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Scientific paper

Synthesis and Antitumor Evaluation of Novel Dihydropyrimidine, Thiazolo[3,2-*a*]pyrimidine and Pyrano[2,3-*d*]pyrimidine Derivatives

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Abstract

A simple and efficient method has been developed for the synthesis of 4,5-dihydro-2-mercapto-4-oxo-6-substituted arylpyrimidine derivatives **2a–e** and their fused rings **3b**, **4b**, **5b**, **6b** and also 1,4-dihydro-2-mercaptopyrimidine derivatives **7a–e**, **9a–e** using triethylamine as a catalyst. The structure of the newly synthesized compounds was confirmed on the basis of their spectral data and elemental analyses. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against six human cancer cell lines and normal fibroblasts. Nine of the tested compounds (*i.e.* **2a**, **2c**, **2d**, **3b**, **4b**, **5b**, **8**, **9a** and **9c**) exhibited significant cytotoxicity against most cell lines. Among these derivatives compounds **2a**, **3b** and **9c** are the most potent, they exhibited cytotoxic effect against the six cancer cell lines with IC_{50} values < 330 nM compared to the standard CHS 828. Normal fibroblast cells (WI38) were affected to a much lesser extent (IC_{50} >10,000 nM). Toxicity of the most potent compounds was measured against shrimp larvae; the results showed that compounds **2a** and **3b** are not toxic towards the tested organisms.

Keywords: 4,5-dihydropyrimidine; 1,4-dihydropyrimidine; thiazolo[3,2-*a*]pyrimidine; pyrano[2,3-*d*]pyrimidine; anticancer activity

1. Introduction

Cancer is one of the most dreadful disease in the world and despite immense advances in the field of basic and clinical research, which have resulted in higher cure rates for a number of malignancies, cancer remains the second leading cause of death in developing as well as developed countries.[1–3] Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited mainly due to the undesirable side effects and this clearly underscores the need of developing novel chemotherapeutic agents for more effective cancer treatments.[4]

Among the wide range of compounds tested as potential anticancer agents, pyrimidine and fused pyrimidine derivatives comprise an important class of therapeutic agents. They were reported as antitumor,[5–10] antimicrobial,[11,12] antiinflammatory,[13] anti HIV,[14] antinociceptive,[15,16] and antioxidant agents.[17,18] Various drugs containing pyrimidine nucleus were synthesized and used as anticancer agents like 5-fluorouracil (5-FU), tegafur and thioguanine (Figure 1).[19]

In recent years dihydropyrimidinones/thiones (DHPMs) and their derivatives have occupied an important position in natural and synthetic organic chemistry because of their wide range of biological activities, such as antioxidant, antiinflammatory, anthelminic, antimicrobial,[20–22] antituberculoses[23] and anticancer.[24]

Although a number of papers have been concerned with the synthesis of pyrimidine derivatives,[17,18] just a few one pot syntheses have been published using aromatic aldehydes, ethyl cyanoacetate and thiourea.[11,20–24] Also, it was reported that 3,4-dihydropyrimidinone/thione have been synthesized by a three-component condensation of aldehydes, ethyl acetoacetate and urea/thiourea using different catalysts and new methods to improve and modify this reaction.[25–29] Moreover, the one step synthesis of 6-amino-5-cyano-4-phenyl-2-mercaptopyrimidine using aldehydes, malononitrile and urea/thiourea in the presence of a catalytic amount of

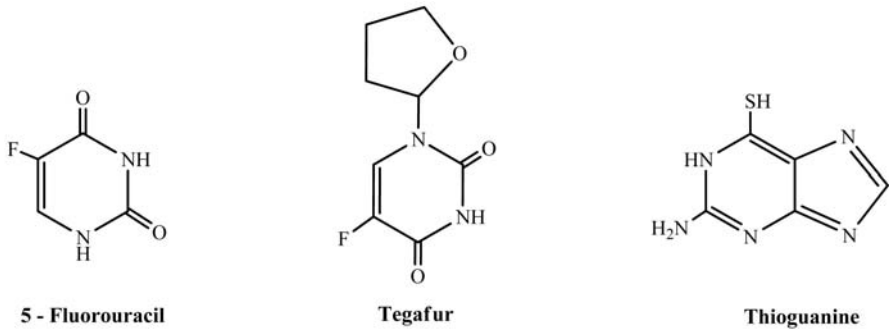


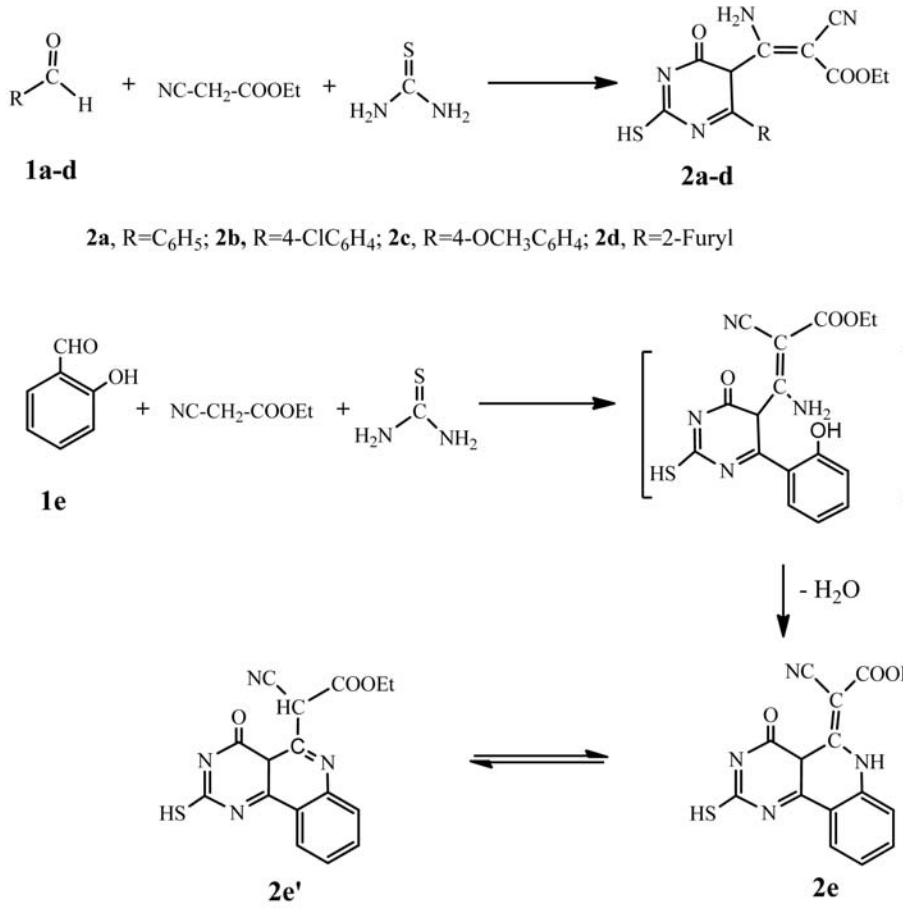
Figure 1. Pyrimidine derivatives as anticancer agents.

phosphorus pentoxide has been reported.[30] Therefore, in the view of the facts mentioned above and to discover potentially active new agents, we have synthesized some new dihydropyrimidine derivatives by a three-component condensation (MCR) of aromatic aldehydes, thiourea and either ethyl cyanoacetate, ethyl acetoacetate or malononitrile using triethylamine under refluxing condition. The cytotoxicity of the newly synthesized products was investigated against six cancer and one normal human cell lines.

2. Results and Discussion

2. 1. Chemistry

In the present work, we have developed an efficient method to generate the dihydropyrimidine derivatives **2a**–**d** and oxopyrimido[5,4-*c*]quinoline **2e** by the cyclization of three components, like aryl aldehydes **1a**–**e**, ethyl cyanoacetate and thiourea in ethanol in the presence of triethylamine in a one-pot reaction (Scheme 1). The structure assigned to the synthesized compounds was



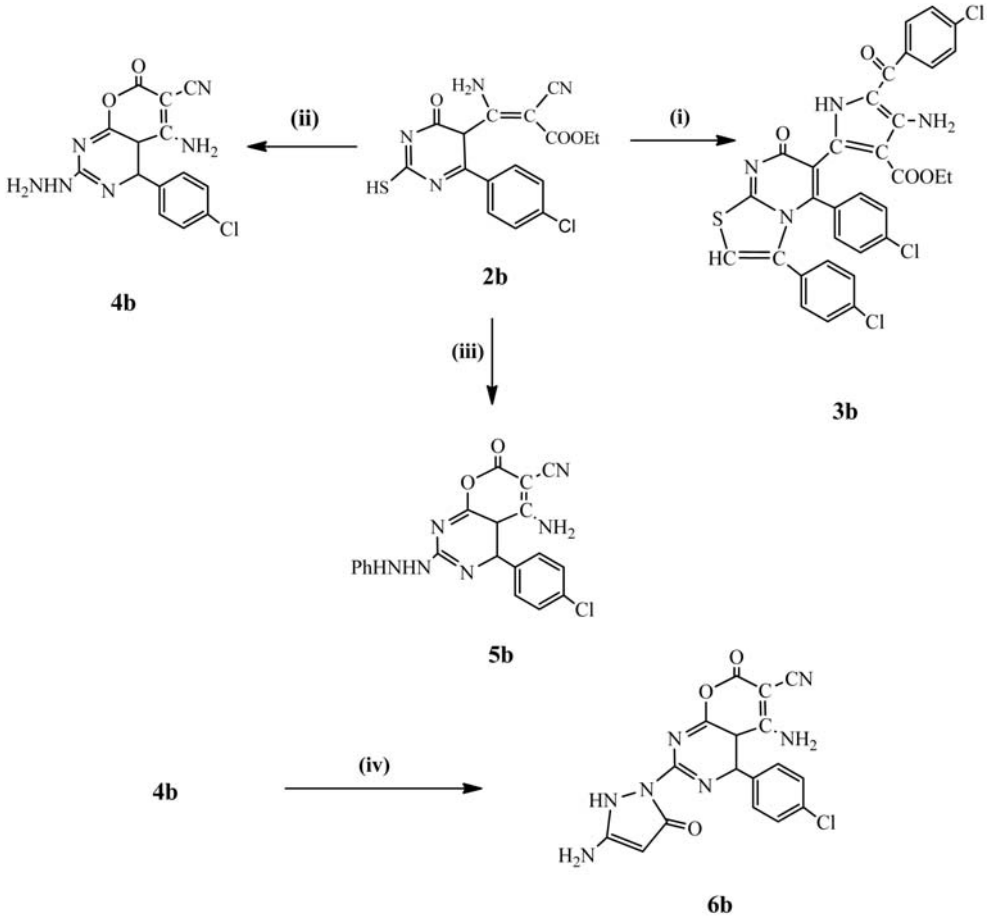
Scheme 1. Reaction of **1a**–**e**, ethyl cyanocetate and thiourea in absolute EtOH, Et_3N, heat 3 h.

substantiated by their analytical and other spectral data. The IR spectrum of the synthesized compound **2a** showed characteristic signals at 1691, 1685 cm^{-1} for two C=O, 2228 cm^{-1} for CN and 3450 cm^{-1} for NH_2. Similarly the ^1H NMR spectrum, showed the presence of a triplet at δ 1.28 ppm and a quartet at δ 4.31 ppm indicating the presence of the ester CH_3 and CH_2 groups respectively, beside the appearance of a singlet at δ 8.41 ppm corresponding to the presence of a CH-pyrimidine moiety, proving its structure.

The formation of the oxopyrimido[5,4-*c*]quinolin[31] **2e** was proved by the presence of two stretching C=O signals at 1727, 1720 cm^{-1} and a CN signal at 2227 cm^{-1}, beside the absence of any signal corresponding to the OH group either in the IR or the ^1H NMR spectra. Moreover, ^1H NMR spectrum showed the presence of the D_2O exchangeable signal at δ 3.83 ppm corresponding to the NH group, the triplet and quartet signals at δ 1.29 and 4.29 ppm corresponding to the CH_3 and CH_2 groups, respectively, the CH pyrimidine signal at δ 8.95 ppm beside the SH signal at δ 10.75 ppm. In addition its ^{13}C NMR spectrum revealed the presence of signals at δ 14.09 (CH_3), 32.04 (CH-CN), 61.05 (CH_2), 76.40 (C-5 pyrimidine), 112.02 (CN), 164.98, 169.8 (2 C=O). The appearance of a signal at δ 32.04 ppm in the ^{13}C NMR spectrum corresponding to the CH-CN proved the formation of the tautomeric form **2e'**.

It was reported that the three-component reaction of aromatic aldehydes, ethyl cyanoacetate and thiourea in ethanol using potassium carbonate afforded 6-oxo-4-substituted aryl-2- sulfanyl-1,6- dihydropyrimidine-5- carbonitrile.[11,20]

Thiazolo[3,2-*a*]pyrimidine **3b** and pyrano[2,3-*d*]pyrimidines[32] **4b**, **5b** (Scheme 2) were obtained from the reaction of 4,5-dihydropyrimidine derivative **2b** with ω-bromo-4-chloroacetophenone, hydrazine hydrate and phenyl hydrazine, respectively. The structure of these compounds was elucidated by IR, ^1H and ^{13}C NMR spectra beside their analytical and mass spectral data. Thus, the IR spectrum of **3b** showed the absence of any CN signal beside the expected signals. Meanwhile, ^1H NMR spectrum showed a singlet at δ 6.09 ppm for CH of the thiazole ring with the disappearance of signals corresponding to SH and pyrimidine H-5. At the same time ^{13}C

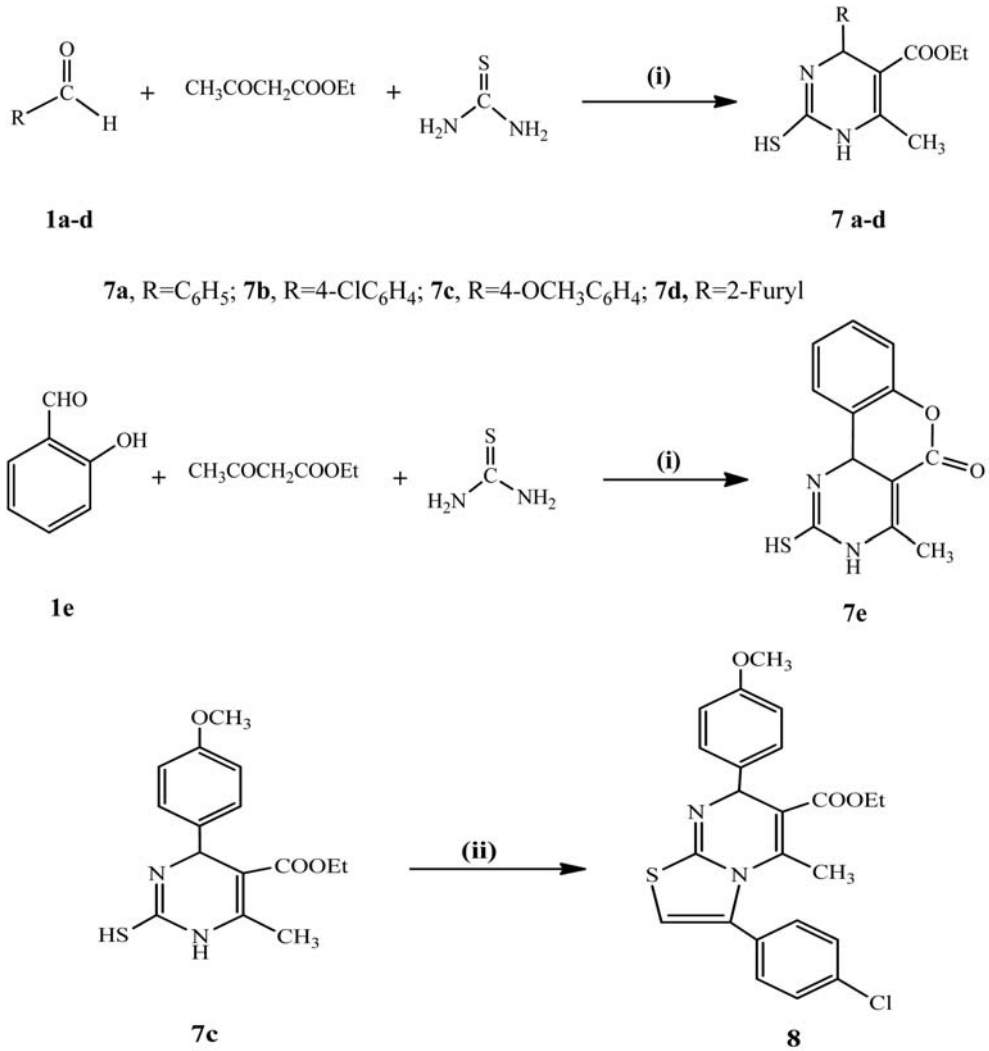


Scheme 2. Reactions of 4,5-dihydropyrimidine **2b** and reaction of **4b** with ethyl cyanoacetate. Reagents and conditions; (i) 4-$ClC_6H_4COCH_2Br$, absolute EtOH, Et_3N, heat 3 h; (ii) $NH_2NH_2.H_2O$, absolute EtOH, heat 2 h; (iii) $PhNHNH_2$, absolute EtOH, heat 2 h; (iv) ethyl cyanoacetate, DMF, heat 4 h.

NMR spectrum exhibited signals at δ 25.70, 53.25 (ethyl group), 96.07 (CH thiazole), 158.45, 159.97 and 162.15 (3 C=O). The IR spectra of pyrano[2,3-*d*]pyrimidines **4b**, **5b** showed the presence of only one stretching C=O signal at 1736 and 1744 cm^{-1}, respectively. Similarly, their ^1H NMR spectra showed the absence of any signals corresponding to ethyl ester, SH, H-5 pyrimidine beside the appearance of signals corresponding to H-4 and H-5 pyrimidine within the aromatic range. Compound **4b** exhibited the presence of two singlets for the two NH_2 at δ 3.73 and 8.69 ppm and a singlet for NH at δ 13.12 ppm (D_2O exchangeable). In addition, for **5b** there is a singlet appearing at δ 3.73 ppm indicating the presence of the NH_2 and the two singlets at δ 6.73, 10.40 (D_2O exchangeable) for the two NH groups of the -NHNHPh moiety. Moreover, the formation of pyrano[2,3-*d*]pyrimidines **4b** and **5b** was confirmed by their ^{13}C NMR spectra. ^{13}C NMR spectrum of **4b** showed signals at δ 65.29, 67.12 (pyrimidine C-4 and C-5), 113.43 (CN), 160.28 (C=O). At the same time ^{13}C NMR spectrum of **5b** showed signals at δ 65.29, 67.4 (pyrimidine C-4 and C-5), 113.43 (CN), 160.28 (C=O).

On the other hand, the hydrazino moiety present in **4b** showed high activity towards cyanomethylene reagents. Thus, its reaction with ethyl cyanoacetate in dimethylformamide solution gave the pyrazole derivative[33] **6b**. The IR spectrum of **6b** showed beside the former data the presence of the two C=O at 1791, 1683 cm^{-1} in addition to the presence of three signals corresponding to two NH_2 groups at δ 3.79, 13.16 ppm and one NH at 8.70 ppm in the ^1H NMR spectrum. Moreover, the ^{13}C NMR spectrum showed beside the expected signals, a signal at δ 96.67 ppm corresponding to the pyrazole CH group, the presence of two CO and two C=N groups at δ 161.95, 162.15, 164.04, 166.38 ppm, respectively.

The reaction of the arylaldehydes **1a**–**e**, ethyl acetoacetate and thiourea in 1,4 dioxane using triethylamine as the catalyst afforded 1,4-dihydromercaptopyrimidines **7a**–**d** and chromeno[4,3-*d*]pyrimidine **7e** (Scheme 3). The



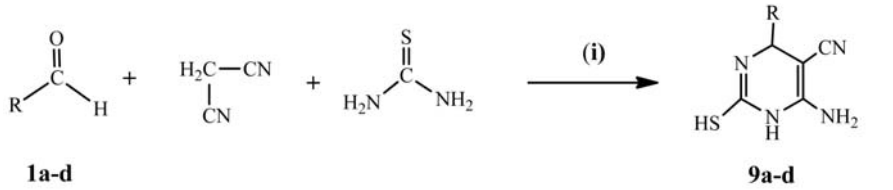
Scheme 3. Reaction of **1a**–**e,** ethyl acetoacetate and thiourea and reaction of **7c** with 4-$ClC_6H_4COCH_2Br$. Reagents and conditions; (i) 1,4-dioxane, Et_3N, heat 6–8 h; (ii) absolute EtOH, Et_3N, heat 1 h.

^{1}H NMR spectrum of **7a** showed beside the expected signals the presence of singlets at δ 5.17, 9.6 and 10.29 ppm corresponding to the presence of CH pyrimidine, NH and SH, respectively. In our work, the pyrimidine derivative exists in the thiol form and not in the thione form as it was reported.[25–29] This was confirmed through the reaction of the pyrimidine derivative **7c** with a-halocarbonyl compounds. Thus, reaction of **7c** with ω-bromo-4-chloroacetophenone afforded thiazolo[3,2-*a*]pyrimidine **8** (Scheme 3). The ^{1}H NMR spectrum of **8** showed the absence of SH and NH signals with the presence of CH-thiazole singlet at δ 7.15 ppm.

The structure of chromeno[4,3-*d*]pyrimidine **7e** was elucidated on the basis of its IR spectrum which showed the absence of OH signal beside the ^{1}H NMR spectrum which revealed the absence of both OH and COOEt signals. Moreover, the ^{13}C NMR spectrum of **7e** showed δ at 26.06 (CH_3), 64.56 (CH pyrimidine) and 160.28 (C=O).

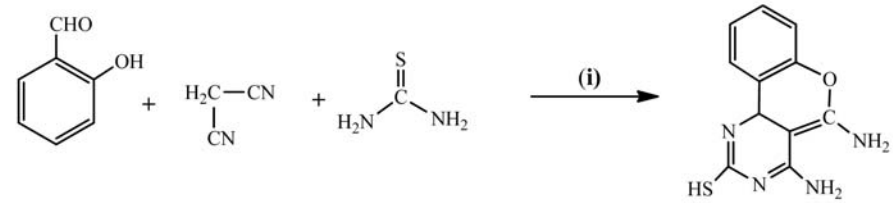
Condensation of each of the arylaldehydes **1a–e** with the malononitrile and thiourea in ethanol in the presence of a catalytic amount of triethylamine produced 1,4-dihydromercaptopyrimidines **9a–d** and chromeno[4,3-*d*]pyrimidine **9e** (Scheme 4).

The structure of these compounds was based on their IR and ^{1}H NMR spectra. Thus, the ^{1}H NMR spectrum of **9a** showed the presence of singlets at δ 7.90 and 10.14 ppm corresponding to the presence of NH and SH, respectively, beside the presence of a multiplet at δ 7.15–7.64 ppm corresponding to the aromatic protons and one pyrimidine proton. In our work we obtained the 1,4-dihydropyrimidine derivatives instead of pyrimidine derivatives as reported.[30] The structure of 1,4-dihydropyrimidine **9a–e** was confirmed by the reaction of **9c** with ω-bromoacetophenone to afford thiazolo[3,2-*a*]pyrimidine **10**. The ^{1}H NMR spectrum of **10** showed beside the expected signals the absence of any SH and NH with the presence of a singlet at δ 5.21 ppm corresponding to the presence of CH-

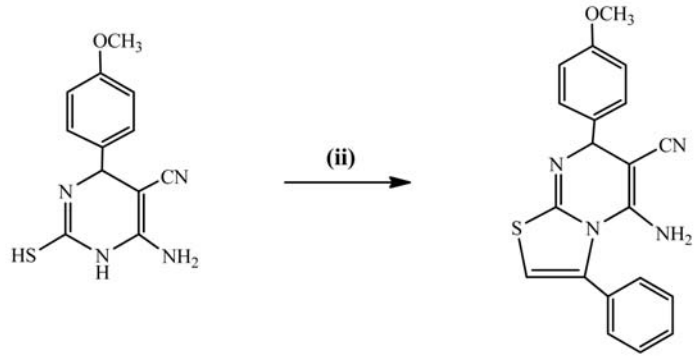


1a-d **9a-d**

9a, R=C_6H_5; **9b**, R=4-ClC_6H_4; **9c**, R=4-$OCH_3C_6H_4$; **9d**, R=2-Furyl



1e **9e**



9c **10**

Scheme 4. Reaction of **1a–e**, malononitrile and thiourea and reaction of **9c** with $C_6H_5COCH_2Br$. Reagents and conditions; (i) absolute EtOH, Et_3N, heat 2 h; (ii) absolute EtOH, Et_3N, heat 2 h.

thiazole. The formation of chromeno[4,3-*d*]pyrimidine derivative **9e** was proved by the absence of any CN and OH signals in the IR spectrum beside the presence of a singlet at δ 6.96 ppm corresponding to the presence of two NH_2 in the ^{1}H NMR spectrum. Moreover, ^{13}C NMR spectrum of **9e** showed signal at δ 61.05 ppm (CH pyrimidine).

2. 2. *In vitro* Cytotoxicity

2. 2. 1. Effect on the Growth of Human Cancer Cell Lines

The heterocyclic compounds prepared in this study were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). For comparison, CHS 828 was used as a standard antitumor drug. The IC_{50} values (the sample concentration that produces 50% reduction in cell growth) were in nanomolar (nM) range and are listed in Table 1. Nine of the tested compounds showed cytotoxic activity with IC_{50} values < 650 nM and the results are represented in Figures 2–4. Compounds **2a**, **3b**, **9c** were found to be the most potent derivatives. All the synthesized compounds were tested for their cytotoxicity against normal fibroblast cells as many anticancer drugs are toxic not only against cancer cells but also normal ones. The results obtained showed that normal fibroblast cells (WI38) were affected to a much lesser extent (IC_{50} >10,000 nM).

2. 2. 2. Structure Activity Relationship

In this study when comparing the structures of the synthesized compounds with their anticancer activity it has been observed that nine of the tested compounds, namely: **2a**, **2c**, **2d**, **3b**, **4b**, **5b**, **8**, **9a** and **9c** showed significant broad cytotoxic effect with IC_{50} values < 650 nM. Normal fibroblast cells (WI38) were affected to a much lesser extent (IC_{50} >10,000 nM). Considering the 4,5-dihydromercaptopyrimidine derivatives **2a**–**e**, the unsubstituted derivative **2a** showed high potency against the six cancer cell lines. It exhibited potent activity against gastric cancer NUGC (IC_{50} 89 nM), colon cancer DLDI (IC_{50} 49 nM) and liver cancer HA22T (IC_{50} 64 nM). Compounds **2c** and **2d** displayed cytotoxicity against three cancer cell lines. Compound **2c** bearing the 4-methoxy moiety was toxic against colon DLD1 (IC_{50} 188 nM), liver cancer HEPG2 (IC_{50} 102 nM) and breast cancer MCF (IC_{50} 239 nM), while **2d**, substituted by a furan group, showed cytotoxic activity against gastric cancer NUGC (IC_{50} 228 nM), colon cancer DLD1 (IC_{50} 126 nM) and nasopharyngeal carcinoma HONE1 (IC_{50} 138 nM). Moreover, **2b** and **2e** demonstrated selective toxicity against gastric cancer NUGC with IC_{50} 323 and 128 nM, respectively. The cytotoxicity of compounds **2a**–**e** might be attributed to the presence of cyanoaminopyrimidine moiety.[34] Comparing the cytotoxicity of the mercaptopyrimidine derivative **2b** and its cyclized product the thiazolo[3,2-*a*]pyrimidine derivative **3b**, it is obvious that **3b** possessed potential cytotoxic effect to all cancer cell lines. It demonstrated significant cytotoxicity against gastric cancer NUGC (IC_{50} 38 nM) compared to the standard CHS 828 (IC_{50} 25 nM).[5]

Pyrano[2,3-*d*]pyrimidines **4b**, **5b** obtained by the reaction of **2b** with hydrazine hydrate and phenyl hydrazine, respectively, showed remarkable increase of the toxicity against most cancer cell lines. Such increase in toxicity can be attributed to the presence of the hydrazino moiety.[35]

The 1,4-dihydropyrimidine derivatives **7a**–**d** are almost devoid of activity. However, compound **7d** substituted by a furan ring was the only active compound, it showed selective cytotoxic activity against nasopharyngeal carcinoma HONE1 with (IC_{50} 44 nM) compared to the standard CHS 828 (IC_{50} 15 nM). Thiazolo[3,2-*a*]pyrimidine **8** obtained by the reaction of **7c** with ω-bromo-4-chloroacetophenone exhibited significant cytotoxic activity against gastric cancer NUGC (IC_{50} 226 nM), colon cancer DLD1 (IC_{50} 254 nM) and liver cancer HA22T (IC_{50} 94 nM). On the other hand, among the1,4-dihydropyrimidine derivatives **9a**–**d**, the 4-methoxy derivative **9c** possessed potent cytotoxic activity against the six cancer cell lines. The latter compound showed cytotoxicity against gastric cancer NUGC (IC_{50} 38 nM), colon cancer DLD1 (IC_{50} 35 nM), liver cancer HA22T (IC_{50} 98 nM) and breast cancer MCF (IC_{50} 103 nM). Moreover, the unsubstituted derivative **9a** was toxic against liver cancer HEPG2, nasopharyngeal carcinoma cancer HONE1 and breast cancer MCF cell lines, while the furano derivative **9d** was toxic only against liver cancer HEPG2 (IC_{50} 128 nM) cell line.[36]

Thus, even though some of the compounds were not the most potent, their specific activity against particular cell lines makes them of interest for further development as anticancer drugs.

2. 2. 3. Toxicity

Bioactive compounds are often toxic to shrimp larvae. In order to establish *in vivo* lethality of these cytotoxic compounds to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay[37] was used. Results were analysed with LC_{50} program to determine LC_{50} values and 95% confidence intervals.[38] Results for the compounds which exhibited optimal cytotoxic effect against cancer cell lines (*i.e.* **2a**, **3b**, **5b**, **7d** and **9c**) are given in Table 2. The shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials,[39] natural and synthetic organic compounds.[37] It has also been shown that *A. salina* toxicity test results have a correlation with rodent and human

acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for the tested compounds.[40]

In order to prevent the toxicity results from possible false effects originating from solubility of compounds and

Table 1. Cytotoxicity of compounds **2a–e**, **3b**, **4b**, **5b**, **6b**, **7a–e**, **8**, **9a–e** and **10** against a variety of cancer cell lines [a] [IC_{50} [b] (nM)]

Compound No.	Cytotoxicity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
2a	89	49	64	238	272	328	NA
2b	323	3129	1155	2424	1242	1252	NA
2c	2179	188	2120	102	2380	239	NA
2d	228	126	1248	2269	138	1170	NA
2e	128	2115	3180	2312	1284	3260	1277
3b	38	364	175	122	206	274	NA
4b	328	608	282	523	201	348	NA
5b	55	146	1413	263	536	285	222
6b	2208	2128	2153	1236	1138	2240	NA
7a	2166	4840	1320	3266	4385	1428	NA
7b	1214	1640	2183	2809	2266	2285	NA
7c	2470	2482	1226	1483	3576	2328	1068
7d	1220	1240	1203	1237	44	2119	NA
7e	1029	2294	2069	2244	3327	2436	NA
8	266	254	94	1679	2114	1277	3679
9a	1242	2140	3220	634	428	256	NA
9b	2213	2146	2120	2110	3290	1368	NA
9c	38	35	98	308	210	103	NA
9d	1122	3224	24241	128	11220	2136	NA
9e	2253	2690	2166	3309	2213	2318	NA
10	2244	1770	3148	2182	1880	1290	1089
CHS 828	25	2315	2067	1245	15	18	NA

[a] NUGC: gastric cancer; DLDI: colon cancer; HA22T: liver cancer; HEPG2: liver cancer; HONE1: nasopharyngeal carcinoma; MCF: breast cancer; WI38: normal fibroblast cells. [b] The sample concentration produces a 50% reduction in cell growth

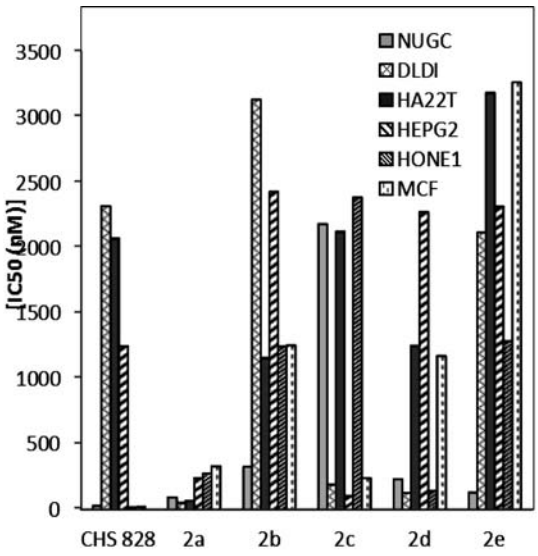


Figure 2. Cytotoxicity of **2a–e** and CHS 828 against NUGC (gastric cancer); DLDI (colon cancer); HA22T (liver cancer); HEPG2 (liver cancer); HONE1 (nasopharyngeal carcinoma); MCF (breast cancer).

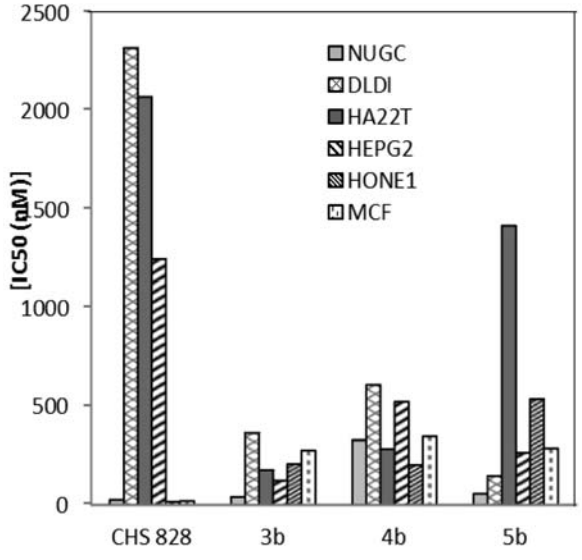


Figure 3. Cytotoxicity of **3b**, **4b**, **5b** and CHS 828 against NUGC (gastric cancer); DLDI (colon cancer); HA22T (liver cancer); HEPG2 (liver cancer); HONE1 (nasopharyngeal carcinoma); MCF (breast cancer).

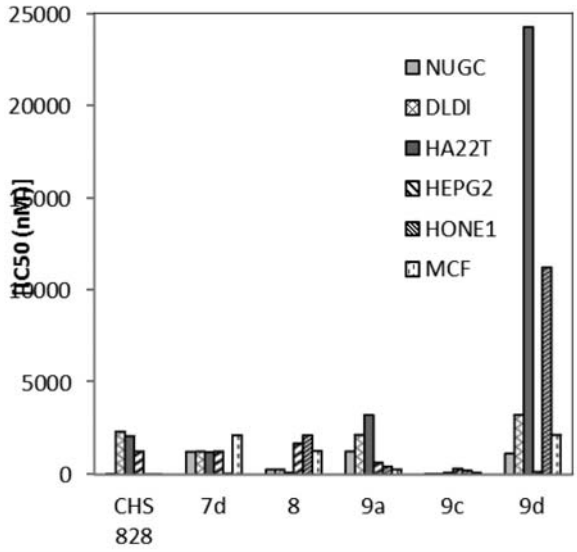


Figure 4. Cytotoxicity of **7d**, **8**, **9a**, **9c**, **9d** and CHS 828 against NUGC (gastric cancer); DLDI (colon cancer); HA22T (liver cancer); HEPG2 (liver cancer); HONE1 (nasopharyngeal carcinoma); MCF (breast cancer).

DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 2 that compounds **2b** and **3b** showed no toxicity against the tested organisms. On the other hand, compound **5b** is very toxic, in addition, compounds **7b**, **9c** are harmful.

3. Experimental

3. 1. Chemistry

All melting points were determined on a Stuart apparatus and the values given are uncorrected. IR spectra (KBr, cm^{-1}) were determined on a Shimadzu IR 435 spectrophotometer (Faculty of Science, Cairo University, Egypt). ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz (Microanalysis Center, Cairo University, Egypt) using TMS as the internal standard. Chemical shift values are recorded in ppm on δ scale. Mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Microanalysis Center, Cairo University, Egypt). Elemental analyses were carried out at the Microanalysis Center, Cairo University, Egypt; found values were within ±0.35% of the theoretical ones. Progress of the reactions was monitored using thin layer chromatography (TLC) sheets coated with UV fluorescent silica gel Merck 60F 254 and were visualized using UV lamp.

General Procedure for the Synthesis of 2a–e

The dihydropyrimidines **2a–e** were synthesized by refluxing the aprropriate arylaldehyde **1a–e** (0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL) for 3 h. The solution was left to cool to room temperature, poured onto ice/water and neutralized with hydrochloric acid. The precipitated solid was filtered off, washed with cold water and crystallized from ethanol.

Ethyl 3-Amino-2-cyano-3-(2-mercapto-4-oxo-6-phenyl-4,5-dihydropyrimidin-5-yl)acrylate (2a). Yield: 65%; m.p.: 296–298 °C; IR (KBr, cm^{-1}): 3450–3320 (NH_2, NH), 3082 (CH, aromatic), 2228 (CN), 1691–1685 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.89 (s, 2H, NH_2, D_2O exchangeable), 4.31 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 7.54–8.07 (m, 5H, CH aromatic), 8.41 (s, 1H, pyrimidine H-5), 13.17 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 342 (M^+, 42). *Anal*. Calcd. for $C_{16}H_{14}N_4O_3S$: C, 56.13; H, 4.12; N, 16.36; S, 9.37. Found: C, 56.43; H, 4.46; N, 16.72; S, 9.59.

Table 2. LC_{50} of compounds **2a**, **3b**, **5b**, **7d** and **9c** against shrimp larvae

Compound No.	Cons. (μg/mL)	Mortality[a]	Toxicity	LC_{50}	Upper 95% lim.	Lower 95% lim.
2a	10	0	Non toxic	996.27	–	–
	100	0				
	1000	5				
3b	10	0	Non toxic	880.42	–	–
	100	1				
	1000	4				
5b	10	1	Very toxic	18.38	–	–
	100	6				
	1000	10				
7d	10	0	Harmful	22.7	210.59	160.22
	100	6				
	1000	8				
9c	10	0	Harmful	420.28	112.23	90.55
	100	5				
	1000	10				

[a] Ten organisms (*A. salina*) tested for each concentration

Ethyl 3-Amino-3-(6-(4-chlorophenyl)-2-mercapto-4-oxo-4,5-dihydropyrimidin-5-yl)-2-cyanoacrylate (**2b**). Yield: 55%; m.p.: 155–157 °C; IR (KBr, cm^{-1}): 3431–3321 (NH_2, NH), 3075 (CH, aromatic), 2221 (CN), 1725–1718 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.86 (s, 2H, NH_2, D_2O exchangeable), 4.31 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 7.67–8.08 (m, 4H, CH aromatic), 8.41 (s, 1H, pyrimidine H-5), 13.09 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 376 (M^+, 34). *Anal*. Calcd. for $C_{16}H_{13}ClN_4O_3S$: C, 51.00; H, 3.48; N, 14.87; S, 8.51. Found: C, 50.67; H, 3.33; N, 14.62; S, 8.73.

Ethyl 3-Amino-2-cyano-3-(-2-mercapto-6-(4-methoxyphenyl)-4-oxo-4,5-dihydropyrimidin-5-yl)acrylate (**2c**). Yield: 63%; m.p.: 116–118 °C; IR (KBr, cm^{-1}): 3418–3324 (NH_2, NH), 3094 (CH, aromatic), 2212 (CN), 1716–1710 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 1.27 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.83 (s, 3H, OCH_3), 3.86 (s, 2H, NH_2, D_2O exchangeable), 4.25 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 7.13–8.09 (m, 4H, CH aromatic), 8.30 (s, 1H, pyrimidine H-5), 13.10 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 372 (M^+, 21). *Anal*. Calcd. for $C_{17}H_{16}N_4O_4S$: C, 54.83; H, 4.33; N, 15.04; S, 8.61. Found: C, 54.69; H, 4.22; N, 15.37; S, 8.93

Ethyl 3-Amino-2-cyano-3-(6-(furan-2-yl)-2-mercapto-4-oxo-4,5-dihydropyrimidin-5-yl)acrylate (**2d**). Yield: 59%; m.p.: 96–98 °C; IR (KBr, cm^{-1}): 3421–3329 (NH_2, NH), 3040 (CH, aromatic), 2221 (CN), 1719–1712 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.89 (s, 2H, NH_2, D_2O exchangeable), 4.31 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 6.92– 8.21 (m, 3H, CH furan), 8.27 (s, 1H, pyrimidine H-5), 13.14 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 332 (M^+, 62). *Anal*. Calcd. for $C_{14}H_{12}N_4O_4S$: C, 50.60; H, 3.64; N, 16.86; S, 9.65. Found: C, 50.38; H, 3.32; N, 16.69; S, 9.95.

Ethyl 2-Cyano-2-(2-mercapto-4-oxo-4,4a-dihydropyrimido[5,4-*c*]quinolin-5(6*H*)-ylidene)acetate (**2e**). Yield: 62%; m.p.: 185–187 °C; IR (KBr, cm^{-1}): 3436 (NH), 3043 (CH, aromatic), 2227 (CN), 1727–1720 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 1.29 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.83 (s, 1H, NH, D_2O exchangeable), 4.29 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 7.41–7.93 (m, 4H, CH aromatic), 8.95 (s, 1H, pyrimidine H-5), 10.75 (s, 1H, SH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 14.09, 32.04, 61.05, 76.40, 112.02, 126.70, 129.25, 130.43, 132.09, 134.80, 162.04, 164.98, 169.8, 172.75, 173.40; MS: *m/z* (%) 340 (M^+, 24). *Anal*. Calcd. for $C_{16}H_{12}N_4O_3S$: C, 56.46; H, 3.55; N, 16.46; S, 9.42. Found: C, 56.66; H, 3.29; N, 16.77; S, 9.29.

Synthesis of Ethyl 4-Amino-2-(3,5-bis(4-chlorophenyl)-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidin-6-yl)-5-(4-chlorobenzoyl)-1*H*-pyrrole-3-carboxylate (3b)

A solution of 4,5-dihydropyrimidine **2b** (2.63 g, 0.01 mol) and ω-bromo-4-chloroacetophenone (2.34 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL) was heated under reflux for 3 h, left to cool to room temperature, poured onto ice/water and neutralized by hydrochloric acid. The precipitated solid was filtered, washed with water and crystalized from ethanol.

Yield: 75%; m.p.: 255–257 °C; IR (KBr, cm^{-1}): 3439–3307 (NH_2, NH), 3046 (CH, aromatic), 1743–1665 (3 C=O); ^{1}H NMR (DMSO-d_6): δ 0.91 (t, 3H, J = 7.2 Hz, CH_2-$\underline{CH_3}$), 3.49 (s, 2H, NH_2, D_2O exchangeable), 4.20 (q, 2H, J = 7.2 Hz, $\underline{CH_2}$-CH_3), 6.09 (s, 1H, CH thiazole), 7.26–8.21 (m, 13H, 12H aromatic and 1H, NH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 25.70, 53.25, 96.07, 114.04, 120.56, 123.09, 124.69, 129.20, 129.53, 131.95, 132.05, 133.14, 134.49, 138.3, 139.22, 140.33, 158.45, 159.97, 162.15, 164.50, 166.39; MS: *m/z* (%) 663 (M^+, 51). *Anal*. Calcd. for $C_{32}H_{21}Cl_3N_4O_4S$: C, 57.89; H, 3.19; N, 8.44; S, 4.83. Found: C, 57.55; H, 3.09; N, 8.09; S, 4.64.

General Procedure for the Synthesis of Pyrano[2,3-*d*] pyrimidine Derivatives 4b, 5b.

A mixture of 4,5-dihydropyrimidine **2b** (2.63 g, 0.01 mol) with either of hydrazine hydrate (0.05 g, 0.01 mol) or phenyl hydrazine (1.08 g, 0.01 mol) in absolute ethanol (40 mL), was heated under reflux for 2 h. The solid was precipitated by cooling to room temperature, filtered off, and crystalized from ethanol.

5-Amino-4-(4-chlorophenyl)-2-hydrazinyl-7-oxo-4a,7-dihydro-4*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**4b**). Yield: 73%; m.p.: 265–267 °C; IR (KBr, cm^{-1}): 3483–3200 (2 NH_2, NH), 3094 (CH, aromatic), 2225 (CN), 1736 (C=O); ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 2H, NH_2, D_2O exchangeable), 7.20–7.91 (m, 6H, 4H aromatic and 2H pyrimidine), 8.69 (s, 2H, NH_2, D_2O exchangeable), 13.12 (s, 1H, NH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 65.29, 67.12, 113.43, 121.38, 127.73, 129.47, 130.59, 148.93, 150.74, 160.28, 163.19, 164.46; MS: *m/z* (%) 330 (M^+, 11). *Anal*. Calcd. for $C_{14}H_{11}ClN_6O_2$: C, 50.84; H, 3.35; N, 25.41. Found: C, 50.53; H, 3.09; N, 25.09.

5-Amino-4-(4-chlorophenyl)-7-oxo-2-(2-Phenylhydrazinyl)-4a,7-dihydro-4*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**5b**). Yield: 76%; m.p.: 149–151°C; IR (KBr, cm^{-1}): 3457–3307 (NH_2, 2 NH), 3092 (CH, aromatic), 2260 (CN), 1744 (C=O); ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 2H, NH_2, D_2O exchangeable), 6.73–7.67 (m, 11H, 9H aromatic and 2H pyrimidine), 7.84 (s, 1H, NH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 65.29, 67.03, 113.43, 118.12, 121.01, 123.37, 124.22, 127.73, 129.47, 130.59, 140.02, 148.93, 150.74, 160.28, 163.23, 164.46; MS: *m/z* (%) 406 (M^+, 13). *Anal*. Calcd. for $C_{20}H_{15}ClN_6O_2$: C, 59.05; H, 3.72; N, 20.66. Found: C, 59.36; H, 3.87; N, 20.82.

Synthesis of 5-Amino-2-(3-amino-5-oxo-2,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-chlorophenyl)-7-oxo-4a,7-dihy-

dro-4*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**6b**).
A mixture of pyrano[2,3-*d*]pyrimidine **4b** (3.3 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) in dimethyl formamide (20 mL) was heated under reflux for 4 h. The solution was left to cool, poured onto ice/water, the solid product was collected by filtration and crystalized from EtOH/DMF.

Yield: 40%; m.p.: 256–258 °C; IR (KBr, cm^{-1}): 3446–3192 (2 NH_2, NH), 3090 (CH, aromatic), 2223 (CN), 1791–1683 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 3.79 (s, 2H, NH_2, D_2O exchangeable), 7.39–7.91 (m, 7H, 4H aromatic, 2H pyrimidine and 1H pyrazole H-4), 8.70 (s, 1H, NH, D_2O exchangeable), 13.16 (s, 2H, NH_2, D_2O exchangeable); ^{13}C HMR (DMSO): δ 66.01, 66.12, 96.07, 114.06, 116.07, 120.56, 124.69, 129.20, 133.14, 138.02, 140.33, 161.95, 162.15, 164.04, 166.38; MS: *m/z* (%) 397 (M^+, 65). *Anal*. Calcd. for $C_{17}H_{12}ClN_7O_3$: C, 51.33; H, 3.04; N, 24.65 Found: C, 51.09; H, 3.1; N, 24.79.

General Procedure for the Synthesis of 7a-e
A solution of the appropriate arylaldehyde **1a**–**e** (0.01 mol), ethyl acetoacetate (1.3 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL) was heated under reflux for 6–8 h, then left to cool. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration and crystallized from ethanol.

Ethyl 2-Mercapto-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (**7a**). Yield: 81%; m.p.: 275–277 °C; IR (KBr, cm^{-1}): 3325 (NH), 3090 (CH, aromatic), 2982 (CH, aliphatic), 1670 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.07 (t, 3H, *J* = 7.2 Hz, CH_2-$\underline{CH_3}$), 2.28 (s, 3H, CH_3), 3.97 (q, 2H, *J* = 7.2 Hz, $\underline{CH_2}$-CH_3), 5.17 (s, 1H, pyrimidine H-4), 7.20–7.37 (m, 5H, CH aromatic), 9.60 (s, 1H, NH, D_2O exchangeable), 10.29 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 276 (M^+, 27). *Anal*. Calcd. for $C_{14}H_{16}N_2O_2S$: C, , 60.85; H, 5.84; N, 10.14; S, 11.60. Found: C, 60.68; H, 5.99; N, 10.38; S, 11.89.

Ethyl 4-(4-Chlorophenyl)-2-mercapto-6-methyl-1,4-dihydropyrimidine-5-carboxylate (**7b**). Yield: 85%; m.p.: 210–212 °C; IR (KBr, cm^{-1}): 3302 (NH), 3094 (CH, aromatic), 2982 (CH, aliphatic), 1731 (C=O); ^{1}H NMR (DMSO-d_6): δ 0.94 (t, 3H, *J* = 7.2 Hz, CH_2-$\underline{CH_3}$), 2.30 (s, 3H, CH_3), 3.91 (q, 2H, *J* = 7.2 Hz, $\underline{CH_2}$-CH_3), 6.11 (s, 1H, pyrimidine H-4), 7.30–7.40 (m, 4H, CH aromatic), 8.38 (s, 1H, NH, D_2O exchangeable), 8.74 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 310 (M^+, 41). *Anal*. Calcd. for $C_{14}H_{15}ClN_2O_2S$: C, 54.10; H, 4.86; N, 9.01; S, 10.32. Found: C, 53.88; H, 4.99; N, 9.35; S, 10.03.

Ethyl 2-Mercapto-4-(4-methoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate (**7c**). Yield: 82%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3380 (NH), 3087 (CH, aromatic), 2983 (CH, aliphatic), 1726 (C=O); ^{1}H NMR (DMSO-d_6): δ 0.90 (t, 3H, *J* = 7.2 Hz, CH_2-$\underline{CH_3}$), 2.12 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.85 (q, 2H, *J* = 7.2 Hz, $\underline{CH_2}$-CH_3), 5.73 (s, 1H, pyrimidine H-4), 6.86–7.25 (m, 4H, CH aromatic), 9.92 (s, 1H, NH, D_2O exchangeable), 12.33 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 306 (M^+, 16). *Anal*. Calcd. for $C_{15}H_{18}N_2O_3S$: C, 58.80; H, 5.92; N, 9.14; S, 10.47. Found: C, 58.93; H, 5.61; N, 9.29; S, 10.09.

Ethyl 4-(Furan-2-yl)-2-mercapto-6-methyl-1,4-dihydropyrimidine-5-carboxylate (**7d**). Yield: 86%; m.p.: 200–202 °C; IR (KBr, cm^{-1}): 3336 (NH), 3089 (CH, aromatic), 2979 (CH, aliphatic), 1723 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.03 (t, 3H, *J* = 7.2 Hz, CH_2-$\underline{CH_3}$), 2.23 (s, 3H, CH_3), 3.95 (q, 2H, *J* = 7.2 Hz, $\underline{CH_2}$-CH_3), 6.30 (s, 1H, pyrimidine H-4), 6.33–7.71 (m, 3H, CH furan), 10.25 (s, 1H, NH, D_2O exchangeable), 10.58 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 266 (M^+, 54). *Anal*. Calcd. for $C_{12}H_{14}N_2O_3S$: C, 54.12; H, 5.30; N, 10.52; S, 12.04. Found: C, 53.92; H, 5.09; N, 10.35; S, 11.98.

2-Mercapto-4-methyl-3*H*-chromeno[4,3-*d*]pyrimidin-5(10b*H*)-one (**7e**). Yield 84%; m.p.: 290–292 °C; IR (KBr, cm^{-1}): 3381 (NH), 3088 (CH, aromatic), 2981 (CH, aliphatic), 1722 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 6.98 (s, 1H, pyrimidine H-4), 7.41–8.40 (m, 4H, aromatic), 8.58 (s, 1H, NH, D_2O exchangeable), 11.19 (s, 1H, SH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 26.04, 64.56, 113.43, 121.38, 123.37, 127.73, 129.47, 130.59, 148.93, 150.74, 160.28, 164.46; MS: *m/z* (%) 246 (M^+, 20). *Anal*. Calcd. for $C_{12}H_{10}N_2O_2S$: C, 58.52; H, 4.09; N, 11.37; S, 13.02. Found: C, 58.28; H, 4.43; N, 11.09; S, 12.89.

Synthesis of Ethyl 3-(4-Chlorophenyl)-7-(4-methoxyphenyl)-5-methyl-7*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate (8).
A mixture of 1,4-dihydropyrimidine **7c** (3.06 g, 0.01 mol) with ω-bromo-4-chloroacetophenone (2.34 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL) was heated under reflux for 1 h, left to cool to room temperature, poured onto ice/water, and neutralized by hydrochloric acid. The precipitated solid was filtered, washed with water and crystalized from ethanol.

Yield: 65%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3022 (CH, aromatic), 2932 (CH, aliphatic), 1702 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.15 (t, 3H, *J* = 7.2 Hz, CH_2-$\underline{CH_3}$), 2.06 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 3.75 (q, 2H, *J* = 7.2 Hz, $\underline{CH_2}$-CH_3), 5.67 (s, 1H, CH pyrimidine), 7.15 (s, 1H, CH thiazole), 7.43–8.58 (m, 8H, CH aromatic); MS: *m/z* (%): 440 (M^+, 100). *Anal*. Calcd. for $C_{23}H_{21}ClN_2O_3S$: C, 62.65; H, 4.80; N, 6.35; S, 7.27. Found: C, 62.33; H, 4.54; N, 6.12; S, 6.99.

General Procedure for the Synthesis of 9a–e
A mixture of the appropriate arylaldehyde **1a**–**e** (0.1 mol),

malononitrile (0.66 g, 0.1 mol) and thiourea (0.76 g, 0.01 mol) were heated under reflux in ethanol (40 mL) containing triethylamine (1.0 mL) for 3 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystalized from ethanol.

6-Amino-2-mercapto-4-phenyl-1,4-dihydropyrimidine-5-carbonitrile (**9a**). Yield: 87%; m.p.: 150–152 °C; IR (KBr, cm^{-1}): 3444–3353 (NH_2, NH), 3063 (CH, aromatic), 2191 (CN), 1633 (C=N); ^{1}H NMR (DMSO-d_6): δ 6.82 (s, 2H, NH_2), 7.15–7.64 (m, 6H, 5H aromatic and pyrimidine H-4), 7.90 (s, 1H, NH, D_2O exchangeable), 10.14 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 230 (M^+, 35). *Anal*. Calcd. for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33; S, 13.92. Found: C, 57.09; H, 4.41; N, 24.68; S, 13.79.

6-Amino-4-(4-chlorophenyl)-2-mercapto-1,4-dihydropyrimidine-5-carbonitrile (**9b**). Yield: 82%; m.p.: 207–209 °C; IR (KBr, cm^{-1}): 3380–3258 (NH_2, NH), 3071 (CH, aromatic), 2190 (CN), 1628 (C=N); ^{1}H NMR (DMSO-d_6): δ 6.80 (s, 2H, NH_2), 7.12–7.64 (m, 5H, 4H aromatic and pyrimidine H-4), 7.84 (s, 1H, NH, D_2O exchangeable), 10.17 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 264 (M^+, 29). *Anal*. Calcd. for $C_{11}H_9ClN_4S$: C, 49.91; H, 3.43; N, 21.16; S, 12.11. Found: C, 49.93; H, 3.08; N, 21.42; S, 12.34.

6-Amino-2-mercapto-4-(4-methoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile (**9c**). Yield: 88%; m.p.: 158–160 °C; IR (KBr, cm^{-1}): 3439–3367 (NH_2, NH), 3073 (CH, aromatic), 2219 (CN), 1605 (C=N); ^{1}H NMR (DMSO-d_6): δ 3.94 (s, 3H, OCH_3), 7.23 (s, 2H, NH_2), 7.24–8.05 (m, 5H, 4H aromatic and pyrimidine H-4), 8.45 (s, 1H, NH, D_2O exchangeable), 10.12 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 260 (M^+, 11). *Anal*. Calcd. for $C_{12}H_{12}N_4OS$: C, 55.37; H, 4.65; N, 21.52; S, 12.32. Found: C, 55.59; H, 4.39; N, 21.55; S, 12.34.

6-Amino-4-(furan-2-yl)-2-mercapto-1,4-dihydropyrimidine-5-carbonitrile (**9d**). Yield 81%; m.p.: 280–282 °C; IR (KBr, cm^{-1}): 3324–3247 (NH_2, NH), 3076 (CH, aromatic), 2217 (CN), 1606 (C=N); ^{1}H NMR (DMSO-d_6): δ 6.46 (s, 2H, NH_2), 6.50–8.08 (m, 4H, 3H furan and pyrimidine H-4), 8.81 (s, 1H, NH, D_2O exchangeable), 10.01 (s, 1H, SH, D_2O exchangeable); MS: *m/z* (%) 220 (M^+, 43). *Anal*. Calcd. for $C_9H_8N_4OS$: C, 49.08; H, 3.66; N, 25.44; S, 14.56. Found: C, 49.42; H, 3.75; N, 25.68; S, 14.42

4,5-Diamino-10b*H*-chromeno[4,3-*d*]pyrimidine-2-thiol (**9e**). Yield 79%, m.p. > 300°C; IR (KBr, cm^{-1}): 3438–3345 (2 NH_2), 3080 (CH, aromatic), 1611 (C=N); ^{1}H NMR (DMSO-d_6): δ 6.96 (br s, 4H, 2 NH_2, D_2O exchangeable), 7.24–8.33 (m, 5H, 4H aromatic and pyrimidine H-4), 8.98 (s, 1H, SH, D_2O exchangeable); ^{13}C HMR (DMSO): δ 61.05, 76.12, 112.02, 129.25, 130.43, 134.80, 164.98, 169,47, 172.23, 173.40; MS: *m/z* (%) 246 (M^+, 19). *Anal*. Calcd. for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09; N, 22.75; S, 13.02. Found: C, 53.82; H, 4.39; N, 22.47; S, 12.74.

Synthesis of 5-Amino-7-(4-methoxyphenyl)-3-phenyl-7*H*-thiazolo[3,2-*a*]pyrimidine-6-carbonitrile (**10**).
A mixture of 1,4-dihydropyrimidine **9c** (2.6 g, 0.01 mol) and ω-bromoacetophenone (2 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL) was heated under reflux for 2 h, left to cool to room temperature, poured onto ice/water, and neutralized by hydrochloric acid. The precipitated solid was filtered, washed with water and crystalized from ethanol.

Yield: 58%; m.p.: 148–150 °C; IR (KBr, cm^{-1}): 3439 (NH_2), 3073 (CH, aromatic), 2223 (CN), 1612 (C=N); ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 5.21 (s, 1H, CH thiazole), 7.12 (s, 2H, NH_2), 7.15–8.15 (m, 10H, 9H aromatic and 1H pyrimidine); MS: *m/z* (%) 360 (M^+, 30). *Anal*. Calcd. for $C_{20}H_{16}N_4OS$: C, 66.65; H, 4.47; N, 15.54; S, 8.90. Found: C, 66.32; H, 4.22; N, 15.67; S, 8.78.

3. 2. *In vitro* Cytotoxic Assay

Chemicals: Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethylsulfoxide (DMSO), CHS 828, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures: were obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and were routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for the six human cancer cell lines followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

4. Conclusion

In this study we synthesized a series of 4,5-dihydro-2-mercapto-4-oxo-6-substituted arylpyrimidine derivati-

ves **2a–e** and their fused analogues **3b**, **4b**, **5b**, **6b**, as well as 1,4-dihydro-2-mercaptopyrimidine derivatives **7a–e** and **9a–e** using triethylamine as a catalyst. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against six human cancer cell lines and normal fibroblast cells. Compounds **2a**, **3b** and **9c** were found to be the most potent derivatives. Toxicity of the most potent compounds was measured against shrimp larvae; the results showed that compounds **2a** and **3b** are non-toxic towards the tested organisms. Most of the compounds that exhibited anticancer activity were nontoxic to normal fibroblast cells suggesting that they may serve as lead compounds for further development of new drugs.

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Povzetek

Razvili smo enostavno in učinkovito metodo za sintezo 4,5-dihidro-2-merkapto-4-okso-6-substituiranih arilpirimidinskih derivatov **2a**–**e** ter njihovih pripojenih analogov **3b**, **4b**, **5b**, **6b** kot tudi 1,4-dihidro-2-merkaptopirimidinskih derivatov **7a**–**e**, **9a**–**e**, ki temelji na uporabi trietilamina kot katalizatorja. Strukture novih produktov smo potrdili na osnovi njihovih spektroskopskih podatkov in elementnih analiz. Za vse pripravljene spojine smo določili njihovo *in vitro* delovanju proti šestim človeških rakastim celičnim linijam in normalnim fibroblastom. Devet izmed preiskovanih spojin (to so **2a**, **2c**, **2d**, **3b**, **4b**, **5b**, **8**, **9a** in **9c**) je izkazovalo občutno citotoksičnost proti večini celičnih linij. Izmed teh derivatov so se spojine **2a**, **3b** in **9c** pokazale kot najbolj učinkovite, saj so se citotoksični efekti proti šestim celičnim linijam pojavili že pri IC_{50} vrednostih < 330 nM, kar je zelo učinkovito v primerjavi s standardom CHS 828. Normalne fibroblastne celice (WI38) pa so bile na preiskovane spojine bistveno manj občutljive (IC_{50} > 10,000 nM). Izmerili smo tudi morebitno toksičnost najbolj učinkovitih spojin pri ličinkah rakcev *Artemia salina*; rezultati so pokazali, da sta spojini **2a** in **3b** za preiskovani organizem nestrupeni.