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Synthesis and Antiproliferative Activity of Basic Thioanalogues of Merbarone

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Abstract—Three series of 5-substituted 1,3-diphenyl-6-(ω-dialkyl- and ω-cyclo-aminoalkyl)thio-2-thiobarbiturates (**11**–**13**) were synthesized as polysubstituted thioanalogues of merbarone, a topoisomerase II inhibitor acting on the catalytic site. To better understand pharmacophore requirements, a forth series of conformationally constrained analogues **14** was also prepared. Derivatives **11b**,**e**, **14b**,**e**,**h**,**i**,**j** were active in the low micromolar concentration range (IC_{50}: 3.3–4.3 μM), whereas compounds **11a**,**c**,**d**,**f**,**h**,**j** and **13a**,**b**,**d**,**g**,**j** and **14a**,**d**,**f** showed IC_{50} values between 10 and 15.5 μM. In constrast, compounds **12a**–**c**,**g**–**j**, **13e**,**f**,**h** and **14k** were inactive. Cytotoxicity data provided from N.C.I. on selected compounds provided evidence that **11b**,**d**, **13d**,**g** and **14b**,**d**,**f**,**h**,**i**,**j** were endowed with potent antiproliferative activity against leukemia and prostate cell lines (GI_{50} up to 0.01 μM). In general, bicyclic derivatives **14** were up to 10-fold more potent than monocyclic counterparts against solid tumor-derived cell lines. SAR studies indicated that, in general, a certain tolerability in length of the alkyl side chains and in shape of distal amines is allowed in the four series, but in the monocyclic derivatives (**11**–**13**) antiproliferative activity was strongly affected by the nature of the 5-substituents ($COOC_2H_5 > COCH_3 \gg C_6H_5$). Compounds **11b** and **14b** were also evaluated against KB cell subclones expressing altered levels of topoisomerases or the multidrug resistance phenotype (MDR). In both cases the above compounds showed a decrease in potency. In enzyme assays, **11b** and **14b** turned out to be inhibitors of topoisonerase II as merbaron.

Introduction

Most of the antitumor agents currently used in the treatment of human malignancies are targeted at topoisomerase II (topo II). The function of this enzyme is that of relaxing a supercoiled DNA by allowing double-stranded DNA (dsDNA) chains to pass one through another following a cleavage generating blunted DNA ends. Notwithstanding DNA breaks, genome integrity is maintained because topo II attaches to the newly generated DNA termini forming transient enzyme–DNA complexes.[1,2]

The topo II inhibitors referred to as 'poisons' (etoposide, doxorubicin, mitoxantrone and amsacrine) convert this crucial enzyme into a potent cellular toxin that eventually drives cells to death.[3,4] In fact, they do not inhibit the topo II cleavage activity, rather they increase the physiologic concentration and/or life-time of the cleavage complexes, thus converting transient DNA breaks into permanent fractures.[3–7] A consequence of this peculiar mode of action is that the higher are the enzyme levels, the more lethal are the effects of topo II poisons.[8,9] This explains why topo II poisons result particularly efficacious against aggressive cancers which possess high enzyme levels,[9,10] whereas they are ineffective against slow growing cancers, which possess low topo II levels.[3,4,11]

Compounds interfering with the catalytic activity of the enzyme [merbarone **1**,[12] fostriecin **2**,[13] aclarubicin,[14] and dexrazoxane (ICRF-187, belonging to bis(2,6-dioxopiperazine derivatives **3**[15,16] (Fig. 1)] are expected to be active against both fast and slow growing cancers.[11,17] Among them, merbarone is particularly interesting for the following reasons: (i) it acts primarily by blocking

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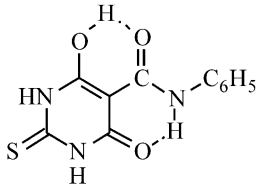
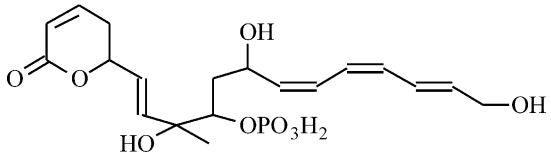
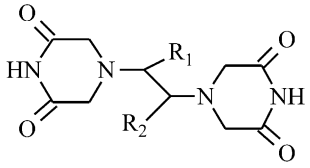


1, Merbarone



2, Fostriecin



3, Bis(2,6-dioxopiperazine) derivatives

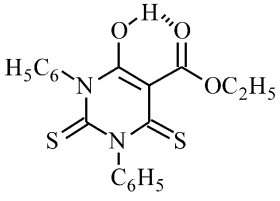
Figure 1. Structures of topisomerase II inhibitors that do not stabilize DNA–topoisomerase II complexes.

topo II-mediated DNA cleavage without intercalating into DNA or binding to the minor groove;[18] (ii) it inhibits in vitro topo II decatenation and relaxation activities[12] and causes G2/M blockade;[19] (iii) it induces apoptosis[20] by a mechanism distinct from that reported for the topo II poisons etoposide and doxorubicin.[21]

In a National Cancer Institute (NCI) screening, merbarone showed antitumor activity against the murine L1210 leukemia model as well as against B16 melanoma and M5076 sarcoma[22] (optimum dose range 50–100 mg/kg). The antitumor efficacy was retained when the tumor implant site was distant from the drug injection site, but, in spite of its effects in ip- and sc-implanted L1210 leukemia models, the drug showed only marginal activity against the ic-implanted tumors. This might indicate that, because of its acidity (pK_a: 4) and high ionizability at physiological pH, merbarone does not efficiently penetrate the CNS. However, thanks to its activity against B16 melanoma and M5076 sarcoma, merbarone was tested in phase I and II clinical trials.[23–26] Although no major antitumor effects were observed, these studies led to the conclusion that the drug is relatively well tolerated with few constitutional symptoms.

Thereby, since merbarone analogues were little investigated,[27] we wish to report the synthesis and antiproliferative activity of three new series of basic merbarone thioanalogues **11**–**13**.

A few years ago, some of us have described the one-pot synthesis of 5-substituted 1,3-diphenyl-2,6-dithiobarbiturates **4** and **5**[28] (Fig. 2), having a variety of substituents not readily available by prior-art synthetic processes at the 1-, 3- and 5-pyrimidine ring positions. **4**, **5** and **6** (here described) share the 2-thiobarbiturate framework with **1**. Owing to the presence of the two phenyl substituents at the 1 and 3 positions, these compounds are more lipophilic than merbarone, as evidenced by their calculated LogP (Fig. 3). The different values can be related to the diverse polarity of the



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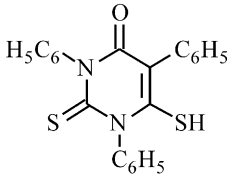

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Figure 2. Starting compounds **4** and **5**[28] for the synthesis of series **11** and **12**.

	LogP[a]	pKa[a]
1	-0.17±1.00	4.50[b]±1.00
4	5.02±1.00	4.50±1.00
5	3.17±0.75	4.50±1.00
6	1.88±1.00	4.50±1.00

Figure 3. Calculated LogP and pK_a of compounds **1**, **4**, **5**, **6**. (a) These values were obtained through the ACD/I-Lab service; (b) Lit[22]: 4.00.

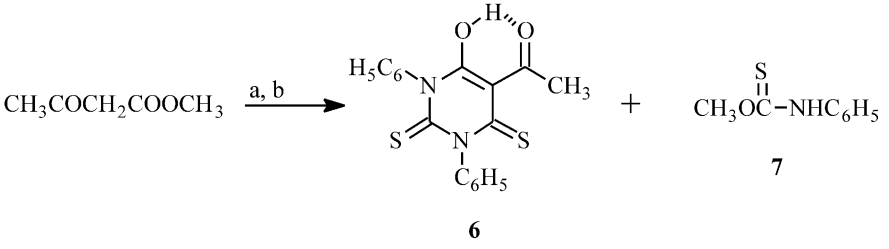
5-substituents (ethoxycarbonyl, phenyl and acetyl), this structural motif providing a wider range of hydrophilic-lipophilic balance. As a consequence, increase in lipophilicity could be expected to facilitate the ability of the derivatives to cross CNS and, in more general terms, biological membranes better than **1**. Nevertheless, **4**, **5**, **6** maintain the same excessive acidity of **1** (see their calculated pK_a, Fig. 3). In order to both suppress acid ionizability and hopefully improve pharmacodynamic and pharmacokinetic properties of title compounds **11**, **12**, **13**, the 6-sulphur atoms of **4**, **5**, and the 4-sulphur atom of **6** were alkylated with ω-chloroalkyl-dialkyl- or-cyclo-amines differing in chain length (two or three carbon atoms) and/or in terminal (cyclo)aliphatic tertiary amine. Some synthetic efforts to prepare the corresponding 6-oxygen analogues failed (data not shown).

These structural modifications were made considering that length, flexibility of the alkyl side chain, basicity and shape of the distal amine may give an important contribution to both the anticancer activity and the solubility of aminoalkyl derivatives under physiological conditions in respect to parent compounds.[29–35] Efforts to introduce a cyano group at the pyrimidine ring 5-position, starting from malononitrile unexpectedly gave bicyclic compound **8**. Interestingly, **8** could be considered, in some extent, as a conformationally constrained molecule, broadly related to merbarone, on the basis of following considerations. **1** (as depicted in Fig. 1), better than **4**[28] and **6** (vide infra), can be conformationally stabilised by two intramolecular H-bonds among the 5-amide moiety and the proximal enolizable carbonyl groups. The result of these interactions is a molecule mimicking a phenalene-like ring system with an outward phenyl moiety. In order to better understand if this conformation is a pharmacophore requirement, starting from **8**, basic congeners **14** were also synthesized and tested.

Chemistry

Starting compounds **4** and **5** were prepared according to previously described synthetic methods,[28] which were partially modified to synthesize **6** (Scheme 1) in order to improve its purity and yield. Firstly, methyl acetoacetate and phenylisothiocyanate (three molar equivalents) were allowed to react under cooling in anhydrous *N*,*N*-dimethylformamide (DMF), in the presence of sodium hydride (one molar equivalent). Then, to complete the reaction, another molar equivalent of sodium hydride was added portionwise at different times (for mechanistic details see ref 28). The reaction product was easily separated from by-product *O*-methyl-*N*-phenylthiocarbamate **7**, owing to its solubility in 1 M sodium carbonate. Compound **6** was better represented by a chelated 6-enol form, rather than by tautomers $\mathbf{6_{1\text{-}3}}$ (Fig. 4), on the basis of the following spectral data. The ^{1}H NMR spectrum exhibited a D_2O exchangeable proton peak as a sharp singlet at δ 17.92, which was assigned to the OH proton of the 6-enol moiety. Comparison of the ^{13}C NMR of **6** with those of **4** and **5**[28] allowed us to attribute the signals at δ 196.94, 187.31, 177.24 and 158.36 to the carbonyl carbon of the 5-acetyl group and to the 2,4-dithione and 6-enol carbons, respectively. As a consequence, tautomer **6** is conformationally stabilized through a strong intramolecular hydrogen bond between the 5-CO group and the 6-OH group. The free rotation of acetyl is hindered by this bond and, consequently, the resonance corresponding to the 6-enol proton occurred at the above high δ value, being the downfield shift a reflection of the deshielding effect of the close carbonyl group. In addition, the lack of OH signal in the IR spectrum could be due to resonance effects which led to a considerable strengthening of the hydrogen bond. Its pattern seemed to be similar to that of enolizable β-dichetones, where the effects described above led to a remarkable decrease in the intensity of the OH bond to such an extent that the identification of the band was rather difficult.[36]

Synthesis of intermediate **8** (Scheme 2) was started by reacting malononitrile and phenylisothiocyanate (one molar equivalent) in anhydrous DMF in the presence of sodium hydride (1.5 molar equivalents). In this way, an equilibrated mixture of monoanion **A** and more reactive dianion **A′** of malononitrile carbothiamide was generated. The reaction of **A** and **A′** with other two molar equivalents of phenylisothiocyanate added in a second time, via the cyclic enaminonitrile **B**, afforded mono/disodium salt **C**, that, following treatment with 1 M acetic acid, yielded **8**. Efforts to isolate intermediate **B** were unsucessfull. It should be noted that, unlike the synthetic procedures for the preparation of **4**, **5**, and **6**, two molar equivalents of sodium hydride gave a more complex reaction pattern. Compound **8** can be depicted in other three tautomeric forms $\mathbf{8_{1\text{-}3}}$ (Fig. 5), whose existence was excluded on the basis of the following



Scheme 1. Synthesis of intermediate **6**. Reagents and reaction conditions: (a) NaH (one equiv), C_6H_5NCS (three equiv), anhydrous DMF, 5 °C, then NaH (another equiv), rt, 15 h; (b) H_2O, 10 M HCl.

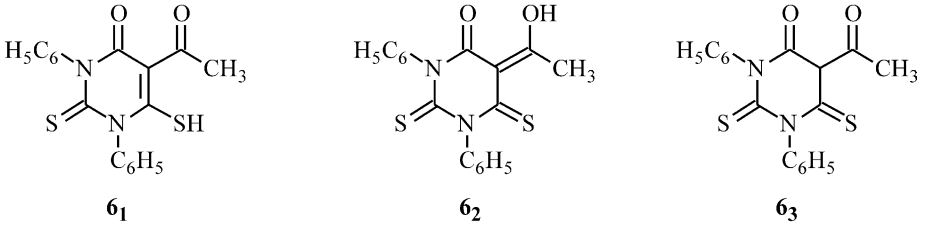
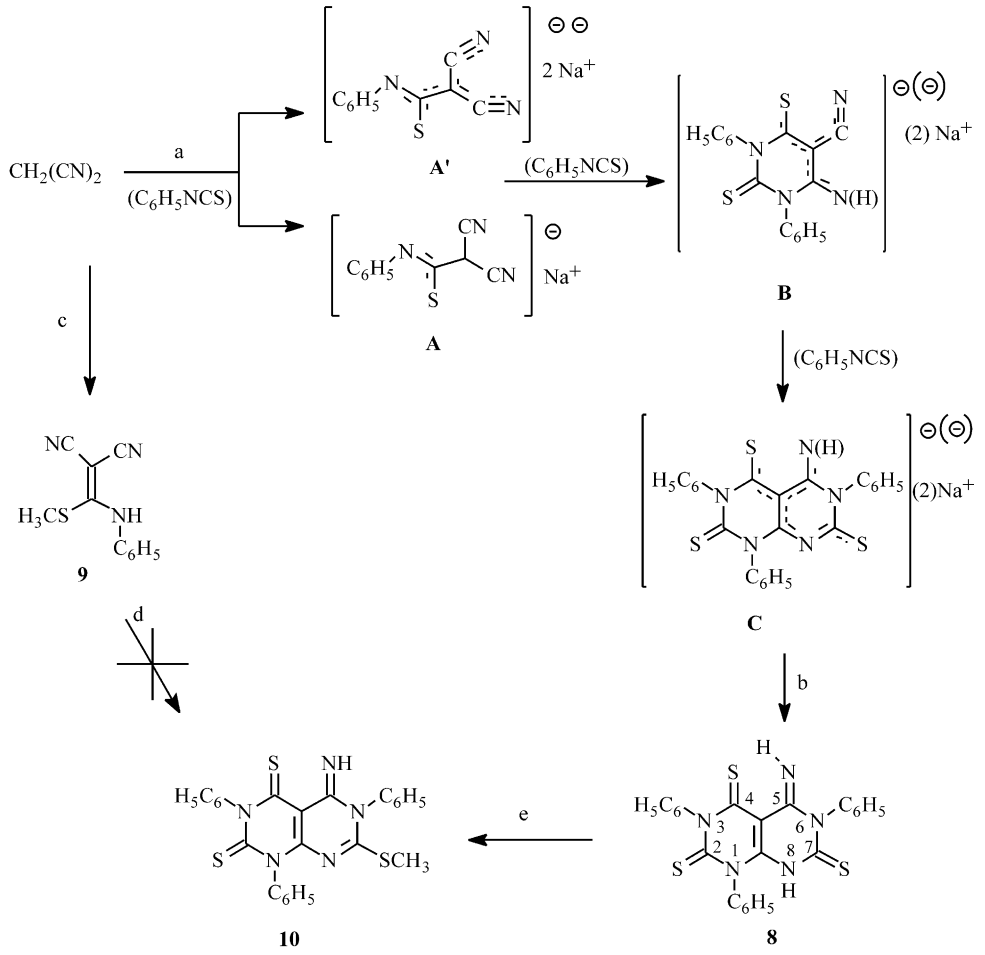


Figure 4. Other three possible tautomers of **6**.



Scheme 2. Synthesis of intermediate **8**, of its thiomethyl derivative **10** and of **9** (with the proposed mechanism for formation of **8**). Reagents and reaction conditions: (a) C_6H_5NCS (1 equiv), NaH (1.5 equiv), anhydrous DMF, 5 °C, then C_6H_5NCS (2 equiv), rt, 15 h; (b) 1M CH_3COOH; (c) C_6H_5NCS, CH_3I, NaH, anhydrous DMF, heat; (d) C_6H_5NCS (2 equivs), NaH, anhydrous DMF; (e) CH_3I, $NaHCO_3$, DMF, heat.

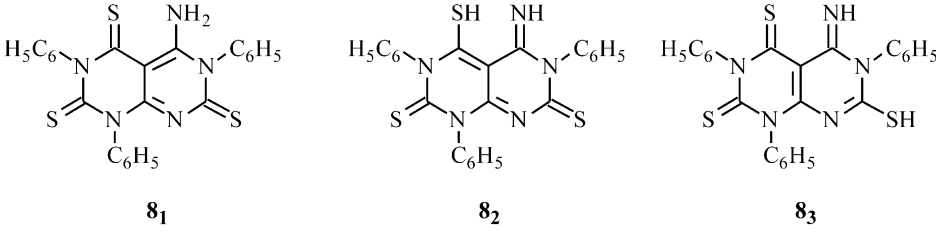


Figure 5. Other three possible tautomers of **8**.

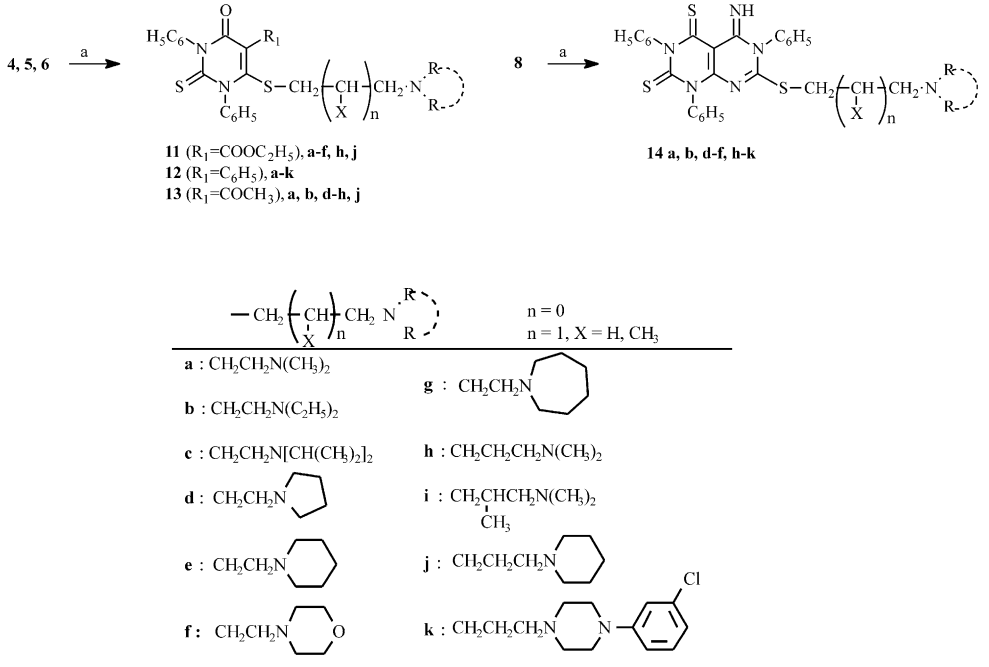
spectral evidences. The ^{13}C NMR spectrum of **8** exhibited the carbon signals at δ 182.63, 180.78 and 176.49 which were assigned to the three thione carbons at positions 4, 7, 2 of the pyrimidopyrimidine scaffold, respectively; while IR spectrum showed no weak SH adsorption within the narrow range of 2600–2550 cm^{-1}. In the ^1H NMR spectrum two D_2O exchangeable proton peaks were present at δ 12.35 and 7.60; the former corresponded to one proton which was assigned to the 5-imine group (its observed high δ value could be due to the hydrogen bond between the imine moiety and the 4-thione group); the latter was attributed to the 8-NH proton. The synthesis of **8** deserves some comments. Since the key intermediate dianions originated from corresponding initial sodium carbothiamides are decisive factors for the synthesis of intermediates **4**, **5** and **6**, it is interesting to note that the reaction gave **8** in good yields, even when the dianion formation was uncompleted. Moreover, if conditions preventing the dianion formation were adopted by using only one molar equivalent of sodium hydride, or by blocking one of the sites where the negative charges are preferentially delocalized, as in the case of thiomethyl derivative **9**, **8** was obtained in poor yield; or **9** did not cyclize to **10**, even though it was allowed to react in DMF solution with one and two molar equivalents of sodium hydride and phenylisothiocyanate, respectively. We prepared **9**[37,38] by an alternative one-pot procedure starting from malononitrile, phenylisothiocyanate and iodomethane in anhydrous DMF in the presence of sodium hydride

(Scheme 2), while **10** was synthesised by methylation of **8** with iodomethane in DMF in the presence of sodium bicarbonate (Scheme 2). According to methods A–F and work up procedure W_A and W_B (see Experimental), intermediates **4, 5**, **6** and **8** were alkylated at the 6-, 4- and 7-sulphur atoms, respectively, using ω-chloroalkyldialkyl- or -cyclo-amines in the presence of diverse bases (A: $NaHCO_3$, B: Triton B, C: sodium hydride, D: K_2CO_3, E: triethylamine, F: 2 equivalents of chloroamine), to afford compounds **11a–f,h,j**, **12a–k**, **13a,b,d–h,j** and **14a,b,d–f,h–k** (Scheme 3). The various synthetic procedures adopted were due to different reactivity of the starting intermediates towards alkylation. In some cases, the reaction gave low to moderate yield or did not occur. Only **5** reacted with each of the relevant ω-chloroalkylamines. Regio-alkylation of the 7-sulphur atom of **8** was exemplified by the ^{13}C NMR spectra of **10** and **14b**, where the 7-carbon signals, (at δ 180.78 in the ^{13}C NMR spectrum of **8**) were found to resonate at δ 169.66 and 169.74, respectively, as a result of the thiourea/isothiourea rearrangement. Similarly, alkylation at position 6 of **4** and at position 4 of **6** was proved by the ^{13}C NMR spectra of **11a** and **13b**, where the 6- and 4-thione carbon signals of the precursors (see ^{13}C NMR spectra of **4**[28] and **6**) were lacking, due to formation of the enethioalkyl substructure. Thus, the 6-carbon signals of **11a** and **13b** were found to resonate at δ 153.36 and 153.66, respectively. Conversely, alkylation of intermediate **5**, as exemplified by the ^{13}C NMR spectrum of **12b**, brought to a slight difference in the chemical shift of the C-6 atoms of **12b** (δ 154.18) and of **5** (δ 152.67),[28] the latter having a stable enethiolic tautomeric form.

Biological results

The first synthesized derivatives **11b,d**, **13d,g**, and **14b,d,f,h,i,j** were evaluated in vitro at N.C.I. against subpanels of nine different types of cell lines derived from human cancers.[39,40] In these assays merbarone was used as reference drug (Table 1). Both monocyclic and bicyclic derivatives showed the same potency against leukemia cell lines (GI_{50} range: 0.01–3 μM), and turned out to be up to 100-fold more potent than merbarone. On the other hand, bicyclic derivatives **14b,d,f,h,i,j** were more potent than monocyclic counterparts **11b,d**, **13d,g** against solid tumor cell lines, showing antiproliferative activity in the concentration range of 1–10 μM against NSCLC, colon, CNS, melanoma, renal, prostate and breast cancer cell lines. By contrast, the monocyclic derivatives exhibited a comparable potency only against prostate cancer cells. Interestingly, series **14** emerged as the most cytotoxic, leading to cell death at concentrations only 2–3-fold higher than those required to inhibit cell growth by 50%.

Meanwhile, new derivatives of series **11**, **13**, and **14**, in addition to a new series of compounds **12**, were synthesized and tested in our laboratory for antiproliferative activity (Table 2). As reference compounds, one derivative of each of the series tested at NCI (e.g., **11b**, **13d** and **14b**) was included in the assays. In general, the IC_{50} values of **11b**, **13d** and **14b** against MT-4 cells (Table 2) were about 10-fold higher than the average GI_{50} values obtained at NCI against the leukemia cell line subpanel (Table 1). Among the new derivatives, none showed antiproliferative potency higher than that of first generation counterparts. Compounds **11a,c,e,f,h,j**, **13a,b,j** and **14e** resulted as potent as **11b**, **13d** and **14b**, respectively, whereas derivatives of series **12** were either marginally active (**12a,b,h–j**) or inactive (**12c–g,k**). Taken together, these data indicate that the antiproliferative activity decreases in the following order: **14** > **11** > **13** > **12** within the series.



Scheme 3. Synthesis of the title compounds. Reagents and reaction conditions: (a) ω-chloroalkyldialkyl/cyclo-amines, various bases and solvents (methods A–F), heat.

Table 1. Antiproliferative, cytostatic, cytocide activities (μM)[a] of selected test compounds against the 9 NCI subpanels of human tumor cells in culture

Type of tumor	**11b**	**11d**	**13d**	**13g**	**14b**	**14d**	**14f**	**14h**	**14i**	**14j**	**1**
Leukemia	0.6–2	1–3	1–3	0.01–nd[b]	0.1–0.4	0.04–0.4	1–2	0.3–2	0.2–1	0.2–2	10–28
	5	5	5	nd[b].	0.3–1.5	0.2–2	4	1–4	0.5–3	0.5–4	50–100
	1–100	10–100	10–100	≥100	1–100	5–100	7–100	6–100	7	4–100	>100
NSCLC[c]	1–20	1–20	1–30	3–40	0.4–30	1–10	1–3	1–2	0.6–2	1–2	8–41
	3–45	4–40	3–100	10–100	2–60	3–20	3–10	4	2–5	3–5	20–100
	10–100	20–100	20–100	20–100	6–100	6–100	6–10	7	5–100	6–100	>100
Colon	1–10	1–20	2–15	4–15	1–2	1–2	1–2	2	1–2	1–2	14–47
	3–40	4–40	4–30	15–40	4	3	4	4	3	4	70–100
	6–100	6–100	7–80	40–100	6–100	6–100	6–10	7	5–100	6–100	>100
CNS	10–30	3–30	15–60	10–50	2–10	2–10	2–8	1–4	1–5	2–5	25–32
	20–100	10–60	30–100	30–100	3–30	4–30	3–20	3–10	3–20	3–15	100
	50–100	30–100	70–100	60–100	7–70	10–60	5–50	5–50	5–50	5–50	>100
Melanoma	1–15	1–10	1–20	1–10	1–4	1–3	1–3	1–2	1–2	1–2	20–50
	3–30	3–30	3–50	4–50	3–15	3–20	3–5	3–5	2–5	2–5	50–100
	10–100	6–70	5–100	10–100	6–100	6–100	6–100	6–10	6–20	5–30	>100
Ovarian	2–50	1–40	2–50	10–50	1–15	2–20	1–3	1–10	1–10	2–20	25–50
	3–100	4–40	5–100	25–100	3–30	3–30	3–30	3–30	3–20	3–30	70–100
	6–100	6–100	10–100	100	5–60	6–60	6–60	5–50	5–50	5–100	>100
Renal	1–15	1–10	1–15	2–6	1–10	1–10	1–3	1–3	1–3	2	20–50
	3∼30	2–20	3–30	3–30	2–20	3–20	3–10	3–5	2–6	3–7	50–100
	5–50	5–50	5–50	6–50	5–50	5–50	5–30	5–10	5–20	5–20	>100
Prostate	2	2	2–6	2–10	2	2	2	2	2	2	14–30
	4	3	6–20	3–20	3	3	3	3	3	3	80–100
	5–15	6	25–50	5–50	6	6	6	6	6	6	>100
Breast	1–30	1–30	1–20	2–40	2–6	2–10	2–4	0;3–3	0.3–3	0.3–3	8–30
	3–100	3–20	3–70	5–100	3–100	3–100	3–100	3–100	1–100	1∼100	70–100
	6–100	6–100	6–100	60–100	6–100	10–100	6–100	6–100	6–100	6–100	>100
MG-MID GI_{50}	4.67	4.16	6.16	9.33	1.82	1.95	2.08	1.66	1.54	1.66	
MG-MID TGI	10.9	9.77	15.84	26.30	4.78	5.12	5.24	3.98	3.71	3.89	
MG-MID LC_{50}	30.9	26.3	40.73	56.23	15.48	17.37	15.13	8.91	10.71	12.30	

[a]In each column, the GI_{50}, TGI, LC_{50} range values (μM) (1st, 2nd, 3rd row for each type of tumor, respectively) of the most active compound, are reported. Likewise, at the bottom of each column are also reported the MG-MID GI_{50}/TGI/LC_{50} (μM) parameters of the compound, evaluating the average antitumor activity against the entire panel of cell lines. These data were generously provided by Developmental Therapeutics Program, N.C.I.
[b]Not determined.
[c]Non-small cell lung cancer.

Multidrug resistance (MDR)[41,42] and atypical topoisomerase-mediated MDR (at-MDR)[17,43] are among the main reasons for chemotherapy failure. Since drug-resistant cell lines have been reported to express partial[17] cross-resistance to merbarone, we deemed it interesting to investigate the susceptibility of drug-resistant KB subclones to the basic merbarone thioanalogues. Therefore, **14b** and **11b**, representative compounds of the two most potent series, were tested against cell lines (KB^{MDR} and KB^{V20C}) over-expressing the drug efflux pump MDR1/P-glycoprotein responsible for the MDR phenotype[44,45] and against an etoposide-resistant KB cell line[46] (KB^{7D}) that, besides a 2-fold decrease in topo II levels, over-expresses a protein, referred to as multidrug-resistance associated protein (MRP), which is known to reduce the uptake of etoposide and other antineoplastic agents (MDR phenotype). Compounds **11b** and **14b** were also evaluated against a camptothecin-resistant KB cell line[47] (KB^{CPT300}) expressing altered levels of topo I (approximately 30% of parental KB cells).

In general, **11b** and **14b** were 2–3-fold less active against the KB resistant subclones (Table 3) than against the parental KB cell line. The sole exception was the KB^{7D} subclone, which was 20-fold less susceptible to the test compounds.

Consistently with previous results,[46] the KB^{7D} cells proved highly resistant to the topo II poisons doxorubicin (56-fold) and etoposide (33-fold) and moderately resistant to the antimitotic drug vincristine (10-fold). By contrast, the KB^{7D} cells showed only marginal resistance (approximately 2-fold) to merbarone, whereas KB^{MDR} cells did not show altered sensitivity to the reference drug.

In order to assess whether the antiproliferative activity of **11b** and **14b** correlated with inhibition of topoisomerase II catalytic activity, as suggested by the above results, a cell-free system (see Experimental) was used.[48] This inhibition effect was compared with that of the standard topo-II inhibitor etoposide (VP16) and Merbarone. Like VP16, **11b** and **14b** were able to inhibit the formation of supercoiled DNA from relaxed DNA (Fig. 6). Interestingly, they turned out to be more potent inhibitors than Merbarone (Fig. 6).

The title compounds were also randomly assayed against HIV-1 infected MT-4 cells for their ability to inhibit the virus-induced cytopathogenicity. However, none of them was able to prevent the HIV-induced cytopathogenicity at non cytotoxic concentrations (data not shown). Furthermore, they were evaluated for their

Table 2. Antiproliferative activity of derivatives **11**, **12**, **13** and **14** against MT-4 cells

Compd	Synthetic route/Work-up procedure[a]	IC_{50} (μM)[b]
11a	A/W_A	12.1±1.4
11b	A/W_A	4.3±0.8
11c	B/W_A	11.0±1.3
11d	A/W_A	ND[c]
11e	A/W_A	10.5±1.3
11f	A/W_A	12.7±1.6
11h	A/W_A	13.0±0.9
11j	A/W_A	11.5±1.5
12a	D/W_A	88.3±5.3
12b	A/W_A	78.5±6.1
12c	A/W_A	>200
12d	B/W_A	>200
12e	A/W_A	>200
12f	D/W_A	>200
12g	B/W_A	>200
12h	A/W_A	63.6±4.9
12i	A/W_A	66.6±5.3
12j	A/W_A	42.4±3.3
12k	B/W_A	>200
13a	E/W_A	10.3±1.4
13b	F/W_A	12.1±1.1
13d	B/W_A	12.0±2.0
13e	A/W_A	>200
13f	A/W_A	>200
13g	F/W_A	ND[c]
13h	E/W_A	35.4±2.7
13j	A/W_A	12.2±1.6
14a	B/W_B	15.5±1.2
14b	B/W_B	3.3±0.6
14d	D/W_B	ND
14e	C/W_B	3.6±0.9
14f	C/W_B	ND[c]
14h	B/W_B	ND[c]
14i	B/W_B	ND[c]
14j	B/W_B	ND[c]
14k	B/W_B	109.4±12

[a]The compounds were prepared and isolated according to methods A–F and work up procedures W_A and W_B, described in Experimental.
[b]IC_{50} (Inhibitory concentration fifty): the values represent the drug concentration (±S.D.) required to reduce cell growth by 50% with respect to untreated controls, as measured by the MTT method.
[c]Not determined.

capability to inhibit the multiplication of various human pathogenic fungi (*Candida albicans, C. parapsilosis, C. paratropicalis, Aspergillus fumigatus and Criptococcus neoformans*) and bacteria (*Staphylococcus aureus*, group D *Streptococcus*, S*almonella* spp and *Shigella* spp). Miconazole and streptomycin were used as reference compounds in antimicotic and antibacterial assays.

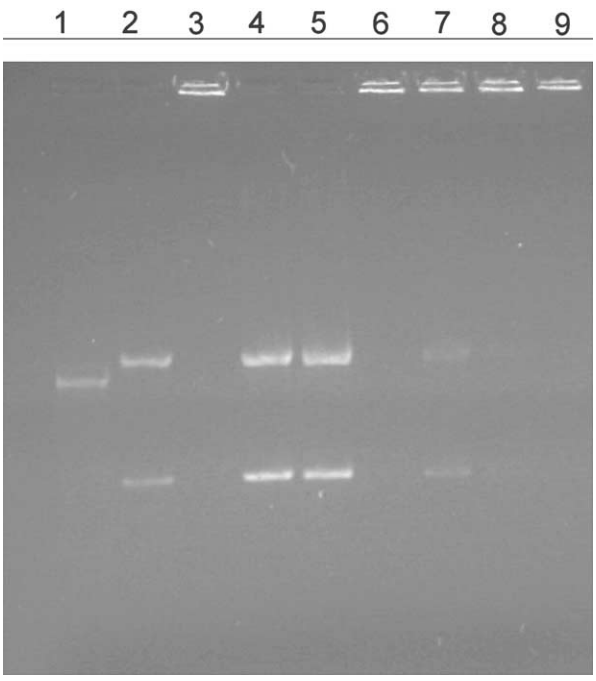


Figure 6. Inhibitory effects of Etoposide, Merbarone, **11b** and **14b** on the catalytic activity of topoisomerase II. The first five lanes represent controls; *lane 1:* Marker Linear KDNA, *lane 2:* Marker Decatenated KDNA, *lane 3:* Kinetoplast DNA (KDNA), *lane 4:* Topo-II activity, *lane 5:* Topo-II activity in the presence of DMSO [0.1%]. *Lane 6:* Topo-II activity in the presence of VP16 [100 μM]; *lane 7:* Topo-II activity in the presence of Merbarone [100 μM]; *lane 8:* Topo-II activity in the presence of **11b** [100 μM]; *lane 9:* Topo-II activity in the presence of **14b** [100 μM].

None of the compounds was active against the tested bacteria and fungi (data not shown).

Discussion

This study is part of an ongoing research program concerning the design of new simple or fused poly-substituted pyrimidines as lead compounds for the development of antitumor agents.

In order to better elucidate pharmacophore requirements, some structure–activity relationships can be made. Many of the active compounds in the tested series have aminoalkyl side chains with different length and/or amine shape, although in some instances diethylamino/pyrrolidino-ethyl, piperidino-ethyl/-propyl appear to

Table 3. Antiproliferative activity of derivatives **11b** and **14b** in parental and drug-resistant KB cell lines

Compd	IC_{50} (μM)[a]				
	KB^{wt}	KB^{MDR}	KB^{V20C}	KB^{7D}	KB^{CPT300}
11b	2.4±0.4	6.6±0.8	7.4±0.9	46.1±4.9	7.5±0.8
14b	0.8±0.2	3.0±0.5	2.9±0.3	18.0±3.1	2.7±0.4
Merbarone	18.3±2.5	20.7±2.7	ND	42.3±3.7	ND
Doxorubicin	0.06±0.01	1.8±0.1	0.35±0.1	2.8±0.3	0.19±0.1
Vincristine	0.006±0.001	0.7±0.2	0.2±0.09	0.05±0.01	0.018±0.01
Etoposide	0.6±0.1	>20	6.2±1.8	>20	1.5±0.2
Camptothecin	0.03±0.01	0.03±0.02	0.09±0.01	0.19±0.07	10.0±2.3

[a]IC_{50} (Inhibitory concentration fifty): values represent the drug concentration (±S.D.) required to reduce cell growth by 50% with respect to untreated controls, as measured by the MTT method.

give the greatest contribution to the antitumor activity. However, in the pyrimidine series the structural requirements of 5-substituent seem to be quite stringent. Thus, the presence of a 5-phenyl ring causes drop or loss of antiproliferative activity as in derivatives **12**, probably owing to capacity of this moiety to elicit unfavorable π–π or lipophilic interactions. Conversely, replacement of the phenyl ring with polar groups (ethoxycarbonyl and acetyl in series **11** and **13**, respectively) capable of electrostatic interactions, enhances activity. In series **13**, cytotoxicity data also reveal a close dependence on length and amine size of the basic side chain. Thus, unlike **11e**,**f**, **13e** and **13f**, bearing the piperidinoethyl and morpholinoethyl moieties, were devoid of activity. Conversely, homologues **13j**,**g** were active. Further comparison of antiproliferative effects induced by homologous extension of the side chains inside the same series indicates that, with the exception of **11e**,**j** and **13d**,**h**, compounds carrying the 3-aminopropyl side chains are either equipotent (**11h**–**11a**, **14j**–**14e**) or more potent than derivatives bearing the 2-aminoethyl moieties [**12h** > **12d**, **12j** ≫ **12e** (inactive), **13j** ≫ **13e** (inactive), **14h** > **14a**)].

Side chain elaboration in terms of steric load shows that: (i) a methyl group at position 2 of the 3-aminopropyl chains does not cause any difference in antiproliferative activity with respect to the unbranched compounds (compare **14i** with **14h** and **12i** with **12h**); (ii) a 3-chlorophenyl substituent at the piperazine 4-position lowers antiproliferative activity, as found in **14k**. This indicates that steric bulk (and/or aromatic character) of substituents on the distal aliphatic amine ring could cause adverse effects on activity.

In conclusion, biological data suggest that, in general, change of the physico-chemical properties of the title compounds, due to increase of lipophilicity and suppression of acid ionizability, resulting from alkylation of the reactive sulphur atoms of key-intermediates **4**, **5**, **6**, and **8** with ω-chloro alkyl amines, are beneficial to activity. Moreover, introduction of conformational constrain, as in series **14**, or replacement of the 5-phenylcarbamoyl moiety of **1** by groups with a higher degree of polarity (ethoxycarbonyl, acetyl), as in series **11** and **13**, are additional structural parameters enhancing potency. These results encourage further investigation in animal models to establish whether also pharmacodinamic and pharmacokinetic properties of the title compounds are improved.

Experimental

Materials and methods

Chemicals (methyl phenylacetate, ethyl malonate, methyl acetoacetate, malonitrile, phenylisothiocyanate, 60% sodium hydride suspension in mineral oil, $NaHCO_3$, K_2CO_3, triethylamine, Triton B, ω-chloroalkylamines) were purchased from Aldrich Chimica, Milan (Italy). Solvents were reagent grade. DMF was dried on molecular sieves. Organic solutions were dried over anhydrous sodium sulphate and concentrated using a rotatory evaporator operating at reduced pressure of about 15–20 Torr. TLC systems for routine monitoring of reaction mixture and confirming the homogeneity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with chloroform-methanol as developing solvents. Developed plates were visualized by UV light and iodine. Melting points were determined on a Fisher–Johns (mp $<300\,^{\circ}C$) or an Electrothermal apparatus (mp $>300\,^{\circ}C$) and are uncorrected. Microanalyses were performed by an EA 1110 Elemental Analyser, FISON Instruments (Milan).

IR spectra were recorded on a Perkin–Elmer 398 spectrometer as KBr discs. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 200 instruments. Chemical shifts were reported in δ (ppm) units relative to the internal reference tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and brs (broad singlet). First order values reported for coupling constants were given in hertz.

Synthesis of 1-(6-hydroxy-1,3-diphenyl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidin-5yl)ethanone (6). 60% sodium hydride dispersion in mineral oil (8.0 g, 0.2 mol) was added in a single portion to a stirred, ice-cooled, anhydrous DMF solution (200 mL) of methyl acetoacetate (23.3 g, 0.2 mol). When hydrogen evolution subsided, neat phenylisothiocyanate (81.23 g, 0.6 mol) was poured into this mixture in a single portion. Then, 60% sodium hydride suspension in mineral oil (8.0 g, 0.2 mol) was added in three portions (5 + 1 + 2 g) after 15 min, 1 and 1.5 h, respectively. The resulting mixture was allowed to react at room temperature for 15 h, treated with ice-cooled water (600 mL) and extracted thoroughly with diethyl ether–petroleum ether (bp 40–70 °C) 2:1 (50 mL×2). The extracts were washed with water (20 mL×5), dried and evaporated under reduced pressure to give an oily residue which slowly crystallized. Recrystallization from diethyl ether-petroleum ether 7:1 afforded a first crop (5.25 g) of *O*-methyl-*N*-phenylcarbamate **7**, mp 96–97 °C.[28] The aqueous solution was cooled with crushed ice and acidified with 10 M HCl (300 mL) to give a red-brown precipitate which was filtered, washed with water and dissolved in dichloromethane. The organic phase was extracted with 1 M sodium carbonate (180 mL×3). During the first extraction the voluminous precipitate formed (sodium salt of **6**) was dissolved upon dilution with water. The dichloromethane extract was then rinsed with water, dried and evaporated under reduced pressure. The residue was chromatographed on neutral alumina (eluents: diethyl ether–dichloromethane 10:1) to yield the bulk of **7** (35.0 g, 74% overall yield). The cooled alkaline aqueous solution furnished by acidification with 10 M HCl a brown precipitate which was filtered, washed and dissolved in dichloromethane. The organic phase was washed with water (100 mL), dried and evaporated in vacuo. The solid residue was crystallized from dichloromethane–methanol to give **6** (63.0 g, 89% yield) as an ochre solid. Mp 238–240 °C, IR (KBr) cm^{-1} 1690;

^{1}H NMR ($CDCl_3$) δ 2.83 (s, 3H, CH_3/acetyl-H), 6.92–7.23 (m, 10H, arom H), 17.92 (sharp s, 1H, exchangeable, enol-H); ^{13}C NMR ($CDCl_3$) δ 28.07, 107.82, 128.1, 128.64, 128.88, 129.51, 139.42, 142.16, 158.36 (6-C), 177.24 (2-C), 187.31 (4-C), 196.94 (CO). Elemental analysis: calcd for $C_{18}H_{14}N_2O_2S_2$; C, 61.00; H, 3.98; N, 7.90; S, 18.09%; found; C, 61.02; H, 3.99; N, 7.98; S, 18.28%.

Synthesis of 5-imino-1,3,6-triphenyl-5,8-dihydropyrimido[4,5-*d*] pyrimidine-2,4,7(1*H*,3*H*,6*H*)-trithione (8). To a stirred, ice-cooled anhydrous DMF solution (150 mL) of malonitrile (6.9 g, 0.1 mol) and phenylisothiocyanate (13.7 g, 0.1 mol), 60% sodium hydride dispersion in mineral oil (6 g, 0.15 mol) and, after 10 min, neat phenylisothiocyanate (27.04 g, 0.2 mol) were added in a single portion. The resulting mixture was stirred at 0–5 °C for 15 min and then at room temperature for 15 h. Reaction was quenched by adding ice-cooled 1 M acetic acid (250 mL). The orange precipitate formed was filtered and dissolved in dichloromethane. The organic phase was washed with water (40 mL), 4 M sodium hydroxyde (20×mL) and dried. Concentration in vacuo yielded **8** (33.1 g, 70.2% yield), which was crystallized from dichlomethane–methanol 3:2 as a yellow-orange solid; mp 314–316 °C; IR (KBr) cm^{-1} 3410, 3280, 1610, 1590, 1540, 1305; ^{1}H NMR (DMSO-d_6) δ 6.90–7.75 (m, 15H, arom H), 7.90 (s, 1H, exchangeable, NH), 12.35 (s, 1H, exchangeable, imine-H); ^{13}C NMR ($CDCl_3$) δ 97.91; 128.06, 128.14, 128.73, 129.01, 129.35, 129.71, 130.68, 137.77, 140.75, 144.08, 149.05, 156.95 (5-C), 176.49 (2-C), 180.78 (7-C), 182.63 (4-C). Elemental analysis: calcd for $C_{24}H_{17}N_5S_3$; C, 61.14; H, 3.64; N, 14.86; S, 20.36%; found; C, 61.00; H, 3.76; N, 14,71; S, 20.16%.

Synthesis of 3-Anilino-2-cyano-3-methylthioacrylonitrile (9). 60% sodium hydride dispersion in mineral oil (0.88 g, 20 mmol) was added in a single portion to a stirred, ice-cooled anhydrous DMF solution (25 mL) of malononitrile (1.32 g, 20 mmol), phenylisothiocyanate (2.70 g, 20 mmol) and iodomethane (2.84 g, 20 mmol). The resulting mixture was stirred at room temperature for 1 h, then at 50–55 °C for 15 min. The solid separated after adding water (150 mL) was filtered, dissolved in dichloromethane and dried. Evaporating in vacuo to dryness gave a residue that was crystallized from methanol–dichloromethane 4:1 to give colorless needles (3.87 g, 90%); mp 178–179 °C (literature values 176 °C,[37] 177 °C[38]).

Synthesis of 5-imino-7-(methylthio)-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (10). A mixture of **8** (4.72 g, 10 mmol), sodium bicarbonate (0.84 g, 10 mmol) and iodomethane (1.42 g, 10 mmol) in DMF (20 mL) was stirred at room temperature for 1 h and then heated at 55 °C for 2 h. After treatment with water (100 mL) the precipitated formed was collected by filtration and dissolved in dichloromethane. The organic phase was dried and evaporated under reduced pressure to give a residue which, after crystallization from dichloromethane–methanol–diethyl ether 1:2:2, afforded **10** (3.7 g, 76%) as a yellow-orange solid; mp 265–267 °C; IR (KBr) cm^{-1} 3180, 1615, 1590, 1485, 1430; ^{1}H NMR δ 1.70 (s, 3H, thiomethyl-H), 6.95–7.30 (m, 15H, arom H), 11.75 (s, 1H, exchangeable, imine-H); ^{13}C NMR ($CDCl_3$) δ 15.41, 128.91, 129.08, 129.16, 129.86, 130.28, 130.60, 137.34, 141.26, 144.72, 150.12, 155.70 (5-C), 169.66 (7-C), 179.69 (2-C), 184.84 (4-C). Elemental analysis: calcd for $C_{25}H_{19}N_5S_3$; C, 61.83; H, 3.94; N, 14.42; S, 19.80%; found C, 61.85; H, 3.93; N, 14.22; S, 19.63%.

General procedure A for the preparation of compounds 11a,b,d,e,f,h,j, 12b–e,h,j, 13e,f,j. To a stirred mixture of **4** (3.85 g, 10 mmol) or **5** (3.89 g, 10 mmol) or **6** (3.54 g, 10 mmol) and sodium bicarbonate (1.0 g, 12 mmol) in DMF (50 mL), the proper ω-chloroalkylamine (12 mmol) was added at room temperature after 15 min. Then, the reaction mixture was heated at 55–60 °C for 4 h under stirring.

Work-up$_A$. The solid, that separated after treatment with water (300 mL), was filtered, dissolved in dichloromethane, dried and evaporated in vacuo to dryness. The oily or solid residues were purified by crystallization from proper solvent mixtures.

Physical and chemical data of compounds 11a,b,d,e,f,h,j

Ethyl 6-[(2-dimethylaminoethyl)thio]-4-oxo-1,3-diphenyl-2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11a). 2.87 g (63% yield), mp 208–210 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1730, 1685; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 8 Hz, CH_3/ethoxy-H), 2.09 (s, 6H, 2 CH_3/dimethylamino-H), 2.40 (t, 2H, J = 7 Hz, S-CH_2/ethyl-H), 2.90 (t, 2H, J = 7 Hz, N-CH_2/ethyl-H), 4.40 (q, 2H, J = 8 Hz, CH_2/ethoxy-H), 7.02–7.74 (m, 10H, arom H). ^{13}C NMR ($CDCl_3$) δ 14.53, 35.80, 45.34, 57.84, 63.02, 119.62, 128.46, 129.32, 129.91, 130.17, 139.64, 141.81,153.36 (6-C), 157.26 (4-C), 163.95 ($COOC_2H_5$), 179.83 (2-C). Elemental analysis: calcd for $C_{23}H_{25}N_3O_3S_2$; C, 64.64; H, 5.53; N, 9.22; S, 14.07%; found C, 64.40; H, 5.50; N, 9.04; S, 13.94%.

Ethyl 6-[(2-diethylaminoethyl)thio]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (11b). 3.96 g (82% yield), mp 159–161 °C (from dichloromethane/2-propanol). IR (KBr) cm^{-1} 1725, 1675; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.0 Hz, 2CH_3/diethyilamino-H), 1.37 (t, 3H, J = 7.0 Hz, CH_3/ethoxy-H), 2.05–3.18 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2CH_2/diethylamino-H), 4.40 (q, 2H, J = 7.0 Hz, CH_2/ethoxy-H), 7.03–7.40 (m, 10H, arom H). Elemental analysis: calcd for $C_{25}H_{29}N_3O_3S_2$; C, 62.09; H, 6.04; N, 8.69; S, 13.26%; found C, 62.08; H, 5.91; N, 8.53; S, 13.35%.

Ethyl 4-oxo-1,3-diphenyl-6-[(2-pyrrolidin-1-ylethyl)thio]-2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11d). 4.24 g (88% yield), mp 190–191 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1720, 1680, ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3H, J = 7.0 Hz, CH_3/ethoxy-H), 1.58–2.08 (m, 4H, 2CH_2/pyrrolidine-H), 2.19–3.12 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2CH_2/pyrrolidine-H), 4.41 (q, 2H, CH_2/ethoxy-H), 7.05–7.80 (m, 10H, arom H). Elemental analysis: calcd for $C_{25}H_{27}N_3O_3S_2$;

C, 62.35; H, 5.65; N, 8.72; S, 13.31%; found C, 62.51; H, 5.54; N, 8.50; S, 13.08%.

Ethyl 4-oxo-1,3-diphenyl-6-[(2-piperidin-1-ylethyl)thio]-2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11e). 4.31 g (87% yield), mp 187–188 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1720, 1685; ^1H NMR($CDCl_3$) δ 1.14–1.89 (m, 9H, CH_3/ethoxy-H and 3 CH_2/piperidine-H), 2.04–2.59 (m, 4H, 2 CH_2/piperidine-H), 2.43 (t, 2H, $J=6.0$ Hz, S-CH_2/ethyl), 2.93 (t, 2H, $J=6.0$, N-CH_2/eyhyl-H), 4.42 (q, 2H, $J=6.0$ Hz, CH_2/ethoxy-H), 7.10–7.84 (m, 10H, arom H). Elemental analysis: calcd for $C_{26}H_{29}N_3O_3S_2$; C, 63.00; H, 5.90; N, 8.48; S, 12.94%; found C, 62.86; H, 5.87; N, 8.36; S, 12.72%.

Ethyl 6-[(2-morpholin-4-ylethyl)thio]-4-oxo-1,3-diphenyl-2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11f). 4.18 g (84% yield), mp 201–202 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1720, 1685; ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, $J=7.0$ Hz, CH_3/ethoxy-H), 2.04–2.69 (m, 6H, S-CH_2/ethyl-H and 2CH_2/morpholine-H) 2.92 (t, 2H, $J=6.0$ Hz, N-CH_2/ethyl-H), 3.19–3.86 (m, 4H, 2CH_2/morpholine-H), 4.40 (q, 2H, $J=7.0$ Hz, CH_2/ethoxy-H), 7.06–7.82 (m, 10H, arom H). Elemental analysis: calcd for $C_{25}H_{27}N_3O_4S_2$; C, 60.34; H, 5.47; N, 8.44; S, 12.89%; found C, 60.40; H, 5.48; N, 8.37; S, 12.97%.

Ethyl 6-[(3-dimethylaminopropyl)thio]-4-oxo-1,3-diphenyl - 2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11h). 3.00 g (64% yield), mp 137–139 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1725, 1680; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, $J=7.0$ Hz, CH_3/ethoxy-H), 1.48–1.91 (m, 2H, CH_2/propyl-H), 1.97–2.38 (m, 2H, S-CH2/propyl-H), 2.13 (s, 6H, 2CH_3/dimethylamino-H), 2.78 (t, 2H, $J=7.0$, N-CH_2/propyl-H), 4.40 (q, 2H, $J=7.0$ Hz, CH2/ethoxy-H), 7.02–7.84 (m, 10H, arom H). Elemental analysis: calcd for $C_{24}H_{27}N_3O_3S_2$; C, 61.38; H, 5.80; N, 8.95; S, 13.65%; found C, 61.19; H, 5.69; N, 8.70; S, 13.44%.

Ethyl 1,3-diphenyl-6-[(3-piperidin-1-ylpropyl)thio]-4-oxo-2 - thioxo - 1,2,3,4 - tetrahydropyrimidine - 5 - carboxylate (11j). 4.08 g (80% yield), mp 143–145 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1725, 1690; ^1H NMR ($CDCl_3$) δ 1.07–1.9 (m, 11H, CH_3/ethoxy-, CH_2/propyl-H and 3CH_2/piperidine-H), 1.99–2.50 (m, 6H, S-CH_2/propyl-H and 2CH_2/piperidine-H), 2.75 (t, 2H, $J=7.0$ Hz, N-CH_2/propyl-H), 4.40 (q, 2H, $J=7.0$ Hz, CH_2/ethoxy-H), 7.05–7.77 (m, 10H, arom H). Elemental analysis: calcd for $C_{27}H_{31}N_3O_3S_2$; C, 63.63; H, 6.13; N, 8.24; S, 12.58%; found C, 63.41; H, 6.07; N, 8.13; S, 12.39%.

Physical and chemical data of compounds 12b,c, e, h, i, j

6-[(2-diethylaminoethyl)thio] -1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12b). 3.66 g (75% yield), mp 190–191 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1670, 1390, 1325; ^1H NMR ($CDCl_3$) δ 0.82 (t, 6H, $J=7.6$ Hz, 2CH_3/diethylamino-H), 2, 25 (q, 4H, $J=7.6$ Hz, 2CH_2/diethylamino-H), 2.27 (m, 4H, S-CH_2, N-CH_2/ethyl-H), 7.23–7.7 (m, 15H, arom H). ^{13}C NMR ($CDCl_3$) δ 12.27, 34.82, 47.08, 51.31, 121.90, 128.62, 128.71, 128.98,129.68, 129.80, 129.98, 131.16, 133.43, 140.51,142.96, 154.18 (6-C), 159.56 (4-C), 163.95, 179.33 (2-C). Elemental analysis: calcd for $C_{28}H_{29}N_3OS_2$; C, 68.96; H, 5.99; N, 8.62; S, 13.15%; found C, 69.11; H, 6.01; N, 8.85; S, 12.94%.

6-[(2-Diisopropylaminoethyl)thio]-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12c). 3.61 g (70% yield), mp 203–204 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1670, 1390, 1320; ^1H NMR ($CDCl_3$) δ 0.77 (d, 12H, $J=6.0$ Hz, 4CH_3/diisopropylamino-H), 2.15–2.4 (m, 4H, S-CH_2, N-CH_2/ethyl-H), 2.35–297 (m, 2H, 2CH/diisopropylamino-H), 7.05–7.72 (m, 15H, arom H). Elemental analysis: calcd for $C_{30}H_{33}N_3OS_2$; C, 69.87; H, 6.45; N, 8.15; S, 12.43%; found C, 70.00; H, 6.45; N, 8.30; S, 12.31%.

1,3,5-Triphenyl-6-[(2-piperidin-1-ylethyl)thio]-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12e). 4.15 g (83% yield), mp 231–233 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1675, 1555, 1390, 1330; ^1H NMR ($CDCl_3$) δ 1.30–1.60 (m, 6H, 3 CH_2/piperidine-H), 1.98–2.35 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/piperidine-H), 7.25–7.67 (m, 15H, arom H). Elemental analysis: calcd for $C_{29}H_{29}N_3OS_2$; C, 69.71; H, 5.85; N, 8.41; S, 12.83%; found C, 69.74; H, 5.99; N, 8.65; S, 12.97%.

6-[(3-Dimethylaminopropyl)thio]-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12h). 3.69 g (78% yield), mp 194–195 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1672, 1560, 1390, 1327; ^1H NMR ($CDCl_3$) δ 1.2–1.75 (m, 2H, CH_2/propyl-H) 1.77–2.28 (m, 4H, S-CH_2, N-CH_2/propyl-H), 2.09 (s, 6H, 2CH_3/dimethylamino-H), 7.3–7.7 (m, 15, arom H). Elemental analysis: calcd for $C_{27}H_{27}N_3OS_2$; C, 68.47; H, 5.75; N, 8.87; S, 13.54%; found C, 68.24; H, 5.75; N, 8.81; S, 13.74%.

6-{[3-(Dimethylamino)-2-methylpropyl]thio}-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12i). 4.34 g (89% yield), mp 168–170 °C (from dichloromethane/methanol). IR(KBr) cm^{-1} 1670, 1555, 1388, 1325; ^1H NMR ($CDCl_3$) δ 0.63 (d, 3H, $J=6.0$ Hz, methyl-H), 1.37–1.88 (m, 3H, CH- and S-CH_2/propyl-H), 1.90–2.10 (m, 2H, N-CH_2/propyl-H), 2.06 (s, 6H, 2CH_3/dimethylamino-H), 7.2–7.73 (m, 15H, arom H). Elemental analysis: calcd for $C_{28}H_{29}N_3OS_2$; C, 68.98; H, 5.99; N, 8.62; S, 13.15%; found C, 68.72; H, 6.11; N, 8.62; S, 12.95%.

1,3,5-triphenyl-6-[(3-piperidin-1-ylpropyl)thio]-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12j). 4.42 g (86% yield), mp 155–156 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1680, 1555, 1385, 1330; ^1H NMR ($CDCl_3$) δ 1.3–1.73 (m, 8H, CH_2/propyl-H and 3CH_2/piperidine-H), 1.82–2.38 (m, 8H, S-CH_2, N-CH_2/propyl-H and 2CH_2/piperidine-H), 7.20–7.65 (m, 15H, arom H). Elemental analysis: calcd for $C_{30}H_{31}N_3OS_2$; C, 70.14; H, 6.08; N, 8.18; S, 12.48%; found C, 70.01; H, 6.06; N, 8.14; S, 12.20%.

Physical and chemical data of compounds 13e,f,j

5-Acetyl-1,3-diphenyl-6-[(2-piperidin-1-ylethyl)thio]-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13e). 2.42 g (52% yield), mp 182–184 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1680, 1385, 1330; ^1H NMR ($CDCl_3$) δ 1.25–1.55 (m, 6H, $3CH_2$/piperidine-H), 2.05–3.0 (m, 8H, S-CH_2, N-CH_2/ethyl-H and $2CH_2$/piperidine-H), 2.60 (s, 3H, acetyl-H), 7.15–7.65 (m, 10H, arom H). Elemental analysis: calcd for $C_{25}H_{27}N_3O_2S_2$; C, 64.49; H, 5.84; N, 9.02; S, 13.77%; found C, 64.26; H, 5.98; N, 8.94; S, 13.45%.

5-Acetyl-6-[(2-morpholin-4-ylethyl)thio]-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13f). 3.60 g (77% yield), mp 240–242 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1704, 1670, 1385, 1320; ^1H NMR ($CDCl_3$) δ 2.08–2.70 (m, 6H, S-CH_2/ethyl-H and $2CH_2$/morpholine-H), 2.65 (s, 3H, acetyl), 2.85 (t, 2H, $J=7.0$ Hz, N-CH_2/ethyl-H), 3.40–3.75 (m, 4H, $2CH_2$/morpholine-H), 7.15–7.70 (m, 10H, arom H). Elemental analysis: calcd for $C_{24}H_{25}N_3O_3S_2$; C, 61.45; H, 5.39; N, 8.99; S, 13.71%; found C, 61.35; H, 5.44; N, 8.74; S, 13.53%.

5-Acetyl-1,3-diphenyl-6-[(3-piperidin-1-ylpropyl)thio]-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13j). 3.60 g (75% yield), mp 127–129 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1705, 1675, 1390, 1325; ^1H NMR($CDCl_3$) δ 1.32–1.90 (m, 8H, CH_2/propyl-H and $3CH_2$/piperidine-H), 2.04–2.45 (m, 6H, S-CH_2/propyl-H and $2CH_2$/piperidine-H), 2.50–2.54 (m, 10H, arom H). Elemental analysis: calcd for $C_{26}H_{29}N_3O_2S_2$; C, 65.11; H, 6.09; N, 8.76; S, 13.77%; found C, 64.95; H, 5.98; N, 8.76; S, 13.57%.

General procedure B for the preparation of compounds 14a,b,h,i,j,k, 11c, 12d,g,k, 13d. A 40% Triton methanol solution (4.4 mL, 10 mmol) was evaporated in vacuo to dryness and the viscous residue was dissolved in DMF (40 mL). To the solution, stirred for 15 min, **8** (4.72, 10 mmol) or **4** (3.85 g, 10 mmol) or **5** (3.89 g, 10 mmol) or **6** (3.54 g, 10 mmol) and the proper ω-chloroalkylamine (12 mmol) were added at room temperature. The mixture was then heated under stirring at 65–70 °C for 4 h. Compounds **11c, 12d,g,k, 13d** were isolated according to work-up$_A$, while those of series **14** as follows:

Work-up$_B$. After treatment with water (200 mL), the oily or solid phase separated was dissolved and extracted with dichlorometane. The combined organic layers were extracted with 1 M HCl (25 mL×3) and discarded. The acid solution was made alkaline with 4 M NaOH (40 mL) and extracted thoroughly with dichloromethane. The combined extract was dried and evaporated in vacuo to dryness to give a residue that was purified by crystallization.

Physical and chemical data of compounds 14a,b,h,i,j,k

7-[(2-Dimethylaminoethyl)thio]-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione (14a). 2.98 g (55% yield), mp 228–230 °C (from dichloromethane/2-propanol). IR (KBr) cm^{-1} 3190, 1620; ^1H NMR ($CDCl_3$) δ 1.86–2.21 (m, 2H, S-CH_2/ethyl-H), 2.01(s, 6H, $2CH_3$/dimethylamino-H), 2.31–2.58 (m, 2H, N-CH_2/ethyl-H), 7.1–7.78 (m, 15H, arom H), 11.45 (s,1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{28}H_{26}N_6S_3$; C, 61.97; H, 4.83; N, 15.48; S, 17.72%; found C, 61.75; H, 4.72; N, 15.29; S, 17.57%.

7-[(2-Diethylaminoethyl)thio]-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione (14b). 1.48 g (26% yield), mp 193–195 °C (from dichloromethane/2-propanol/petroleum ether). IR (KBr) cm^{-1} 3190, 1625; ^1H NMR ($CDCl_3$) δ 0.86 (t, 6H, $J=7.0$ Hz, 2 CH_3/diethylamino-H), 2.29 (q, 4H, $J=7.0$ Hz, 2 CH_2/diethylamino-H), 1.98–2.63 (m, 4H, S-CH_2, N-CH_2/ethyl-), 7.05–7.78 (m, 15H, arom H), 11.50 (s, 1H, exchangeable, imine-H). ^{13}C NMR ($CDCl_3$) δ 31.93, 45.67, 57.67, 104.31, 128.91, 129.01, 129.09, 129.19, 129.99, 130.28, 130.53, 130.64, 137.34, 141.35, 144.75, 150.16, 155.78 (4-C), 169.74 (2-C), 176.73 (7-C), 184.73 (5-C). Elemental analysis: calcd for $C_{30}H_{30}N_6S_3$; C, 63.13; H, 5.30; N, 14.72; S, 16.85%; found C, 63.18; H, 5.26; N, 14.74; S, 16.50%.

7-[(3-Dimethylaminopropyl)thio]-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (14h). 2.81 g (54% yield), mp 218–220 °C (from dichloromethane/2-propanol/petroleum ether). IR (KBr) cm^{-1} 3190, 1620; ^1H NMR ($CDCl_3$) δ 1.05–1.45 (m, 2H, methylene-H), 1.67–260 (m, 4H, S-CH_2, CH_2/N-propyl-H), 2.10 (s, 6H, dimethylamino-H), 7.0–7.8(m, 15H, arom H), 11.65 (s, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{29}H_{28}N_6S_3$; C, 62.56; H, 5.07; N, 15.09; S, 17.27%; found C, 62.53; H, 5.11; N, 14.97; S, 17.10%.

7-{[3-(dimethylamino)-2-methylpropyl]thio}-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione (14i). 1.48 g (26% yield), mp 234–236 °C (from dichloromethane/petroleum ether). IR (KBr) cm^{-1} 3190, 1620; ^{1}HNMR ($CDCl_3$) δ 0.63 (d, 3H, $J=6.0$ Hz, methyl-H), 1.50–1.93 (m, 3H, methine-H and S-CH_2/propyl-H), 2.06 (s, 6H, 2 CH_3/dimethylamino-H), 2.35–2.65 (m, 2H, N-CH_2/propyl-H), 6.95–7.73 (m, 15H, arom H), 11.55 (brs, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{30}H_{30}N_6S_3$; C, 63.13; H, 5.30; N, 14.72; S, 16.85%; found C, 62.93; H, 5.23; N, 14.56; S, 16.51%.

5-Imino-1,3,6-triphenyl-7-[(3-piperidin-1-ylpropyl)thio]-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (14j). 4.00 g (67% yield), mp 201–203 °C (from dichloromethane/2-propanol/petroleum ether). IR (KBr) cm^{-1} 3185, 1620; ^1H NMR ($CDCl_3$) δ 1.06–1.66 (m, 8H, CH_2/propyl-H and 3 CH_2/piperidine-H), 1.78–2.61 (m, 8H, S-CH_2, N-CH_2/propyl-H and 2 CH_2/piperidine-H), 11.45 (s, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{32}H_{32}N_6S_3$; C, 64.40; H, 5.40; N, 14.08; S, 16.12%; found C, 64.21; H, 5.44; N, 13.98; S, 16.28%.

7-({3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}thio)-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*] pyrimi-

dine-2,4(1*H*,3*H*)-dithione (14k). 3.19 g (45% yield), mp 160–162 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 3190, 1620; ^1H NMR ($CDCl_3$) δ 1.06–1.42 (m, 2H, CH_2/propyl-H), 1.86–2.66 (m, S-CH_2, N-CH_2/propyl-H and piperazine-H), 2.96–3.26 (m, 4H, piperazine-H), 6.56–7.76 (m, 19H, arom H), 11.62 (brs, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{37}H_{34}ClN_7S_3$; C, 62.74; H, 4.84; N, 13.84; S, 13.58%; found C, 62.94; H, 4.64; N, 13.99; S, 13.82%.

Physical and chemical data of compound 11c

Ethyl 6-[(2-diisopropylaminoethyl)thio]-4-oxo-1,3-diphenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (11c). 3.07 g (60% yield), mp 182–184 °C (from dichloromethane/2-propanol/petroleum ether). IR (KBr) cm^{-1} 1725, 1690; ^1H NMR ($CDCl_3$) δ 0.85 (d, 12H, $J=8.0$ Hz, 4 CH_3/diisoproylamine-H),1.35 (t, 3H, $J=7.0$ Hz, CH_3/ethoxy-H), 2.30–3.05 (m, 6H, S-CH_2, N-CH_2/ethyl-H and 2 CH/diisopropylamine-H), 4.35 (q, 2H, $J=7.0$ Hz, CH_2/ethoxy-H), 7.05–7.65 (m, 10H, arom H). Elemental analysis: calcd for $C_{27}H_{33}N_3O_3S_2$; C, 63.38; H, 6.50; N, 8.21; S, 12.53%; found C, 63.54; H, 6.50; N, 8.36; S, 12.61%.

Physical and chemical data of compounds 12d,g,k

1,3,5-Triphenyl-6-[(2-pyrrolidin-1-ylethyl)thio]-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12d). 4.47 g (92% yield), mp 234–235 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1670, 1555, 1390, 1320; ^1H NMR ($CDCl_3$) δ 1.50–2.02 (m, 4H, 2 CH_2/pyrrolidine-H), 2.04–2.45 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/pyrrolidine-H), 7.20–7.74 (m, 15H, arom H). Elemental analysis: calcd for $C_{28}H_{27}N_3OS_2$; C, 69.25; H, 5.90; N, 8.95; S, 13.20%; found C, 69.07; H, 5.58; N, 8.63; S, 12.50%.

6-[(2-Azepan-1-ylethyl)thio]-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12g). 4.37 g (85% yield), mp 196-197 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1669, 1555, 1390, 1325; ^1H NMR ($CDCl_3$) δ 1.17–1.75 (m, 8H, 4 CH_2/homopiperidine-H), 2.05–2.54 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/homopiperidine-H), 7.25–7.67 (m, 15H, arom H). Elemental analysis: calcd for $C_{30}H_{31}N_3OS_2$; C, 70.14; H, 6.08; N, 8.18; S, 12.48%; found C, 69,95; H, 6.02; N, 8.14; S, 12.19%.

6-({3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}thio)-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12k). 3.44 g (55% yield), mp 116–118 °C (from acetonitrile/methanol). IR (KBr) cm^{-1} 1665, 1590; ^1H NMR ($CDCl_3$) δ 1.1–1.5 (m, 2H, CH_2/propyl-H), 1.83–2.65 (m, 8H, S-CH_2, N-CH_2/-propyl-H and 2 CH_2/piperazine-H), 2.83-3.17 (m, 4H, piperazine-H), 6.73–7.6 (m, 19H, arom H). Elemental analysis: calcd for $C_{35}H_{33}ClN_4OS_2$; C, 67.24; H, 5.32; N, 8.96; S, 10.26%; found C, 66.99; H, 5.42; N, 9.16; S, 10.40%.

Physical and chemical data of compound 13d

5-acetyl-1,3-diphenyl-6-[(2-pyrrolidin-1-ylethyl)thio]-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13d). 2.98 g (66% yield), mp 170–172 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1680, 1380, 1330; ^1H NMR ($CDCl_3$) δ 1.57–1.9 (m, 4H, 2 CH_2/pyrrolidine-H), 2.22–2.53 (m, 6H, S-CH_2/ethyl-H and 2 CH_2/pyrrolidine-H), 2.59 (s, 3H, CH_3/acetyl-H), 2.82 (t, 2H, $J=7.0$ Hz, N-CH_2/ethyl-H), 7.2–7.65 (m, 10H, arom H). Elemental analysis: calcd for $C_{24}H_{25}N_3O_2S_2$; C, 63.83; H, 5.58; N, 9.30; S, 14.20%; found C, 63.67; H, 5.52; N, 9.20; S, 14.40%.

Procedure C for the preparation of compounds 14e,f. To a stirred mixture of **8** (4.72 g, 10 mmol) and the proper ω-chloroalkylcycloalkylamine (12 mmol) in anhydrous DMF (30 mL), 60% sodium hydride dispersion in mineral oil (0.4 g, 10 mmol) was added. The reaction mixture was heated at 60–65 °C under stirring, and, after 4 h, treated according to Work-up$_B$.

Physical and chemical data of compounds 14e,f

5-Imino-1,3,6-triphenyl-7-[(2-piperidin-1-ylethyl)thio]-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (14e). 3.32 g (57% yield), mp 213–215 °C (from dichloromethane/diethylether). IR (KBr) cm^{-1} 3190, 1620; ^1H NMR ($CDCl_3$) δ 1.30–1.78 (m, 6H, 3 CH_2/piperidine-H), 1.92–2.7 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/piperidine-H), 7.05–7.85 (m, 15H, arom H), 11.57 (brs, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{31}H_{33}N_6S_3$; C, 63.89; H, 5.19; N, 14.42; S, 16.50%; found C, 63.89; H, 5.14; N, 14.20; S, 16.40%.

5-Imino-7-[(2-morpholin-4-ylethyl)thio]-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (14f). 3.68 g (63% yield), mp 238–240 °C (from dichloromethane/diethylether). IR (KBr) cm^{-1} 3.200, 1620; ^1H NMR ($CDCl_3$) δ 1.61–2.71 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/morpholine-H), 3.40–3.85 (m, 4H, morpholine-H), 6.95–8.0 (m, 15H, arom H), 11.58 (s, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{30}H_{28}N_6OS_3$; C, 61.62; H, 4.83; N, 14.37; S, 16.45%; found C, 61.60; H, 4.83; N, 14.13; S, 16.28%.

Procedure D for the preparation of compounds 14d, 12a,f. To a stirred mixture of **8** (4.72, 10 mmol) or **5** (3.89 g, 10 mmol) and anhydrous potassium carbonate (1.52 g, 11 mmol) in DMF (50 mL), the proper ω-chloroalkylamine (12 mmol) was added in a single portion after 15 min at room temperature. The reaction mixture was then heated at 60–65 °C and, after 4 h, was treated according to Work-up$_B$ for **14d** or Work-up$_A$ for **12a**,**f**.

5-Imino-1,3,6-triphenyl-7-[(2-pyrrolidin-1-ylethyl)thio]-5,6-dihydropyrimido[4,5-*d*] pyrimidine-2,4(1*H*,3*H*)-dithione (14d). 3.97 g (70% yield), mp 231–233 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 3190, 1620; ^1H NMR ($CDCl_3$) δ 1.5–1.9 (m, 4H, 2 CH_2/pyrrolidine-H), 2.05–2.69 (m, 8H, S-CH_2, CH_2-N/ethyl-H and 2 CH_2/pyrrolidine-H), 7.10–7.72 (m, 15H, arom H), 11.54 (brs, 1H, exchangeable, imine-H). Elemental analysis: calcd for $C_{30}H_{28}N_6S_3$; C, 65.35; H, 4.96; N, 14.78; S, 16.91%; found C, 63.15; H, 4.96; N, 14.62; S, 16.70%.

Physical and chemical data of compounds 12a,f

6-[(2-Dimethylaminoethyl)thio]-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12a). 2.39 g (52% yield), mp 200–201 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1673; ^1H NMR ($CDCl_3$) δ 1.95 (s, 6H, 2 CH_3/dimethylamino-H), 2.07–2.35 (m, 4H, S-CH_2, N-CH_2/ethyl-H), 7.2–7.68 (m, 15H, arom H). Elemental analysis: calcd for $C_{26}H_{25}N_3OS_2$; C, 67.94; H, 5.48; N, 9.14; S, 13.95%; found C, 67.75; H, 5.35; N, 9.11; S, 13.76%.

6-[(2-Morpholin-4-ylethyl)thio]-1,3,5-triphenyl-2-thioxo-2,3-dihydropyrimidin-4-(1*H*)-one (12f). 4.66 g (93% yield), mp 228–229 °C (from dichloromethane/methanol). IR (KBr) cm^{-1} 1672, 1590, 1390, 1327; ^1H NMR ($CDCl_3$) δ 1.98–2.38 (m, 8H, S-CH_2, N-CH_2/ethyl-H and 2 CH_2/morpholine-H), 3.40–3.75 (m, 4H, 2 CH2/morpholino-H), 7.25–7.75(m, 15H, arom H). Elemental analysis: calcd for $C_{28}H_{27}N_3O_2S_2$; C, 67.04; H, 5.42; N, 8.38; S, 12.78%; found C, 67.07; H, 5.37; N, 8.51; S, 12.50%.

Procedure E for the preparation of compounds 13a,h. To a stirred mixture of **6** (3.54, 10 mmol) and triethylamine (1.01 g, 10 mmol) in 1,4-dioxane (80 mL), the proper ω-chloroalkylamine (12 mmol) was added in a single portion, after 15 min at room temperature. The reaction mixture was heated at 85–90 °C for 4 h and then treated according to Work-up$_A$.

Physical and chemical data of compounds 13a,h

5-Acetyl-6-[(2-dimethylaminoethyl)thio]-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13a). 2.68 g (63% yield), mp 186–187 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1673; ^1H NMR ($CDCl_3$) δ 2.06 (s, 6H, 2 CH_3/dimethylamino-H), 2.37 (t, 2H, $J = 6.0$ Hz, S-CH_2/ethyl-H), 2.58 (s, 3H, CH_3/acetyl-H), 2.85 (t, 2H, $J = 6.0$ Hz, N-CH_2/ethyl-H), 7.15–7.65 (m, 10H, arom H). Elemental analysis: calcd for $C_{22}H_{23}N_3O_2S_2$; C, 62.06; H, 5.45; N, 9.87; S, 15.07%; found C, 61.90; H, 5.38; N, 9.71; S, 14.80%.

5-Acetyl-6-[(3-dimethylaminopropyl)thio]-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13h). 2.81 g (64% yield), mp 163–164 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1705, 1678, 1390, 1330; ^1H NMR ($CDCl_3$) δ 1.3–1.85 (m, 2H, CH_2/propyl-H) 2.15 (s, 6H, 2 CH_3/dimethylamino-H), 2.0–2.25 (m, 2H, S-CH_2/propyl-H), 2.6 (s, 3H, acetyl-H), 7.3–7.7 (m, 15, arom H). Elemental analysis: calcd for $C_{23}H_{25}N_3O_2S_2$; C, 62.84; H, 5.73; N, 9.56; S, 14.59%; found C, 62.66; H, 5.55; N, 9.50; S, 14.29%.

Procedure F for the preparation of compounds 13b,g. A stirred solution of **6** (3.54 g, 10 mmol) and the proper ω-chloroalkylamine (24 mmol) in DMF (40 mL) was heated at 70–75 °C for 4 h and then treated according to Work-up$_A$.

Physical and chemical data of compounds 13b,g

5-acetyl-6-[(2-diethylaminoethyl)thio]-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13b). 1.81 g (40% yield), mp 127–129 °C (from dichloromethane/ethanol). IR (KBr) cm^{-1} 1715, 1680; ^1H NMR ($CDCl_3$) δ 0.92 (t, 6H, $J = 7.0$ Hz, 2 CH_3/dietyhlamino-H), 2.58 (s, 3H, acetyl-H), 7.15–765 (m, 10H, arom H). ^{13}C NMR ($CDCl_3$) δ 32.34, 37.34, 45.34, 57.64, 125.01, 128.45, 129.36, 129.84, 129.95, 130.24, 139.61, 141.81, 153.66 (6-C), 157.99 (4-C), 179.64 (2-C), 198.23 ($COCH_3$). Elemental analysis: calcd for $C_{24}H_{27}N_3O_2S_2$; C, 63.55; H, 6.00; N, 9.26; S, 14.14%; found C, 63.55; H, 5.84; N, 9.01; S, 13.98%.

5-acetyl-6-[(2-azepan-1-ylethyl)thio]-1,3-diphenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (13g). 1.97 g (41% yield), mp 150–152 °C (from dichloromethane/2-propanol). IR (KBr) cm^{-1} 1702, 1675, 1386, 1325; ^1H NMR ($CDCl_3$) δ 1.47 (m, 8H, homopiperidine-H), 1.92–2.94 (m, 8H, S-CH_2/,N-CH_2/ethyl- and homopiperidine-H), 2.57 (s, 3H, acetyl-H), 7.07–7.78 (m, 10H, arom H). Elemental analysis: calcd for $C_{26}H_{29}N_3O_2S_2$; C, 65.11; H, 6.09; N, 8.76; S, 13.37%; found C, 65.34; H, 6.05; N, 8.83; S, 13.32%.

Antiproliferative assays performed at NCI. The NCI high-flux anticancer drug screen[39,40] utilized a panel of 60 human tumor cell lines in culture derived from nine clinically isolated neoplastic diseases, namely non-small cell lung, colon, CNS, ovarian, renal, prostate, breast cancer, melanoma and leukemia. Cell lines were exposed to test agents in 96-well plates for the last 48 of a 72 h incubation and were stained for total protein with sulforhodamine B according to the standard NCI protocol. The cytotoxic effects of each test agent were evaluated through the growth inhibition parameters GI_{50}, TGI and LC_{50}, which represent the molar drug concentration required to produce half (GI_{50}), or total (TGI) growth inhibition, and 50% of cytocidal effect (LC_{50}), respectively. A standard dose–response curve for individual agent, tested at concentrations of 10^{-8} to 10^{-4} M, was provided for each of the 60 cell lines. The relative responsiveness of each cell line compared with the average responsiveness of all of the lines was provided in graphic form as mean-graph mid-point (MG-MID). Compounds which did not inhibit cell growth by 50% at 10^{-4} M were still employed in the calculation of cytotoxicity.

Assays performed in our laboratory

Test compounds were dissolved in DMSO at an initial concentration of 200 μM and were then serially diluted in culture medium. Tumor cell growth at each drug concentration was expressed as percentage of untreated controls and the concentrations resulting in 50% (EC_{50} and IC_{50}, respectively) growth inhibition was determined by linear regression analysis.

Cells. MT4 lymphoblastoid T cells were from American Type Culture Collection (ATCC, USA). The nasopharingeal carcinoma KB cell line and the resistant mutant subclones KB^{MDR} KB^{V20C}, KB^{7D} and KB^{CPT300} were generous gift of Prof. Y. C. Cheng, Yale University, USA. All resistant KB cell lines were maintained in

growth medium supplemented with 0.02 μM vincristine for KB^{V20C}, 7 μM etoposide for KB^{7D}, 0.3 μM camptothecin for KB^{CPT300}, 0.09 μM doxorubicin for KB^{MDR}. MT-4 [grown in RPMI 1640 containing 10% foetal calf serum (FCS), 100 UI/mL penicillin G and 100 μg/mL streptomicin] were also used for anti-HIV-1 assays. All cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco).

Antiproliferative and antiviral assays. Cytotoxicity of compounds, based on the viability of mock-infected cells as monitored by the MTT method, was evaluated in parallel with their antiviral activity. Activity against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells.[49] Briefly, 50 μL of RPMI 10% FCS containing 1×10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 μL of medium and serial dilutions of test compounds. 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were then added. After a 4 day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[50]

Exponentially growing cells were resuspended at a density of 1×10^5 cells/mL in RPMI containing serial dilutions of the test drugs. Cell viability was determined after 4 days at 37 °C by MTT method.[51] Activity against surface adherent cells was evaluated in exponentially growing cultures seeded at 5×10^4 cells/mL which were allowed to adhere for 16 h to cultures plates before addition of the drugs. Cell viability was determined by the MTT method 4 days later. For antiproliferative assays in KB resistant subclones, stock cell lines were cultured in absence of drugs for 3 days before seeding for compound testing.

Topo II catalytic assay. Inhibition of Topo II catalytic activity was evaluated by using a Topoisomerase-II kit (TopoGEN, INC, Columbus, Ohio). Purified human topoisomerase -II (TopoGEN, Columbus, Ohio) was employed as a source for topo-II in the topo-II assay kit. Assays were performed according to the manufacturer's instructions in the presence and absence of different concentrations of the compounds. Reaction products were analysed on a 1% agarose gel in the presence of 0.5 μg/mL ethidium bromide as required by the manufacturer's instructions.

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