Synthesis of thieno[2,3-*d*]pyrimidines, thieno[2,3-*d*]triazinones and thieno[2,3-*e*]diazepinones of anticipated anti-cancer activity

M.M. Kandeel, Ashraf A. Mounir, Hanan M. Refaat* and Asmaa E. Kassab

Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

A novel series of 4-aminohexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidines, hexahydrocycloocta[4,5]thieno[2,3-*d*]-1,2,3-triazin-4-one and its N-3 substituted derivatives in addition to 3-aryl hexahydrocycloocta[4,5] thieno[2,3-*e*]-1,4-diazepin-5-ones were synthesised. Also, 2-(N-ethylcarbamothioylamino) hexahydrocycloocta[b] thiophene-3-carbonitrile and 19-imino tetradecahydrocycloocta[4′,5′] thieno[2′,3′:4,5]pyrimido[1,6-*a*] cycloocta[4,5]thieno [3,2-*e*]pyrimidine-9-thione were prepared. Almost all the synthesised compounds exhibited anti-tumour activity against human colon carcinoma (HCT 116) cell line *in vitro*. Five compounds (IC_{50}: 15.92, 22.59, 25.85, 27.40 and 29.70 μM, respectively) exhibited 2.16 to 1.15 fold more potent antitumour activity than imatinib (IC_{50}: 34.40 μM).

Keywords: hexahydrocycloocta[4,5]thieno[2,3-*d*]pyrimidines, hexahydrocycloocta[4,5]thieno[2,3-*d*]-1,2,3-triazin-4-ones, hexahydrocycloocta[4,5] thieno[2,3-*e*]-1,4-diazepin-5-ones, antitumour activity

The thieno[2,3-*d*]pyrimidine system is an integral part of numerous potential cancer chemotherapeutic agents,[1,2] expressing appreciable anti-cancer activity via inhibition of various enzymes.[3–11] Certain thieno[2,3-*d*] pyrimidines were originally prepared as bioisosteres of gefitinib (Iressa®)[12] and erlotinib (Tarceva®)[13] (Fig. 1), drugs that have been approved for the treatment of gastrointestinal stromal tumours and lung cancer.

We have previously synthesised various hexahydrocycloocta [4,5]thieno[2,3-*d*]pyrimidine and pyrimidin-4-one derivatives that could be considered as analogues of gefitinib (M. M. Kandeel, A. A. Mounir, H. M. Refaat, A. E. Kassab, in preparation). This synthesis was motivated by the fact that several reports support the anti-cancer activity for thieno[2,3-*d*]pyrimidines fused with various five, six and seven membered cycloalkyl moieties and / or substituents,[14–19] but there are no reports on the anti-cancer evaluation of thieno[2,3-*d*]pyrimidines fused with a hexahydrocycloocta-ring. All the synthesised compounds exhibited anti-tumour activity against human colon carcinoma (HCT 116) cell line *in vitro* and several compounds exhibited 4.3 to 1.3 fold more potent anti-tumour activity than imatinib (Fig. 1).

With the same purpose, our group considered the design and synthesis of various 2-substituted and 4-substituted hydrazinyl thieno[2,3-*d*]pyrimidines as promising anti-tumour agents. Some products demonstrated 2.89 to 1.07 fold more potent antitumour activity than imatinib. Moreover, the thienotriazolopyrimidine derivatives were prepared as structure analogues to 4-hydrazinyl thieno[2,3-*d*]pyrimidines and were found to be of moderate activity.

Encouraged by the above findings and in continuation of our research programme concerned with the modification of certain anti-cancer thieno[2,3-*d*]pyrimidines structurally related to gefitinib, we report here the synthesis of a new series of thieno[2,3-*d*]pyrimidines, thieno[2,3-*d*]triazin-4-ones and thieno[2,3-*e*]-1,4-diazepin-5-ones. Three elements in the target compounds are varied while reserving the hexahydrocycloocta-ring as the cycloalkyl moiety (Fig. 2):

(1) The N-3 substituents in pyrimidine; (2) 4-Amino moiety;

3- The size of the annulated ring (pyrimidine, triazin-4-one and diazepin-5-one).

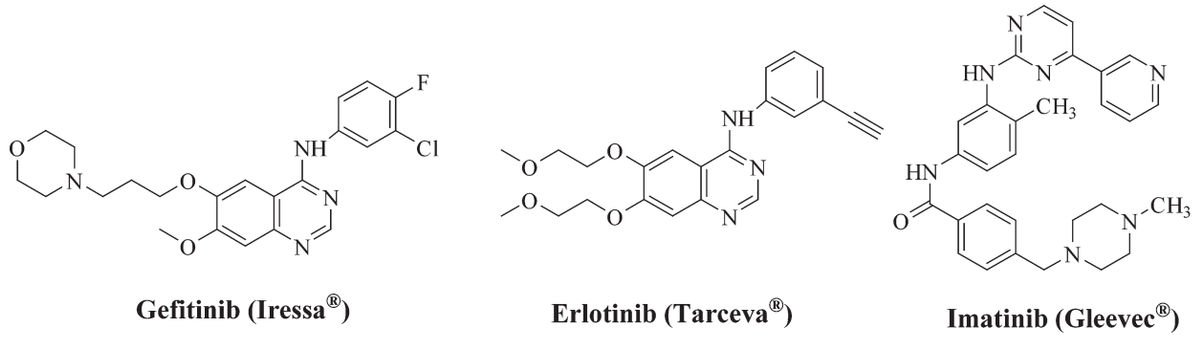
In the present work, we report the synthesis of the hitherto unknown title compounds and evaluate their *in vitro* anticancer activity.

Results and discussion

The synthetic route to the target compounds is outlined in Schemes 1 and 2. 2-Amino-4,5,6,7,8,9-hexahydrocycloocta[*b*] thiophene-3-carbonitrile (**1**) and 2-amino-4,5,6,7,8,9,10-hexah ydrocycloocta[b]thiophene-3-carboxamide (**6**) were selected as our primary starting materials for this series of reactions, and were prepared via reported procedures.[22]

4-Amino thieno[2,3-*d*]pyrimidine **2** was obtained by condensing the amino cyano derivative **1** with formamide. The IR spectrum of the title compound indicated the presence of a forked absorption band at 3394 and 3305 cm^{-1} corresponding to NH_2 group. While, the ^1H NMR spectrum showed an exchangeable singlet signal at δ 6.83 indicating the two NH_2 protons, in addition to a singlet signal at δ 8.18 corresponding to the C2–H proton.

Compounds **5a–d** were prepared by reacting the amino-cyano derivative **1** with the selected isothiocyanate in ethanol in the presence of a catalytic amount of triethylamine. The IR spectra of **5a–d** showed the presence of two absorption bands



Gefitinib (Iressa®) **Erlotinib (Tarceva®)** **Imatinib (Gleevec®)**

Fig. 1 Structures of clinically potent anticancer pyrimidines.

* Correspondent. E-mail: lgchen@tju.edu.cn

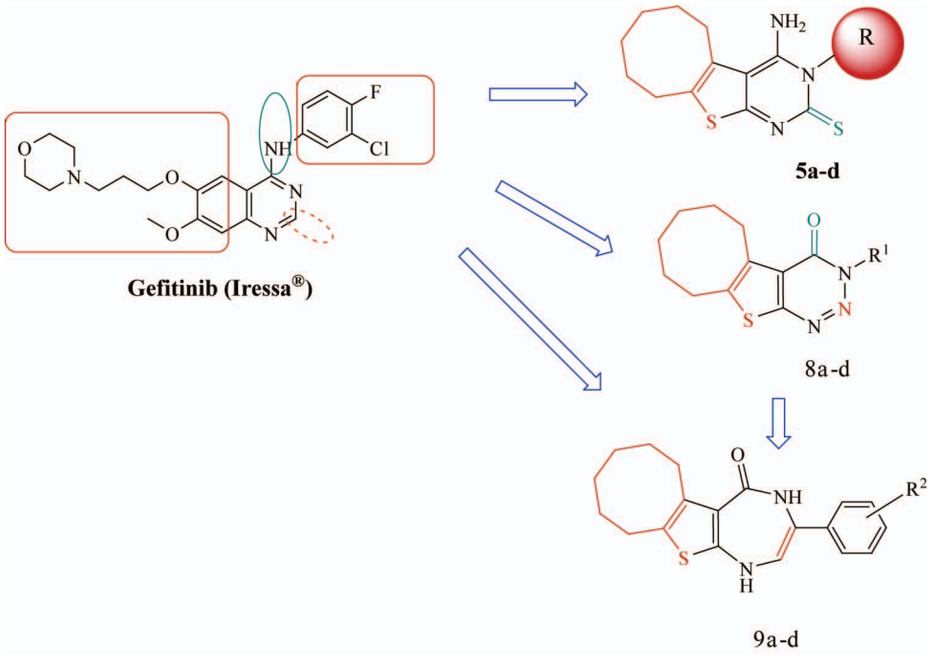
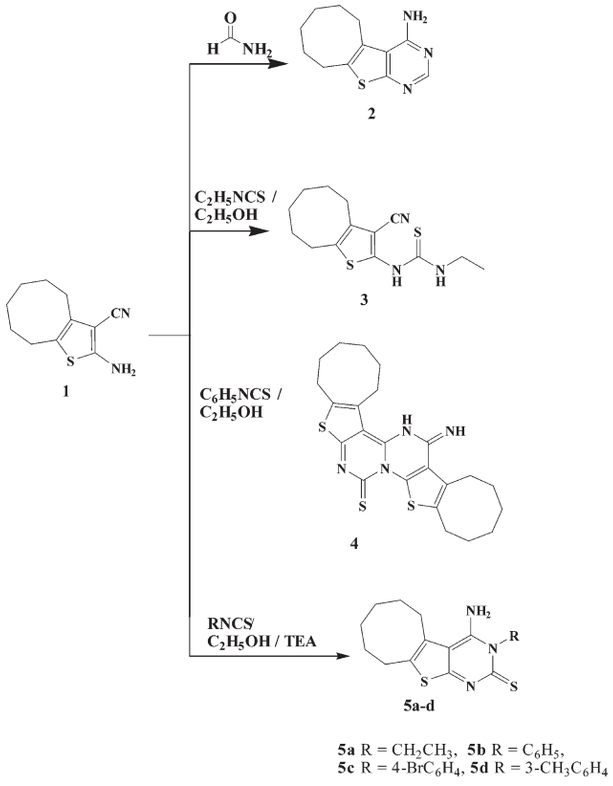
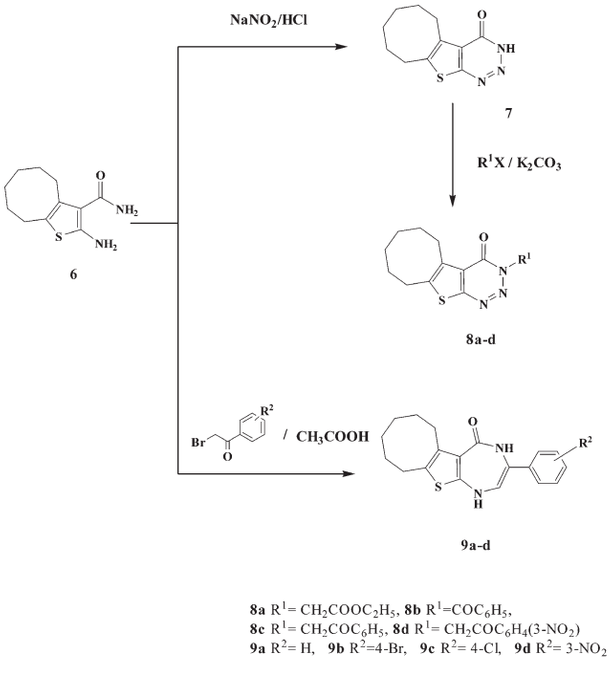


Fig. 2 Strategies for structural modifications of gefitinib.



Scheme 1 Synthesis of compounds **2–5**.



Scheme 2 Synthesis of compounds **7–9**.

at 3480-3437 and 3460–3440 cm^{-1} corresponding to the NH_2 group, in addition to the C=S group which appeared at 1273–1193 cm^{-1}. While, ^1H NMR spectra revealed the presence of one exchangeable singlet signal at δ 7.62–8.46 corresponding to NH_2 protons in addition to the characteristic signals of the N-CH_2CH_3 (**5a**) at δ 1.24 and 4.60–5.00 and the signals corresponding to the N-substituted phenyl groups (**5b–d**) at δ 6.72–7.50.

When **1** was heated with ethyl isothiocyanate in ethanol without the addition of triethylamine, the thioureido derivative **3** was produced rather than the cyclised form identical to **5a**. The structure of compound **3** was confirmed by IR and ^1H NMR spectra. The IR spectrum proved useful in tracing the absence of the absorption band corresponding to an NH_2 group but showed two absorption bands at 3331 and 3265 cm^{-1} corresponding to the two NH groups, and another two bands at 2216 and 1247 cm^{-1} corresponding to CN and C=S groups, respectively. In addition, the ^1H NMR spectrum of the product revealed the presence of two exchangeable singlet signals at δ 7.38 and 9.23 due to two NH protons instead of NH_2 protons.

In an attempt to prepare the N-phenyl analogue of compound **5**, by reacting **1** with phenyl isothiocyanate in ethanol, the hexacyclothienopyrimidine **4** was the sole product. The IR spectrum of compound **4** illustrated the appearance of two absorption bands at 3288 and 3209 cm^{-1} due to the two NH groups, in addition, another absorption band appeared at 1327 cm^{-1} corresponding to the C=S group. Further evidence was obtained from the ^1H NMR spectrum which revealed the absence of signals due to aromatic protons at δ 6–9. In addition, two exchangeable singlet signals at δ 7.62 and 8.85 appeared corresponding to two NH protons. Moreover, the mass spectrum of **4** displayed a peak at *m/z* 454 (M^+). A literature survey revealed that an analogous compound was produced under similar reaction conditions.[21] A suggested mechanism for the formation of the title compound is shown in Fig. 3.

The second approach is concerned with the synthesis of thieno[2,3-*d*]triazin-4-ones and thieno[2,3-*e*]-1,4-diazepin-5-ones. A literature survey revealed that the thienotriazines[22–26] and the thienodiazipinones [27] ring systems were scarcely mentioned in the literature. Reaction of o-amino amide derivative **6** with sodium nitrite under acidic conditions yielded the corresponding thienotriazinone derivative **7** which was reacted with the appropriate halogen compound in dry acetone and in the presence of anhydrous potassium carbonate to yield **8a–d**. The IR spectrum of **7** revealed the presence of an absorption band at 3200 cm^{-1} due to the NH group and a band at 1665 cm^{-1} corresponding to the C=O group. While the ^1H NMR spectrum showed an exchangeable singlet signal at δ 13.72 due to the NH proton. Moreover, the mass spectrum displayed three molecular ion peaks at *m/z* 235 (M^+), 236 ($M+1^{\cdot+}$) and 237 ($M+2^{\cdot+}$).

The IR spectra of **8a–d** lacked the presence of an absorption band due to the NH group and showed two absorption bands at 1755–1668 cm^{-1} due to two C=O groups. While, the ^1H NMR spectra showed the disappearance of the signal of the NH proton and displayed the characteristic signals corresponding to the different NR^1 group.

Reacting the amino amide **6** with the selected phenacyl bromide in glacial acetic acid afforded the desired 1,4-diazepinones **9a–d**. The structure of these compounds was substantiated by elemental analysis and spectral data. The IR spectra of **9a–d** showed the presence of two absorption bands at 3445–3387 and 3248–3109 cm^{-1} corresponding to two NH groups and only one band at 1659–1647 cm^{-1} due to one C=O group, whereas the ^1H NMR spectra of **9a–d** revealed the presence of an C2–H proton signal in the aromatic range. The mass spectrum of **9d** (R^1 = 3-NO_2) displayed three molecular ion peaks at *m/z* 369 (M^+), 370 ($M+1^{\cdot+}$) and 371 ($M+2^{\cdot+}$).

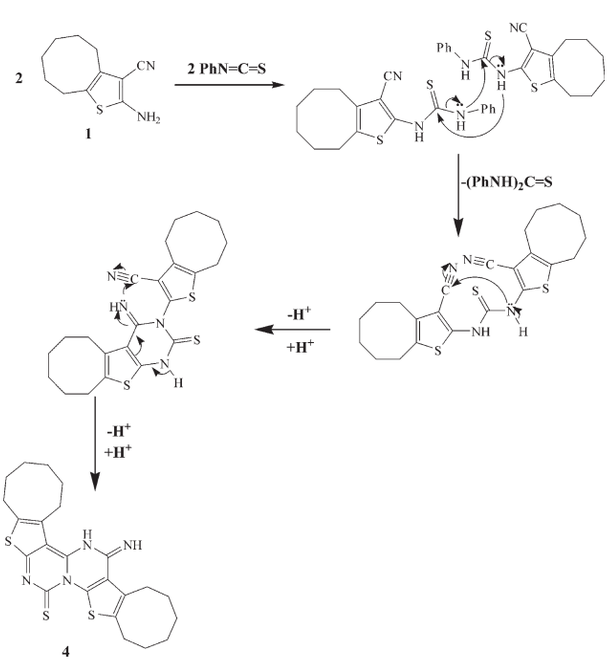


Fig. 3 Formation of compound **4**.

Cytotoxic activity

The *in vitro* growth inhibitory activity of all the prepared compounds was evaluated using colon carcinoma cell line (HCT 116). For comparison purposes, the cytotoxicity of imatinib (Gleevec®) (Fig. 1), a standard anti-tumour drug used for the treatment of gasterointestinal tract tumours,[28,29] was evaluated under the same conditions. The IC_{50} values (dose of the compound which caused a 50% reduction of survival values) are shown in Table 1. The results are represented graphically in Fig. 4. From the analysis of Table 1, it was found that almost all the compounds showed significant anti-tumour activities. Interestingly, compounds **4**, **3**, **8d**, **9a** and **2** (IC_{50}: 15.92, 22.59, 25.85, 27.40 and 29.70 μM, respectively) exhibited 2.16 to 1.15 fold more potent anti-tumour activity than imatinib (IC_{50}: 34.40 μM) and were the most active among their analogues. Further, compounds **5a**, **9b**, **5c** and **9d** (IC_{50}: 45.32, 45.86,

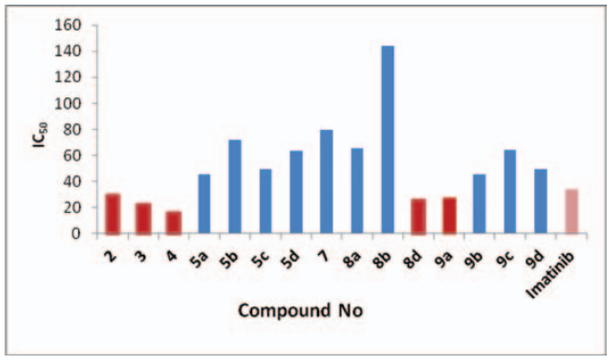


Fig. 4 Cytotoxicity of **2–9d** and imatinib against (HCT 116) cell line.

Table 1 IC_{50} values[a] of compounds **2–9d** and Imatinib against colon carcinoma cell line (HCT 116)

Compound	[a] IC_{50} (μM)
2	**29.70**
3	**22.59**
4	**15.92**
5a	45.32
5b	71.74
5c	49.24
5d	63.57
7	79.90
8a	65.34
8b	143.78
8c	–
8d	**25.85**
9a	**27.40**
9b	45.86
9c	64.36
9d	49.26
Imatinib	34.40

[a] IC_{50} (μM): dose of the compound which caused 50% reduction of survival.
Values were calculated from dose–response curves done in triplicate for each compound.

49.24 and 49.26μM, respectively) showed comparable cytotoxicity to imatinib. Compounds **5d**, **9c**, **8a**, **5b**, **7** and **8b** (IC_{50}: 63.57, 64.36, 65.34, 71.74, 79.90 and 143.78 μM) were less active than imatinib and compound **8c** was inactive.

Interestingly, the hexacyclicthienopyrimidine **4** and thiophene-3-carbonitrile **3** were the most potent derivatives of all the synthesised compounds. A literature survey revealed few compounds with structure resemblance to **3** and **4** [2,24,30,31] possessing significant *in vitro* cytotoxic agents.

Among the 4-amino thieno[2,3-*d*]pyrimidines **2** and **5a–d**, the unsubstituted analogue **2**, was the most active, while the aliphatic derivative **5a** (N-ethyl) manifested comparable activity to imatinib. Compounds **5b–d**, with N-phenyl or N-substituted phenyl, exhibited moderate potency.

The structure–activity relationship of the thienotriazinones was studied in view of the N-3 substituents (R^1). It was observed that the derivative with R^1 = (3-nitrophenyl)carbonyl methyl **8d** demonstrated privileged activity over imatinib, whereas **8b**, R^1 = ethoxycarbonylmethyl and **7**, R^1 = H exhibited moderate activity. Unexpectedly, the thienotriazinone **8c**, R^1 = phenylcarbonyl -methyl failed to show significant inhibition at the concentrations tested while compound **8b**, R^1 = benzoyl showed poor activity.

In the thienodiazepinone **9a–d** series, the 3-phenyl analogue (R^2 = H) **9a** showed more pronounced activity than imatinib. Whereas the alteration of R^2 (when R^2 = Cl, Br or NO_2) had little influence on their activity.

The preliminary encouraging results of biological screening of the thienotriazinones and thienodiazepinones showed that both new ring systems could offer an excellent framework in this field and that by proper substitution; potent anti-tumour agents may be discovered. This may be due to their structure resemblance to thieno[2,3-*d*]pyrimidines.

Experimental

Melting points were obtained on a Griffin apparatus and are uncorrected. Microanalyses for C, H and N were carried out at the Microanalytical Center, Cairo University. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr discs. ^{1}H NMR spectra were performed on a Joel NMR FXQ-200 MHz spectrometer, using TMS as the internal standard. Mass spectra were recorded on a GCMP-QP1000 EX mass spectrometer. Progress of the reactions was monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and was visualised by UV lamp.

4-Amino-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidine (**2**): A solution of 2-Amino-4,5,6,7,8,9-hexahydrocycloocta [*b*]thiophene-3-carbonitrile (**1**) (0.21 g, 0.001 mol) in formamide (10 mL) was refluxed for 2 h. The reaction mixture was cooled; the formed solid was filtered, washed with ethanol (10 mL), dried and crystallised from ethanol. M.p. 208–210 °C; yield 65%; IR (KBr) ν_{max}: 3394, 3305 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.13–1.25 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.55–1.65 (m, 4H, $2CH_2$), 2.85 (t, 2H, *J* = 6.0 Hz, CH_2), 2.97 (t, 2H, *J* = 6.0 Hz, CH_2), 6.83 (s, 2H, NH_2, D_2O exchangeable) and 8.18 (s, 1H, C2-H) ppm; MS [*m/z*,%]: 235 [M+H+1$^{\gamma\cdot+}$ and M+2$^{\gamma\cdot+}$, 11.54], 234 [M+H$^{\gamma\cdot+}$ and M+1$^{\gamma\cdot+}$, 100] and 233 [M$^{\cdot+}$, 6.09]. Anal. Calcd for $C_{12}H_{15}N_3S_2$ (233.32): C, 61.76; H, 6.48; N, 18.00. Found: C, 61.60; H, 6.50; N, 18.19%.

2-(N-Ethylcarbamothioylamino)-4,5,6,7,8,9-hexahydrocyclo-octa[b] thiophene-3-carbonitrile (**3**): Ethyl isothiocyanate (0.44 g, 0.005 mol) was added dropwise to a well stirred solution of **1** (1 g, 0.005 mol) in absolute ethanol (5 mL). The mixture was then refluxed on a steam bath for 6 h and left overnight at room temperature. The separated solid was filtered, dried and crystallised from ethanol. M.p. 162–164 °C; yield 50%; IR (KBr) ν_{max}: 3331, 3265 (2NH), 2216 (CN) and 1247 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.31 (t, 3H, *J* = 7.2 Hz, $CH_2C\boldsymbol{H_3}$), 1.26-1.35 (m, 2H, CH_2), 1.38-1.50 (m, 2H, CH_2), 1.60-1.70 (m, 4H, $2CH_2$), 2.68 (t, 2H, *J* = 6.3 Hz, CH_2), 2.73 (t, 2H, *J* = 6.3 Hz, CH_2), 3.63 (q, 2H, *J* = 7.2 Hz, $C\boldsymbol{H_2}CH_3$), 7.38 (s, 1H, NH, D_2O exchangeable) and 9.23 (s, 1H, NH, D_2O exchangeable) ppm; MS [*m/z*,%]: 295 [M+H+1$^{\gamma\cdot+}$ and M+2$^{\gamma\cdot+}$, 51.40], 294 [M+H$^{\gamma\cdot+}$ and M+1$^{\gamma\cdot+}$, 100] and 293 [M$^{\cdot+}$, 52.42]. Anal. Calcd for $C_{14}H_{19}N_3S_2$ (293.43): C, 57.30; H, 6.52; N, 14.32. Found: C, 57.58; H, 6.50; N, 14.54.

19-Imino-1,2,3,4,5,6,12,13,14,15,16,17,18,19-tetradeca-hydrocycloocta [4′,5′] thieno[2′,3′:4,5]pyrimido[1,6-a] cycloocta[4,5]thieno[3,2-e] pyrimidine-9-thione (**4**): Phenyl isothiocyanate (0.68 g, 0.005 mol) was added dropwise to a well stirred solution of **1** (1 g, 0.005 mol) in absolute ethanol (5 mL). The mixture was then refluxed on a steam bath for 6 h and left at room temperature overnight. The separated solid was filtered, washed with ethanol (10 mL) dried and crystallised from dimethylformamide. M.p. 230–232 °C; yield 50%; IR (KBr) ν_{max}: 3288, 3209 (2NH), 1629 (C=N) and 1327 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.15–1.26 (m, 4H, $2CH_2$), 1.42–1.50 (m, 4H, $2CH_2$), 1.60–1.75 (m, 8H, $4CH_2$), 2.86 (t, 2H, *J* = 5.7 Hz, CH_2), 2.91 (t, 2H, *J* = 5.7 Hz, CH_2), 3.10 (t, 2H, *J* = 5.7 Hz, CH_2), 3.26 (t, 2H, CH_2), 7.62 (s, 1H, NH, D_2O exchangeable) and 8.85 (s, 1H, NH, D_2O exchangeable) ppm; MS [*m/z*,%]: 454 [M$^{\cdot+}$, 6.49] and 78 [$C_6H_6^{\gamma\cdot+}$, 100]. Anal. Calcd for $C_{23}H_{26}N_4S_3$ (454.66): C, 60.75; H, 5.76; N, 12.32. Found: C, 61.05; H, 5.58; N, 12.15%.

Preparation of 4-amino-3-substituted-3H-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine-2-thiones (**5a–d**)*; general procedure*

The appropriate isothiocyanate (0.005 mol) was added to **1** (1 g, 0.005 mol) dissolved in absolute ethanol (25 mL) in the presence of triethylamine (4 drops). The reaction mixture was heated under reflux for 10 h, cooled and the separated solid was filtered, dried and crystallised from dimethylformamide.

4-Amino-3-ethyl-3H-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d] pyrimidine-2-thione (**5a**): M.p. 210–212 °C; yield 50%; IR (KBr) ν_{max}: 3437, 3440 (NH_2), 1645(C=N) and 1273(C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.15–1.30 (m, 2H, CH_2), 1.24 (t, 3H, $CH_2C\boldsymbol{H_3}$), 1.40–1.50 (m, 2H, CH_2), 1.55–1.65 (m, 4H, $2CH_2$), 2.75–2.80 (m, 2H, CH_2), 2.90–3.00 (m, 2H, CH_2), 4.60–5.00 (m, 2H, $C\boldsymbol{H_2}CH_3$), 7.62 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal. Calcd for $C_{14}H_{19}N_3S_2$ (293.43): C, 57.30; H, 6.52; N, 14.32. Found: C, 57.24; H, 6.39; N, 13.97%.

4-Amino-3-phenyl-3H-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno [2,3-d]pyrimidine-2-thione (**5b**): M.p. 234–236 °C; yield 50%; IR (KBr) ν_{max}: 3452, 3440 (NH_2), 1600 (C=N) and 1219 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.10–1.15 (m, 2H, CH_2), 1.45–1.50 (m, 2H, CH_2), 1.60–1.70 (m, 4H, $2CH_2$), 2.90–2.95 (m, 2H, CH_2), 3.10–3.20 (m, 2H, CH_2), 6.86 (t, 1H, *J* = 7.5 Hz, ArH), 6.97 (t, 2H, *J* = 7.5 Hz, ArH), 7.50 (d, 2H, *J* = 7.5 Hz, ArH) and 8.31 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal. Calcd for $C_{18}H_{19}N_3S_2$ (341.47): C, 63.30; H, 5.60; N, 12.30. Found: C, 63.20; H, 5.50; N, 12.00%.

4-Amino-3-(4-bromophenyl)-3H-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine-2-thione (**5c**): M.p. 208–210 °C; yield 46%; IR (KBr) ν_{max}: 3452, 3450 (NH_2), 1597 (C=N), 1222 (C=S), 825 (P-substitution) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.00–1.15 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 1.52–1.55 (m, 2H, CH_2), 1.60–1.66 (m, 2H, CH_2), 2.85–2.95 (m, 2H, CH_2), 3.06–3.20 (m, 2H, CH_2), 6.99 (d, 2H, *J* = 9.0 Hz, ArH), 7.40 (d, 2H, *J* = 9.0 Hz, ArH) and 8.46 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal. Calcd for $C_{18}H_{18}BrN_3S_2$ (420.36): C, 51.42; H, 4.31; N, 9.99. Found: C, 52.00; H, 4.05; N, 11.49%.

4-Amino-3-(3-methylphenyl)-3H-5,6,7,8,9,10-hexahydrocycloocta [4,5]thieno[2,3-d]pyrimidine-2-thione (**5d**): M.p. 209–211 °C; yield 30%; IR (KBr) ν_{max}: 3480, 3460 (NH_2), 1610 (C=N) and 1193 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.10–1.20 (m, 2H, CH_2), 1.45–1.53 (m, 2H, CH_2), 1.55–1.65 (m, 4H, $2CH_2$), 2.14 (s, 3H, CH_3), 2.91 (t, 2H, CH_2), 3.16 (t, 2H, CH_2), 6.72–6.80 (m, 2H, ArH), 7.35 (d, 1H, ArH), 7.42 (s, 1H, ArH) and 8.20 (s, 2H, NH_2, D_2O exchangeable) ppm; MS [*m/z*,%]: 356 [M+H$^{\gamma\cdot+}$, 15.43] and 340 [M-NH$^{\gamma\cdot+}$ and or M-$CH_3^{\gamma\cdot+}$, 100]. Anal. Calcd for $C_{19}H_{21}N_3S_2$ (355.50): C, 64.18; H, 5.95; N, 11.81. Found: C, 64.30; H, 5.60; N, 12.00%.

5,6,7,8,9,10-Hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-one (**7**): A solution of sodium nitrite (1.12 g, 0.016 mol in 11.2 ml water) was added dropwise with cooling and stirring to a suspension of 2-amino-4,5,6,7,8,9,10-hexahydrocycloocta[b]thiophene-3-carboxamide (**6**) (1 g, 0.004 mol) in hydrochloric acid (6 N, 23 mL) over a period of 15 minutes. The mixture was further stirred at room temperature for 30 minutes and left overnight; the formed solid was filtered, washed with water (20 mL), dried and crystallised from ethyl acetate. M.p. 120–122 °C; yield 77%; IR (KBr) ν_{max}: 3200 (NH) and 1665 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.25–1.40 (m, 2H, CH_2), 1.42–1.55 (m, 2H, CH_2), 1.70–1.85 (m, 4H, $2CH_2$), 2.98 (t, 2H, *J* = 6.2 Hz, CH_2), 3.16 (t, 2H, *J* = 6.2 Hz, CH_2) and 13.72 (s, 1H, NH, D_2O exchangeable) ppm; MS [*m/z*,%]: 237 [M+2$^{\gamma\cdot+}$, 2.50], 236 [M+1$^{\gamma\cdot+}$,

2.70], 235 [$M^{\cdot+}$, 18.60], 207 [$M\text{-}CO^{\cdot+}$, 37.20] and 164 [$C_{10}H_{12}S^{\cdot+}$, 100]. Anal. Calcd for $C_{11}H_{13}N_3OS$ (235.29): C, 56.14; H, 5.56; N, 17.85. Found: C, 56.30; H, 5.40; N, 17.39%.

Preparation of 3-substituted-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-ones **(8a–d)**; *general procedure*
A mixture of the triazine **7** (0.28 g, 0.001 mol), anhydrous potassium carbonate (0.35 g, 0.002 mol) and the selected halogen compound (0.01 mol) in dry acetone (10 mL) was heated under reflux for 8 h. The reaction mixture was then cooled, treated with ice cold water (50 mL) and the separated solid was filtered, washed with water (20 mL), dried and crystallised from ethanol.

3-Ethoxycarbonylmethyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-one (**8a**): M.p. 108–110 °C; yield 88%; IR (KBr) ν_{max}: 1751 and 1678 (2C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, *J* = 7.0 Hz, $CH_2C\boldsymbol{H_3}$), 1.30–1.42 (m, 2H, CH_2), 1.45–1.55 (m, 2H, CH_2), 1.70–1.82 (m, 4H, $2CH_2$), 2.99 (t, 2H, *J* = 5.8 Hz, CH_2), 3.14 (t, 2H, *J* = 5.8 Hz, CH_2), 4.26 (q, 2H, *J* = 7.0 Hz, $C\boldsymbol{H_2}CH_3$) and 5.14 (s, 2H, NCH_2CO) ppm; Anal. Calcd for $C_{15}H_{19}N_3O_3S$ (321.38): C, 56.05; H, 5.95; N, 13.07. Found: C, 55.90; H, 5.80; N, 13.27%.

3-Benzoyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-one (**8b**): M.p. 104–106 °C; yield 50%; IR (KBr) ν_{max}: 1755 and 1668 (2C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.30–1.55 (m, 4H, $2CH_2$), 1.65–1.75 (m, 4H, $2CH_2$), 2.90–3.10 (m, 4H, $2CH_2$), 7.60–7.75 (m, 3H, ArH) and 8.15–8.25 (m, 2H, ArH) ppm. Anal. Calcd for $C_{18}H_{17}N_3O_2S$ (339.40): C 63.69; H, 5.05; N, 12.38. Found: C, 63.80; H, 5.00; N, 12.86%.

3-Phenylcarbonylmethyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-one (**8c**): M.p. 170–172 °C; yield 50%; IR (KBr) ν_{max}: 1690 and 1676 (2C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25–1.40 (m, 2H, CH_2), 1.45–1.55 (m, 2H, CH_2), 1.65–1.80 (m, 4H, $2CH_2$), 2.90–3.05 (m, 2H, CH_2), 3.07–3.20 (m, 2H, CH_2), 5.85 (s, 2H, NCH_2CO), 7.45–7.70 (m, 3H, ArH) and 8.00–8.13 (m, 2H, ArH) ppm. Anal. Calcd for $C_{19}H_{19}N_3O_2S$ (353.42): C, 64.56; H, 5.41; N, 11.88. Found: C, 64.60; H, 4.71; N, 11.69%.

3-(3-Nitrophenyl)carbonyl-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]-1,2,3-triazin-4(3H)-one (**8d**): M.p. 98–100 °C; yield 51%; IR (KBr) ν_{max}: 1668 (2C=O), 1529 and 1348 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.25–1.30 (m, 2H, CH_2), 1.35–1.45 (m, 2H, CH_2), 1.55–1.65 (m, 2H, CH_2), 1.66–1.73 (m, 2H, CH_2), 3.05–3.10 (m, 4H, $2CH_2$), 6.14 (s, 2H, NCH_2CO), 7.92 (t, 1H, ArH), 8.53–8.58 (m, 2H, ArH) and 8.79 (s, 1H, ArH) ppm; MS [*m/z*,%]: 399 [$M+1^{\cdot+}$, 0.04], 398 [$M^{\cdot+}$, 0.21] and 150 [$C_7H_4NO_3^{\cdot+}$, 100]. Anal. Calcd for $C_{19}H_{18}N_4O_4S$ (398.42): C, 57.27; H, 4.55; N, 14.06. Found: C, 57.40; H, 4.60; N, 14.17%.

Preparation of 3-aryl-6,7,8,9,10,11-hexahydro-1H-cycloocta[4,5]thieno[2,3-e]-1,4-diazepin-5-(4H)-ones **(9a–d)**; *general procedure*
The selected phenacyl bromide (0.004 mol) was added to a solution of amino amide **6** (1 g, 0.004 mol) in glacial acetic acid (20 mL). The reaction mixture was heated under reflux for 10 h, and then cooled; the separated solid was filtered, washed with ethanol (20 mL), dried and crystallised from dimethylformamide.

3-Phenyl-6,7,8,9,10,11-hexahydro-1H-cycloocta[4,5]thieno[2,3-e]-1,4-diazepin-5-(4H)-one (**9a**): M.p. >300 °C; yield 50%; IR (KBr) ν_{max}: 3445, 3180 (2NH) and 1655 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.25–1.35 (m, 2H, CH_2), 1.40–1.46 (m, 2H, CH_2), 1.60–1.66 (m, 4H, $2CH_2$), 2.88 (t, 2H, *J* = 5.7 Hz, CH_2), 3.08 (t, 2H, *J* = 5.7 Hz, CH_2), 7.48–7.56 (m, 4H, 3ArH and C_2-H), 8.09 (d, 2H, *J* = 8.1 Hz, ArH) and 12.47 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{19}H_{20}N_2OS$ (324.43): C, 70.33; H, 6.21; N, 8.63. Found: C, 70.40; H, 6.30; N, 8.89%.

3-(4-Bromophenyl)-6,7,8,9,10,11-hexahydro-1H-cycloocta[4,5]thieno[2,3-e]-1,4-diazepin-5-(4H)-one (**9b**): M.p. >300 °C; yield 60%; IR (KBr) ν_{max}: 3402, 3109 (2NH) and 1647(C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.25–1.30 (m, 2H, CH_2), 1.35–1.46 (m, 2H, CH_2), 1.55–1.65 (m, 4H, $2CH_2$), 2.88 (t, 2H, *J* = 6.9 Hz, CH_2), 3.07 (t, 2H, *J* = 6.6 Hz, CH_2), 7.71–7.74 (m, 3H, C_2-H and 2ArH), 8.05 (d, 2H, *J* = 8.7 Hz, ArH) and 12.54 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{19}H_{19}BrN_2OS$ (403.33): C, 56.57; H, 4.74; N, 6.94. Found: C, 56.09; H, 4.27; N, 6.64%.

3-(4-Chlorophenyl)-6,7,8,9,10,11-hexahydro-1H-cycloocta[4,5]thieno[2,3-e]-1,4-diazepin-5-(4H)-one (**9c**): M.p. >300 °C; yield 64%; IR (KBr) ν_{max}: 3422, 3113 (2NH) and 1651(C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.20–1.30 (m, 2H, CH_2), 1.35–1.46 (m, 2H, CH_2), 1.53–1.66 (m, 4H, $2CH_2$), 2.85–2.90 (m, 2H, CH_2), 3.05–3.10 (m, 2H, CH_2), 7.58 (d, 2H, *J* = 8.8 Hz, ArH), 7.93 (s, 1H, C_2-H), 8.13 (d, 2H, *J* = 8.8 Hz, ArH) and 12.55 (s, 1H, NH, D_2O exchangeable) ppm. Anal. Calcd for $C_{19}H_{19}ClN_2OS$ (358.87): C, 63.58; H, 5.33; N, 7.80. Found: C, 63.60; H, 5.40; N, 7.95%.

3-(3-Nitrophenyl)-6,7,8,9,10,11-hexahydro-1H-cycloocta[4,5]thieno[2,3-e]-1,4-diazepin-5-(4H)-one (**9d**): M.p. >300 °C; yield 80%; IR (KBr) ν_{max}: 3387, 3248 (2NH), 1659 (C=O), 1528 and 1346 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.25–1.30 (m, 2H, CH_2), 1.35–1.46 (m, 2H, CH_2), 1.55–1.70 (m, 4H, $2CH_2$), 2.90 (t, 2H, *J* = 6.0 Hz, CH_2), 3.07 (t, 2H, *J* = 6.0 Hz, CH_2), 7.79–7.85 (m, 2H, ArH and C_2-H), 8.38 (d, 1H, *J* = 8.4 Hz, ArH), 8.54 (d, 1H, *J* = 8.1 Hz, ArH), 8.94 (s, 1H, ArH), 12.15 (br s, 1H, NH, D_2O exchangeable) and 12.82 (s, 1H, NH, D_2O exchangeable) ppm; MS [*m/z*,%]: 371 [$M+2^{\cdot+}$, 2.49], 370 [$M+1^{\cdot+}$ and $M+H^{\cdot+}$, 2.07], 369 [$M^{\cdot+}$, 1.76], 341 [$M\text{-}CO^{\cdot+}$, 0.58], 194 [$C_{10}H_{14}N_2S^{\cdot+}$, 2.26], 179 [$C_{10}H_{13}NS^{\cdot+}$, 10.80], 164 [$C_{10}H_{12}S^{\cdot+}$, 7.12] and 41 [$C_3H_5^{\cdot+}$, 100]. Anal. Calcd for $C_{19}H_{19}N_3O_3S$ (369.42): C, 61.76; H, 5.18; N, 11.37. Found: C, 61.79; H, 4.53; N, 11.06%.

Cytotoxic activity studies
Anti-cancer activity studies were carried out at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

Compounds **2–9d** were tested at concentrations between 1 and 10 μg mL^{-1} using SulfoRhodamine-B (SRB) assay for cytotoxic activity against human colon tumour cell line (HCT116). Imatinib which is a 2-substituted aminopyrimidine derivative was chosen as a reference standard anti-cancer drug because it showed potency against gasterointestinal tract tumours.[28,29]

The potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*[34] Cells were plated in 96 multiwell plates (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment to the wall of the plate. Different concentrations of the compounds (0, 1, 2.5, 5 and 10 μg mL^{-1}) were added to the cell monolayer and triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °c in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SulfoRhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relationship between the surviving fraction and the drug concentration was plotted to obtain the survival curve of each tumour cell line after the specified compound was administered.

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