchangeable singlet signals at δ = 12.20 and 9.97 ppm due to NH protons. Next, the reaction with ethyl chloroformate gave the product **15** that showed absorption band of carbonyl group (C═O) at υ = 1727 cm^{-1}, and ^1H NMR showed bands at δ = 4.10 and 1.25 ppm, corresponding to protons of ethyl group and molecular ion peak at *m/z* 329. The ^1H NMR of compound **16** showed singlet signals at δ = 11.42 ppm of (NH) exchangeable with D_2O and two singlet signals at δ = 2.64 and 2.24 ppm corresponding to six protons of two CH_3 groups.

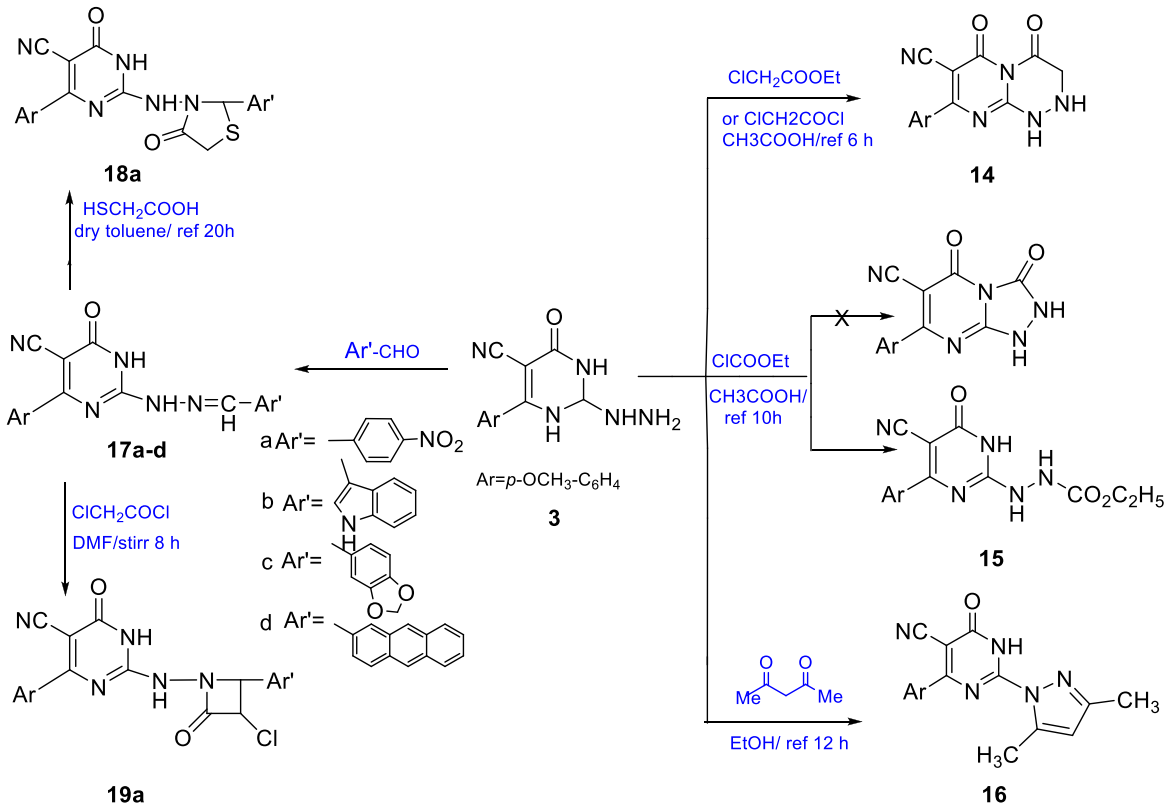
A series of 4-(4-methoxyphenyl)-6-oxo-2-(2-(substituted benzylidene)hydrazinyl)-1,6-dihydropyrimidine-5-carbonitrile **17a–d** derivatives were prepared from the reaction of **3** with different aromatic aldehydes in glacial acetic acid. The IR spectra of **17a–d** derivatives showed the disappearance of NH_2 band and appearance of a singlet signal corresponding to N═CH at δ = 9.41–8.01 ppm, respectively, in ^1H NMR is an indication about the structures and mass spectra showed the molecular ion peaks of **17a–d** derivatives at 390, 384, 389, and 445, respectively (Scheme 3).-.

Oxothiazolidine derivative **18a** and oxoazetidine derivative **19a** were formed when Schiff base **17a** was allowed to react with thioglycolic acid and/or chloroacetyl chloride, respectively. The IR spectrum of **18a** exhibited strong absorption bands at υ = 1693 and 1650 cm^{-1} for two carbonyl groups. ^1H NMR of compound **18a** showed singlet signals at δ = 5.24 and 3.58 ppm due to C—H and —CH_2 thiazolidinyl. Also, the IR of compound **19a** showed absorption bands at υ = 1691 and 1650 cm^{-1} of two carbonyl, oxoazetidine ring was unstable so easily broken and return to Schiff base so the mass spectrum showed the parent peak at *m/z* 390 (Scheme 3).

On the other hand, the synthesis of the novel compounds thiazolo pyrimidine derivatives **20** and **21** were achieved by reacting compound **1** with chloro acetic acid and/or chloroacetyl chloride. The structures of compounds **20** and **21** were confirmed by IR that showed the complete absence of NH absorption band and the appearance of the carbonyl absorption bands of oxo-thiazolo ring at υ = 1736 and 1730 cm^{-1}, respectively, as well as appearance of the singlet signal of ($COCH_2$) in ^1H NMR spectrum.

Moreover, 3-imino-thiazolo pyrimidine derivative **22** and 4-imino-pyrimido thiazine derivative **23** were prepared by refluxing **1** with chloroacetonitrile and/or acrylonitrile in ethanol and few drops of triethylamine. The proposed structures of compounds **22** and **23** were in accordance with the ^1H NMR spectra that showed a singlet signal at δ = 3.66 ppm of SCH_2 in compound **22**, also two triplet signals at δ = 3.51 and 3.03 ppm for CH_2—CH_2 protons in compound **23**.

Scheme 3 [Color figure can be viewed at wileyonlinelibrary.com]



Finally, treatment of compound **1** with oxalyl chloride in tetrahydrofurane afforded trioxo thiazolo pyrimidine derivative **24** *via* nucleophilic attacks upon the carbonyl carbon atom of oxalyl chloride. Compound **24** was confirmed *via* the appearance of two bands at υ = 1754 and 1739 cm^{-1} of the two carbonyl groups of the thiazolidinone ring (Scheme 4).

Antimicrobial Activity. ***Antibacterial activity evaluation.*** The antimicrobial activities of the novel compounds have been screened using agar diffusion method [24,25]. The synthesized compounds have been tested for their antibacterial activities against *S. aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *C. albicans* (yeast), and *A. niger* (fungus). The obtained results are compiled in

Scheme 4 [Color figure can be viewed at wileyonlinelibrary.com]

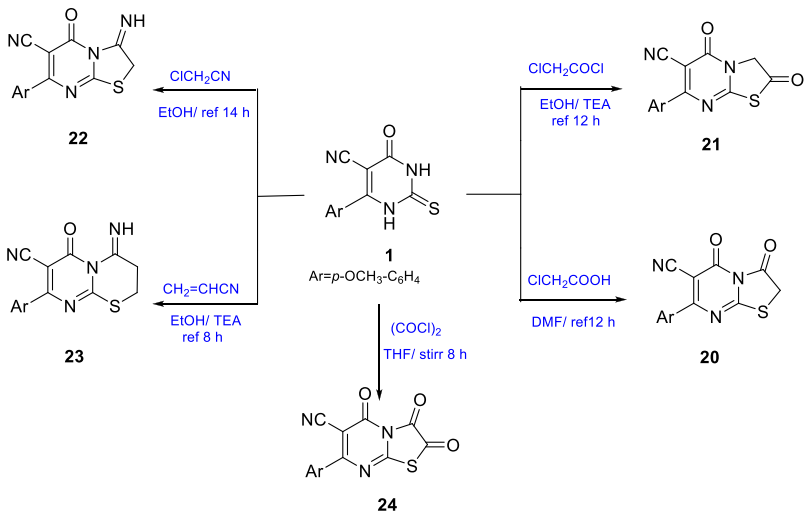


Table 1

Antimicrobial activity of synthesized compounds.

Sample name	Clear zone (ϕmm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
2	20	19	18	15
5	28	30	16	14
6	14	18	18	0
7	16	14	14	0
8	14	18	18	0
9	14	16	15	0
10	15	18	14	0
11	17	14	17	0
12	14	21	15	14
13	25	23	26	0
14	13	0	15	13
15	21	22	16	16
16	29	31	31	13
17a	13	13	0	0
17b	18	16	0	0
17c	15	19	0	15
17d	22	23	23	10
18a	13	13	0	0
19a	14	15	14	16
20	18	20	16	14
22	22	24	15	14
23	14	16	17	14
24	10	15	15	0
Streptomcin	30	28	25	15
Cyclohexamide	00	00	22	37

Table 1. It is obvious that most of the compounds show moderate to high antimicrobial activities. In particular, compounds **5**, **13**, **16**, **17d**, and **22** showed remarkable antimicrobial activity, which could consider that those compound are having the chance to be candidates as antimicrobial agents.

Pharmacological Activity. ***Determination of total antioxidant capacity.*** The total antioxidant capacity of the tested compounds was estimated *via* phosphomolybdenum antioxidant technique. The total antioxidant capacity values equivalent to ascorbic acid in mg ascorbic acid equivalent (AAE)/g compound are presented in Table 2. The obtained values were varied and ranged from 121.65 to 824.0 mg AAE/g compound. Moreover, the results revealed that compounds **7**, **5**, **6**, and **9** showed the most potent activity followed by compounds **24**, **22**, **17d**, **19a**, **15**, **14**, **16**, **17a**, **18a**, **17b**, and **17c** having moderate activity, respectively. On the other hand, compounds **20**, **2**, **23**, and **8** showed the lowest activity, respectively.

From the aforementioned results, we can conclude that the tested compounds have different abilities on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acid pH, which may be due to the variation in the chemical structures of tested compounds and to the presence or absence of certain substituents like carbonyl, hydroxyl, methoxy, and carboxylic.

Cytotoxic activity of some compounds against human tumor cells. The prepared compounds were tested for cytotoxic activities against three human tumor cells. The best results

Table 2

Total antioxidant capacity values of the synthesized compounds.

Compound No.	Total antioxidant capacity (mg AAE/g compound)
2	218.64 ± 2.01
5	560.47 ± 2.95
6	547.78 ± 3.40
7	824.0 ± 4.04
8	121.65 ± 3.25
9	510.32 ± 5.10
11	317.47 ± 3.49
12	303.00 ± 4.03
13	281.81 ± 3.25
14	421.82 ± 2.95
15	431.65 ± 4.50
17a	409.03 ± 3.40
17b	381.57 ± 2.02
17c	373.42 ± 2.35
17d	446.61 ± 2.02
18a	397.24 ± 3.40
19a	432.63 ± 1.23
20	238.45 ± 3.49
22	452.30 ± 4.50
23	134.96 ± 2.02
24	481.51 ± 4.89

Results are (means ± SD) (n = 3). AAE, ascorbic acid equivalent.

were observed for compounds **13**, **14**, **15**, and **17b**, which were found very strong cytotoxic compounds. Compounds **11**, **16**, **17a**, and **17d** were also considered strongly cytotoxic. Finaly, compounds **2**, **9**, **10**, **12**, **20**, **22**, and **23** were moderate in their cytotoxic activity, and compounds **5**, **6**, **7**, **8**, **17c**, and **18a** showed weak cytotoxicity (Table 3).

EXPERIMENTAL

All melting points were measured on a Gallenkamp electric melting point apparatus (Germany) uncorrected.-The infrared spectra were recorded in potassium bromide disks on a pyeUnicam SP-3-300 (Cambridge, England) and Shimadzu FTIR 8101 PC infrared spectrophotometers (Shimadzu Corp., Kyoto, Japan).

The ^{1}H NMR and ^{13}C NMR were recorded at a Varian Mercury VX-300 MHz and 75 MHz (Varian, Inc., Palo Alto, CA), respectively, using TMS as internal standard in deuterated chloroform ($CDCl_3$) or deuterateddimethylsulphoxide (DMSO-d_6). Chemical shifts are measured in ppm. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were carried out at the microanalytical center of Cairo University. All the reactions and the purity of the new compounds were followed and checked by TLC using TLC aluminum sheets silica gel F_{254}.

Table 3

Cytotoxic activity of some compounds against human tumor cells.

Compounds	*In vitro* cytotoxicity IC_{50} (μM)		
	HePG2	HCT-116	MCF-7
DOX	4.50 ± 0.2	5.23 ± 0.3	4.17 ± 0.2
2	21.31 ± 1.8	23.90 ± 2.0	27.43 ± 2.5
5	68.81 ± 4.1	65.16 ± 3.9	58.12 ± 4.2
6	93.35 ± 5.3	>100	84.25 ± 5.1
7	78.56 ± 4.5	81.14 ± 4.7	71.36 ± 4.4
8	61.34 ± 3.6	44.06 ± 3.2	48.71 ± 3.9
9	49.27 ± 2.9	41.62 ± 3.0	33.54 ± 3.1
10	24.75 ± 2.1	31.48 ± 2.4	28.67 ± 2.7
11	13.62 ± 1.2	14.28 ± 1.5	17.09 ± 1.6
12	32.69 ± 2.5	38.55 ± 2.8	42.28 ± 3.6
13	3.48 ± 0.2	6.45 ± 0.4	7.28 ± 0.6
14	11.35 ± 1.0	9.14 ± 0.8	7.91 ± 0.7
15	5.49 ± 0.3	8.23 ± 0.7	9.34 ± 0.9
16	19.12 ± 1.6	22.41 ± 1.8	25.11 ± 2.2
17a	9.80 ± 0.8	12.72 ± 1.3	14.50 ± 1.4
17b	7.74 ± 0.5	10.53 ± 1.1	11.87 ± 1.2
17c	85.92 ± 4.7	89.26 ± 4.8	76.80 ± 4.6
17d	15.07 ± 1.4	18.79 ± 1.6	20.58 ± 1.8
18a	73.55 ± 4.3	69.24 ± 4.1	63.84 ± 4.2
20	28.13 ± 2.4	35.07 ± 2.7	30.59 ± 2.8
22	46.20 ± 2.8	52.39 ± 3.5	55.36 ± 4.0
23	54.01 ± 3.1	62.48 ± 3.9	36.03 ± 3.3

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

2-(Ethylthio)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2). An equimolar mixture of pyrimidine **1** (0.01 mol, 2.59 g) and ethyl iodide (0.01 mol, 1.56 mL) in ethanol containing sodium acetate was heated under reflux for 10 h. The reaction mixture was cooled and poured onto ice. The formed precipitate was filtered, dried, and crystallized from benzene to give **2**.

Yield (71%), mp 244–246°C. *Anal*. Calcd for $C_{14}H_{13}N_3O_2S$ (287): C, 58.53; H, 4.52; N, 14.63. Found: C, 58.26; H, 4.32; N, 14.83. FTIR (KBr) (cm^{-1}): 3134 (NH), 2941, 2849 (C—H aliphatic), 2224 (CN), 1673 (C=O), 1602 (C=C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 13.60 (s, 1H, NH, D_2O exchangeable), 8.01 (d, J = 6.82 Hz, 2H, Ar—H), 7.13 (d, J = 6.82 Hz, 2H, Ar—H), 3.86 (s, 3H, OCH_3), 3.23 (q, 2H, CH_2), 1.34 (t, J = 6.82 Hz, 3H, CH_3). MS, *m/z* (%): 287 ($M^{\cdot+}$, 100%).

2-Hydrazinyl-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3). A mixture of pyrimidine **1** (0.01 mol, 2.59 g) and hydrazine hydrate (0.04 mol, 2 mL) in ethanol was heated under reflux for 8 h. The formed precipitate was filtered, dried, and crystallized from dioxan to give **3**.

Yield (52%), mp 236–240°C. *Anal*. Calcd for $C_{12}H_{11}N_5O_2$ (257): C, 56.03; H, 4.28; N, 27.23. Found: C, 56.47; H, 4.45; N, 26.83. FTIR (KBr) (cm^{-1}): 3326, 3276 (NH_2), 3186, 3111 (NH), 2208 (CN), 1686 (C=O). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 10.15 (s, 1H, NH, D_2O exchangeable), 8.2 (s, 1H, NH, D_2O exchangeable), 7.83–6.99 (m, 4H, Ar—H), 6.8 (s, 2H, NH_2, D_2O exchangeable), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 257 ($M^{\cdot+}$, 100%).

Ethyl 2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-2-yl)thio)acetate (4). A mixture of pyrimidine **1** (0.01 mol, 2.59 g) and ethyl chloroacetate (0.01 mol, 1.22 mL) in acetone containing potassium carbonate anhydrous was heated under water steam for 7 h. The reaction mixture was allowed to cool and then acidified by HCl after pouring into water/ice. The formed precipitate was filtered, dried, and crystallized from benzene to give **4**.

Yield (86%), mp 220–222°C. *Anal*. Calcd for $C_{16}H_{15}N_3O_4S$ (345): C, 55.65; H, 4.38; N, 12.17. Found: C, 55.33; H, 3.98; N, 11.95. FTIR (KBr) (cm^{-1}): 3079 (NH), 2219 (CN), 1726, 1667 (C=O). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 13.7 (s, 1H, NH, D_2O exchangeable), 7.96 (d, J = 6.82 Hz, 2H, Ar—H), 7.08 (d, J = 6.82 Hz, 2H, Ar—H), 4.11(q, 2H, CH_2), 4.01 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 1.12 (t, J = 6.82 Hz, 3H, CH_3). MS, *m/z* (%): 344 ($M^+ - 1$, 100%).

6-(4-Methoxyphenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (5). A mixture of pyrimidine **1** (0.01 mol, 2.59 g), ammonia solution (12.5 mL), and hydrogen peroxide (3.5 mL) was stirred at room temperature for 4 h. The

reaction mixture was left overnight; the formed precipitate was filtered, dried, and crystallized from ethanol to give **5**.

Yield (42%), mp over 300°C. *Anal*. Calcd for $C_{12}H_9N_3O_3$ (243): C, 59.25; H, 3.70; N, 17.28. Found: C, 59.48; H, 4.09; N, 16.85. FTIR (KBr) (cm^{-1}): 3138 (NH), 2221 (CN), 1684, 1659 (C═O). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.52 (s, 1H, NH, D_2O exchangeable), 10.5 (s, 1H, NH, D_2O exchangeable), 7.34–6.88 (m, 4H, Ar─H), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 243 ($M^{\cdot+}$, 16%), 242 (100%).

6-(4-Methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimine-5-carboxylic acid (6). A solution of pyrimidine **1** (0.01 mol, 2.59 g) in sulfuric acid 70% (30 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool and then neutralized by NH_4OH after pouring onto ice. The formed precipitate was filtered, dried, and crystallized from ethanol to give **6**.

Yield (50%), mp 146–148°C. *Anal*. Calcd for $C_{12}H_{10}N_2O_4S$ (278): C, 51.79; H, 3.59; N, 10.07. Found: C, 51.39; H, 3.19; N, 10.47. FTIR (KBr) (cm^{-1}): broad band at 3394 (OH, NH), 1709, 1651 (C═O). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.52 (s, 1H, OH), 11.82 (s, 1H, NH, D_2O exchangeable), 10.15 (s, 1H, NH, D_2O exchangeable), 7.98–7.15 (m, 4H, Ar─H), 3.81(s, 3H, OCH_3). MS, *m/z* (%): 278 ($M^{\cdot+}$, 20%).

2,2′-Disulfanediylbis(4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile) (7). A solution of iodine (0.01 mol, 2.34 g) in potassium iodide solution (0.01 mol, 1.66 g) was added to compound **1** (0.01 mol, 2.59 g) in 10% sodium hydroxide solution (20 mL) and was stirred at room temperature for 4 h. The formed precipitate was collected and crystallized from ethanol to give **7**.

Yield (12%), mp 286–289°C. *Anal*. Calcd for $C_{24}H_{16}N_6O_4S_2$ (516): C, 55.81; H, 3.10; N, 16.27. Found: C, 56.11; H, 3.34; N, 15.97. FTIR (KBr) (cm^{-1}): 3148 (NH), 2205 (CN), 1673 (C═O). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.52 (s, 2H, 2NH, D_2O exchangeable), 8.18–7.08 (m, 8H, Ar─H), 3.86 (s, 6H, OCH_3). MS, *m/z* (%): 516 ($M^{\cdot+}$, 6), 257 (14).

4-Chloro-6-(4-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (8). A mixture of pyrimidine **1** (0.01 mol, 2.59 g), phosphorous oxychloride (10 mL), and phosphorous pentachloride (1.5 g) was heated under water steam for 8 h. The reaction mixture was allowed to cool and then poured onto ice. The formed precipitate was filtered, dried, and crystallized from ethanol to give **8**.

Yield (92%), mp 172–174°C. *Anal*. Calcd for $C_{12}H_8N_3OSCl$ (277): C, 51.98; H, 2.88; N, 15.16. Found: C, 51.64; H, 3.09; N, 14.93. FTIR (KBr) (cm^{-1}): 3127 (NH), 2222 (CN), 1624 (C═N), 1262 (C═S). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.52 (s, 1H, 1NH, D_2O exchangeable), 8.2–7.7 (m, 4H, Ar─H), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 245 ($M^{\cdot+}$−S, 100%).

3-(4-Methoxyphenyl)-10-oxo-1-thioxo-2,10-dihydro-1*H*-pyrimido[6,1-b]quinazoline-4-carbonitrile (9). A mixture of **8** (0.01 mol, 2.77 g) and anthranilic acid (0.01 mol, 1.37 g) in ethanol was heated under reflux for 6 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **9**.

Yield (25%), mp 249–250°C. *Anal*. Calcd for $C_{19}H_{12}N_4O_2S$ (360): C, 63.33; H, 3.33; N, 15.55. Found: C, 62.92; H, 3.64; N, 15.85. FTIR (KBr) (cm^{-1}): 3180 (NH), 2209 (CN), 1673 (C═O), 1605 (C═C), 1256 (C═S). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 11.93 (s, 1H, 1NH, D_2O exchangeable), 8.65–7.11 (m, 8H, Ar─H), 3.86 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6), δ ppm 169.53, 162.18, 161.59, 160.36, 158.33, 140.85, 133.47, 133.26, 131.12, 130.99, 130.54, 128.37, 122.35, 122.02, 120.75, 120.53, 120.48, 113.88, 55.45. MS, *m/z* (%): 360 ($M^{\cdot+}$, 100%).

4-((4-Acetylphenyl)amino)-6-(4-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (10). A mixture of **8** (0.01 mol, 2.77 g) and *p*-amino acetophenone (0.01 mol, 1.35 g) in ethanol containing drops of Conc HCl was heated under reflux for 7 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **10**.

Yield (60%), mp 284–286°C. *Anal*. Calcd for $C_{20}H_{16}N_4O_2S$ (376): C, 63.82; H, 4.25; N, 14.89. Found: C, 63.59; H, 4.05; N, 15.29. FTIR (KBr) (cm^{-1}): 3307, 3127 (NH), 2212 (CN), 1677 (C═O), 1602 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 14.14 (s, 1H, NH, D_2O exchangeable), 11.20 (s, 1H, NH, D_2O exchangeable), 7.87–6.81 (m, 8H, Ar─H), 3.86 (s, 3H, OCH_3), 1.07 (s, 3H, CH_3). MS, *m/z* (%): 376 ($M^{\cdot+}$, 100%).

6-(4-Methoxyphenyl)-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11). A mixture of **8** (0.01 mol, 2.77 g) and thiourea (0.01 mol, 0.76 g) in ethanol containing drops of glacial acetic acid (five drops) was heated under reflux for 15 h. The formed precipitate was filtered, dried, and crystallized from butanol to give **11**. Yield (73%).

A mixture of **8** (0.01 mol, 2.77 g) and phosphorus pentasulphide (0.01 mol, 2.22 g) in dry toluene was heated under reflux for 2 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **11**.

Yield (60%), mp 291–293°C. *Anal*. Calcd for $C_{12}H_9ON_3S_2$ (275): C, 52.36; H, 3.27; N, 15.27. Found: C, 52.68; H, 2.87; N, 14.87. FTIR (KBr) (cm^{-1}): 3127 (NH), 2224 (CN), 1606 (C═C), 1256, 1232 (C═S). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 14.13 (s, 1H, NH, D_2O exchangeable), 8.05 (s, 1H, NH, D_2O exchangeable), 7.69 (d, J = 6.82 Hz, 2H, Ar─H), 7.12 (d, J = 6.82 Hz, 2H, Ar─H), 3.87 (s, 3H, OCH_3). MS, *m/z* (%): 275 ($M^{\cdot+}$, 100%).

7-(4-Methoxyphenyl)-5-thioxo-5,6-dihydrotetrazolo[1, 5-c]pyrimidine-8-carbonitrile (12). A mixture of **8** (0.01 mol, 2.77 g) and sodium azide (0.01 mol, 0.65 g) in glacial acetic acid was heated under reflux for 5 h. The

reaction mixture was allowed to cool and then poured into ice/water. The formed precipitate was filtered, dried, and crystallized from ethanol to give **12**.

Yield (51%), mp over 300°C. *Anal*. Calcd for $C_{12}H_8N_6OS$ (284): C, 50.70; H, 2.81; N, 29.57. Found: C, 52.09; H, 2.40; N, 29.29. FTIR (KBr) (cm^{-1}): 3166 (NH), 2220 (CN), 1604 (C═C), 1258 (C═S). ^1H NMR (DMSO-d_6): δ_H (ppm) 13.02 (s, 1H, NH, D_2O exchangeable), 7.99 (d, J = 6.82 Hz, 2H, Ar—H), 7.32 (d, J = 6.82 Hz, 2H, Ar—H), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 285 ($M^{\cdot+}$+1, 100%).

4-Hydrazinyl-6-(4-methoxy phenyl)-2-thioxo-1,2-dihydro-pyrimidine-5-carbonitrile (13). A mixture of **8** (0.01 mol, 2.77 g) and hydrazine hydrate (0.04 mol, 2 mL) in ethanol was heated under reflux for 7 h. The reaction mixture was allowed to cool. The formed precipitate was filtered, dried, and crystallized from ethanol to give **13**.

Yield (55%), mp 224–226°C. *Anal*. Calcd for $C_{12}H_{11}N_5OS$ (273): C, 52.74; H, 4.02; N, 25.64. Found: C, 53.25; H, 3.85; N, 26.96. FTIR (KBr) (cm^{-1}): 3333, 3314, 3289, 3170 (NH_2, NH), 2194 (CN), 1609 (C═C), 1265 (C═S). ^1H NMR (DMSO-d_6): δ_H (ppm) 13.15 (s, 1H, NH, D_2O exchangeable), 8.29 (s, 1H, NH, D_2O exchangeable), 7.69 (d, J = 6.82 Hz, 2H, Ar—H), 7.12 (d, J = 6.82 Hz, 2H, Ar—H), 4.74 (s, 2H, NH_2, D_2O exchangeable), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 273 ($M^{\cdot+}$, 18%), (271, 100%).

8-(4-Methoxyphenyl)-4,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrimido[2,1-c][1,2,4]triazine-7-carbonitrile (14). A mixture of **3** (0.01 mol, 2.57 g) and ethylchloroacetate or chloroacetyl chloride (0.01 mol) in glacial acetic acid was heated under reflux for 6 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **14**.

Yield (28%), mp 270–272°C. *Anal*. Calcd for $C_{14}H_{11}N_5O_3$ (297): C, 56.56; H, 3.70; N, 23.56. Found: C, 56.81; H, 3.35; N, 23.26. FTIR (KBr) (cm^{-1}): 3261, 3087 (NH), 2218 (CN), 1695, 1665 (C═O), 1605 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 12.20 (s, 1H, 1NH, D_2O exchangeable), 9.97 (s, 1H, 1NH, D_2O exchangeable), 7.90 (d, J = 6.82 Hz, 2H, Ar—H), 7.05 (d, J = 6.82 Hz, 2H, Ar—H), 3.83 (s, 3H, OCH_3), 1.91 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6), δ ppm 169.67, 162.21, 161.67, 156.88, 148.54, 130.14, 128.11, 117.22, 113.63, 91.3, 55.34, 40.28, 40.00, 20.89. MS, *m/z* (%): 299 ($M^{\cdot+}$ +2, 51.7), (257, 100%).

Ethyl 2-(5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydro-pyrimidine-2-yl)hydrazine-1-carboxylate (15). A mixture of **3** (0.01 mol, 2.57 g) and ethylchloroformate (0.02 mol, 2.16 mL) in glacial acetic acid was heated under reflux for 10 h. The reaction mixture was allowed to cool and then poured onto ice/water. The formed precipitate was filtered, dried, and crystallized from ethanol to give **15**.

Yield (50%), mp 154–156°C. *Anal*. Calcd for $C_{15}H_{15}N_5O_4$ (329): C, 54.71; H, 4.55; N, 21.27. Found: C, 54.98; H, 4.32; N, 21.53. FTIR (KBr) (cm^{-1}): broad band centered at 3232 (3NH), 2223 (CN), 1727, 1651 (C═O), 1605 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 12.36 (s, 1H, NH, D_2O exchangeable), 10.25 (s, 1H, NH, D_2O exchangeable), 9.50 (s, 1H, NH, D_2O exchangeable), 7.89 (d, J = 6.82 Hz, 2H, Ar—H), 7.09 (d, J = 6.82 Hz, 2H, Ar—H), 4.10 (q, 2H, CH_2), 3.86 (s, 3H, OCH_3), 1.25 (t, J = 6.82 Hz, 3H, CH_3). MS, *m/z* (%): 329 ($M^{\cdot+}$, 100%).

2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (16). A mixture of hydrazino **3** (0.01 mol, 2.57 g) and acetyl acetone (0.01 mol, 0.98 mL) in absolute ethanol was heated under reflux for 12 h. The formed precipitate was filtered, dried, and crystallized from dioxan to give **16**.

Yield (72%), mp 230–232°C. *Anal*. Calcd for $C_{17}H_{15}N_5O_2$ (321): C, 63.55; H, 4.67; N, 21.80. Found: C, 63.83; H, 4.76; N, 21.65. FTIR (KBr) (cm^{-1}): 3339 (NH), 2218 (CN) 1669 (C═O), 1593 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 11.42 (s, 1H, NH, D_2O exchangeable), 8.03 (d, J = 6.82 Hz, 2H, Ar—H), 7.13 (d, J = 6.82 Hz, 2H, Ar—H), 6.30 (s, 1H, CH═C pyrazol), 3.88 (s, 3H, OCH_3), 2.64 (s, 3H, CH_3), 2.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6), δ ppm 167.73, 162.25, 161.65, 152.46, 148.54, 143.81, 130.57, 127.13, 116.02, 114.01, 112.15, 91.31, 55.43, 40.33, 40.06, 14.88, 13.29. MS, *m/z* (%): 321 ($M^{\cdot+}$, 100%).

4-(4-Methoxyphenyl)-6-oxo-2-(2-(4-nitrobenzylidene) hydrazinyl)-1,6-dihydropyrimidine-5-carbonitrile (17a). A mixture of **3** (0.01 mol, 2.57 g) and *p*-nitrobenzaldehyde (0.01 mol, 1.51 mL) in glacial acetic acid was heated under reflux for 6 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **17a**.

Yield (85%), mp over 300°C. *Anal*. Calcd for $C_{19}H_{14}N_6O_4$ (390): C, 58.46; H, 3.58; N, 21.53. Found: C, 58.67; H, 3.31; N, 21.73. FTIR (KBr) (cm^{-1}): 3353, 3264 (NH), 2214 (CN), 1655 (C═O), 1606 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 12.48 (s, 1H, 1NH, D_2O exchangeable), 11.26 (s, 1H, 1NH, D_2O exchangeable), 8.38 (s, 1H, CH═N), 8.27–8.21 (m, 4H, Ar—H), 7.92 (d, J = 6.82 Hz, 2H, Ar—H), 7.08 (d, J = 6.82 Hz, 2H, Ar—H), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6), δ ppm 169.67, 162.21, 161.71, 158.13, 147.85, 144.21, 139.91, 130.20, 128.78, 123.4 (2), 123.1 (2), 117.15, 113.63 (2), 91.3, 55.36, 40.05. MS, *m/z* (%): 390 ($M^{\cdot+}$, 93), (268, 100%).

2-(2-((1*H*-indol-3yl)methylene)hydrazinyl)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (17b). A mixture of **3** (0.01 mol, 2.57 g) and indol-3-aldehyde (0.01 mol, 1.44 g) in glacial acetic acid was heated under reflux for 6 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **17b**.

Yield (79%), mp over 300°C. *Anal*. Calcd for $C_{21}H_{16}N_6O_2$ (384): C, 65.62; H, 4.16; N, 21.87. Found: C, 66.91; H, 4.47; N, 21.66. FTIR (KBr) (cm^{-1}): 3378, 3269, 3142 (NH), 2217 (CN), 1667 (C═O), 1633 (C═N), 1603 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.20 (s, 1H, NH, D_2O exchangeable), 11.72 (s, 1H, 1NH, D_2O exchangeable), 11.42 (s, 1H, NH, D_2O exchangeable), 8.44 (s, 1H, CH═N), 8.37–7.07 (m, 9H, 8Ar─H + CH indolyl), 3.85 (s, 3H, OCH_3). MS, *m/z* (%): 384 ($M^{\cdot+}$, 72), (142, 100%).

2-(2-(Benzo[*d*][1,3]dioxol-5-yl)methylene)hydrazinyl)-4-(4-methoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (17c). A mixture of **3** (0.01 mol, 2.57 g) and piperonal (0.01 mol, 1.5 g) in glacial acetic acid was heated under reflux for 6 h. The formed precipitate was filtered, dried, and crystallized from ethanol to give **17c**.

Yield (43%), mp over 300°C. *Anal*. Calcd for $C_{20}H_{15}N_5O_4$ (389): C, 61.69; H, 3.85; N, 17.99. Found: C, 62.25; H, 3.51; N, 18.30. FTIR (KBr) (cm^{-1}): 3213, 3142 (NH), 2207 (CN), 1662 (C═O), 1630 (C═N), 1601 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 12.28 (s, 2H, 2NH, D_2O exchangeable), 8.07 (s, 1H, CH═N), 8.02–6.92 (m, 7H, Ar─H), 6.07 (s, 2H, OCH_2O), 3.83 (s, 3H, OCH_3). MS, *m/z* (%): 389 ($M^{\cdot+}$, 100%).

2-(2-(Anthracen-1-ylmethylene)hydrazinyl)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (17d). A mixture of **3** (0.01 mol, 2.57 g) and anthraldehyde (0.01 mol, 2.06 g) in butanol (3 mL) was fused at temperature not more than 200°C. The formed precipitate was filtered, dried, and crystallized from ethanol to give **17d**.

Yield (59%), mp 282–284°C. *Anal*. Calcd for $C_{27}H_{19}N_5O_2$ (445): C, 72.80; H, 4.26; N, 15.73. Found: C, 73.11; H, 4.46; N, 15.45. FTIR (KBr) (cm^{-1}): 3342, 3285 (NH), 2212 (CN), 1655 (C═O), 1633 (C═N), 1603 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 11.25 (s, 1H, NH, D_2O exchangeable), 10.62 (s, 1H, NH, D_2O exchangeable), 9.41 (s, 1H, CH═N), 8.71–7.04 (m, 13H, Ar─H), 3.85 (s, 3H, OCH_3). MS, *m/z* (%): 445 ($M^{\cdot+}$, 40).

4-(4-Methoxyphenyl)-2-((2-(4-nitrophenyl)-4-oxothiazolidin-3yl)amino)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (18a). A mixture of hydrazinyl **17a** (0.01 mol, 4.61 g) and thioglycolic acid (0.01 mol, 0.92 mL) in dry toluene was heated under reflux for 20 h. The reaction mixture was concentrated and was allowed to cool. The formed precipitate was filtered, dried, and crystallized from ethanol to give **18a**.

Yield (69%), mp over 300°C. *Anal*. Calcd for $C_{21}H_{16}N_6O_5S$ (464): C, 54.31; H, 3.44; N, 18.10. Found: C, 54.70; H, 3.34; N, 17.86. FTIR (KBr) (cm^{-1}): 3142, 3105 (NH), 2223 (CN), 1693, 1650 (C═O), 1619 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 11.32 (s, 1H, NH, D_2O exchangeable), 10.52 (s, 1H, NH, D_2O exchangeable), 8.26–7.07 (m, 8H, Ar─H), 5.24 (s, 1H, CH thiazolidinyl), 3.84 (s, 3H, OCH_3), 3.58 (s, 2H, CH_2 thiazolidinyl). MS, *m/z* (%): 466 ($M^{\cdot+}$+2, 60).

2-((3-Chloro-2-(4-nitrophenyl)-4-oxoazetidine-1yl)amino)-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (19a). A mixture of **17a** (0.01 mol, 4.61 g) and chloroacetyl chloride (0.01 mol, 11.2 mL) in dimethylformamide was stirred for 8 h, and the reaction mixture was left overnight. The reaction mixture was poured onto cold water. The formed precipitate was filtered, dried, and crystallized from ethanol to give **19a**.

Yield (30%), mp over 300°C. *Anal*. Calcd for $C_{21}H_{15}N_6O_5Cl$ (466): C, 54.07; H, 3.21; N, 18.02. Found: C, 54.31; H, 3.39; N, 17.68. FTIR (KBr) (cm^{-1}): 3228, 3109 (NH), 2219 (CN), 1691, 1650 (C═O), 1604 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 11.52 (s, 1H, NH, D_2O exchangeable), 10.25 (s, 1H, NH, D_2O exchangeable), 8.26–6.88 (m, 8H, Ar─H), 5.64 (s, 1H, CHCl), 5.02 (s, 1H, NCH), 3.84 (s, 3H, OCH_3). MS, *m/z* (%): 390 ($M^{\cdot+}$, 100%).

7-(4-Methoxyphenyl)-3,5-dioxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (20). A mixture of pyrimidine **1** (0.01 mol, 2.59 g) and chloroacetic acid (0.01 mol, 0.95 mL) in DMF (30 mL) was heated under reflux for 12 h. The reaction mixture was concentrated, was allowed to cool, and was poured onto ice. The formed precipitate was filtered, dried, and crystallized from ethanol to give **20**.

Yield (50%), mp over 300°C. *Anal*. Calcd for $C_{14}H_9N_3O_3S$ (299): C, 56.18; H, 3.01; N, 14.04. Found: C, 56.40; H, 3.41; N, 14.34. FTIR (KBr) (cm^{-1}): 2230 (CN), 1736, 1672 (C═O), 1605 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 8.02–7.34 (m, 4H, Ar─H), 4.20 (s, 2H, SCH_2), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 299 ($M^{\cdot+}$, 7), (134, 100%).

7-(4-Methoxyphenyl)-2,5-dioxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (21). A mixture of pyrimidine **1** (0.01 mol, 2.59 g), chloroacetyl chloride (0.01 mol, 11.2 mL) in ethanol (30 mL), and triethylamine were heated under reflux for 12 h. The reaction mixture was concentrated, was allowed to cool, and was poured onto ice/HCl. The formed precipitate was filtered, dried, and crystallized from ethanol to give **21**.

Yield (43%), mp over 300°C. *Anal*. Calcd for $C_{14}H_9N_3O_3S$ (299): C, 56.18; H, 3.01; N, 14.04. Found: C, 56.32; H, 3.21; N, 14.27. FTIR (KBr) (cm^{-1}): 2219 (CN), 1730, 1673 (C═O), 1604 (C═C). ^{1}H NMR (DMSO-d_6): δ_H (ppm) 8.02–7.34 (m, 4H, Ar─H), 5.11 (s, 2H, CH_2C═O), 3.86 (s, 3H, OCH_3). MS, *m/z* (%): 299 ($M^{\cdot+}$, 25).

3-Imino-7-(4-methoxyphenyl)-5-oxo-2,5-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (22). To-a solution of pyrimidine **1** (0.01 mol, 2.59 g) in ethanol (30 mL), chloroacetonitrile (0.01 mol, 0.75 mL) was added dropwise with continuous stirring during 30 min in the presence of drops of triethylamine, and then the

mixture was heated under reflux for 14 h. The reaction mixture was neutralized by HCl after pouring onto ice/water. The formed precipitate was filtered, dried, and crystallized from ethanol to give **22**.

Yield (53%), mp 154–156°C. *Anal*. Calcd for $C_{14}H_{10}N_4O_2S$ (298): C, 56.37; H, 3.35; N, 18.79. Found: C, 56.60; H, 3.15; N, 19.11. FTIR (KBr) (cm^{-1}): 3437 (NH), 2218 (CN), 1678 (C═O), 1601 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 10.25 (s, 1H, NH, D_2O exchangeable), 8.02–7.34 (m, 4H, Ar—H), 3.86 (s, 3H, OCH_3), 3.66 (s, 2H, SCH_2). MS, *m/z* (%): 298 ($M^{\cdot+}$, 100%).

4-Imino-8-(4-methoxyphenyl)-6-oxo-3,4-dihydro-2*H*,6*H*-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile (23). A mixture of pyrimidine **1** (0.01 mol, 2.59 g) and acrylonitrile (0.02 mol, 1.06 mL) in the presence of triethylamine was heated under reflux in absolute ethanol. The reaction mixture was poured onto diluted HCl. The formed precipitate was filtered, dried, and crystallized from ethanol to give **23**.

Yield (86%), mp 232–234°C. *Anal*. Calcd for $C_{15}H_{12}N_4O_2S$ (312): C, 57.69; H, 3.84; N, 17.94. Found: C, 58.05; H, 3.99; N, 17.78. FTIR (KBr) (cm^{-1}): 3187 (NH), 2216 (CN), 1652 (C═O), 1601 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 9.52 (s, 1H, NH, D_2O exchangeable), 8.00 (d, J = 6.82 Hz, 2H, Ar—H), 7.11 (d, J = 6.82 Hz, 2H, Ar—H), 3.85 (s, 3H, OCH_3), 3.51 (t, J = 6.82 Hz, 2H, $SCH_2\underline{CH_2}$), 3.03 (t, J = 6.82 Hz, 2H, $S\underline{CH_2}CH_2$). ^{13}C NMR (DMSO-d_6), δ ppm 166.30, 164.31, 162.22, 161.41, 130.70, 127.11, 120.44, 120.41, 118.92, 116.03, 113.99, 91.3, 55.47, 25.96, 17.55. MS, *m/z* (%): 312 ($M^{\cdot+}$, 31), (259, 100%).

7-(4-Methoxyphenyl)-2,3,5-trioxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carbonitrile (24). A solution of pyrimidine **1** (0.01 mol, 2.59 g) in (15 mL) tetrahydrofuran was stirred at room temperature for 10 min. The solution of oxalyl chloride (0.01 mol, 1.27 mL) in dry tetrahydrofuran (15 mL) was added dropwise in the presence of triethylamine during 30 min with continuous stirring. After this, the mixture was stirred for 8 h and was left overnight. The solid obtained was filtered, dried, and crystallized from THF to give **24**.

Yield (60%), mp 251–252°C. *Anal*. Calcd for $C_{14}H_7N_3O_4S$ (313): C, 53.67; H, 2.23; N, 13.41. Found: C, 53.86; H, 2.43; N, 13.01. FTIR (KBr) (cm^{-1}): 2237 (CN), 1754, 1714, 1657 (C═O), 1604 (C═C). ^1H NMR (DMSO-d_6): δ_H (ppm) 7.61 (d, J = 6.82 Hz, 2H, Ar—H), 7.06 (d, J = 6.82 Hz, 2H, Ar—H), 3.80 (s, 3H, OCH_3). MS, *m/z* (%): 313 ($M^{\cdot+}$, 100%).

Experimental for Antimicrobial Activity. The samples were prepared by dissolving 2 mg in 2 mL of DMSO, and 100 μL (containing 100 μg) was used in this test. The antimicrobial activity of different samples was investigated by the agar cup plate method. Four different test microbes, namely *S. aureus* (G+ve), *P. aeruginosa* (G-ve), *C. albicans* (yeast), and *A. niger* (fungus), were used. Nutrient agar plates were heavily seeded uniformly with 1 mL of 10^5–10^6 cells/mL in case of bacteria and yeast. A Czapek–Dox agar plate seeded by the fungus was used to evaluate the antifungal activities. Then, a hole was made in media by gel cutter (Cork borer no.4) in sterile condition. Then, one drop of melted agar was poured into the hole and allowed to solidify to make a base layer. After that, specific amount of culture filtrate (0.1 mL) was poured into the hole. Then, plates were kept at low temperature (4°C) for 2–4 h to allow maximum diffusion. The plates were then incubated at 37°C for 24 h for bacteria and at 30°C for 48 h in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once, and mean of reading was recorded [24,25].

Experimental for Antioxidant Activity. ***Determination of total antioxidant capacity.*** The antioxidant activity of each compound was determined according to phosphomolybdenum method using ascorbic acid as standard. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green colored [phosphate = Mo (V)] complex at acidic pH with a maximal absorption at 695 nm. In this method, 0.5 mL of each compound (100 μg/mL) in methanol was combined in dried vials with 5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The vials containing the reaction mixture were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample, and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the sample was expressed as the number of AAE [26].

Cytotoxicity Assay. ***Materials and methods.*** Cell line hepatocellular carcinoma (HEPG-2), mammary gland (MCF-7), and colorectal carcinoma (HCT-116). 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 RPMI-1640 medium, MTT, and DMSO (Sigma Co., St. Louis, USA), fetal bovine serum (GIBCO, UK).

MTT Assay. The aforementioned cell lines were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. 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 seedes 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 were treated with different concentration 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 incubated for 4 h. Dimethyl sulfoxide (DMSO) in a 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, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) × 100 [27,28].

CONCLUSION

6-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile was utilized as building blocks to construct new polyfunctionally substituted pyrimidine-2-thione derivatives. Some of the products exhibited promising antimicrobial and pharmacological activities.

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