Synthesis of Some Novel Thieno[3,2-*d*]pyrimidines as Potential Cytotoxic Small Molecules against Breast Cancer

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A variety of novel thieno[3,2-*d*]pyrimidines with different decorating functional groups were synthesized as a part of a study aiming to enrich the arsenal of chemotherapeutic agents for the treatment of cancer. The design of synthetic molecules based on DNA-interchelating properties by hydrogen bond formation. The reported compounds herein are: 4-aminothienopyrimidine derivatives 4a, b and their 4-substituted phenylamino analogues 8a, b; 4-thienopyrimidin-4-ones 5a, b; *N*-alkyl thienopyrimidin-4-ones 6a–g; 4-chloro-thienopyrimidines 7a, b and thienopyrimidoquinazolinones 9a, b which are the structural mimics of 8a, b. The synthesized molecules were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF-7). Biological screening revealed varying cytotoxic potencies of the tested molecules compared with Doxorubicin as a reference drug. The cytotoxicity results from the study suggested that the synthesized molecules are potential antitumor agents and compound 4a was the most potent with an IC_{50} 2.04 nM.

Key words heterocycle; thieno[3,2-*d*]pyrimidine; cytotoxic activity; breast cancer

The development of novel chemotherapeutic agents has significantly progressed within the last 60 years, success in developing non-cytotoxic "targeted" drugs with fewer side effects has occurred only in the last decade. Despite these advances, the treatment of most types of solid tumors (*e.g.*, breast and ovarian) is still a problem and survival rates remain significantly low.[1)] Doxorubicin (DOX) is an antitumour antibiotic and essential component of the chemotherapy of a large number of solid tumors, including breast cancer, soft tissue sarcomas, and aggressive lymphomas. Despite their long-standing clinical utilization, the mechanism of action is still unclear and subject to controversy. From a chemical point of view, DOX is composed of a tetracyclic ring linked to the amino sugar; the mode of action of DOX is intercalation with guanine base of DNA.[2)] DOX exerted its action by intercalating DNA and consequently inhibiting the macromolecular biosynthesis machinery and topoisomerase II (topo II).[3)] Topoisomerases are crucial for the several DNA functions (*e.g.*, replication and transcription). The main pharmacophoric feature of DOX and other synthetic interclators is the hydrogen bonding formation with DNA.[4)] Mitoxantrone was a synthetic analog of DOX and is active in breast cancer with a similar mode of action. Isosteric substitution of one or more carbons of the benzene rings of Mitoxantrone with nitrogen atom has been employed as a strategy for the design of pixantrone (*simplified analogue of mitoxantrone* with an increased affinity to DNA due to the presence of hydrogen bonding sites).[5–7)] Guided with this bioisosteric modification, fused hetrocylic ring core could be used instead of the planner polycyclic ring system in the design of novel synthetic molecules as DNA intercalators.

In addition, Pyrimidine is one of the most important pharmacophoric ring exhibiting remarkable pharmacological activities as it is one of the essential building blocks of nucleic acids, DNA and RNA.[8)] Moreover thiophene ring is also known to possess anticancer activity.[9,10)] From biological point of view, fused hetero-aromatic ring systems are often of much greater interest than their constituting monocyclic fragments. Thienopyrimidines occupies a special position among them as they serve as structural analogues for biogenic purines and potential nucleic acid antimetabolites.[11)] Literature screening revealed that many thienopyrimidine derivatives A, B and C have been developed and showed pronounced cytotoxic activity.[12–20)] In particular, they are currently an important group of compounds that have anticancer activity especially against solid tumors (*e.g.*, breast and ovarian).[21–24)] Several mechanisms have been reported for the cytotoxic activity of thienopyrimidines including: inhibition of protein kinases (PKs) either as competitive or noncompetitive inhibitors. It was reported that they exerted their action by inhibiting tyrosine kinases (TKs) that represents a major advance in the treatment of solid tumors.[25,26)] Thienopyrimidine derivatives D have been shown to prevent angiogenesis of tumor cells *via* their potent inhibition of vascular endothelial growth factor receptor (VEGFR).[27)] Moreover, thienopyrimidine derivatives E exhibited inhibition of DNA replication during cell division through the inhibition of cycle protein kinase (Cdc7) and thienopyrimidine derivative **F** might be also apoptosis inducer in human breast cancer cells.[28,29)] Beside that, there are certain groups of antitumor drugs working as topo II inhibitors that they differ from topo II poisons (*DNA intercalators*) in interfering with the steps of the catalytic cycle as they targeted the N-terminal ATPase domain of topo II leading to inhibition of DNA synthesis. These groups have been synthesized to mimic ATP, where they usually consist of a heterocyclic ring system that occupied the purine binding site and it served as a scaffold for side chains that occupied the adjacent hydrophobic regions I and II.[30–36)]

In the light of mentioned findings, and applying the concept of pharmacophoric hybridization, a number of small synthetic molecules containing DNA-interchelating fragments and kinase inhibition properties was designed as potential cytotoxic agents. The work reported the synthesis and cytotoxic activity of some novel small synthetic molecules in the aim of exploring structure–activity relationship (SAR) of thienopyrimidine ring for inhibition of DNA synthesis by comparison between

The authors declare no conflict of interest.

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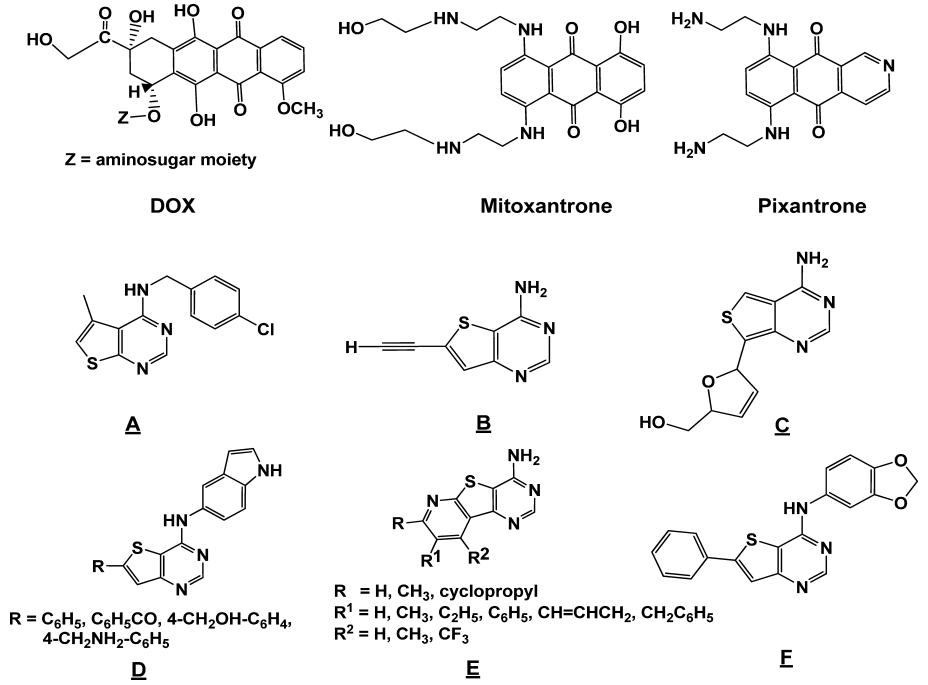


Fig. 1. Structures of DOX, Mitoxantrone, Pixantrone and Some of the Literature Active Thienopyrimidines as Cytotoxic Molecules

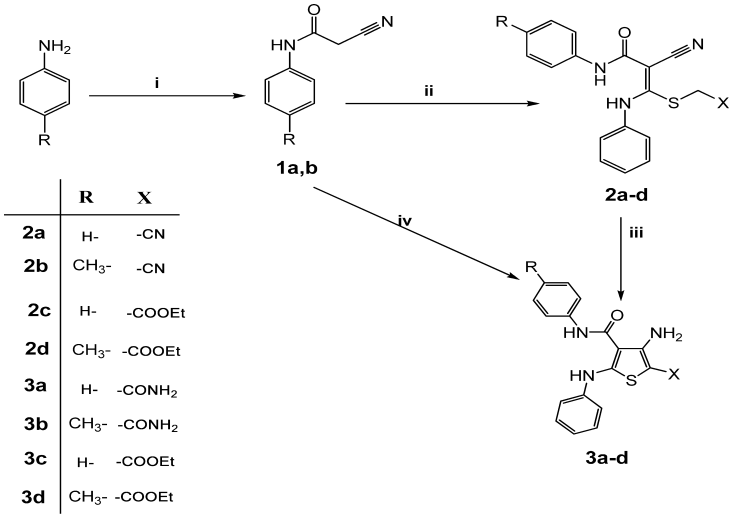
different function groups on the cytotoxicity, where 4-aminothienopyrimidines **4a**, **b** and their 4-substituted phenylamino derivatives **8a**, **b** were built to study the effect of substitution on NH_2 group by polar group (*benzoic acid moiety*) that can form additional hydrogen bond on cytotoxicity, also planar tetracyclic compounds **9a**, **b** which structurally related to the synthetic intercalators were synthesized as structural mimics of **8a**, **b** to explore structural rigidity. In parallel, a series of molecules was synthesized to compare the difference in activity between the NH_2 group of derivatives **4a**, **b** and other function groups present in thienopyrimidin-4-ones **5a**, **b** and 4-chlorothienopyrimidines **7a**, **b**. In this context, *N*-alkyl thienopyrimidin-4-ones **6a**–**g** were synthesized as the structural extension that could form extra hydrogen bond formation sites in hetrocyclic core. The synthetic molecules probably displayed both DNA intercalators and topo inhibition structural features, consequently inhibition of DNA functions.

Results and Discussion

Chemistry The synthetic strategies adopted for constructing all the target molecules are illustrated in (Figs. 2–7). Ethyl cyanooacetate reacts with the appropriate aromatic amine to give cyanoacetamides **1a**, **b**.[37] It was reported that the reaction of the acidic methylene compounds with phenyl isothiocyanate in a basic medium such as potassium hydroxide gave the suitable α-halo active methylene compound.[38] The alkaline solution of the **1a**–**b** in *N*,*N*-dimethylformamide (DMF) was treated with phenyl isothiocyanate followed by reaction with either chloroacetonitrile to afford **2a**, **b** or ethyl chloroacetate to give **2c**, **d**. ^{1}H-NMR spectra revealed the presence of a singlet peak at δ 3.62–4.00 ppm corresponding to CH_2 protons, two exchangeable singlet signals at δ 8.80–9.58 and 10.16–11.42 ppm corresponding to the two NH protons. In addition, compounds **2c**, **d** showed a triplet signal at δ 1.15 ppm and a quartet signal at δ 4.04 ppm corresponding to CH_3 and CH_2 protons of the ethoxide group, respectively. The key intermediate thiophene derivatives **3a**–**d** were prepared either from the cyanoacetamides **1a**, **b** adopting Gewald precautions or by reacting the acyclic methylthio compounds **2a**–**d** with sodium ethoxide in ethanol.[39] The latter procedure was found to afford a better yield than the former method. The IR spectra of **3a**–**d** showed absorption bands at the range 3356–3200 cm^{-1} due to two NH and NH_2 groups. Compounds **3c**, **d** showed the disappearance of C≡N group absorption band of the precursor **2c**, **d**. The ^{1}H-NMR spectra of **3b**–**d** lacked the presence of the singlet signal corresponding to CH_2 protons of its precursor **2b**–**d** but showed an additional exchangeable singlet signal corresponding to NH_2 protons at δ 6.41–6.68 ppm. Compounds **3b**, **d** displayed a singlet signal at δ 2.25–2.26 ppm indicating CH_3 protons of *p*-tolyl moiety.

4-Aminothienopyrimidines can be obtained from the corresponding *o*-aminocyano derivatives by reaction a mixture of formamide and formic acid in DMF[40] or formamide only.[41] The desired 4-aminothienopyrimidines **4a**, **b** were obtained by treating of the *o*-aminocyanothiophenes **3a**, **b** with excess formamide. ^{1}H-NMR spectra revealed the presence of the signal due to NH_2 protons in the aromatic region in addition to two exchangeable singlet signals appearing at δ 11.38–11.41 and 11.99–12.08 ppm corresponding to two NH protons. Also, a singlet signal appeared at δ 8.43 corresponding to C2 proton of thienopyrimidine ring. Moreover, the mass spectrum of **4b** displayed three molecular ion peaks at *m*/*z* 376, 375 and 374 corresponding to $(M+1)^{+\cdot}$, $(M)^{+\cdot}$ and $(M-1)^{+\cdot}$, respectively.

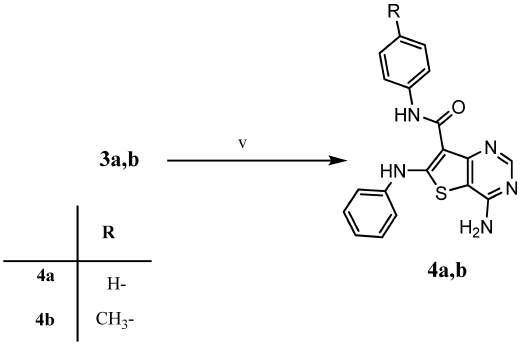
Synthesis of thienopyrimidinones from the corresponding *o*-aminoesters *via* reaction with excess formamide was reported in literature.[42,43] Fusion of the parent *o*-aminoesters **3c**, **d** with excess formamide gave the thieno[3,2-*d*]pyrimidinones **5a**, **b**. The IR spectra of **5a**, **b** indicated the presence of absorption bands at 3438–3436, 3236–3182 and 1666–1639 cm^{-1} corresponding to OH, three NH and two C=O groups, respectively. ^{1}H-NMR spectra revealed the disappearance of the signals of ethoxy protons of the parent ester **3c**, **d** whereas the



	R	X
2a	H-	-CN
2b	CH_3-	-CN
2c	H-	-COOEt
2d	CH_3-	-COOEt
3a	H-	-$CONH_2$
3b	CH_3-	-$CONH_2$
3c	H-	-COOEt
3d	CH_3-	-COOEt

Reagents and conditions: (i) $NCCH_2COOC_2H_5$,reflux, 5h; (ii) iia- KOH/DMF, r.t., 3h; iib- PhNCS, r.t., 3h; iic- $ClCH_2X$, r.t., 24h; (iii) EtONa/EtOH, reflux, 30min. and (iv) iva-EtONa /EtOH,reflux, 30 min; ivb- PhNCS; 70°C, 30min. ivc- $ClCH_2CN$, 60°C, 30min.;ivd- EtONa, reflux. 3h.

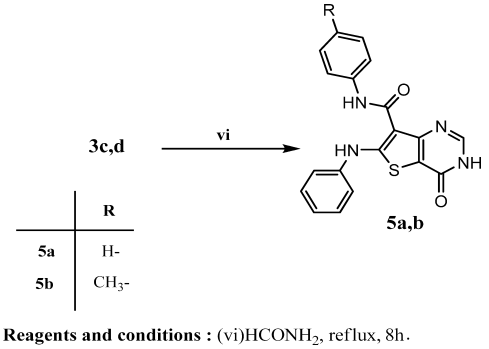
Fig. 2. Synthesis of the Intermediates **2a–d** and **3a–d**



	R
4a	H-
4b	CH_3-

Reagents and conditions: (v)$HCONH_2$, reflux, 2h.

Fig. 3. Synthesis of 4-Aminothienopyrimidine Derivatives **4a**, **b**



	R
5a	H-
5b	CH_3-

Reagents and conditions : (vi)$HCONH_2$, reflux, 8h.

Fig. 4. Synthesis of the Thienopyrimidinones **5a**, **b**

appearance of two exchangeable singlet signals at δ 11.43 and 11.47–11.53 ppm and one singlet signal at δ 12.61–12.69 ppm indicated NH protons and OH protons, respectively. Additionally, C2 proton of thienopyrimidine appeared as a singlet signal at δ 8.39 and 8.36 ppm for compounds **5a** and 5**b**. The mass spectrum of 5**a** showed a molecular ion peak at *m*/*z* 362 (M^{+}) (46.73).

Moreover, conversion of thienopyrimidinone derivatives into the corresponding *N*-alkyl derivatives could be achieved through reaction of the thienopyrimidinones with the respective alkyl halide in ethanolic solution of sodium ethoxide.[44] Reacting thienopyrimidinones **5a** or **5b** with ethanolic potassium hydroxide solution followed by the addition of the appropriate alkyl halide afforded the corresponding *N*-alkyl derivatives **6a**–**g**. IR spectra of compounds **6a**–**g** showed an absorption band at 3510 cm^{-1} indicating OH group. ^{1}H-NMR spectrum of **7a** showed a triplet signal at δ 1.30 ppm and a quartet signal at δ 4.02 ppm corresponding to CH_3 and CH_2 protons of the ethoxide moiety. ^{1}H-NMR spectra of **6b**–**g** showed two singlet signals at δ 4.86–4.93 and δ 8.61–8.65 ppm indicating CH_2 and C2 protons, respectively. Also, three exchangeable singlet signals appeared at δ 9.21–10.46, 10.19–11.35 and 11.28–11.50 ppm corresponding to three NH protons. In addition, OH proton of **6e** and **6g** appeared as an exchangeable singlet signal at δ 11.45 and 11.49 ppm, sequentially. The mass spectra of **6a**, **b** and **6g** displayed molecular ion peaks at *m*/*z* 404, 495 and 525, respectively.

Conversion of thienopyrimidinones to the corresponding chlorosubstituted thienopyrimidine derivatives could be achieved by using phosphorus oxychloride.[45] Chlorination of thienopyrimidinones **5a**, **b** with excess phosphorus oxychloride gave the 4-chlorothienopyrimidine derivatives **7a**, **b**. Compounds **7a**, **b** showed the disappearance of OH group absorption band at 3236–3182 cm^{-1} of the precursor **5a**, **b**.

It has been reported that nucleophilic substitution of chlorosubstituted derivatives with different aromatic amines could be operated in DMF.[46] Reacting equimolar amounts of **7a**, **b** with anthranilic acid in DMF containing catalytic amounts of anhydrous potassium carbonate afforded the 4-anilino derivatives **8a**, **b**. ^{1}H-NMR spectra of **8a**, **b** proved the structure as it showed an exchangeable singlet signal at δ 13.09 ppm corresponding to acidic OH, whereas, the mass spectrum of **8a** displayed a molecular ion peak at *m*/*z* 481.

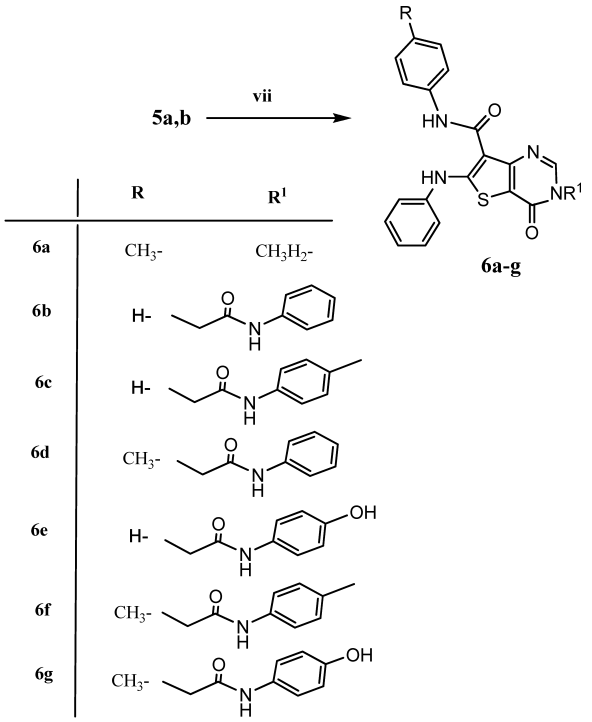
Cyclization of the latter with excess thionyl chloride furnished the thienopyrimidoquinazolinones **9a**, **b**. IR spectra showed two absorption bands at the range 3432–3224 cm^{-1} due

to two NH groups. ^{1}H-NMR spectrum lacked the presence of the signals corresponding to NH, OH protons of **8a**, **b**. Mass spectrum of **9b** demonstrated three molecular ion peaks at *m*/*z* 478 ($M+1^{+\cdot}$), 477 ($M^{+\cdot}$) and 476 ($M-1^{+\cdot}$).

Biological Evaluation The antitumor screening of novel synthesized compounds was carried in National Center Institute (NCI), Department of Cancer Biology, Cairo University, Cairo, Egypt.

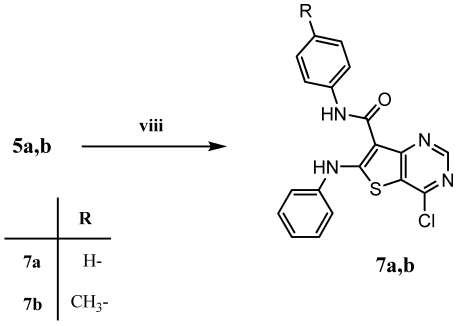
Preliminary *in Vitro* Antitumor Screening Most of the newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line MCF7. DOX was one of the most effective anticancer agents and therefore was used as a reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of MCF7. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. It was observed from the results obtained that majority of the test compounds showed significant cytotoxic activities against MCF7 with IC_{50} values ranging between 2.04 and 35.36 nM (Fig. 8). Eight compounds **4a**, **b**, **5a**, **b**, **8a**, **b** and **9a**, **b** exhibited enhanced antitumor activities compared with that of DOX. Compounds **7a** (IC_{50}=*8.21 nM*) was found to be nearly as active as DOX. Compounds **6a**, **6e** and **7b** showed a moderate activity, while compounds **6b**–**d** and **6f**, **g** were less active than the reference drug.

SAR Structurally, the biologically screened molecules belong to a structure category of compounds with thienopyrimidine heterocyclic core that have hydrogen bond acceptor donor pairs at 6 and 7 positions but with different functional groups decoration at 3 and 4 positions These molecules are classified to four types of thienopyrimidine derivatives: first, 4-aminothienopyrimidine derivatives, **4a**, **b** and their 4-phenylamino analogues **8a**, **b**; second, rigid derivatives **9a**, **b** which are the structural mimics of **8a**, **b**; third, 4-chlorothienopyrimidines **7a**, **b**, thienopyrimidine-4-ones **5a**, **b** and *N*-alkyl thienopyrimidine-4-ones **6a**–**g**. Examining the activity of the molecules, we can deduce that 4-aminothienopyrimidine derivatives **4a**, **b** represent the most potent cytotoxic molecules, especially **4a** with an IC_{50} of 2.04 nM. Substitution of amino group with



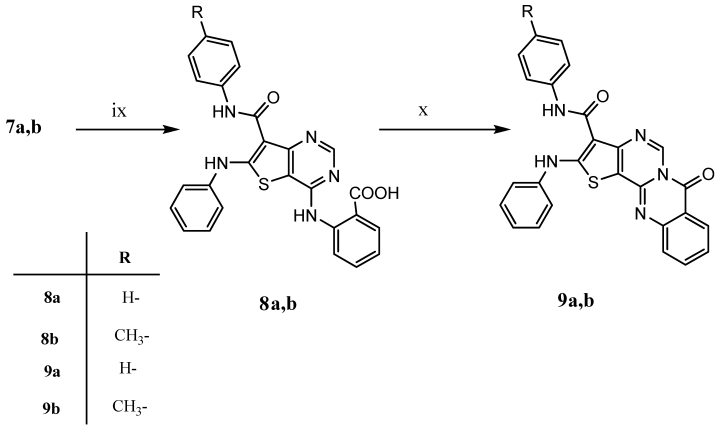
Reagents and conditions : (vii)R^1Cl,KOH/EtOH, reflux, 12h.

Fig. 5. Synthesis of *N*-Alkyl Thienopyrimidine-4-ones **6a**–**g**



Reagents and conditions : (viii) $POCl_3$, reflux, 8h.

Fig. 6. Synthesis of 4-Chlorothienopyrimidines **7a**, **b**



Reagents and conditions : (ix) anthranilic acid, K_2CO_3/DMF, reflux, 24 h;(x) $SOCl_2$, reflux, 4 h

Fig. 7. Synthesis of 4-Substituted Phenylaminothienopyrimidines **8a**, **b** and Thienopyrimidoquinazolinones **9a**, **b**

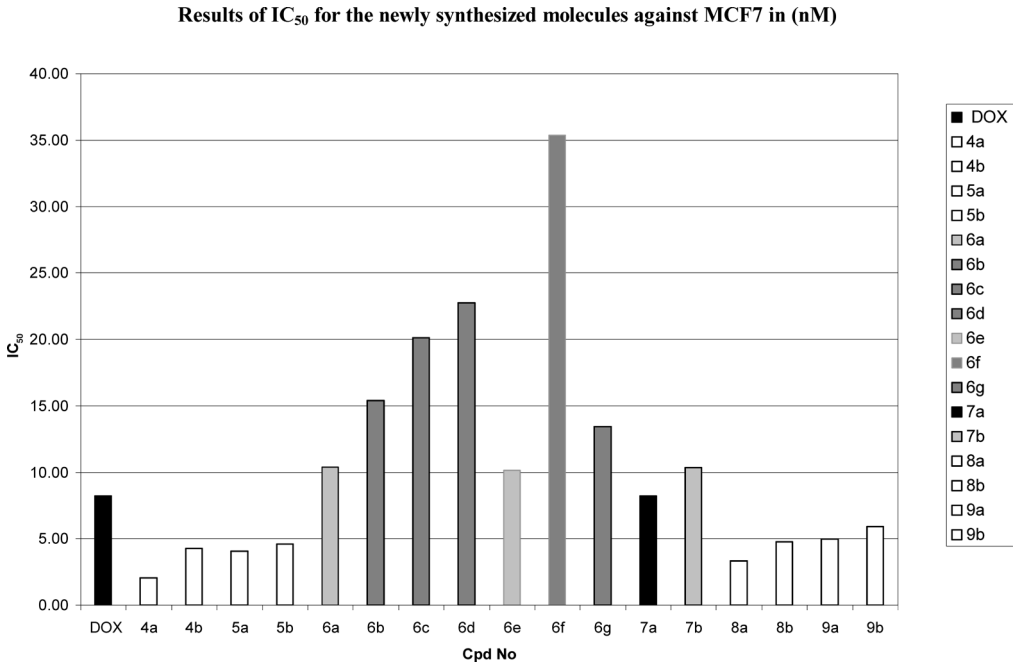


Fig. 8. Results of the *in Vitro* Cytotoxic Activity of the Synthesized Molecules and DOX against MCF7 Cells

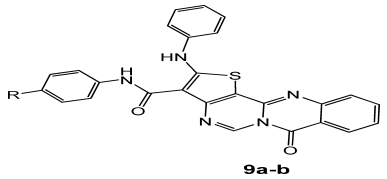
Table 1. Results of the *in Vitro* Cytotoxic Activity of **4a**, **b**, **8a**, **b** and **7a**, **b** against MCF7 Cells

4a,b 7a,b and 8a,b

Cpd. No.	R	R^1	IC_{50} (nM[a)])
4a	H–	$–NH_2$	2.04±0.10
4b	CH_3–	$–NH_2$	4.28±0.18
7a	H–	–Cl	8.21±011
7b	CH_3–	–Cl	10.35±0.15
8a	H–	$–NHC_6H_4COOH$	3.34±0.16
8b	CH_3–	$–NHC_6H_4COOH$	4.76±0.15

a) The values given are means of three experiments.

Table 2. Results of the *in Vitro* Cytotoxic Activity of **9a**, **b** against MCF7 Cells



9a-b

Cpd. No.	R	IC_{50} (nM[a)])
9a	H–	4.96±0.14
9b	CH_3–	5.91±0.11

a) The values given are means of three experiments.

aromatic carboxylic acid moiety slightly diminished its activity (**4a**, **b** compared with **8a**, **b**). Moreover, replacement of the amino group with chloride radical **7a**, **b** decreased the activity (Table 1).

Cyclization of 4-substituted aminothienopyrimidines **8a**, **b** afforded mimic structures **9a**, **b** and increased the activity, probably due geometrical similarity with DOX (Table 2).

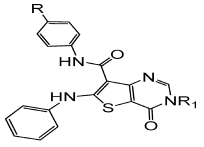
Thienopyrimidinones **5a**, **b** displayed a potent activity, although extending the structure of **5a**, **b** by a polar chain was hypothesized to increase the activity by increasing the number of hydrogen bonding sites in the molecules, our results were to the contrary of this hypothesis, where the substitution of **5a** or **5b** with ethyl or alkyl aryl amide radicals to produce 3-alkyl thienopyrimidinones **6a**–**g** decreased the cytotoxic activity. Order of activity for alkyl substitution 4-pyrimidinones was $H>C_2H_5>CH_2CONHPh$ (Table 3).

Substituting by methyl group on *p*-position of phenyl amide moiety at 7 position decreased activity. The cytotoxic activity of the synthesized molecules may be attributed to the following suggested explanations, the compounds may be targeted both the mentioned modes of actions for the reported leads molecules as intercalators (**5a**–**g** and **9a**, **b**) or kinase inhibitors (**4a**, **b**, **7a**, **b** and **8a**, **b**). Further studies are still needed to determine the exact mechanism of the antitumor action as well as to explore the SAR of other positions of the nucleus.

Conclusion

In summary, a series of thieno[3,2-*d*]pyrimidine derivatives were designed and synthesized as small synthetic molecules with antitumor activity. The results of cytotoxic screening were in good agreement with our design rationale except compounds **6a**–**g** as the additional hydrogen bond formation moiety introduced in 3 position of the fused ring decreased their activity. All the target molecules were evaluated for their *in vitro* cytotoxicity against MCF7. The cytotoxicity results concluded that there are three levels of activity compared with the reference, compounds **4a**, **b**, **5a**, **b**, **8a**, **b** and **9a**, **b**, exhibited significant cytotoxic activity which was even higher than that of DOX. Compound **4a** was the most potent with IC_{50} 2.04 nM. Compound **7a** was equipotent as DOX. Compounds **6a**, **6e** and **7b** showed moderate activity. Compounds **6b**–**d** and **6f**, **g** were

Table 3. Results of the *in Vitro* Cytotoxic Activity of **5a**, **b** and **6a**–**g** against MCF7 Cells



5a-b and 6a-g

Cpd. No.	R	R^1	IC_{50} (nM[a)])
5a	H–	H–	4.08±0.08
5b	CH_3–	H–	4.62±0.12
6a	CH_3–	CH_3CH_2–	10.41±0.11
6b	H–	C_6H_5–NHCOCH$_2$–	15.38±0.15
6c	H–	4-CH_3–C_6H_4–NHCOCH$_2$–	20.14±0.14
6d	CH_3–	C_6H_5–NHCOCH$_2$–	22.75±0.15
6e	H–	4-OH–C_6H_4–NHCOCH$_2$–	10.13±0.13
6f	CH_3–	4-CH_3–C_6H_4–NHCOCH$_2$–	35.36±0.16
6g	CH_3–	4-OH–C_6H_4–NHCOCH$_2$–	13.43±0.13

a) The values given are means of three experiments.

less active DOX. The study suggested that the newly synthesized molecules are potential antitumor agents but further studies are needed to investigate the mechanism of action of these cytotoxic molecules.

Experimental

Chemistry Melting points (°C, uncorrected) were determined in open capillaries on a Graffin melting point apparatus. IR spectra were determined as KBr discs on Shimadzu IR 435 Spectrophotometer and values were represented in cm^{-1}. ^{1}H-NMR spectra (in DMSO-d_6) were carried out on Varian Gemini 300 MHZ Spectrophotometer, at nuclear magnetic resonance, Cairo University, Egypt, using TMS as internal standard and chemical shifts were recorded in ppm on δ scale.^{13}C-NMR were carried out on Bruker AC 200 (200 MHz) spectrometer, Institute for synthetic chemistry, Vienna. University of Technology, Vienna, Austria Electron impact Mass Spectra were recorded on Hewlett Packard 5988 Spectrometer. Element analysis was carried out at the Micro analytical Center, Cairo University, Giza, Egypt. All compounds were within ±0.5% of the theoretical values. Pre-coated silica gel plates (silica gel 0.25 mm, MERCK 60F 254, Germany) were used for thin layer chromatography, benzene–ethyl acetate (8.5 : 1.5 mL) mixture was used as a developing solvent system and the spots were visualized by UV lamp. Compounds **1a**, **b** and **3a** are synthesized according to reported methods.[37,47)]

***N*-Aryl-2-cyano-3-phenylamino-3-(substituted methylthio)-acrylamides (2a–d)** General Procedure: To a cold suspension of finely grinded KOH (1.4 g, 0.025 mol) in DMF (20 mL), *N*-aryl-2-cyanoacetamides **1a** or **1b** (0.025 mol) was added and the mixture was stirred at room temperature for 3 h. Phenyl isothiocyanate (3.4 g, 0.025 mol) was added and the mixture was stirred at room temperature for other 3 h. The active methylene chloro derivative (0.025 mol) was then added and the mixture was left on stirring for additional 24 h at room temperature. The mixture was triturated with cold water (*ca.* 50 mL). The resultant solid product was collected by filtration, dried, crystallized from ethanol to give **2a**–**d**.

2-Cyano-3-cyanomethylthio-*N*-phenyl-3-(phenylamino)-acrylamide (**2a**): Yield, 0.07 g (84%); mp 186–187°C; *Anal.* Calcd for $C_{18}H_{14}N_4OS$ (334.39): C, 64.65; H, 4.22; N, 16.75. Found: C, 64.90; H, 4.33; N, 16.94. IR (KBr, cm^{-1}): 3404–3296 (2NH), 2181 (C≡N), 1636 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 4.00 (s, 2H, CH_2), 6.97–7.28 (m, 3H, ArH), 7.30–7.44 (m, 3H, ArH), 7.55–7.66 (m, 2H, ArH), 7.69 (d, J=2.7 Hz, 2H, ArH), 8.90 (s, 1H, NH, D_2O exchangeable), 10.18 (s, 1H, NH–CO, D_2O exchangeable).

2-Cyano-3-cyanomethylthio-3-(phenylamino)-*N*-*p*-tolyl Acrylamide (**2b**): Yield, 0.08 g (92%); mp 180–182°C; *Anal.* Calcd for $C_{19}H_{16}N_4OS$ (348.42): C, 65.50; H, 4.63; N, 16.08. Found: C, 65.26; H, 4.36; N, 15.67. IR (KBr, cm^{-1}): 3411–3255 (2NH), 2183 (C≡N), 1645 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3 of *p*-tolyl moiety), 3.99 (s, 2H, CH_2), 7.05–7.10 (m, 2H, ArH), 7.37–7.44 (m, 3H, ArH), 7.52–7.54 (m, 2H, ArH), 7.64 (d, J=2.7 Hz, 2H, ArH), 8.80 (s, 1H, NH, D_2O exchangeable), 10.16 (s, 1H, NH–CO, D_2O exchangeable); MS *m/z* (%): 349 [M+1$^{+\cdot}$, 2.54], 348 [M$^{+\cdot}$, 8.23], 77 [$C_6H_5^{+\cdot}$, 100].

(2-Cyano-1-phenylamino-2-phenylcarbamoyl-vinylsulfanyl)-acetic Acid Ethyl Ester (**2c**): Yield, 0.08 g (84%); mp 202–204°C; *Anal.* Calcd for $C_{20}H_{19}N_3O_3S$ (381.45): C, 62.97; H, 5.02; N, 11.02; Found: C, 63.26; H, 5.08; N, 11.33. IR (KBr, cm^{-1}): 3442–3345 (2NH), 2200 (C≡N), 1728–1656 (2C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.13–1.18 (t, J=6.9 Hz, 3H, –OCH$_2$C<u>H</u>$_3$), 3.64 (s, 2H, CH_2), 4.00–4.08 (q, J=6.9 Hz, 2H, –OC<u>H</u>$_2$CH$_3$), 7.04–7.09 (m, 2H, ArH), 7.18–7.23 (m, 1H, ArH), 7.26–7.46 (m, 3H, ArH), 7.51 (d, J=7.8 Hz, 2H, ArH), 7.55 (d, J=7.8 Hz, 2H, ArH), 9.58 (s, 1H, NH, D_2O exchangeable), 11.34 (s, 1H, NH, D_2O exchangeable); MS *m/z* (%): 382 [M+1$^{+\cdot}$, 13.90], 381 [M$^{+\cdot}$, 28.60], 77 [100].

(2-Cyano-1-phenylamino-2-*p*-tolyl carbamoyl-vinylsulfanyl)-acetic Acid Ethyl Ester (**2d**): Yield, 0.07 g (71%); mp 200–202°C; *Anal.* Calcd for $C_{21}H_{21}N_3O_3S$ (395.47): C, 63.78; H, 5.35; N, 10.63; Found: C, 63.91; H, 5.49; N, 10.85; IR (KBr, cm^{-1}): 3412–3340 (2NH), 2192 (C≡N), 1745–1654 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.13–1.17 (t, J=7.2 Hz, 3H, –OCH$_2$C<u>H</u>$_3$) 2.24 (s, 3H, CH_3 of *p*-tolyl moiety), 3.62 (s, 2H, CH_2), 3.99–4.07 (q, J=7.2 Hz, 2H, –OC<u>H</u>$_2$CH$_3$), 7.05–7.19 (m, 3H, ArH), 7.28–7.41 (m, 4H, ArH), 7.49–7.52 (t, J=8.4 Hz, 2H, ArH), 9.50 (s, 1H, NH, D_2O exchangeable), 11.42 (s, 1H, NH, D_2O exchangeable); MS *m/z* (%): 396 [M+1$^{+\cdot}$, 8.40], 395 [M$^{+\cdot}$, 19.50], 215 [100].

4-Amino-*N*-aryl-5-substituted-2-phenylaminothiophene-3-carboxamides (3a–d) General Procedure. Method A: To a well stirred sodium ethoxide solution (prepared from sodium (0.46 g, 0.02 mol) in 20 mL absolute ethanol), *N*-aryl-2-cyano-3-phenylamino-3-(substituted methylthio)-acrylamides **2a**–**d** (0.005 mol) was added. The reaction mixture was heated under reflux for half an hour and set aside to cool at room temperature. The separated solid was filtered, washed with ethanol and crystallized from ethanol to give **3a**–**d**.

Method B: An ethanolic solution of sodium ethoxide (prepared from sodium (2.3 g, 0.1 mol) in 40 mL absolute ethanol) was added dropwise to a well stirred solution of *N*-aryl-2-cyanoacetamides **1a** or **1b** (0.1 mol) in absolute ethanol (20 mL). After complete addition, the reaction mixture was stirred at room temperature for 2 h. Phenyl isothiocyanate (13.5 g, 0.1 mol) was added and the mixture was heated on a water-

bath at 70°C for half an hour then cooled to 25°C. Chloroacetonitrile (7.55 g, 0.1 mol) was then added, and the temperature was raised to 60°C for half an hour more, followed by addition of an ethanolic solution of sodium ethoxide (prepared from sodium (1.15 g, 0.05 mol) in 10 mL absolute ethanol) and heating was continued for additional 3 h. After cooling, water was added and the separated product was filtered and crystallized from ethanol to afford **3a**, **b**.

4-Amino-5-cyano-*N*-*p*-tolyl-2-phenylaminothiophene-3-carboxamide (**3b**): Yield, 26.20 g (75%); mp 280–282°C; *Anal.* Calcd for $C_{19}H_{16}N_4OS$ (348.42): C, 65.50; H, 4.63; N, 16.08; Found: C, 65.61; H, 4.42; N, 16.46. IR (KBr, cm^{-1}): 3356–3224 (2NH, NH_2), 2186 (C≡N), 1644 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3 of *p*-tolyl moiety), 6.41 (s, 2H, NH_2, D_2O exchangeable), 7.06–7.11 (m, 3H, ArH), 7.26–7.37 (m, 4H, ArH), 7.47 (d, J=8.1 Hz, 2H, ArH), 9.66 (s, 1H, NH, D_2O exchangeable), 9.72 (s, 1H, NH–CO, D_2O exchangeable); MS *m*/*z* (%): 349 [M+1]$^{+\cdot}$, 20.50], 348 [M]$^{+\cdot}$, 82.60], 107 [$C_7H_9N^{+\cdot}$, 100].

3-Amino-5-phenylamino-4-phenyl Carbamoyl Thiophene-2-carboxylic Acid Ethyl Ester (**3c**): Yield, 24.22 g (63%); mp 245–247°C; *Anal.* Calcd for $C_{20}H_{19}N_3O_3S$ (381.45): C, 62.97; H, 5.02; N, 11.02; Found: C, 62.87; H, 5.39; N, 11.24. IR (KBr, cm^{-1}): 3356–3316 (2NH, NH_2), 1663–1638 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.19–1.23 (t, J=6.9 Hz, 3H, –OCH$_2$C<u>H</u>$_3$), 4.11–4.18 (q, J=6.9 Hz, 2H, –OC<u>H</u>$_2$CH$_3$), 6.68 (s, 2H, NH_2, D_2O exchangeable), 7.03–7.10 (m, 3H, ArH), 7.28–7.39 (m, 5H, ArH), 7.62 (d, J=7.5 Hz, 2H, ArH), 9.69 (s, 1H, NH, D_2O exchangeable), 9.72 (s, 1H, NH–CO, D_2O exchangeable); MS *m*/*z* (%): 382 [M+1]$^{+\cdot}$, 18.60], 381 [M]$^{+\cdot}$, 67.70], 242 [100].

3-Amino-5-phenylamino-4-*p*-tolyl Carbamoyl Thiophene-2-carboxylic Acid Ethyl Ester (**3d**): Yield, 27.42 g (61%); mp 258–260°C; *Anal.* Calcd for $C_{21}H_{21}N_3O_3S$ (395.47): C, 63.78; H, 5.35; N, 10.63; Found: C, 63.90; H, 5.00; N, 10.51. IR (KBr, cm^{-1}): 3340–3262 (2NH, NH_2), 1667–1625 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.19–1.23 (t, J=6.9 Hz, 3H, –OCH$_2$C<u>H</u>$_3$), 2.26 (s, 3H, CH_3 of *p*-tolyl moiety), 4.11–4.18 (q, J=7.2 Hz, 2H, –OC<u>H</u>$_2$CH$_3$), 6.68 (s, 2H, NH_2, D_2O exchangeable), 7.09–7.12 (m, 3H, ArH), 7.30–7.39 (m, 4H, ArH), 7.50 (d, J=8.4 Hz, 2H, $H_{2,6}$), 9.63 (s, 1H, NH, D_2O exchangeable), 9.69 (s, 1H, NH–CO, D_2O exchangeable); MS *m*/*z* (%): 397 [M+2]$^{+\cdot}$, 11.50], 396 [M+1]$^{+\cdot}$, 16.20], 395 [M]$^{+\cdot}$, 82.00], 242 [100].

4-Amino-*N*-aryl-6-phenylaminothieno[3,2-*d*]pyrimidine-7-carboxamides (4a, b) General Procedure: A mixture of 4-amino-*N*-aryl-5-cyano-2-phenylamino-thiophene-3-carboxamides **3a** or **3b** (0.1 mol) and formamide (30 mL) was heated under reflux for 2 h in an oil bath at 160°C. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (*ca.* 80 mL) and the formed precipitate was collected by filtration, washed with water, dried and crystallized from the proper solvent to give **4a**, **b**.

4-Amino-*N*-phenyl-6-phenylaminothieno[3,2-*d*]pyrimidine-7-carboxamide (**4a**): Yield, 23.50 g (65%); mp 297–299°C; *Anal.* Calcd for $C_{19}H_{15}N_5OS$ (361.42): C, 63.14; H, 4.18; N, 19.38; Found: C, 63.00; H, 4.00; N, 19.01. IR (KBr, cm^{-1}): 3450–3250 (2NH, NH_2), 1687 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 7.08–7.12 (m, 3H, ArH), 7.21–7.35 (m, 5H, ArH+NH_2, D_2O exchangeable), 7.37–7.51 (m, 2H, ArH), 7.72 (d, J=8.4 Hz, 2H, ArH), 8.43 (s, 1H, C2-H), 11.38 (s, 1H, NH, D_2O exchangeable), 12.08 (s, 1H, NHCO, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 116.3 (s), 119.2 (s), 122.5 (s), 123.5 (s), 125.6 (s), 128.9 (s), 129.1 (s), 134.4 (s), 137.3 (s), 140.2 (s), 145.8 (s), 154.2 (s), 160.2 (s), 163.2 (s), 166.8 (s).

4-Amino-6-phenylamino-*N*-*p*-tolyl Thieno[3,2-*d*]pyrimidine-7-carboxamide (**4b**): Yield, 22.50 g (60%); mp 280–282°C; *Anal,* Calcd for $C_{20}H_{17}N_5OS$ (375.45): C, 63.98; H, 4.56; N, 18.65; Found: C, 63.90; H, 4.40; N, 18.41. IR (KBr, cm^{-1}): 3422–3220 (2NH, NH_2), 1644 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3 of *p*-tolyl moiety), 7.17–7.20 (m, 3H, ArH), 7.26–7.33 (m, 4H, ArH+NH_2, D_2O exchangeable), 7.49–7.51 (m, 2H, ArH), 7.61 (d, J=8.4 Hz, 2H, ArH), 8.45 (s, 1H, C2-H), 11.40 (s, 1H, NH, D_2O exchangeable), 11.95 (s, 1H, NHCO, D_2O exchangeable); MS *m*/*z* (%): 376 [M+1]$^{+\cdot}$, 9.89], 375 [M]$^{+\cdot}$, 21.00], 107 [100].

***N*-Aryl-4-oxo-6-phenylamino-3,4-dihydrothieno[3,2-*d*]-pyrimidine-7-carboxamides (5a, b)** General Procedure: A suspension of ethyl 3-amino-4-arylcarbamoyl-5-phenylamino-thiophene-2-carboxylates **3c** or **3d** (0.01 mol) and formamide (30 mL) was heated under reflux for 8 h in an oil bath at 160–180°C. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (*ca.* 80 mL) and the formed precipitate was collected by filtration, washed with water, dried and crystallized from chloroform to afford **5a**, **b**.

4-Oxo-*N*-phenyl-6-phenylamino-3,4-dihydrothieno[3,2-*d*]-pyrimidine-7-carboxamide (**5a**): Yield, 2.16 g (60%); mp 257–259°C; *Anal.* Calcd for $C_{19}H_{14}N_4O_2S$ (362.41): C, 62.97; H, 3.89; N, 15.46; Found: C, 63.02; H, 4.19; N, 15.26. IR (KBr, cm^{-1}): 3438–3232 (2NH), 1642 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 7.08–7.13 (m, 2H, ArH), 7.21–7.26 (m, 1H, ArH), 7.35–7.39 (m, 2H, ArH), 7.49–7.51 (m, 2H, ArH), 7.71 (d, J=8.7 Hz, 2H, ArH), 8.39 (s, 1H, C2-H), 11.43 (s, 1H, NH, D_2O exchangeable), 11.53 (s, 1H, NH–CO, D_2O exchangeable), 12.69 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 117.3 (s), 120.9 (s), 123.2 (s), 126.8 (s), 128.0 (s), 129.1 (s), 130.5 (s), 137.3 (s), 140.2 (s), 144.5 (s), 147.1 (s), 155.1 (s), 160.2 (s), 161.8 (s), 165.4 (s); MS *m*/*z* (%): 363 [M+1]$^{+\cdot}$, 10.82], 362 [M]$^{+\cdot}$, 46.73], 269 [100].

4-Oxo-6-phenylamino-*N*-*p*-tolyl-3,4-dihydrothieno[3,2-*d*]-pyrimidine-7-carboxamide (**5b**): Yield, 2.45 g (65%); mp 262–264°C; *Anal.* Calcd for $C_{20}H_{16}N_4O_2S$ (376.43): C, 63.81; H, 4.28; N, 14.88; Found: C, 64.09; H, 4.31; N, 14.61. IR (KBr, cm^{-1}): 3436–3236 (2NH), 1639 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3 of *p*-tolyl moiety), 7.18–7.24 (m, 3H, ArH), 7.48–7.50 (m, 4H, ArH), 7.58 (d, J=8.7 Hz, 2H, ArH), 8.36 (s, 1H, C2-H), 11.43 (s, 1H, NH, D_2O exchangeable), 11.47 (s, 1H, NH–CO, D_2O exchangeable), 12.61 (s, 1H, NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 14.2 (q, CH_3), 116.3 (s), 119.5 (s), 123.2 (s), 126.1 (s), 127.9 (s), 128.3 (s), 130.9 (s), 138.4 (s), 140.9 (s), 144.1 (s), 147.6 (s), 156.6 (s), 161.0 (s), 162.1 (s), 167.9 (s).

***N*-Aryl-4-oxo-3-substituted-6-phenylaminothieno[3,2-*d*]-pyrimidine-7-carboxamides (6a–g)** General Procedure: To a solution of potassium hydroxide (1.12 g, 0.02 mol) in absolute ethanol (20 mL), *N*-aryl-4-oxo-6-phenylamino-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamides **5a** or **5b** (0.001 mol) was added and the mixture was left on stirring at room temperature for 2 h. The appropriate alkyl halide (0.02 mol) was then added and the reaction mixture was heated under reflux for 20 h. After cooling to room temperature, the reaction mixture was poured onto crushed ice (20 g). The precipitated solid was filtered, washed with water, dried and crystallized from the proper solvent to yield **6a**–**g**.

3-Ethyl-4-oxo-6-phenylamino-*N*-*p*-tolyl-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6a**): Yield, 0.23 g (57%); mp 270–272°C; *Anal.* Calcd for $C_{22}H_{20}N_4O_2S$ (404.48): C, 65.33; H, 4.98; N, 13.85; Found: C, 65.29; H, 4.90; N, 14.10. IR (KBr, cm^{-1}): 3395–3264 (2NH), 1648 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.28–1.32 (t, J=6.3 Hz, 3H, $-OCH_2\underline{CH_3}$), 2.28 (s, 3H, CH_3 of *p*-tolyl moiety), 4.01–4.03 (q, J=6.3 Hz, 2H, $-O\underline{CH_2}CH_3$), 7.16–7.23 (m, 5H, ArH), 7.46–7.51 (m, 2H, ArH), 7.55 (d, J=6.9 Hz, 2H, ArH), 8.65 (s, 1H, C2-H), 11.23 (s, 1H, NH, D_2O exchangeable), 11.44 (s, 1H, NH–CO, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 13.4 (q, CH_3), 20.3 (q, CH_3), 36.3 (t, CH_2), 115.9 (s), 119.3 (s), 122.9 (s), 126.5 (s), 126.9 (s), 129.3 (s), 131.2 (s), 138.2 (s), 140.6 (s), 143.2 (s), 147.4 (s), 156.5 (s), 160.9 (s), 161.7 (s), 166.4 (s); MS *m*/*z* (%): 405 [M+1]$^{+\cdot}$, 5.73], 404 [M]$^{+\cdot}$, 19.90], 107 [100].

4-Oxo-*N*-phenyl-6-phenylamino-3-phenylcarbamoylmethyl-3,4-dihydro-thieno[3,2-*d*]pyrimidine-7-carboxamide (**6b**): Yield, 0.38 g (78%); mp 354–356°C; *Anal.* Calcd for $C_{27}H_{21}N_5O_3S$ (495.55): C, 65.44; H, 4.27; N, 14.13; Found: C, 65.22; H, 4.42; N, 13.77. IR (KBr, cm^{-1}): 3421–3276 (3NH), 1670–1647 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 4.93 (s, 2H, CH_2), 7.05–7.16 (m, 3H, ArH), 7.24–7.42 (m, 6H, ArH), 7.50 (d, J=4.2 Hz, 2H, ArH), 7.59 (d, J=8.1 Hz, 2H, ArH), 7.73 (d, J=7.8 Hz, 2H, ArH), 8.65 (s, 1H, C2-H), 10.46 (s, 1H, NH, D_2O exchangeable), 11.35 (s, 1H, NH–CO, D_2O exchangeable), 11.46 (s, 1H, NH–CO, D_2O exchangeable); MS *m*/*z* (%): 496 [M+1]$^{+\cdot}$, 14.66], 495 [M]$^{+\cdot}$, 46.17], 269 [100].

4-Oxo-*N*-phenyl-6-phenylamino-3-(*p*-tolylcarbamoylmethyl)-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6c**): Yield, 0.32 g (63%); mp >300°C; *Anal.* Calcd for $C_{28}H_{23}N_5O_3S$ (509.58): C, 66.00; H, 4.55; N, 13.74; Found: C, 66.03; H, 4.26; N, 13.67. IR (KBr, cm^{-1}): 3408–3281 (3NH), 1665–1648 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3 of *p*-tolyl moiety), 4.90 (s, 2H, CH_2), 7.10–7.15 (m, 3H, ArH), 7.23–7.26 (m, 1H, ArH), 7.36–7.49 (m, 8H, ArH), 7.72 (d, J=7.8 Hz, 2H, ArH), 8.63 (s, 1H, C2-H), 10.36 (s, 1H, NH, D_2O exchangeable), 11.34 (s, 1H, NH–CO, D_2O exchangeable), 11.46 (s, 1H, NH–CO); ^{13}C-NMR (DMSO-d_6) δ: 20.9 (q, CH_3), 52.3 (t, CH_2), 115.9 (s), 118.1 (s), 119.4 (s), 121.9 (s), 125.4 (s), 126.6 (s), 127.0 (s), 129.6 (s), 131.4 (s), 132.2 (s), 139.1 (s), 141.3 (s), 143.5 (s), 147.8 (s), 155.4 (s), 156.9 (s), 161.2 (s), 162.3 (s), 166.1 (s), 167.8 (s); MS *m*/*z* (%): 509 [M]$^{+\cdot}$, 42.20], 310 [100].

4-Oxo-6-phenylamino-3-phenylcarbamoylmethyl-*N*-*p*-tolyl-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6d**): Yield, 0.37 g (73%); mp 298–300°C; *Anal.* Calcd for $C_{28}H_{23}N_5O_3S$ (509.58): C, 66.00; H, 4.55; N, 13.74; Found: C, 65.98; H, 4.65; N, 13.51. IR (KBr, cm^{-1}): 3436–3279 (3NH), 1670–1645 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3 of *p*-tolyl moiety), 4.93 (s, 2H, CH_2), 7.07–7.10 (t, J=7.8 Hz, 2H, ArH), 7.19–7.35 (m, 6H, ArH), 7.49–7.51 (m, 2H, ArH), 7.57–7.63 (m, 4H, ArH), 8.65 (s, 1H, C2-H), 10.45 (s, 1H, NH, D_2O exchangeable), 11.28 (s, 1H, NH–CO, D_2O exchangeable), 11.50 (s, 1H, NH–CO, D_2O exchangeable).

3-(4-Hydroxyphenylcarbamoylmethyl)-4-oxo-*N*-phenyl-6-phenylamino-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6e**): Yield, 0.36 g (70%); mp 337–9°C; *Anal.* Calcd for $C_{27}H_{21}N_5O_4S$ (511.55): C, 63.39; H, 4.14; N, 13.69; Found: C, 63.51; H, 4.16; N, 13.38. IR (KBr, cm^{-1}): 3510 (OH), 3399–3279 (3NH), 1660–1643 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 4.86 (s, 2H, CH_2), 6.69 (d, J=7.2 Hz, 2H, ArH), 7.10–7.25 (m, 4H, ArH), 7.34–7.48 (m, 6H, ArH), 7.71 (d, J=7.2 Hz, 2H, ArH), 8.62 (s, 1H, C2-H), 9.21 (s, 1H, NH, D_2O exchangeable), 10.18 (s, 1H, NH–CO, D_2O exchangeable), 11.34 (s, 1H, NH–CO, D_2O exchangeable), 11.45 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 50.6 (t, CH_2), 114.3 (s), 117.5 (s), 120.3 (s), 123.1 (s), 125.1 (s), 126.9 (s), 127.3 (s), 130.1 (s), 131.1 (s), 131.9 (s), 138.3 (s), 140.6 (s), 143.2 (s), 147.1 (s), 155.8 (s), 156.2 (s), 160.9 (s), 161.7 (s), 165.2 (s), 167.1 (s).

4-Oxo-6-phenylamino-*N*-*p*-tolyl-3-(*p*-tolylcarbamoylmethyl)-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6f**): Yield, 0.38 g (73%); mp >300°C; *Anal.* Calcd for $C_{29}H_{25}N_5O_3S$ (523.61): C, 66.52; H, 4.81; N, 13.38; Found: C, 66.29; H, 5.06; N, 13.14. IR (KBr, cm^{-1}): 3453–3200 (3NH), 1665–1644 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3 of *p*-tolyl moiety), 2.28 (s, 3H, CH_3 of *p*-tolyl moiety), 4.89 (s, 2H, CH_2), 7.10–7.26 (m, 5H, ArH), 7.45–7.48 (m, 4H, ArH), 7.59 (d, J=8.1 Hz, 4H, ArH), 8.61 (s, 1H, C2-H), 10.36 (s, 1H, NH), 11.24 (s, 1H, NH), 11.50 (s, 1H, NH).

3-(4-Hydroxyphenylcarbamoylmethyl)-4-oxo-6-phenylamino-*N*-*p*-tolyl-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (**6g**): Yield, 0.39 g (74%); mp 298–300°C; *Anal.* Calcd for $C_{28}H_{23}N_5O_4S$ (525.58): C, 63.99; H, 4.41; N, 13.33; Found: C, 64.37; H, 4.48; N, 13.33; IR (KBr, cm^{-1}): 3515 (OH), 3401–3270 (3NH), 1665–1640 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3 of *p*-tolyl moiety), 4.87 (s, 2H, CH_2), 6.70 (d, J=8.7 Hz, 2H, ArH), 7.19–7.28 (m, 5H, ArH), 7.36 (d, J=8.7 Hz, 2H, ArH), 7.47–7.51 (m, 2H, ArH), 7.62 (d, J=8.7 Hz, 2H, ArH), 8.63 (s, 1H, C2-H), 9.22 (s, 1H, NH, D_2O exchangeable), 10.19 (s, 1H, NH–CO, D_2O exchangeable), 11.28 (s, 1H, NH-CO, D_2O exchangeable), 11.49 (s, 1H, OH, D_2O exchangeable); MS *m*/*z* (%): 525 [M]$^{+\cdot}$, 16.97] and 283 [100].

***N*-Aryl-4-chloro-6-phenylaminothieno[3,2-*d*]pyrimidine-7-carboxamides (7a, b)** General Procedure: A mixture of phosphorus oxychloride (15 mL) and *N*-aryl-4-oxo-6-phenylamino-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamides **5a** or **5b** (0.001 mol) and was heated under reflux in an oil bath at 120°C for 8 h. Excess phosphorus oxychloride was distilled off under vacuum and the residual syrup was poured while stirring over crushed ice. The formed precipitate was collected by filtration, washed with water, dried and crystallized from benzene to afford **7a**, **b**.

4-Chloro-*N*-phenyl-6-phenylaminothieno[3,2-*d*]pyrimidine-7-carboxamide (**7a**): Yield, 0.26 g (68%); mp 182–184°C; *Anal.* Calcd for $C_{19}H_{13}ClN_4OS$ (380.85): C, 59.92; H, 3.44; N, 14.71; Found: C, 60.02; H, 3.70; N, 15.00. IR (KBr, cm^{-1}): 3413–3249 (2NH), 1661 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 7.23–7.34 (m, 2H, ArH), 7.36–7.47 (m, 2H, ArH), 7.62 (d, J=8.4 Hz, 2H, ArH), 7.74–7.79 (m, 2H, ArH), 7.95 (d, J=8.4 Hz, 2H, ArH), 9.11 (s, 1H, C2-H), 10.42 (s, 2H, 2NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 117.3 (s), 120.9 (s), 122.5 (s), 123.2 (s), 126.5 (s), 127.9 (s), 129.1 (s), 134.4 (s), 137.3 (s), 140. 2(s), 145.8 (s), 154.2 (s), 160.2 (s), 163.2 (s), 166.2 (s); MS *m*/*z* (%): 380 [M]$^{+\cdot}$, 9.52], 362 [100].

4-Chloro-6-phenylamino-*N*-*p*-tolylthieno[3,2-*d*]pyrimidine-7-carboxamide (**7b**): Yield, 0.23 g (59%); mp 160–162°C; *Anal.* Calcd for $C_{20}H_{15}ClN_4OS$ (394.88): C, 60.83; H, 3.83; N, 14.19; Found: C, 60.74; H, 4.09; N, 14.07. IR (KBr, cm^{-1}): 3420–3245 (2NH), 1680 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3 of *p*-tolyl moiety), 7.22–7.27 (m, 3H, ArH), 7.60 (m, 2H, ArH), 7.75 (m, 2H, ArH), 7.94 (d, J=8.4 Hz, 2H, ArH), 9.10 (s, 1H, C2-H), 10.43 (s, 2H, 2NH, D_2O exchangeable); ^{13}C-NMR

Table 4. The Surviving Fraction of MCF7 Cells for 4 Concentrations of Each Compound (μg/mL) and Their IC_{50} (μg/mL)

Cpd. No.	The surviving fraction at each concentration (μg/mL)				IC_{50} (μg/mL)
	1	2.5	5	10	
DOX	0.360±0.014	0.317±0.026	0.343±0.018	0.370±0.033	4.40±0.09
4a	0.210±0.012	0.155±0.011	0.095±0.018	0.142±0.022	0.74±0.10
4b	0.638±0.042	0.465±0.021	0.243±0.011	0.165±0.016	1.81±0.07
5a	0.523±0.012	0.423±0.031	0.187±0.023	0.143±0.019	1.47±0.04
5b	0.910±0.015	0.685±0.045	0.504±0.030	0.356±0.031	3.91±0.11
6a	0.472±0.020	0.419±0.019	0.361±0.022	0.380±0.023	4.21±0.13
6b	0.572±0.009	0.373±0.012	0.410±0.014	0.259±0.021	7.62±0.12
6c	0.672±0.031	0.470±0.032	0.438±0.030	0.471±0.032	10.26±0.21
6d	0.735±0.012	0.360±0.020	0.314±0.013	0.295±0.021	11.59±0.22
6e	0.523±0.013	0.387±0.011	0.313±0.020	0.324±0.015	5.18±0.06
6f	0.734±0.015	0.626±0.019	0.307±0.023	0.272±0.024	18.51±0.22
6g	0.521±0.022	0.360±0.010	0.346±0.030	0.453±0.031	7.05±0.10
7a	0.309±0.021	0.229±0.014	0.250±0.016	0.339±0.033	3.58±0.08
7b	0.455±0.017	0.467±0.020	0.193±0.012	0.273±0.042	4.08±0.10
8a	0.694±0.018	0.326±0.015	0.233±0.025	0.268±0.031	1.60±0.07
8b	0.531±0.012	0.350±0.030	0.346±0.017	0.453±0.013	2.35±0.08
9a	0.815±0.013	0.405±0.033	0.224±0.011	0.123±0.030	2.29±0.10
9b	0.307±0.027	0.226±0.017	0.240±0.020	0.339±0.018	2.82±0.12

(DMSO-d_6) δ: 20.4 (q, CH_3), 115.9 (s), 120.3 (s), 123.1 (s), 124.2 (s), 126.2 (s), 128.1 (s), 129.3 (s), 134.3 (s), 136.3 (s), 141.1 (s), 146.8 (s), 155.1 (s), 161.4 (s), 163.2 (s), 166.4 (s).

***N*-Aryl-4-(2-carboxy-phenylamino)-6-phenylaminothieno-[3,2-*d*]pyrimidine-7-carboxamides (8a, b)** General Procedure: A mixture of the respective *N*-aryl-4-chloro-6-phenylamino-thieno[3,2-*d*]pyrimidine-7-carboxamides **7a** or **7b** (0.01 mol), anthranilic acid (1.4 g, 0.01 mol) and anhydrous potassium carbonate (1.4 g, 0.01 mol) in DMF (15 mL) was heated under reflux for 24 h. After cooling at room temperature, the reaction mixture was poured onto crushed ice (20 g). The precipitated solid was filtered, washed with water, dried and crystallized from the proper solvent to yield **8a**, **b**.

4-(2-Carboxy-phenylamino)-*N*-phenyl-6-phenylaminothieno-[3,2-*d*]pyrimidine-7-carboxamide (**8a**): Yield, 2.91 g (61%); mp >300°C; *Anal.* Calcd for $C_{26}H_{19}N_5O_3S$ (481.53): C, 64.85; H, 3.98; N, 14.54; Found: C, 64.59; H, 3.63; N, 14.37. IR (KBr, cm^{-1}): 3545–3260 (OH, 3NH), 1679–1629 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 7.16–7.42 (m, 4H, ArH), 7.54–7.74 (m, 6H, ArH), 7.93–7.98 (m, 4H, ArH), 8.45 (s, 2H, C2-H, NH, D_2O exchangeable), 10.23 (s, 2H, 2NH, D_2O exchangeable), 13.09 (s, 1H, OH, D_2O exchangeable); MS *m/z*: 481 [$M^{+\cdot}$, 10.97], 344 [100].

4-(2-Carboxy-phenylamino)-6-phenylamino-*N*-*p*-tolylthieno-[3,2-*d*]pyrimidine-7-carboxamide (**8b**): Yield, 2.57 g (52%); mp 280–282°C; *Anal.* Calcd for $C_{27}H_{21}N_5O_3S$ (495.55): C, 65.44; H, 4.27; N, 14.13; Found: C, 65.18; H, 4.15; N, 13.97; IR (KBr, cm^{-1}): 3545 3260 (OH, 3NH), 1680 1634 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3 of *p*-tolyl moiety), 7.05 (d, *J*=8.4 Hz, 2H, ArH), 7.14 7.20 (m, 3H, ArH), 7.55 7.65 (m, 6H, ArH), 7.89 (d, *J*=8.1 Hz, 2H, ArH), 8.20 (s, 2H, C2-H+ NH, D_2O exchangeable), 8.53 (s, 1H, NH, D_2O exchangeable), 10.80 (s, 1H, NH, D_2O exchangeable), 13.09 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 23.4 (q, CH_3), 117.3 (s), 119.1 (s), 120.2 (s), 120.9 (s), 122.5 (s), 123.2 (s), 126.5 (s), 127.9 (s), 129.1 (s), 132.4 (s), 135.3 (s), 137.3 (s), 134.4 (s), 140.2 (s), 145.3 (s), 145.8 (s), 154.2 (s), 160.2 (s), 163.2 (s), 167.2 (s).

2-Phenylamino-3-*N*-arylcarbamoylthieno[3′,2′-4,5]-pyrimido[3,4-*b*]quinazolin-7-ones (9a, b) General Procedure: A mixture of *N*-aryl-4-(2-carboxy-phenylamino)-6-phenylamino-thieno[3,2-*d*]pyrimidine-7-carboxamides **8a** or **8b** (0.01 mol) and excess thionyl chloride (10 mL) was heated under reflux for 4 h. Distillation of excess thionyl chloride under vacuum afforded the target compounds **9a**, **b** which were crystallized from the appropriate solvent.

2-Phenylamino-3-*N*-phenylcarbamoylthieno[3′,2′-4,5]-pyrimido[3,4-*b*]quinazolin-7-one (**9a**): Yield, 2.50 g (54%); mp >300°C; *Anal.* Calcd for $C_{26}H_{17}N_5O_2S$ (463.51): C, 67.37; H, 3.70, N, 15.11; Found: C, 67.20; H, 3.80; N, 14.86; IR (KBr, cm^{-1}): 3413–3300 (2NH), 1650–1636 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 7.14–7.21 (m, 4H, ArH), 7.32–7.37 (m, 2H, ArH), 7.60–7.68 (m, 5H, ArH), 7.91–7.94 (m, 3H, ArH), 8.26 (s, 1H, C5-H), 10.74 (s, 2H, 2NH, D_2O exchangeable).

2-Phenylamino-3-*N*-*p*-tolylcarbamoylthieno[3′,2′-4,5]-pyrimido[3,4-*b*]quinazolin-7-one (**9b**): Yield, 2.32 g (49%); mp >300°C; *Anal.* Calcd for $C_{27}H_{19}N_5O_2S$ (477.54): C, 67.91; H, 4.01; N, 14.67; Found: C, 68.20; H, 4.28; N, 14.73; IR (KBr, cm^{-1}): 3432–3224 (2NH), 1660–1640 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3 of *p*-tolyl moiety), 7.04–7.20 (m, 5H, ArH), 7.59–7.65 (m, 4H, ArH), 7.89 (d, *J*=8.7 Hz, 4H, ArH), 8.22 (s, 1H, C5-H), 10.79 (s, 2H, 2NH, D_2O exchangeable); MS *m/z*: 478 [$M+1^{+\cdot}$, 1.71], 477 [$M^{+\cdot}$, 5.20], 442 [100].

Biological Evaluation

Materials and Methods The human breast adenocarcinoma cell line (MCF7) was obtained as a gift from NCI, MD, U.S.A. All chemicals and solvents were purchased from Sigma-Aldrich. The cytotoxicity calculated using transformed curves (Graph pad prism software, version 5.01, 2007, GPW5-739310-RAG-3437).

Measurement of Potential Cytotoxicity The cytotoxic activity of the newly synthesized compounds was measured *in vitro* on MCF7 using Sulforhodamine-B stain (SRB) assay applying the method of Skehan *et al.*[48] Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. Test compounds were dissolved in

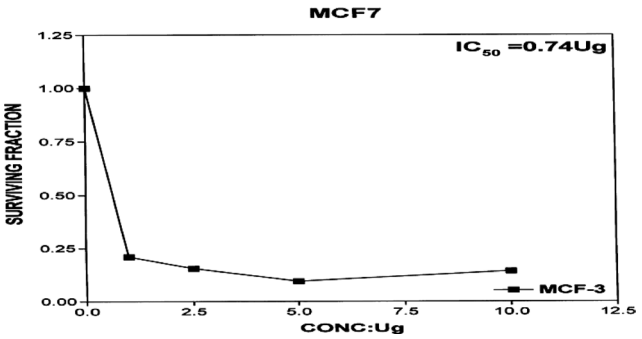


Fig. 9. Survival Curve of MCF7 Cells at 4 Concentrations (μg/mL) for 4-Aminothienopyrimidine **4a**

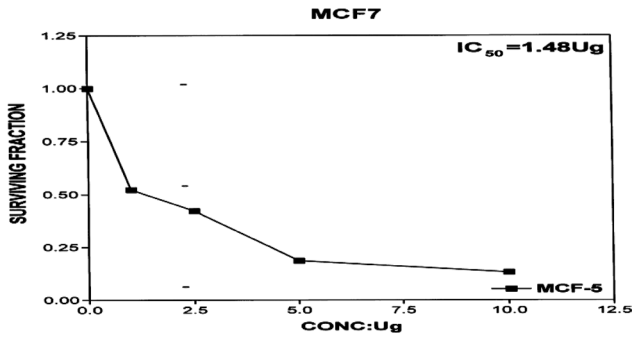


Fig. 10. Survival Curve of MCF7 Cells at 4 Concentrations (μg/mL) for Thienopyrimidine-4-ones **5a**

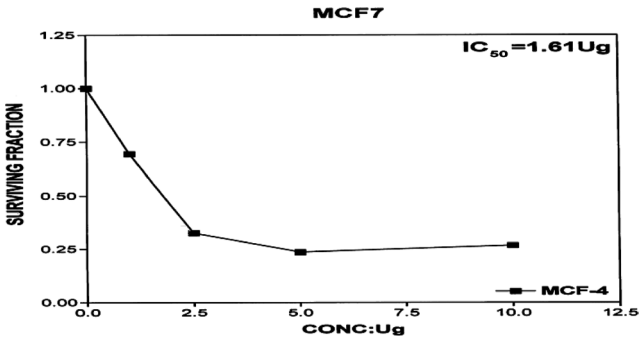


Fig. 11. Survival Curve of MCF7 Cells at 4 Concentrations (μg/mL) for 4-Substituted Phenylaminothienopyrimidine **8a**

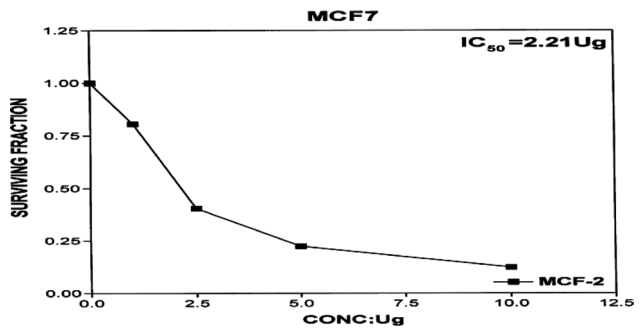


Fig. 12. Survival Curve of MCF7 Cells at 4 Concentrations (μg/mL) for Thienopyrimidoquinazolinone **9a**

dimethyl sulfoxide (DMSO) and diluted with saline to the appropriate volume. Different concentrations of the test compound (0, 2.5, 5, 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37°C in atmosphere of 5% CO_2. After 48 h, cells were fixed with trichloroacetic acid, washed with water and stained for 30 min with 0.4% (w/v) Sulforhodamine-B stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris ethylenediamine tetraacetic acid (EDTA) buffer colour intensity which was measured in enzyme-linked immunosorbent assay (ELISA) reader. The percentage of cell survival was calculated as follows: survival fraction=optical density (OD) (treated cells)/OD (control cells) and the results are showed in (Table 4).

The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell viability was calculated. Each concentration was repeated 3 times and the mean of results are given in (Tables 1–4).

Acknowledgments We are grateful to Prof. Dr. Samia Shouman, Professor of Pharmacology and all members of the Department of Cancer Biology, National Cancer Institute, Cairo, Egypt, for carrying out the cytotoxic activity. Abd El Hameid is gratefully acknowledging OEAD, Austria for providing postdoctoral fellowship to Institute for synthetic chemistry, Vienna, University of Technology, Vienna, Austria during this work.

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