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Novel Reaction Products from Thiobarbituric Acid of Biological Interest

The reactions of 1,2-dihydro-2-thioxo-4,6(*1H,5H*)pyrimidinedione (**1)** with some π-deficients have led to the formation of unexpected heterocyclic products such as anilinomethylenethioxopyrimidinedione, 2,2′-bis(pyrimidinecarbothioamide), allylthioxopyrimidinecarbothioamide, indenopyranopyrimidine and benzofuropyrimidine derivatives. A possible mechanism for the formation of these products is discussed, and the biological activity is determined.

Keywords: Thiobarbituric acid; π-Deficients; Biological activity

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Introduction

1,2-Dihydro-2-thioxo-4,6(*1H,5H*)pyrimidinedione (thiobarbituric acid, **1**) is well known as one of the established class of pyrimidinethiols. These compounds and their derivatives are useful intermediates in pyrimidine chemistry, since the sulfur substitute can be easily removed to give ring-fused pyrimidines [1−4], or can be displaced by a variety of nucleophilies [5-8]. The particularly interesting thiobarbituric acid (**1**) is accessible to nucleophilic attacks at C-5 and/or C-2 [9−21], condensation with aldehydes [22−24], synthesis of dyes [25−29], as well as condensation with benzoin to give thioxofuro[2,3-*d*]pyrimidines [15]. Furthermore, the pyrimidine-2-thiole moiety is present in several compounds of biological and medicinal interest [30-34].

Our long-term and continuing interest in chemical reactions induced by donor-acceptor interactions via charge-transfer (CT)-complexation is part of our systematic efforts to obtain new heterocyclic systems [35−39]. Recently, we described the reaction of 2-thioxo-1,2,4-tetrahydropyrimidine with tetracyanoethylene, dicyanomethyleneindane-1,3-dione as well as 1,4-benzo-and naphthoquinones to synthesize several heterocyclic compounds [40].

Results and discussion

Synthesis and spectroscopic characterization

In this paper, we describe the reaction of thiobarbituric acid (**1)** as an active donor with some selective π-deficient compounds. It has been reported that thiobarbituric acid (**1)** and phenylisocyanate (**2**) stirred in pyridine at 75−80 °C gave pyrimidine carboxanilide (**3**) [41, 42].

In the present work, we describe the results obtained by refluxing equimolar amounts of compound **1** and **2** in DMF; 5-(anilinomethylene)-2-thioxohexahydro-4,6-pyrimidinedione (**4**) [43] was obtained in 87% yield. The formation of **4** can be rationalized in terms of the Vilsmeier-Haack reaction as illustrated in Scheme 1. The structural assignment of **4** was established from comparison of its IR, NMR, and mass spectra as well as melting point with those of an authentic sample prepared according to reference [43].

Interestingly, on reacting **1** with *N*-phenyl-1,2,4-triazoline-3,5-dione (**9**) in DMF, compound **4** was also obtained (Scheme 1). This could be rationalized in terms of the decomposition of **9** in high boiling solvents to give **2** in high yield [44], which then reacts with **1** to give **4** (Scheme 1).

On the basis of structural features, phenyl isothiocyanate (**10**) should react with **1** in a similar manner as **2**. However, refluxing equimolar amounts of phenyl isothiocyanate (**10**) and **1** in DMF afforded an interesting and unexpected compound; 2,2′-bis(N5-phenyl-4,6-dihydroxy-5-pyrimidine-carbothioamide) (**11**). As shown in the proposed mechanism (Scheme 2), a nucleophilic addition of pyrimidine-CH_2 on **10** gave **12** followed by dimerization with elimination of sulfur and dehydro-genationto afford the product **11**. Compound **11** displayed characteristic IR absorptions at ν = 3410−3100 cm^{-1} (OH and NH groups). The ^{13}C-NMR spectrum of **11** showed absorption signals at δ = 151.6 for pyrimidine-C-2, δ = 197.0 for C=S, and δ = 172.2 for pyrimidine C-4 and C-6. The ^{1}H-NMR spectrum clearly shows aromatic protons at the expected position. Furthermore, phenyl-NH resonates as broad sin-

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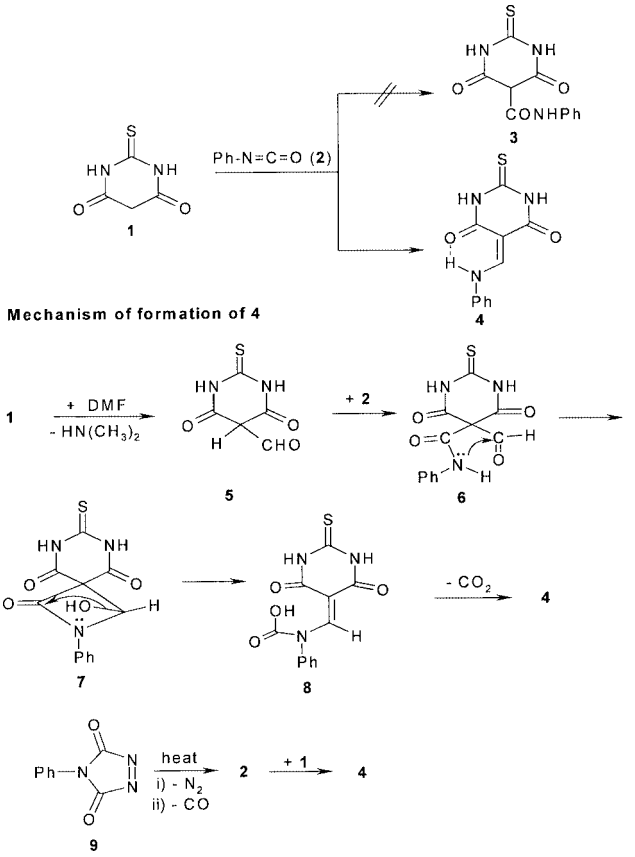
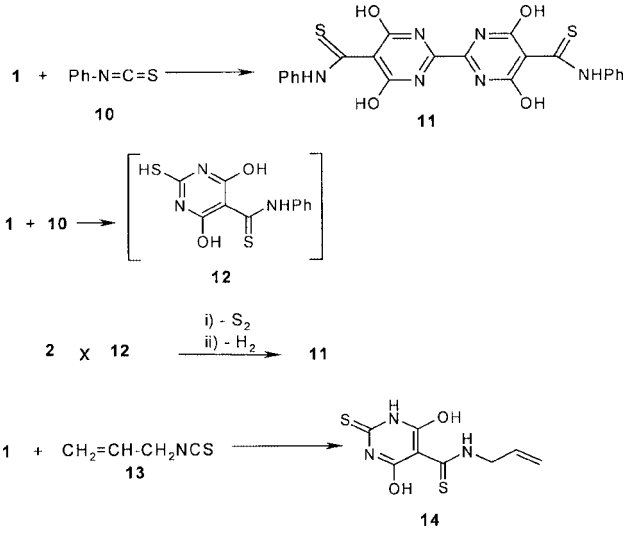
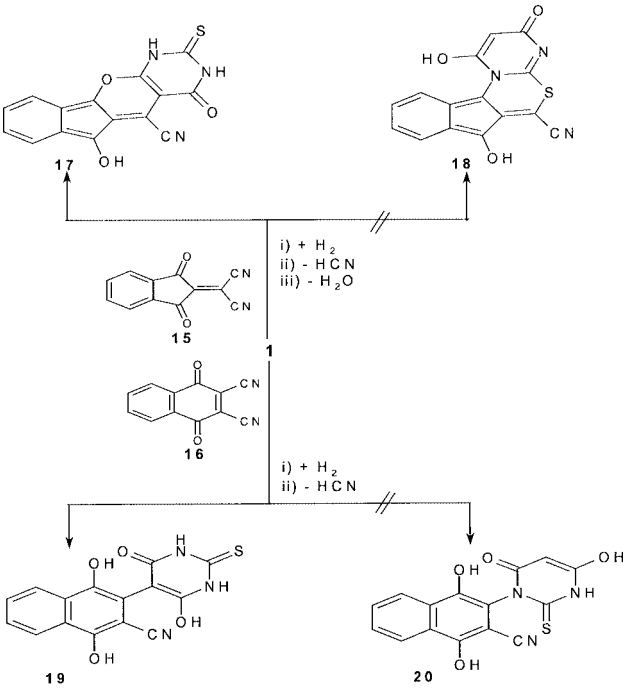


Scheme 1.



Scheme 2.

glet at δ = 9.20. The molecular ion peak in the mass spectrum and satisfactory elemental analysis are also a further support for the structure of **11**.



Scheme 3.

In a different manner compound **1** reacted with allyl isothiocyanate (**13**) to give N5-allyl-4,6-dihydroxy-2-thioxo-1,2-dihydro-5-pyrimidinecarbothioamide (**14**) (Scheme 2). The IR spectrum of **14** showed characteristic absorptions at ν = 3444-3132 cm^{-1} for OH and NH groups. The ^{1}H-NMR spectrum showed signals at δ = 5.90 due to olefinic-CH, 5.20 for olefinic-CH_2, 4.20 for CH_2-N and at d = 8.20 for aliphatic-NH in addition to the pyrimidine-NH at δ = 12.50. The ^{13}C-NMR spectrum, analytical data and mass spectrum support the proposed structure **14**.

Continuation of our studies on the reactions of 2-dicyanomethyleneindane-1,3-dione (**15**) with compounds containing active methylene groups such as *N*-arylisoindolines [45], arylaminomethylbenzimidazole-2-thiols [46], thioxopyrimidine derivatives [40], benzimidazolylacetonitrile [47], and 2-mercaptobenzazoles [48] prompted us to investigate the behavior of 2-dicyanomethyleneindane-1,3-dione (**15**) and its facile isomer 2,3-dicyano-1,4-naphthoquinone [49, 50] (**16**) towards **1**. In the present investigation both compounds, 6-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydroindeno[2′,1′:5,6]pyrano[2,3-*d*]pyrimidine-5-carbonitrile (**17**) and 1,4-dihydroxy-3-(6-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-2-naphthonitrile (**19**), were rather formed than **18** and **20** (Scheme 3).

The ^{1}H-NMR spectrum of **17** clearly shows the presence of pyrimidine-NHs at δ 11.50 and 12.15 ppm, in

addition to the aromatic protons. The ^{13}C-NMR of **17** shows signals at 105.20 (C-5), 117.20 (CN), 150.10 (C-6), 148.52, 157.34, 171.36 (C-10*b*, C-5*a*, C-11*a*), 167.20 (C = O) and 178.00 (C = S), in addition to the aromatic carbons. Compound **18** could be ruled out, owing to the presence of C = S signal in the ^{13}C-NMR and pyrimidine-NH's in the ^{1}H-NMR spectra.

The ^{1}H-NMR spectrum of **19** displayed two broad singlets at 11.80 and 12.15 ppm due to pyrimidine-NH's, in addition to the aromatic protons. In its ^{13}C-NMR spectrum, C-6 and C-5 resonate at δ 166.44 and 105.00 respectively; further peaks at δ 143.20, 149.90 (Ar-*C*-OH), 167.34 (C = O) and 182.50 (C = S) were also observed. Consequently, compound **20** could be eliminated on the basis of ^{1}H-NMR spectrum of **19**, which clearly confirms the presence pyrimidine-NHs and absence of pyrimidine-CH.

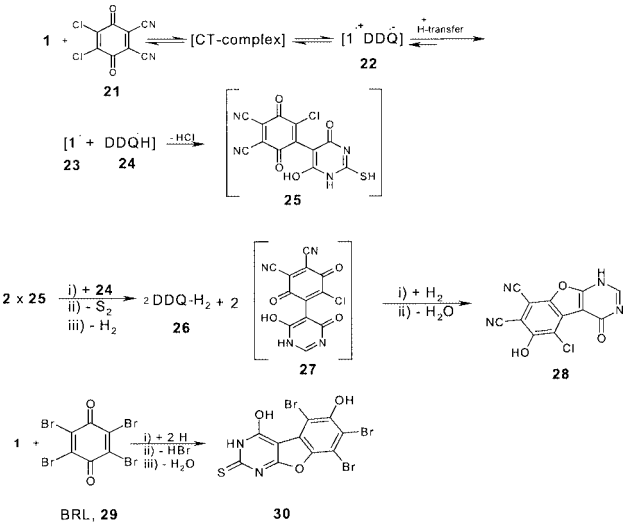
From the above findings it can be concluded that the pyrimidine-CH_2 is the more reactive center in thiobarbituric acid (**1**). Moreover, it is interesting to explore the different behavior of both isomers **15** and **16** towards **1**.

Interestingly, the interaction between 2,3-dichlro-5,6-dicyano-1,4-benzoquinone (DDQ, **21**) and **1** resulted in formation of benzofuropyrimidine derivative **28**, in addition to DDQ-H_2 (**26**).

The IR spectrum of **28** shows two sharp absorptions at 1690 and 2220 cm^{-1} assigned to carbonyl and cyano groups, respectively, as well as hydroxyl group at 3460 cm^{-1}. The ^{1}H-NMR spectrum of **28** clearly shows the presence of pyrimidine-CH at d 9.25 and pyrimidine-NH at 11.88. The ^{13}C-NMR (DMSO-d_6) of **28** shows signals at 125.60, 132.60, 153.80, 155.90 and 156.50 for (C-4*a*, C-11*a*, C-10*b* and C-6), respectively, 137.60 (C-2) and 164.20 (C = O). The molecular formula of **28** was supported by elemental analysis and mass spectrum which gave the expected molecular ion peak as base peak.

A proposed mechanism for the formation of the reaction products is illustrated in Scheme 4. Initially a blue-colored CT-complex was formed, which gradually changed into ion pair **22** containing **1** cation radical and DDQ anion radical. Hydrogen proton transfer from the cation radical to DDQ anion radical generates **23** and **24**. The presence of semiquinone **24** induces the dehydrogenation of **25,** which is followed by dimerization to give the intermediate **27** after split off sulphur [37, 48, 51] (identified experimentally). The latter abstracts a molecule of hydrogen from **1** and eliminates another from H_2O to give 5-chloro-6-hydroxy-4-oxo-1,4-dihydrobenzo[4,5]furo[2,3-*d*]pyrimidine-7,8-dicarbonitriles (**28)**.

On the other hand, the interaction of **1** with BRL (**29**) afforded 5,7,8-tribromo-4,6-dihydroxy-2,3-dihydrobenzo[4,5]furo[2,3-*d*]pyrimidine-2-thione (**30**) (Scheme 4). The gross formula, $C_{10}H_3Br_3N_2O_3S$ of **30** was confirmed by the mass spectrum, which exhibited the molecular ion at m/z 470 (100 %). The IR spectrum showed absorptions at 3430−3250 cm^{-1} (OH and NH). The ^{1}H-NMR displayed one broad singlet at 11.80 ppm for pyrimidine-NH. In the ^{13}C-NMR spectrum, C-6 and C-4 resonate at δ 165.30 and 162.40 ppm respectively, 156.80 (C-8*a*), 172.14 (C-9*a*) and 182.20 (C = S).



Scheme 4.

Biological properties

In vitro test for cytotoxic activity [54]

All compounds were tested against tumor cells and were found to be inactive with the exception of the following two compounds (Table 1).

In vitro disease-oriented primary antitumor screen [55]

In the routine stage screening each agent is tested over a broad concentration range against every cell line in the current panel. The parameter used is GI_{50}, which is the $\log_{10}$ concentration at which the PG is +50. As explained in 'Experimental', only the tested compounds **17** and **28** show activity against cell lines for leukemia, colon cancer, breast cancer, ovarian cancer, renal cancer, and prostate cancer**.** In addition,

Table 1.

Compound No.	% inhibition of cell viability			
	Dose 100 μg/0.1 mL	**Dose** 75 μg/0.1 mL	**Dose** 50 μg/0.1 mL	**Dose** 25 μg/0.1 mL
17	100	100	100	100
28	100	100	75	60

compound **17** shows activity against a CNS cancer cell line.

Antihyperlipedmic activity [56]

Using triton-induced hyperlipidemia animal design model, where the systemic administration of triton to mice results in a biphasic elevation of plasma cholesterol and triglycerides. The mechanism of triton-induced hypercholesterolemia in phase I is thought to be due to increased hepatic synthesis of cholesterol through the ability of triton to interfere with the uptake of plasma lipids by the tissue. Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while drugs interfering with cholesterol excretion and metabolism were active in phase II. In the present investigation, both compounds, **11** and **19,** were found to act in phases I and II to lower the cholesterol serum level.

General hypnotic activity [57]

On performing the potentiation of thiopental sleeping time *in vivo* method for the evaluation of the hypnotic activity, it was found that compound **4** is a very short acting general hypnotic at a dose level of 35 mg/kg and lasted for 10 min, while compound **14** is a short acting, general hypnotic (dose level 40 mg/kg) and lasted for 30 min.

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Experimental

Apparatus and chemical methods

Melting points are uncorrected. Elemental analyses were measured in the Microanalytical Center at Cairo University. IR spectra were recorded on 320 FT. IR and Shimadzu 408 spectrophotometer using KBr pellets. MS: Finnigan MAT 8430 at 70 eV (Finnigan, Bremen, Germany). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (101 MHz): Bruker AM-400 (Bruker, Billerica, MA, USA) with DMSO-d_6 as solvent, with tetramethylsilane (TMS) as internal standard. Preparative layer chromatography (plc) used air-dried 1.00 mm thick layers of slurryly applied silica gel Merck PF_{254} (Merck, Darmstadt, Germany) on 48 cm wide and 20 cm high glass plates, using the solvents listed. Zones were detected by quenching of indicator fluorescence upon exposure to 254 nm UV light and eluted with acetone.

Starting materials

1,2-Dihydro-2-thioxo-4,6(*1H,5H*)pyrimidinedione (thiobarbituric acid, **1**), phenyl isocyanate (**2**), phenyl isothiocyanate (**10**), allyl isothiocyanate (**13**), and N-phenyl-1,2,4-triazoline-3,5-dione (**9**) were purchased from Aldrich (Aldrich, Munich, Germany) and Fluka (Switzerland) and used as received. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, **21**), 2,3,5,6-tetrabromo-1,4-benzoquinone (BRL, **29**), 2-dicyanomethylene-indane-1,3-dione (**15**), 2,3-dicyano-1,4-naphthoquinone (**16**) were purified and prepared as described before [35].

Chemical Reactions

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with phenyl isocyanate* **2**

A solution of phenyl isocyanate **2** (0.12 g, 1 mmol) in 5 mL DMF was added to a stirred solution of **1** (0.144 g, 1 mmol) in 15 mL of DMF. The mixture was refluxed for 12h. A yellow precipitate was formed which was filtered and washed with ethanol. Recrystallization from DMF/EtOH afforded pure yellow crystals of 5-(anilinomethylene)-2-thioxohexahydro-4,6-pyrimidinedione **4**, 0.215 g (87 %), m.p. 343-45 °C [43].

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with N-phenyl-1,2,4-triazoline-3,5-dione* **9**

A mixture of **1** (0.144 g, 1 mmol) and N-phenyl-1,2,4-triazoline-3,5-dione (**9**) (0.18 g, 1 mmol) was refluxed in 20 mL of DMF for 48h. Compound **4** was precipitated, filtered and recrystallized from DMF/EtOH (0.15 g, 61 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with phenyl isothiocyanate* **10**

To a solution of **1** (0.144 g. 1 mmol) in 15 mL DMF, a solution of phenyl isothiocyanate (**10**) (0.135 g, 1 mmol) in 5 mL DMF was added. The reaction mixture was refluxed for 20 h. An

orange precipitate was formed and recrystallized from DMF/EtOH to give orange crystals of 2,2′-bis(N5-phenyl-4,6-dihydroxy-5-pyrimidinecarbothioamide) **11** (0.25 g, 74 %), 275−77 °C; IR (KBr): ν_{max} 3410−3100 cm^{-1} (OH, NH) and 1600 (C=N); ^{1}H-NMR (DMSO-d_6, 400 MHz): d 7.42-7.86 (m, 10H, Ar-H), 9.20 (br, s, 1H, Ph-NH); ^{13}C-NMR (DMSO-d_6, 100.6 MHz): d 124.5 (Ph-2 C), 124.7 (2 pyrimidine-C-5), 125.3 (Ph-4 C), 128.6 (Ph-2 C), 128.8 (Ph-2 C), 139.4 (2 Ar-C-NH), 151.6, 172.2, 173.2 (2 pyrimidine-C-2, C-4, C-6), 197.0 (2 CS); MS (70 eV): *m/z* (%) 492 (M^+,11), 338 (96), 246 (36), 228 (29), 92 (100), 77 (44), 57 (38) (Found: C, 5.81; H, 3.14; N, 16.94; S, 13.16. $C_{22}H_{16}N_6O_4S_2$ requires C, 53.65; H, 3.27; N, 17.06; S, 13.02 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with allyl isothiocyanate* **13**

In a stirred solution of **1** (0.144 g, 1mmol) in 15 mL DMF a solution of allyl isothiocyanate (**13**) (0.119 g, 1 mmol) in 5 mL DMF was added. The reaction mixture was refluxed for 8 h. A reddish-brown precipitate was formed. The precipitate was collected and purified by dissolving it in the minimum amount of acetone and then applying it for chromatographic (plc). Elution with chloroform/methanol (10:1) gives only one zone which was recrystallized from methanol to give a reddish-brown crystals of N5-allyl-4,6-dihydroxy-2-thioxo-1,2-dihydro-5-pyrimidinecarbothio-amide **14** (0.18 g, 75 %), m.p. 238−240 °C; IR (KBr): ν_{max} 3444-3132 cm^{-1} (OH, NH), 2890 (aliphatic- CH); ^{1}H-NMR (400 MHz, DMSO-d_6) d 4.20 (d, 2H, CH_2-N), 5.20 (m, 2H, CH_2), 5.90 (m, 1H, CH), 8.20 (br, s, 1H, NH), 12.50 (br, s, 1H, pyrimidine-NH); ^{13}C-NMR(DMSO-d_6, 100.6 MHz) δ 51.10 (CH_2-N), 67.60 (pyrimidine-C-5), 114.40 (CH_2=), 133.80 (CH), 171.60 (pyrimidine-C-4 and C-6), 181.60 (pyrimidine-C-2), 191.20 (C=S); MS (70 eV), *m/z* (%) 243 (M^+, 24), 225 (100), 209 (5), 186 (15), 100 (9), 56 (19) (Found: C, 39.61; H, 3.65; N, 17.17;S, 26.42. $C_8H_9N_3O_2S_2$ requires C,39.49; H, 3.73; N, 17.27; S, 26.36 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with 2-dicyanomethyleneindane-1,3-dione* **15**

A solution of 2-dicyanomethyleneindane-1,3-dione (**15**) (0.416 g, 2 mmol) in 10 mL DMF was added to a solution of **1** (0.144 g, 1mmol) in 10 mL DMF. The reaction mixture was refluxed for 4h to give a brown precipitate. The precipitate was dissolved in 5 mL DMF and then applied on plc using chloroform/methanol (5:1) as eluent to give only one zone characterized with a brown color. Recrystallization from DMF gave 6-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydroindeno-[2′,1′:5,6]-pyrano[2,3-d]pyrimidinyl-5-carbonitrile **17** (0.21 g, 69 %), m.p. 310−12 °C; IR (KBr): ν_{max} 3440-3220 cm^{-1} (OH, NH), 2210 (CN) and 1690 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.00-7.45 (m, 4H, Ar-H), 11.50 (br, s, 1H, pyrimidine-NH), 12.15 (br, s, 1H, pyrimidine-NH); ^{13}C-NMR (DMSO-d_6, 100.6 MHz), δ 105.20 (C-5), 107.90, 108.10, 108.40, 113.10 (Ph-C), 129.50 (2 Ph-C), 117.20 (CN), 150.10 (C-6), 148.52, 157.34, 171.36 (C-10 b, C-5a, C-11 a), 167.20 (C-4) and 178.00 (C-2); MS (70 eV), *m/z* (%) 309 (M^+, 100), 292 (26), 279 (18), 266 (22), 250 (18), 226 (60), 210 (22), 196 (32), 168 (8), 152 (16), 76 (20) and 44 (62) (Found: C, 58.19; H, 2.33; N, 13.64; S, 10.29. $C_{15}H_7N_3SO_3$ requires,C, 58.25; H,,2.28; N, 13.59; S, 10.37 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with 2,3-dicyano-1,4-naphtho-quinone* **16**

A solution of 2,3-dicyano-1,4-naphthoquinone (**16**) (0.416 g, 2 mmol) in 10 mL DMF was added dropwise to a solution of **1** (0.14 g, 1 mmol) in 10 mLDMF at room temperature. The reaction mixture becomes pale blue and later turns into violet color. It was left standing for 48h, then concentrated and the residue was purified by plc using toluene/ethyl acetate (2:1) to give 1,4-dih-ydroxy-3-(6-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-2-naphthonitrile **19** (0.25 g, 77 %) as violet crystals from acetone, m.p. 323−25 °C; IR (KBr): ν_{max} 3450-3260 cm^{-1} (OH, NH), 2220 (CN) and 1695 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.30−7.52 (m, 4H, Ar-H), 11.80 (br, s, 1H, pyrimidine-NH), 12.15 (br, s, 1H, pyrimidine-NH); ^{13}C-NMR (DMSO-d_6, 100.6 MHz) d 89.90, 115.70, 116.92, 120.82, 121.30, 122.94, 126.32, 128.12, 129.96 (Ph-C and CN), 143.20, 149.90 (Ar-*C*-OH), 105.00 (C-5), 166.44 (C-6), 167.34 (C-4) and 182.50 (C-2); MS (70 eV), *m/z* (%) 327 (M^+, 100), 298 (28), 266 (30), 250 (22), 234 (14), 144 (26), 128 (34), 77 (12) (Found: C, 55.12; H, 2.69; N, 12.91; S, 9.68. $C_{15}H_{19}N_3O_4S$ requires C, 55.04; H, 2.77; N, 12.84; S, 9.80 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with 2,3-dicyano-5,6-dichloro-1,4-benzoquinone* **21**

A solution of 2,3-dicyano-5,6-dichloro-1,4-benzoquinone (**21**) (0.454 g, 2 mmol) in 10 mL DMF was added dropwise to a solution of **1** (0.144 g, 1 mmol) in 10 mL DMF at room temperature. The reaction mixture becomes deeply blue and later turns into yellow color. It was left standing for 48h. The mixture was concentrated and the residue was separated by plc using toluene/ethyl acetate (1:1) into three zones. The fastest migrating zone contained sulphur, the second zone contained 5-chloro-6-hydroxy-4-oxo-1,4-dihydrobenzo[4,5]furo-[2,3-d]pyrimidine-7,8-dicarbonitrile **28**, and finally the slowest one contained DDQ-H_2 (**26**). Compound **28** was recrystallized from methanol to give a yellow crystals (0.18 g, 63 %), m.p.280 °C (decomp.); IR (KBr): ν_{max} 3460 cm^{-1} (OH), 2220 (CN) and 1690 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 9.25 (s, 1H, pyrimidine-CH), 11.88 (br, s, 1H, pyrimidine-NH); ^{13}C-NMR (DMSO-d_6, 100.6 MHz), δ 116.80,119.30 (Ph-*C*-Cl, Ph-*C*-CN and CN), 125.60, 132.60, 153.80, 155.90, 156.50 (C-4a, C-4b, C-11a, C-10b and C-6), 137.60 (C-2) and 164.0 (C-4); MS (70 eV): *m/z* (%) 288 (30), 286 (M^+, 100), 284 (32), 266 (34), 246 (24), 220 (32), 204 (34), 176 (18), 142 (16), 106 (30) and 90 (18) (Found: C, 50.35; H, 1.12; N, 19.42; Cl, 12.29. $C_{12}H_3ClN_4O_3$ requires C, 50.28; H, 1.05; N, 19.55; Cl, 12.37 %).

Reaction of 1,2-Dihydro-2-thioxo-4,6(1H,5H)pyrimidinedione **1** *with 2,3,5,6-tetrabromo-1,4-benzoquinone (BRL)* **29**

A mixture of **1** (0.144 g, 1 mmol) and 2,3,5,6-tetrabromo-1,4-benzoquinone (BRL, **29**) (0.424 g, 1 mmol) in 20 mL glacial acetic acid was refluxed for 5h. The reaction mixture was cooled and added to ice-cold water (200 mL), to give a yellow precipitate. The precipitate was washed several times with water and recrystallized from DMF to afford a brown crystals of 5,7,8-tribromo-4,6-dihydroxy-2,3-dihydrobenzo[4,5]furo-[2,3-d]pyrimidine-2-thione **30**: (0.37 g, 78 %), m.p. 350 °C; IR (KBr): ν_{max} 3430-3250 cm^{-1} (OH, NH); ^{1}H-NMR (DMSO-d_6, 400 MHz), δ 11.80 (br, s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 100.6 MHz) δ 121.20, 125.44, 127.34 (Ph-C and Ph-*C*-Br), 156.8 (C-8a), 165.30 (C-6), 162.40 (C-4), 172.14 (C-9a) and 182.20 (C-2); MS (70 eV): m/z (%) 474 (20), 472 (94), 470 (M^+, 100), 468 (20), 465 (35), 390 (22), 388 (44), 350 (36), 270 (18), 210 (40), 150 (30), 82 (65) and 60 (54) (Found: C, 25.63; H, 0.58; N, 6.09; S, 6.75. $C_{10}H_3Br_3N_2O_3S$ requires C, 25.51; H, 0.64; N, 5.95; S, 6.81 %).

Biological properties
In vitro test for cytotoxic activity

A set of sterile test tubes was used where 2.5×10^5 tumor cells per mL (Ehrlich ascites carcinoma) were suspended in phosphate buffer saline, then 0.1 mL equivalent to 100 mg of the compound suspended in water and Tween 80 were added. The test was incubated for two hours at 37 °C. Trypan blue exclusion test was then carried out to calculate the percentage of non viable cells after two hours in cubation. Compounds producing more than 70% non viable cells or less than 30% viable cells were considered active.

In vitro disease-oriented primary antitumor screen

In the routine stage screening, each agent is tested over a broad concentration range against every cell line in the current panel. All lines are incubated onto a series of standard 96 well microtitre plates on day 0, in the majority of cases at 20,000 cells/well, then pre-incubated in absence of drug for 24 hours. Tested drugs are then added in five to ten fold dilutions starting from the highest concentration and incubated for further 48 hours. Following this, the cell are fixed *in situ*, washed and dried, sulforhodanine B (SRB) was added, the bound stain is solubilized and measured spectrophotometrically on automatic plate readers.

Antihyperlipedmic activity

Male mice weighing 20-25 g are starved for 20 hours and then injected intravenously with 200mg/kg triton WR 1339 [isooctylpolyoxyethylenephenol]. Serum cholesterol level increases sharply 2-3 times after 24 hours (phase I). The hypercholesterolemia decreases nearly to control level within the next 24 hours (phase II). The tested agent and the gum accacia (used to solubilize the drug) are administrated simultaneously with the triton injection. Serum cholesterol analysis are made 5, 24, and 48 hours after triton injection.

The parameter used here is GI_{50} (Table 2) which is the $\log_{10}$ concentration at which the PG is + 50 (Table 3).

General hypnotic activity

On performing the potentiation of thiopental sleeping time *in vivo* method for evaluation of the hypnotic activity, that depend on injection of the tested compounds **4** and **14** or the standard 60 min before *i.v.* injection of 25 mg/kg thiopental to mice with a weight between 18 and 22 g . The animals are placed on their backs and the reappearance of the righting reflux is observed.

Toxicological Studies (Tab. 4)

Table 2.

Cell line	Compound	GI_{50}
Leukemia	**17**	5.22^{E-05}
CCRF-CEM	**28**	3.38^{E-05}
SR	**17**	4.05^{E-05}
	28	3.85^{E-05}
HL-60	**17**	4.70^{E-05}
	28	3.81^{E-05}
MOLT-4	**17**	9.42^{E-05}
	28	7.16^{E-05}
RPMI-8226	**17**	4.70^{E-05}
	28	9.24^{E-05}
Colon cancer	**17**	4.97^{E-05}
Colo 201	**28**	4.44^{E-05}
Breast cancer	**17**	7.58^{E-05}
T-47 D	**28**	6.18^{E-05}
Ovarian cancer	**17**	3.54^{E-05}
IGR OV1	**28**	2.98^{E-05}
Renal cancer	**17**	3.81^{E-05}
786-O	**28**	2.96^{E-05}
Prostate cancer	**17**	6.23^{E-05}
PC-3	**28**	5.31^{E-05}
CNS cancer		
SF − 539	**17**	4.36^{E-05}
SNB − 19	**17**	8.16^{E-05}
SNB − 57	**17**	8.31^{E-05}
U 251	**17**	5.17^{E-05}

Table 3.

Agent	% of serum cholesterol to normal control value			
	0 h	6 h	24 h	48 h
Control	100	100	100	100
Triton	100	228	231	226
Clofiberate	100	104	98	98
Compound **11**	100	103	88	82
Compound **19**	100	106	91	86

Table 4.

	Compound	Dose
ED50	**4**	20 mg/kg
	14	23 mg/kg
D50	**4**	1130 mg/kg
	14	1210 mg/kg

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