Synthesis, antitumor and antibacterial activities of some novel tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivatives

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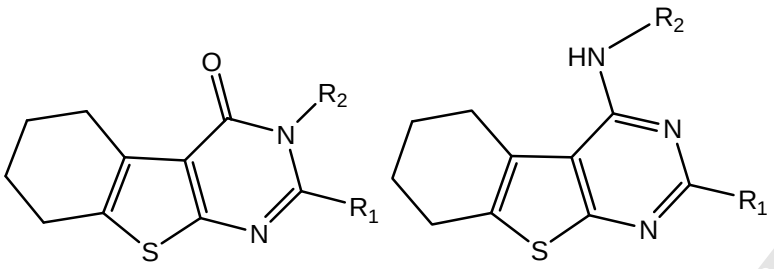
Highlights

- Two series of fused thieno[2,3-d]pyrimidines were synthesized.
- They were tested for their antitumor and antibacterial activities.
- 3-Acridinyl derivatives had potent antitumor and antibacterial activities.

Graphical abstract



3a-z **6a-c**

R_1= 3,4,5-trimethoxyphenyl, styryl**, R_2=** $CSNH_2$, $CONH_2$, CONHOH, heteroalicyclic, aryl, heteroaryl rings

The target compounds **3a-z** and **6a-c** were prepared and tested for their antitumor and antibacterial activities. Compounds **3z** and **3r** exerted the highest activity against MCF-7 and HEPG-2 cell lines, respectively.

Synthesis, antitumor and antibacterial activities of some novel tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivatives

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Abstract

Two series of new tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines namely 2,3-disubstituted derivatives **3a-z** and 2,4-disubstituted ones **6a-c** were prepared and tested for their antitumor and antibacterial activities. The structures of the prepared compounds were confirmed by spectral and elemental analyses. Compound **3z** exhibited the highest antitumor activity against breast MCF-7 with IC_{50}= 0.19 μM compared to Doxorubicin (IC_{50}= 5.46 μM), while **3r** was the most active one against liver HEPG-2 cancer cell line with IC_{50}= 1.29 μM as regard to Doxorubicin (IC_{50}= 7.36 μM). Concerning the antibacterial activity, compounds **3m** and **3z** exerted remarkable activity against the tested bacterial species compared to Ampicillin, whereas compound **6c** showed good activity against only Gram positive species.

Keywords: thieno[2,3-*d*]pyrimidines, antitumor activity, antibacterial activity, MCF-7, HEPG-2.

1. Introduction

Cancer is a major health problem worldwide. The relative mortality rate caused by cancer is still very high in the developed countries, accounting for more than 20% of all deaths. Among the various types of malignant tumors, breast cancer is the second leading cause of death in women [1,2]. In the US and other western countries, alcohol-related cirrhosis, and possibly nonalcoholic fatty liver disease associated with obesity, account for the majority of liver cancer cases. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are associated with less than half of liver cancer cases in the US, although they are the major risk factors for the disease worldwide [1]. Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited mainly due to undesirable side effects and a limited choice of available anticancer drugs [3]. This clearly underlies the urgent need for developing novel chemotherapeutic agents with more potent antitumor activities.

Many anticancer agents which act as tyrosine kinase inhibitors comprised the pyrimidine group as a core moiety. This could be exemplified by different quinazoline

derivatives such as gefitinib (Iressa^{TM}) [4] and tandutinib (MLN518) (phase II clinical trials) [5] (Fig.1). In addition, the thieno[2,3-d]pyrimidine ring, which is considered as a bioisostere of quinazoline, was used as a core for mechanism-based design and synthesis of diversity of compounds of potential antitumor activity [6-19]. Furthermore, the thienopyrimidine nucleus was adopted as a scaffold for many antimicrobial [20-26], antiviral [20, 26-28], antiinflammatory [29] antidiabetic [30], antioxidant [31] and anxiolytic agents [32]. In literature, it was documented that fusion of the thieno[2,3-d]pyrimidine core with five, six or seven-membered cycloalkyl lipophilic moieties conserved the antitumor activity [9, 14-17] as revealed by the 4-substituted-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine **I** which was described to exert high anticancer activity [15]. Moreover, Wang *et al.* [7] reported that the presence of 3,4,5-trimethoxyphenyl moiety at position 2 in compound **4a** was critical for its cytotoxic activity (Fig.1). Therefore, our research herein focused on the synthesis of two novel series including 2,3- and 2,4-disubstituted tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines hoping to obtain new derivatives with dual antitumor and antimicrobial activities. The synthetic pathway was planned in such a way to accommodate a 3,4,5-trimethoxyphenyl or styryl moiety at position 2 of the pyrimidine ring, in addition the 3- and 4- substituents were selected to incorporate well documented pharmacophoric moieties of interest such as amide, thioamide, N-hydroxyamide, aryl and heteroaryl moieties that proved to contribute to antitumor and antibacterial activities such as benzoic acid [33], benzenesulfonamide [34-36], indole [37,38], benzothiazole [39-41] and acridine [42-45]. The synthesized compounds were screened for their antitumor and antibacterial activities to explore the effect of these structural modifications on the activity aiming to obtain new candidates of remarkable activities and minimal side effects.

2- Results and discussion

2.1. Chemistry

The target compounds were prepared as outlined in Schemes 1 and 2. The starting materials ethyl-2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate **1a** and the 3-carboxamide derivative **1b** were prepared following the method of Gewald [46-48] via the reaction of cyclohexanone and sulfur with either ethyl cyanoacetate or

cyanoacetamide, respectively in the presence of diethylamine. Acylation procedure described by Amr *et al.* [32] was applied to react **1a** with either 3,4,5-trimethoxybenzoyl chloride or cinnamoyl chloride in dry benzene to afford **2a,b**, respectively (Scheme 1). ^{1}H NMR spectrum of **2a** revealed the presence of singlet signal at 7.25 ppm corresponding to the two aromatic protons of 3,4,5-trimethoxyphenyl in addition to the triplet quartet pattern at 1.38 and 4.36 ppm of the ethyl group and two singlets at 3.91 and 3.96 ppm attributed to the protons of the three methoxy groups. Similarly, ^{1}H NMR spectrum of **2b** showed signals corresponding to the aromatic protons in addition to the olefinic ones which were attributed to the styryl moiety at 6.75-7.81 ppm. Cyclization of **2a,b** into 2,3-disubstituted-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **3a-z** was adopted using the reported reaction conditions [49]. This was achieved by refluxing **2a,b** with the appropriate amines in the presence of phosphorus oxychloride and phosphorus pentoxide, the latter was added to accelerate the reaction for producing **3a-z** (Scheme 1). ^{1}H NMR spectra of **3a-z** revealed the disappearance of the triplet quartet pattern of the ethyl moiety and the presence of extra signals corresponding to the introduced groups (cf experimental section). Reaction of the 3-carboxamide derivative **1b** with 3,4,5-trimethoxybenzaldehyde or cinnamaldehyde afforded **4a** [7,8] or **4b** [50,51], respectively according to the reported procedures. Likewise, **1b** was reacted with 2-nitro cinnamaldehyde or α-methyl cinnamaldehyde in methanol and in the presence of catalytic amount of hydrochloric acid to give compounds **4c** or **4d**, respectively (Scheme 2). IR spectra of compounds **4c,d** showed bands at 3363-3383 and 1639 cm^{-1} corresponding to the NH and C=O groups, respectively. ^{1}H NMR spectrum of **4d** revealed two singlets at 3.49 and 8.26 ppm related to CH_3 and olefinic protons, respectively in addition to the other signals corresponding to the 2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine nucleus. The 4-chloro derivatives **5a,b** were obtained via the reaction of **4a,b** with phosphorus oxychloride [7,8,50]. Reaction of **5a,b** with hydroxyurea or acriflavine was carried out in the domestic microwave under solvent free condition to yield the corresponding 4-substituted derivatives **6a-c** (Scheme 2). Characterization of the newly prepared derivatives **6a-c** was verified by IR spectra that revealed the presence of broad bands at 3406-3200 and 3314-3190 cm^{-1} corresponding to two NH and OH groups in compounds **6a,b** and NH and NH_2 groups in compound **6c**, respectively. ^{1}H

NMR spectra of compounds **6a,b** showed two exchangeable signals at 6.50 and 7.80, 8.80 ppm correlated to OH and two NH protons, respectively, in addition to the usual signals of the nucleus. Additionally, the presence of a singlet signal at 4.10 ppm and two exchangeable signals at 5.40 and 9.80 ppm corresponding to CH_3, NH_2 and NH moieties, respectively along with the extra aromatic signals of the acridinyl nucleus were observed.

2.2. Antitumor activity

All the prepared target compounds **3a-z** , **4b-d** and **6a-c** were screened for their antitumor activity against breast MCF-7 and liver HEPG-2 cancer cell lines at the Pharmacology Unit of National Research Center according to Skehan *et al* method [52]. The obtained results (Table 1), revealed that the 2-styryl derivatives **3n-z** exerted more remarkable activity against both cell lines than the corresponding 3,4,5-trimethoxyphenyl derivatives **3a-m**.

Regarding the 2-(3,4,5-trimethoxy)phenyl derivatives **3a-m**, the 3-thioamide derivative **3a** exerted weak activity against MCF-7 cancer cell line that increased in the bioisostere carboxamide derivative **3b**. Introduction of a polar hydroxyl group in compound **3c** led to a decrease in the antitumor activity against MCF-7 cell line. On the other hand, compound **3c** possessed higher activity against HEPG-2 cancer cell line than the carboxamide **3b**, while the thioamide analog **3a** was inactive. In addition, it was apparent that the presence of a sulfamoyl moiety in compound **3e** produced a relatively higher activity against MCF-7 compared to the carboxylic acid derivatives **3d**, **3f** and **3g,** whereby the activity was reversed as regard to the liver cell line. Furthermore, substitution at position 3 with a five membered heterocyclic ring such as isoxazolidinone as in **3h**, showed a decrease in the antitumor activity against both cell lines. Ring expansion of the substituent at position 3 to pyrimidinedione as in **3i** led to a sharp decrease in the antitumor activity. Moreover, the antitumor activity augmented, especially against HEPG-2 cell line, by the incorporation of a benzothiazol-2-yl moiety at position 3 as in **3j**. Also, it was noticed that the presence of an electron withdrawing group at position 5 of the benzothiazol-2-yl group as in **3l** was more favorable for enhancing the antitumor activity than the electron donating one as in **3k**. Interestingly, the hybridization of the 2-(3,4,5-trimethoxy)phenyl-5,6,7,8-

tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine with acridinyl nucleus resulted in compound **3m** that exhibited a prominent increase in the antitumor activity against both cell lines (IC_{50}= 0.86, 5.50 μM compared to Doxorubicin IC_{50}= 5.46, 7.36 μM, respectively) (Table 1).

Concerning the antitumor activity of the 2-styryl derivatives **3n-z**, it was found that the 3-thioamide **3n** and its carboxamide analog **3o** were nearly equipotent showing moderate antitumor activity against both cell lines. On the other hand, the activity significantly decreased in the N-hydroxyl derivative **3p.** Furthermore, the sulfonamido derivative **3r** exhibited higher activity than the carboxylic acid candidates **3q**, **3s** and **3t** against MCF-7 and HEPG-2 as revealed from its IC_{50} values (0.43 and 1.29 μM, respectively) which were in the same time more potent than those expressed by Doxorubicin (IC_{50}=5.46 and 7.36 μM, respectively). The incorporation of isoxazolidinone ring as in **3u** revealed mild activity against MCF-7 cell line and a remarkably higher activity against HEPG-2 (IC_{50}= 6.86 μM) even higher than Doxorubicin. Derivative **3v**, carrying the larger pyrimidinedione ring, demonstrated a slight increase in the activity against the MCF-7 cell line. Additionally, it was observed that the 3-benzothiazol-2-yl derivative **3w** and its 5-nitro analog **3y** were more potent than Doxorubicin against MCF-7 as expressed by their IC_{50} values (4.30 and 1.23 μM, respectively). Interestingly, it was noticed that the 3-acridin-3-yl derivative **3z** revealed the highest activity against breast cancer cell line (IC_{50}=0.19 μM) which was considered to be the most active among all the tested compounds. Prominent activity of **3z** against the liver cell line (IC_{50}=5.82 μM) was also remarked. Considering the 3-unsubstituted-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-ones **4b-d**, they revealed moderate to weak activity against both cell lines. It was noteworthy that the incorporation of hydroxy urea moiety at position 4 of the 2-(3,4,5-trimethoxyphenyl) and 2-styryl derivatives as in **6a** and **6b**, respectively resulted in improved activity against both cell lines, especially HEPG-2 (IC_{50}= 2.32 and 3.27 μM, respectively). Finally, incorporation of the acridin-3-yl amino moiety at position 4 of the pyrimidine ring as in compound **6c** contributed to a distinctive increase in the antitumor activity against MCF-7 and HEPG-2 (IC_{50}= 0.58, 1.36 μM, respectively).

2.3. Antibacterial activity

All the target compounds **3a-z**, **4b-d** and **6a-c** were evaluated *in vitro* for their antibacterial activities at the Microanalytical Center, Faculty of Science, Cairo University according to the reported procedure [53]. They were tested against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 12600) as Gram positive strains and *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) as Gram negative strains. The obtained results were represented in Table 2, where it could be noticed that the tested compounds showed mild activity in comparison to the reference drug Ampicillin. Only compounds **3m** and **3z** containing the 3-acridin-3-yl moiety revealed broad spectrum activity comparable to that of Ampicillin. Furthermore, their MIC values against *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the broth microdilution method [54] were illustrated in Table 3. On the other hand, the 2-styryl derivatives **3q**, **3u** and **3v** were equipotent to Ampicillin against *Staphylococcus aureus.* Additionally, 3-carboxamide-2-(3,4,5-trimethoxyphenyl) derivative **3b** was selective against *Pseudomonas aeruginosa.* Moreover, the presence of acridinylamino moiety at position-4 of the 2-styryl derivative **6c** shifted the activity against only Gram positive bacteria.

3. Conclusion

A series of novel 2,3-disubstituted-4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines **3a-z** were prepared via reaction of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate **1a** with the appropriate acid chloride to obtain **2a,b.** The latter were further reacted with different amines in the presence of phosphorus oxychloride and phosphorus pentoxide to give **3a-z**. Additionally, reaction of the 3-carboxamide derivative **1b** with the appropriate aldehyde produced compounds **4a-d**. Chlorination of **4a,b** yielded **5a,b** which reacted with hydroxyurea or acriflavine to afford **6a-c**.

The target compounds **3a-z**, **4b-d** and **6a-c** were screened for their antitumor activity against MCF-7 breast and HEPG-2 liver cancer cell lines and for their antibacterial activity against G (+) and G (-) bacteria. It was concluded that the presence of the styryl moiety at position 2 was more favorable for the antitumor activity than the

3,4,5-trimethoxyphenyl group. In addition, substitution at position 3 with *p*-sulfamoylphenyl **3r**, benzothiazol-2-yl **3w**, 5-nitrobenzothiazol-2-yl **3y** or 6-amino-10-methyl-acridin-3-yl **3m**, **3z** was essential for increased activity. Compound **3z** exhibited the highest antitumor activity against MCF-7 with IC_{50}= 0.19 μM, in addition to compounds **3m**, **3r**, **3w**, **3y** and **6c** that showed higher activity than that expressed by Doxorubicin (IC_{50}= 5.46 μM). Compound **3r** was the most active one against liver HEPG-2 cancer cell line (IC_{50}= 1.29 μM), in addition to compounds **3j**, **3m**, **3u**, **3z** and **6a-c** that demonstrated distinct activity compared with Doxorubicin (IC_{50}= 7.36 μM). Furthermore, the 3-acridin-3-yl derivatives **3m** and **3z** exerted remarkable activity against the tested bacterial species indicating dual antitumor and antibacterial activities, while the 4-acridinyl amino derivative **6c** showed good activity against only the G (+) species. Finally, it could be concluded that some of the newly prepared 2,3- and 2,4-disubstituted derivatives exerted remarkable antitumor and antibacterial activities compared to the 3-unsubstituted ones.

4. Experimental

4.1. Chemistry

Melting points were recorded on Gallenkamp electric melting point apparatus. IR spectra cm^{-1} (KBr) were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer. NMR spectra were recorded on JEOL-ECA500 (^{1}H: 500, ^{13}C: 125 MHz) using TMS as an internal reference. Mass spectra (EI) were recorded on 70 eV with Kratos MS equipment and a Varian MAT311A Spectrometer. Elemental analyses were performed at the Regional center for mycology and biotechnology, Al-Azhar University, Egypt. Reactions were monitored on Merck silicagel 60 F254 aluminum sheets. TLC spots were visualized by inspection of plates under UV light (254 and 365 nm). All commercial reagents were obtained either from Aldrich, or Oxford and used without further purification.

Compounds **1a,b** [46-48], **4a** [7, 8], **4b** [50, 51], **5a** [7, 8]**, 5b** [50] were prepared according to the reported procedures

4.1.1. *General procedure for the preparation of (**2a,b**)*

A mixture of equimolar amounts of **1a** and the appropriate acid chloride (4 mmol) in dry benzene (10 ml) was refluxed for 3 h. The solid separated on cooling was filtered, washed and crystallized from ethanol.

*4.1.1.1. Ethyl-2-(3,4,5-trimethoxybenzamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (**2a**)*

Yellow crystals, mp 170-172^{o}C , 93 % yield; IR (cm^{-1}): *ν* 3400 (NH), 3050 (CH aromatic), 2943-2843 (CH aliphatic), 1786 (C=O ester), 1713 (C=O amide), 1686 (NH bending), 1589-1466 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.38 (t, *J*= 7.20 Hz, 3H, CH_2CH_3), 1.80-1.81 (m, 4H, $2CH_2$), 2.68 (br.s, 2H, CH_2), 2.80 (br.s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 4.36 (q, *J*= 7.20 Hz, 2H, CH_2CH_3), 7.25 (s, 2H, aromatic protons), 12.32 (s, 1H, NH); MS (*m/z*, %): 419 (M^+, 5.00), 420 (1.74), 195 (28.47), 63 (100); Anal. Calcd. for $C_{21}H_{25}NO_6S$ (419.49): C, 60.13; H, 6.01; N, 3.34; found: C, 60.18; H, 5.98; N, 3.42.

4.1.1.2. *Ethyl-2-cinnamamido-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (**2b**)*

Yellow crystals, mp 157-159^{o}C , 90 % yield; IR (cm^{-1}): *ν* 3237 (NH), 3050-3020 (CH aromatic), 2974-2839 (CH aliphatic), 1665 (C=O ester), 1651 (C=O amide), 1626 (NH bending), 1599-1497 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.53 (t, *J*= 7.65 Hz, 3H, CH_2CH_3), 1.86-1.87 (m, 4H, $2CH_2$), 2.78-2.79 (m, 2H, CH_2), 2.94-2.95 (m, 2H, CH_2), 4.54 (q, *J*= 7.65 Hz, 2H, CH_2CH_3), 6.75 (d, *J*=16.05 Hz, 1H, *CH*=CH-ph), 7.41-7.58 (m, 5H, 5 aromatic protons), 7.81 (d, *J*= 16.05 Hz, 1H, CH=*CH*-ph), 12.13 (s, 1H, NH); MS (*m/z*, %): 355 (M^+, 2.82), 148 (100); Anal. Calcd. for $C_{20}H_{21}NO_3S$ (355.45): C, 67.58; H, 5.95; N, 3.94; found: C, 67.65; H, 5.98; N, 4.11.

*4.1.2. General procedure for preparation of (**3a-z**)*

To a solution of **2a,b** (5 mmol) in phosphorus oxychloride (10 ml) was added a solution of the appropriate amine (5 mmol) and phosphorus pentoxide (5 mmol) in phosphorus oxychloride (5 ml), and the mixture was refluxed for 5h. After cooling, the

reaction mixture was poured onto ice/water and neutralized with 5% sodium hydroxide solution; the formed precipitate was filtered, washed with water and crystallized from the ethanol.

*4.1.2.1. 4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidine-3 (4H)-carbothioamide (**3a**)*

Brown crystals, mp 155-156^{o}C , 80 % yield; IR (cm^{-1}): *v* 3441-3390 (NH_2), 3036-3001 (CH aromatic), 2940-2835 (CH aliphatic), 1748 (C=O), 1593 (NH bending), 1558-1454 (C=C), 1223 (C=S); ^{1}H NMR (C*D*Cl$_3$): δ 1.42 (s, 2H, NH_2), 1.87-1.91 (m, 4H, 2CH_2), 2.79 (br.s., 2H, CH_2), 2.94 (br.s., 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 7.49 (s, 2H, aromatic protons); ^{13}C NMR (C*D*Cl$_3$): δ 22.7, 23.0, 24.5, 26.6 (4C of cyclohexene), 56.3 (2OCH_3), 61.0 (OCH_3), 105.2 (2C *o*-OCH_3), 113.1, 127.6, 127.8, 141.7, 141.9, 147.6, 153.4 (aromatic carbons + thiophene carbons), 163.2 (C2 of pyrimidine), 169.1 (C=O), 180.0 (C=S); Anal. Calcd. for $C_{20}H_{21}N_3O_4S_2$ (431.53): C, 55.67; H, 4.91; N, 9.74; found: C, 55.65; H, 4.98; N, 9.91.

*4.1.2.2. 4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidine-3 (4H)-carboxamide (**3b**)*

Yellow crystals, mp 230-231^{o}C, 90 % yield; IR (cm^{-1}): *v* 3280 (NH_2), 3050 (CH aromatic), 2940-2839 (CH aliphatic), 1760, 1680 (2C=O), 1632 (NH bending), 1589-1458 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.66 (s, 2H, NH_2), 1.86-1.90 (m, 4H, 2CH_2), 2.79 (br.s., 2H, CH_2), 2.95 (br.s., 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.97 (s, 6H, 2 OCH_3), 7.50 (s, 2H, aromatic protons); Anal. Calcd. for $C_{20}H_{21}N_3O_5S$ (415.46): C, 57.82; H, 5.09; N, 10.11; found: C, 57.78; H, 5.14; N, 10.23.

*4.1.2.3. N-Hydroxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidine-3 (4H)-carboxamide (**3c**)*

Grey crystals, mp 167-168^{o}C , 77 % yield; IR (cm^{-1}): *v* 3300, 3280 (NH, OH), 3050 (CH aromatic), 2940-2839 (CH aliphatic), 1750, 1680 (2C=O), 1632 (NH bending), 1589-1458 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.84-1.89 (m, 4H, 2CH_2), 2.79 (br.s., 2H, CH_2), 2.94 (br.s., 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.96 (s, 6H, 2 OCH_3), 6.30 (s, 1H, OH), 7.50

(s, 2H, aromatic protons), 12.23 (s, 1H, NH); Anal. Calcd. for $C_{20}H_{21}N_3O_6S$ (431.46): C, 55.67; H, 4.91; N, 9.74; found: C, 55.73; H, 4.96; N, 9.89.

*4.1.2.4. 4-[4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-3 (4H)-yl] benzoic acid (**3d**)*

Light brown Crystals, mp 220-222^{o}C , 86 % yield; IR (cm^{-1}): *ν* 3221 (OH carboxylic), 3050 (CH aromatic), 2997-2851 (CH aliphatic), 1748 (2C=O), 1597-1462 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.88 (s, 4H, 2CH$_2$), 2.78-2.80 (m, 2H, CH$_2$), 2.95-2.98 (m, 2H, CH$_2$), 3.80 (s, 3H, OCH$_3$), 3.92 (s, 6H, 2 OCH$_3$), 7.20-7.80 (m, 6H, aromatic protons), 11.30 (s, 1H, OH); MS (*m/z*, %): 491 (M^{+}-1, 2.62), 373 (49.13), 195 (100); Anal. Calcd. for $C_{26}H_{24}N_2O_6S$ (492.54): C, 63.40; H, 4.91; N, 5.69; found: C, 63.42; H, 4.97; N, 5.88.

*4.1.2.5. 4-(4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-3 (4H)-yl) benzenesulfonamide (**3e**)*

Greyish white Crystals, mp 170-172^{o}C , 80 % yield; IR (cm^{-1}): *ν* 3476-3391 (NH$_2$), 3036 (CH aromatic), 2997-2835 (CH aliphatic), 1748 (C=O), 1593 (NH bending), 1558-1454 (C=C), 1323, 1180 (S=O); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.86 (s, 4H, 2CH$_2$), 2.76-2.78 (m, 2H, CH$_2$), 2.98-3.02 (m, 2H, CH$_2$), 3.88 (s, 3H, OCH$_3$), 3.95 (s, 6H, 2 OCH$_3$), 7.20-8.10 (m, 6H, aromatic protons), 8.50 (s, 2H, NH$_2$); ^{13}C NMR (C*D*Cl$_3$): *δ* 22.0, 22.9, 25.2, 25.3 (4C of cyclohexene), 56.5 (2OCH$_3$), 61.1 (OCH$_3$), 105.3 (2C *o*-OCH$_3$), 116.2, 124.9, 132.3, 134.8, 142.0, 153.3, 155.3 (aromatic carbons + thiophene carbons), 158.3 (C=O), 162.4 (C2 of pyrimidine); Anal. Calcd. for $C_{25}H_{25}N_3O_6S_2$ (527.61): C, 56.91; H, 4.78; N, 7.96; found: C, 56.88; H, 4.82; N, 8.10.

*4.1.2.6. 2-(4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin -3 (4H)-yl)-3-phenylpropanoic acid (**3f**)*

Yellowish white Crystals, mp 177-178^{o}C , 69 % yield; IR (cm^{-1}): *ν* 3275 (OH carboxyic), 3036 (CH aromatic), 2997-2835 (CH aliphatic), 1748 (C=O), 1632 (C=O), 1593-1454 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.87-1.90 (m, 4H, 2CH$_2$), 2.74-2.76 (m, 2H,

CH_2), 2.90-2.93 (m, 2H, CH_2), 3.40-3.55 (m, 2H, CH_2), 3.70-3.72 (m, 1H, CH), 3.85 (s, 3H, OCH_3), 3.95 (s, 6H, 2 OCH_3), 7.20-7.50 (m, 7H, aromatic protons), 11.30 (s, 1H, OH); MS (*m/z*, %): 520.10 (M^+, 8.18), 521.55 (M^++1, 1.38), 373 (62.38), 195 (90.89), 135 (100); Anal. Calcd. for $C_{28}H_{28}N_2O_6S$ (520.60): C, 64.60; H, 5.42; N, 5.38; found: C, 64.57; H, 5.46; N, 5.54.

*4.1.2.7. 3-(1H-indol-2-yl)-4-(4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro benzo[4,5]thieno[2,3-d]pyrimidin -3 (4H)-yl) propanoic acid (**3g**)*

Brown Crystals (from charcoal/ethanol), mp > 300^{o}C , 88 % yield; IR (cm^{-1}): *v* 3329 (NH), 3167 (OH carboxylic), 3059 (CH aromatic), 2920-2847 (CH aliphatic), 1670 (2C=O), 1601 (NH bending), 1535-1458 (C=C); ^{1}H NMR (C*D*Cl_3): δ 1.87-1.88 (m, 4H, $2CH_2$), 2.76-2.78 (m, 2H, CH_2), 2.92-2.95 (m, 2H, CH_2), 3.42-3.53 (m, 2H, CH_2), 3.69-3.71 (m, 1H, CH), 3.89 (s, 3H, OCH_3), 3.95 (s, 6H, 2 OCH_3), 6.70-7.40 (m, 7H, aromatic protons), 11.10 (s, 1H, OH), 12.07 (s, 1H, NH); ^{13}C NMR (DMSO): δ 22.8, 23.1, 24.3, 25.3 (4C of cyclohexene), 26.3 (*CH_2*CH), 56.5 (CH_2*CH*), 56.6 (2OCH_3), 60.8 (OCH_3), 105.4 (2C *o*-OCH_3), 104.9, 112.9, 116.2, 120.0, 123.0, 124.9, 126.0, 127.8, 131.7, 132.0, 135.0, 139.0, 141.3, 147.1, 153.7 (aromatic carbons + thiophene carbons + indol carbons), 158.3 (C=O), 167.1 (C2 of pyrimidine), 168.4 (COOH); MS (*m/z*, %): 559.20 (M^+, 23.54), 202.20 (29.30), 90.50 (100); Anal. Calcd. for $C_{30}H_{29}N_3O_6S$ (559.63): C, 64.39; H, 5.22; N, 7.51; found: C, 64.41; H, 5.25; N, 7.67.

*4.1.2.8. 3-(3-Oxoisoxazolidin-4-yl)-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo [4,5]thieno [2,3-d]pyrimidin -4 (3H)-one (**3h**)*

Yellow Crystals, mp 187-188^{o}C , 90% yield; IR (cm^{-1}): *v* 3310 (NH), 3040 (CH aromatic), 2936-2839 (CH aliphatic), 1748 (C=O), 1660 (C=O), 1632 (NH bending), 1593-1454 (C=C); ^{1}H NMR (C*D*Cl_3): δ 1.87 (s, 4H, $2CH_2$), 2.68-2.72 (m, 2H, CH_2), 2.96-2.98 (m, 2H, CH_2), 3.48-3.60 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.90 (s, 6H, 2 OCH_3), 4.05-4.10 (m, 1H, CH), 7.50 (s, 2H, aromatic protons), 12.23 (s, 1H, NH); ^{13}C NMR (C*D*Cl_3): δ 22.9, 23.1, 24.5, 26.5 (4C of cyclohexene), 56.4 (3OCH_3), 60.6, 61.0 (2C of isoxazolidine), 104.9 (2C *o*-OCH_3), 112.0, 127.1, 127.9, 141.8, 148.1, 153.4 (aromatic carbons + thiophene carbons), 163.1 (C=O), 167.0 (C=O of isoxazolidinone);

Anal. Calcd. for $C_{22}H_{23}N_3O_6S$ (457.50): C, 57.76; H, 5.07; N, 9.18 ; found: C, 57.72; H, 5.13; N, 9.26.

*4.1.2.9. 6-(4-Oxo-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin -3 (4H)-yl)pyrimidine-2,4(1H, 3H)-dione (**3i**)*

Yellow Crystals, mp 173-175^{o}C , 90 % yield; IR (cm^{-1}): *ν* 3356, 3271 (2NH), 3078 (CH aromatic), 2936-2835 (CH aliphatic), 1748 (C=O), 1728, 1655 (2C=O of pyrimidine), 1636 (NH bending), 1589-1462 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.87-1.90 (m, 4H, 2CH$_2$), 2.79 (s, 2H, CH$_2$), 2.96 (s, 2H, CH$_2$), 3.89 (s, 3H, OCH$_3$), 3.95 (s, 6H, 2 OCH$_3$), 6.50 (s, 1H, =CH of pyrimidinedione), 7.50 (s, 2H, aromatic protons), 10.90 (s, 1H, NH), 11.1 (s, 1H, NH); Anal. Calcd. for $C_{23}H_{22}N_4O_6S$ (482.51): C, 57.25; H, 4.60; N, 11.61; found: C, 57.31; H, 4.57; N, 11.83.

*4.1.2.10. 3-(Benzo[d]thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro benzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3j**)*

Brown Crystals, mp 190-191^{o}C , 85 % yield; IR (cm^{-1}): *ν* 3050 (CH aromatic), 2940-2835 (CH aliphatic), 1748 (C=O), 1593-1454 (C=C); ^{1}H NMR (DMSO-d_6): *δ* 1.66-1.72 (m, 4H, 2CH$_2$), 2.63-2.68 (s, 4H, 2CH$_2$), 3.71 (s, 3H, OCH$_3$), 3.80 (s, 6H, 2 OCH$_3$), 7.20 (br.s., 2H, benzothiazole protons), 7.34 (s, 2H, trimethoxyphenyl protons), 8.00 (br.s., 1H, benzothiazole proton), 8.38 (s, 1H, benzothiazole protons); MS (*m/z*, %): 505.55 (M^{+}, 4.75), 373.15 (20.78), 195 (69.32), 149 (100); Anal. Calcd. for $C_{26}H_{23}N_3O_4S_2$ (505.61): C, 61.76; H, 4.59; N, 8.31; found: C, 61.80; H, 4.61; N, 8.49.

*4.1.2.11. 3-(5-Methylbenzo[d]thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3k**)*

Yellow crystals, mp 163-165^{o}C , 65 % yield; IR (cm^{-1}): *ν* 3036 (CH aromatic), 2997-2839 (CH aliphatic), 1748 (C=O), 1593-1454 (C=C); ^{1}H NMR (C*D*Cl$_3$): *δ* 1.60 (s, 3H, CH$_3$), 1.86-1.88 (m, 4H, 2CH$_2$), 2.78-2.82 (m, 2H, CH$_2$), 2.98-3.10 (m, 2H, CH$_2$), 3.88 (s, 3H, OCH$_3$), 3.95 (s, 6H, 2 OCH$_3$), 7.20-7.90 (m, 5H, aromatic protons); Anal.

Calcd. for $C_{27}H_{25}N_3O_4S_2$ (519.64): C, 62.41; H, 4.85; N, 8.09; found: C, 62.47; H, 4.93; N, 8.18.

*4.1.2.12. 3-(5-Nitrobenzo[d]thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3l**)*

Greyish white crystals, mp 180-182^{o}C , 91 % yield; IR (cm^{-1}): *v* 3036 (CH aromatic), 2997-2835 (CH aliphatic), 1748 (C=O), 1632 (C=N), 1593-1454 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.86-1.88 (m, 4H, 2CH$_2$), 2.75-2.77 (m, 2H, CH$_2$), 2.96-2.99 (m, 2H, CH$_2$), 3.82 (s, 3H, OCH$_3$), 3.96 (s, 6H, 2 OCH$_3$), 7.49-8.40 (m, 5H, aromatic protons); ^{13}C NMR (C*D*Cl$_3$): δ 22.1, 22.8, 25.3, 29.8 (4C of cyclohexene), 56.5 (2OCH$_3$), 61.1 (OCH$_3$), 105.5 (2C *o*-OCH$_3$), 106.5, 110.7, 116.2, 125.1, 127.4, 132.3, 134.8, 142.0, 153.1, 154.7 (aromatic carbons + thiophene carbons + benzothiazole carbons), 158.4 (C=O), 162.5 (C2 of pyrimidine); MS (*m/z*, %): 551 (M^{+}+1, 5.34), 373.65 (10.11), 195.35 (78.66), 80.45 (100); Anal. Calcd. for $C_{26}H_{22}N_4O_6S_2$ (550.61): C, 56.72; H, 4.03; N, 10.18; found: C, 56.73; H, 4.11; N, 10.26.

*4.1.2.13. 3-(6-Amino-10-methylacridin-3-yl)-2-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3m**)*

Brown crystals, mp 213-215^{o}C , 93 % yield; IR (cm^{-1}): *v* 3364, 3217 (NH$_2$), 3040 (CH aromatic), 2940-2835 (CH aliphatic), 1748 (C=O), 1639 (NH$_2$ bending), 1601 (C=N), 1562-1497 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.86-1.92 (m, 4H, 2CH$_2$), 2.66-2.70 (m, 4H, 2CH$_2$), 3.93 (s, 3H, OCH$_3$), 3.95 (s, 6H, 2 OCH$_3$), 4.00 (s, 3H, CH$_3$), 5.50 (s, 1H, NH), 7.11-7.58 (m, 9H, aromatic protons); Anal. Calcd. for $C_{33}H_{31}N_4O_4S^{+}$ (579.69): C, 68.37; H, 5.39; N, 9.66; found: C, 68.44; H, 5.45; N, 9.78.

*4.1.2.14. (E)-4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-3(4H)-carbothioamide (**3n**)*

Brown crystals, mp 117-119^{o}C , 69 % yield; IR (cm^{-1}): *v* 3383, 3348 (NH$_2$), 3032 (CH aromatic), 2927-2851 (CH aliphatic), 1680 (C=O), 1643 (NH$_2$ bending), 1535-1447 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.40 (s, 2H, NH$_2$), 1.84-1.86 (m, 4H, 2CH$_2$), 2.68-2.70 (m, 2H, CH$_2$), 2.98-3.08 (m, 2H, CH$_2$), 6.90 (d, *J*= 16 Hz, 1H, *CH*=CH-ph), 7.26-7.50 (m, 5H, aromatic protons), 7.95 (d, *J*=16 Hz, 1H CH=*CH*-ph); MS (*m/z*, %): 367 (M^{+}, 1.51),

204 (1.34), 104 (100); Anal. Calcd. for $C_{19}H_{17}N_3OS_2$ (367.49): C, 62.10; H, 4.66; N, 11.43; found: C, 62.14; H, 4.69; N, 11.51.

*4.1.2.15. (E)-4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-3(4H)-carboxamide (**3o**)*

Green crystals, mp 130-131^{o}C , 88 % yield; IR (cm^{-1}): *v* 3426, 3356 (NH_2), 3040 (CH aromatic), 2870-2735 (CH aliphatic), 1651 (2C=O), 1581 (NH_2 bending), 1492-1480 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.70 (s, 2H, NH_2), 1.84 (s, 4H, $2CH_2$), 2.77 (s, 2H, CH_2), 2.90 (s, 2H, CH_2), 6.90 (d, *J*= 15 Hz, 1H, *CH*=CH-ph), 7.29-7.39 (m, 5H, aromatic protons), 7.90 (d, *J*=15 Hz, 1H, CH=*CH*-ph); ^{13}C NMR (C*D*Cl$_3$): δ 22.1, 22.9, 25.3, 29.8 (4C of cyclohexene), 116.6 (*CH*=CH-ph), 118.4, 127.5, 128.1, 128.8, 130.0, 132.6, 134.8, 141.9, 158.6 (aromatic carbons + thiophene carbons + CH=*CH*-ph), 155.2 (C2 of pyrimidine), 162.3 ($CONH_2$), 169.8 (C=O); MS (*m/z*, %): 352 (M^++1, 8.67), 204 (24.70), 105 (97.97), 77 (100); Anal. Calcd. for $C_{19}H_{17}N_3O_2S$ (351.42): C, 64.94; H, 4.88; N, 11.96; found: C, 64.99; H, 4.86; N, 12.04.

*4.1.2.16. (E)-N-Hydroxy-4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidine-3(4H)-carboxamide (**3p**)*

Grey crystals, mp 123-125^{o}C , 92 % yield; IR (cm^{-1}): *v* 3410 (OH), 3287 (NH), 3032 (CH aromatic), 2936-2855 (CH aliphatic), 1690 (2C=O), 1631 (NH bending), 1539 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.85-1.87 (m, 4H, $2CH_2$), 2.67-2.78 (m, 2H, CH_2), 3.00-3.10 (m, 2H, CH_2), 6.35 (s, 1H, OH), 6.92 (d, *J*= 16 Hz, 1H, *CH*=CH-ph), 7.30-7.50 (m, 5H, aromatic protons), 7.90 (d, *J*=16 Hz, 1H, CH=*CH*-ph), 12.10 (s, 1H, NH); Anal. Calcd. for $C_{19}H_{17}N_3O_3S$ (367.42): C, 62.11; H, 4.66; N, 11.44; found: C, 62.14; H, 4.69; N, 11.46.

*4.1.2.17. (E)-4-(4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-3 (4H)-yl) benzoic acid (**3q**)*

Yellowish brown crystals, mp 143-145^{o}C , 90 % yield; IR (cm^{-1}): *v* 3200 (OH carboxylic), 3055-3028 (CH aromatic), 2924-2851 (CH aliphatic), 1659 (2C=O), 1604 (C=N), 1560-1450 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.83-1.88 (m, 4H, $2CH_2$), 2.70-2.78 (m,

2H, CH_2), 2.80-2.87 (m, 2H, CH_2), 6.75 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.40-7.59 (m, 5H, aromatic protons), 7.80 (d, *J*=16.05 Hz, 1H, CH=*CH*-ph), 7.99 (d, *J*= 7.65 Hz, 2H, aromatic protons), 8.25 (d, *J*= 7.65 Hz, 2H, aromatic protons), 11.28 (s, 1H, OH); ^{13}C NMR (C*D*Cl_3): δ 22.1, 22.9, 23.0, 25.3 (4C of cyclohexene), 111.1 (*CH*=CH-ph), 116.5, 118.4, 127.4, 127.9, 128.1, 128.8, 130.4, 132.4, 134.8, 135.1, 141.9, 142.4, 155.3 (aromatic carbons + thiophene carbons + CH=*CH*-ph), 158.9 (C=O), 163.6 (C2 of pyrimidine), 170.4 (COOH); Anal. Calcd. for $C_{25}H_{20}N_2O_3S$ (428.50): C, 70.07; H, 4.70; N, 6.54; found: C, 70.11; H, 4.76; N, 6.58.

*4.1.2.18. (E)-4-(4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-3 (4H)-yl) benzenesulfonamide (**3r**)*

Black crystals, mp 150-151^{o}C , 70 % yield; IR (cm^{-1}): *v* 3356-3156 (NH_2), 3059-3028 (CH aromatic), 2928-2855 (CH aliphatic), 1680 (C=O), 1634 (NH_2 bending), 1597-1535 (C=C), 1312, 1161 (S=O); ^{1}H NMR (C*D*Cl_3): δ 1.83-1.86 (m, 4H, $2CH_2$), 2.72-2.78 (m, 2H, CH_2), 2.95-3.00 (m, 2H, CH_2), 6.80 (d, *J*= 16 Hz, 1H, *CH*=CH-ph), 7.35-7.86 (m, 9H, aromatic protons), 7.93 (d, *J*=16 Hz, 1H, CH=*CH*-ph), 8.50 (s, 2H, NH_2); MS (*m/z*, %): 463 (M^+, 32.05), 282 (30.67), 63.75 (100); Anal. Calcd. for $C_{24}H_{21}N_3O_3S_2$ (463.57): C, 62.18; H, 4.57; N, 9.06; found: C, 62.22; H, 4.61; N, 9.14.

*4.1.2.19. (E)-4-(4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-3 (4H)-yl) phenylpropanoic acid (**3s**)*

Brown crystals, mp 119-121^{o}C , 93 % yield; IR (cm^{-1}): *v* 3271-3159 (OH carboxylic), 3059-3028 (CH aromatic), 2928-2851 (CH aliphatic), 1680 (C=O), 1650 (C=O), 1632 (C=N), 1562-1454 (C=C); ^{1}H NMR (C*D*Cl_3): δ 1.85 (s, 4H, $2CH_2$), 2.68-2.78 (m, 2H, CH_2), 2.85-2.94 (m, 2H, CH_2), 3.79-3.80 (m, 2H, CH_2CH), 3.98-4.10 (m, 1H, CH_2CH), 6.74 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.39-7.56 (m, 7H, aromatic protons), 7.81 (d, *J*=16.05 Hz, 1H, CH=*CH*-ph), 7.96-8.25 (m, 3H, aromatic protons), 11.25 (s, 1H, OH carboxylic); ^{13}C NMR (C*D*Cl_3): δ 22.0, 22.9, 24.5, 25.2 (4C of cyclohexene), 26.5 (CH_2CH), 55.5 (CH_2CH), 111.5 (*CH*=CH-ph), 118.4, 127.5, 128.1, 128.3, 128.7, 128.9, 130.4, 132.6, 134.5, 141.9, 155.3 (aromatic carbons + thiophene carbons + CH=*CH*-ph), 158.9 (C=O), 163.6 (C2 of pyrimidine), 169.8 (COOH); Anal.

Calcd. for $C_{27}H_{24}N_2O_3S$ (456.56): C, 71.03; H, 5.30; N, 6.14; found: C, 71.02; H, 5.37; N, 6.08.

*4.1.2.20. (E)-3-(1H-indol-2-yl)-4-(4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -3 (4H)-yl) propanoic acid (**3t**)*

Brown crystals (from charcoal/ethanol), mp > 300^{o}C , 83 % yield; IR (cm^{-1}): *v* 3375 (NH), 3175 (OH carboxylic), 3059-3032 (CH aromatic), 2924-2851 (CH aliphatic), 1748 (2C=O), 1624 (NH bending), 1539-1497 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.82 (s, 4H, 2CH$_2$), 2.68-2.78 (m, 2H, CH$_2$), 2.85-2.95 (m, 2H, CH$_2$), 3.73-3.75 (m, 2H, CH$_2$), 4.20-4.25 (m, 1H, CH), 6.63 (d, *J*= 15.30 Hz, 1H, *CH*=CH-ph), 7.39-7.58 (m, 8H, aromatic protons), 7.81 (d, *J*=15.30 Hz, 1H, CH=*CH*-ph), 7.97-7.98 (m, 2H, aromatic protons), 11.26 (s, 1H, OH carboxylic), 12.06 (s, 1H, NH); Anal.Calcd.for $C_{29}H_{25}N_3O_3S$ (495.59): C, 70.28; H, 5.08; N, 8.48; found: C, 70.31; H, 5.14; N, 8.62.

*4.1.2.21. (E)-3-(3-Oxoisoxazolidin-4-yl)-2-styryl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3u**)*

Grey crystals, mp 140-142^{o}C , 90 % yield; IR (cm^{-1}): *v* 3406 (NH), 3063-3032 (CH aromatic), 2924-2851 (CH aliphatic), 1670 (2C=O), 1636 (NH bending), 1558-1458 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.84 (s, 4H, 2CH$_2$), 2.77 (s, 2H, CH$_2$), 3.05 (m, 2H, CH$_2$), 3.72-3.74 (m, 1H, 1H of CH$_2$), 3.89-3.90 (m, 1H, 1H of CH$_2$), 4.23-4.36 (m, 1H, CH), 6.28 (s, 1H, NH), 6.91 (d, *J*= 16.80 Hz, 1H, *CH*=CH-ph), 7.23-7.59 (m, 5H, aromatic protons), 7.90 (d, *J*=16.85 Hz, 1H, CH=*CH*-ph); Anal. Calcd. for $C_{21}H_{19}N_3O_3S$ (393.46): C, 64.10; H, 4.87; N, 10.68; found: C, 64.08; H, 4.89; N, 10.77.

*4.1.2.22. (E)-6-(4-Oxo-2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-3(4H)-yl)pyrimidine-2,4(1H, 3H)-dione (**3v**)*

Brown crystals, mp 110-112^{o}C , 66 % yield; IR (cm^{-1}): *v* 3375-3348 (2NH), 3063 (CH aromatic), 2928-2855 (CH aliphatic), 1659 (C=O), 1640 (2C=O), 1580 (NH bending), 1535-1450 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.86 (s, 4H, 2CH$_2$), 2.79 (s, 2H, CH$_2$), 2.98 (m, 2H, CH$_2$), 6.20 (s, 1H, =CH of pyrimidinedione), 6.90 (d, *J*= 16 Hz, 1H, *CH*=CH-ph), 7.30-7.50 (m, 5H, aromatic protons), 7.90 (d, *J*=16 Hz, 1H, CH=*CH*-ph), 10.8 (s, 1H, NH), 11.20 (s, 1H, NH); ^{13}C NMR (C*D*Cl$_3$): δ 22.0, 22.9, 24.2, 25.3 (4C of

cyclohexene), 74.5, 112.8, 117.8, 126.6, 126.9, 127.7, 128.9, 129.3, 131.6, 146.4, 146.9 (aromatic carbons + thiophene carbons + pyrimidinedione carbons + olefinic carbons), 162.9, 167.3 (2C=O of pyrimidinedione), 168.1 (C=O); Anal. Calcd. for $C_{22}H_{18}N_4O_3S$ (418.47): C, 63.14; H, 4.34; N, 13.39; found: C, 63.22; H, 4.37; N, 13.52.

*4.1.2.23. (E)-3-(Benzo[d]thiazol-2-yl)-2-styryl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d] pyrimidin -4 (3H)-one (**3w**)*

Brown crystals, mp 100-103^{o}C , 60 % yield; IR (cm^{-1}): *v* 3059-3028 (CH aromatic), 2920-2851 (CH aliphatic), 1748 (C=O), 1663 (C=N), 1628-1493 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.83-1.85 (m, 4H, 2CH$_2$), 2.65-2.75 (m, 2H, CH$_2$), 2.80-2.90 (m, 2H, CH$_2$), 6.90 (d, *J*= 15 Hz, 1H, *CH*=CH-ph), 7.28-7.90 (m, 10H, aromatic protons + CH=*CH*-ph); ^{13}C NMR (DMSO-d_6): δ 22.7, 23.0, 25.8, 26.9 (4C of cyclohexene), 111.0 (*CH*=CH-ph), 127.5, 127.6, 127.8, 129.1, 130.1, 132.0, 135.4, 137.9, 145.1, 152.8, 157.9 (aromatic carbons + benzothiazole carbons + CH=*CH*-ph), 166.3 (C=O); Anal. Calcd. for $C_{25}H_{19}N_3OS_2$ (441.57): C, 68.00; H, 4.34; N, 9.52; found: C, 68.03; H, 4.34; N, 9.63.

*4.1.2.24. (E)-3-(5-Methylbenzo[d]thiazol-2-yl)-2-styryl-5,6,7,8-tetrahydrobenzo [4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3x**)*

Grey crystals, mp 155-156^{o}C , 90 % yield; IR (cm^{-1}): *v* 3063 (CH aromatic), 2936-2859 (CH aliphatic), 1643 (C=O), 1535-1458 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.83-1.85 (m, 4H, 2CH$_2$), 2.10 (s, 3H, CH$_3$), 2.78-2.79 (m, 2H, CH$_2$), 2.93-2.96 (m, 2H, CH$_2$), 6.80 (d, *J*= 15 Hz, 1H, *CH*=CH-ph), 7.29-7.90 (m, 9H, aromatic protons + CH=*CH*-ph); Anal. Calcd. for $C_{26}H_{21}N_3OS_2$ (455.59): C, 68.54; H, 4.65; N, 9.22; found: C, 68.60; H, 4.72; N, 9.31.

*4.1.2.25. (E)-3-(5-Nitrobenzo[d]thiazol-2-yl)-2-styryl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin -4 (3H)-one (**3y**)*

Green crystals, mp 133-135^{o}C , 90 % yield; IR (cm^{-1}): *v* 3063-3032 (CH aromatic), 2932-2855 (CH aliphatic), 1651 (C=O), 1631 (C=N), 1580-1454 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.87-1.89 (m, 4H, 2CH$_2$), 2.78-2.80 (m, 2H, CH$_2$), 2.95-3.02 (m, 2H, CH$_2$), 6.90 (d, *J*= 16 Hz, 1H, *CH*=CH-ph), 7.35-8.60 (m, 9H, aromatic protons +

CH=*CH*-ph); Anal. Calcd. for $C_{25}H_{18}N_4O_3S_2$ (486.57): C, 61.71; H, 3.73; N, 11.51; found: C, 61.69; H, 3.76; N, 11.73.

*4.1.2.26. (E)-3-(6-Amino-10-methylacridin-3-yl)-2-styryl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidin -4 (3H)-one (**3z**)*

Brown crystals, mp 260-261^{o}C , 90 % yield; IR (cm^{-1}): *v* 3360, 3217 (NH_2), 3050 (CH aromatic), 2920-2851 (CH aliphatic), 1640 (C=O), 1636 (NH_2 bending), 1605 (C=N), 1493 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.87-1.90 (m, 4H, 2CH_2), 2.77-2.79 (m, 2H, CH_2), 2.92-2.94 (m, 2H, CH_2), 3.49 (s, 3H, CH_3), 3.78 (s, 2H, NH_2), 6.75 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.40-7.57 (m, 12H, aromatic protons), 7.83 (d, *J*=16.05 Hz, 1H, CH=*CH*-ph); ^{13}C NMR (C*D*Cl$_3$): δ 23.0, 24.5, 25.3, 26.3 (4C of cyclohexene), 111.3 (*CH*=CH-ph), 114.1, 116.6, 118.4, 119.4, 119.8, 127.3, 127.5, 128.1, 128.3, 128.8, 129.1, 134.4, 134.8, 135.1, 142.0, 142.4, 144.1, 149.7, 155.3 (aromatic carbons + thiophene carbons + CH=*CH*-ph), 162.7 (C=O), 163.6 (C2 of pyrimidine); Anal. Calcd. for $C_{32}H_{27}N_4OS$+ (515.65): C, 74.54; H, 5.28; N, 10.87; found: C, 74.61; H, 5.33; N, 10.96.

*4.1.3. General procedure for the preparation of (**4a-d**)*

To a mixture of equimolar amounts of **2b** and the appropriate aldehyde (5 mmol) in methanol (10 ml), hydrochloric acid (5 drops) was added and the solution was stirred at room temperature for 5 h. The obtained precipitate was filtered, washed and crystallized from ethanol.

*4.1.3.1. 2-(3,4,5-Trimethoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H) one (**4a**)[7, 8]*

^{1}H NMR (C*D*Cl$_3$): δ 1.87-1.89 (m, 4H, 2CH_2), 2.79 (s, 2H, CH_2), 2.94 (s, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.99 (s, 6H, 2OCH_3), 7.49 (s, 2H, aromatic protons), 12.23 (s, 1H, NH).

*4.1.3.2. (E)-2-Styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)one (**4b**)[50, 51]*

IR (cm^{-1}): *v* 3363(NH), 3039 (CH olifenic and aromatic), 2927 (CH aliphatic), 1647 (C=O), 1616 (C=N); ^{1}H NMR (DMSO-d_6): δ 1.73-1.76 (m, 4H, 2CH_2), 2.70 (s, 2H,

CH_2), 2.84 (m, 2H, CH_2), 6.94 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.20-7.59 (m, 5H, aromatic protons), 7.84 (d, *J*=16.05 Hz, 1H, CH=*CH*-ph), 12.30 (s, 1H, NH); Anal. calcd. for $C_{18}H_{16}N_2OS$ (308.40): C, 70.10; H, 5.23; N, 9.08; found: C, 70.23; H, 5.16; N, 9.20.

*4.1.3.3. (E)-2-(2-Nitrostyryl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H) one (**4c**)*

Orange crystals, mp 208-210^{o}C, 90% yield; IR (cm^{-1}): *v* 3363(NH), 3066 (CH olefinic and aromatic), 2931 (CH aliphatic), 1639 (C=O), 1616 (C=N); ^{1}H NMR ($CDCl_3$): δ 1.86-1.87 (m, 4H, $2CH_2$), 2.78-2.79 (s, 2H, CH_2), 2.94-2.95 (m, 2H, CH_2), 6.75 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.39-7.50 (m, 4H, aromatic protons), 7.81 (d, *J*=16.05 Hz, 1H, CH=*CH*-ph), 12.10 (s, 1H, NH); Anal. calcd. for $C_{18}H_{15}N_3O_3S$ (353.39): C, 61.18; H, 4.28; N, 11.89; found: C, 61.22; H, 4.17; N, 11.54.

*4.1.3.4. (E)-2-(1-Phenylprop-1-en-2-yl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-4(3H) one (**4d**)*

Yellow crystals, mp 240-243^{o}C, 90% yield; IR (cm^{-1}): *v* 3383(NH), 3070 (CH olefinic and aromatic), 2931 (CH aliphatic), 1639 (C=O), 1616 (C=N); ^{1}H NMR ($CDCl_3$): δ 1.86-1.88 (m, 4H, $2CH_2$), 2.69-2.78 (s, 2H, CH_2), 2.95 (s, 2H, CH_2), 3.49 (s, 3H, CH_3), 7.40-8.04 (m, 5H, aromatic protons), 8.26 (s, 1H, =CH-ph), 12.15 (s, 1H, NH); Anal. calcd. for $C_{19}H_{18}N_2OS$ (322.42): C, 70.78; H, 5.63; N, 8.69; found: C, 70.69; H, 5.60; N, 8.71.

*4.1.4. General procedure for the preparation of (**6a-c**)*

A mixture of equimolar amounts of **5a,b** and the appropriate amine (5 mmol) was heated in domestic microwave at 1000 W under solvent free condition for 45 seconds. The obtained product was crystallized from ethanol.

*4.1.4.1. (E)-1-Hydroxy-3-[2-(3,4,5-trimethoxyphenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-d]pyrimidin-4-yl]urea (**6a**)*

Yellow crystals, mp >300^{o}C, 93% yield; IR (cm^{-1}): *v* 3406-3237 (2NH, OH), 3050 (CH aromatic), 2936-2839 (CH aliphatic), 1655 (C=O), 1630 (NH bending), 1589-

1485 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.86-1.88 (m, 4H, 2CH$_2$), 2.77-2.79 (m, 2H, CH$_2$), 2.96-2.99 (m, 2H, CH$_2$), 3.90 (s, 3H, OCH$_3$), 4.00 (s, 6H, 2OCH$_3$), 6.5 (s, 1H, OH), 7.70 (s, 2H, aromatic protons), 7.80 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6): δ 22.6, 23.1, 24.5, 26.3 (4C of cyclohexene), 56.5 (2OCH$_3$), 60.7 (OCH$_3$), 106.7, 130.0, 131.1, 131.6, 136.3, 141.5, 153.7 (aromatic carbons + thiophene carbons), 150.7 (C=O), 157.2 (C4 of the pyrimidine), 165.5 (C2 of the pyrimidine); Anal. Calcd. for $C_{20}H_{22}N_4O_5S$ (430.48): C, 55.80; H, 5.15; N, 13.02; found: C, 55.78; H, 5.14; N, 13.15.

*4.1.4.2. (E)-1-Hydroxy-3-(2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)urea (**6b**)*

Brown crystals, mp 225-227 ^{o}C, 92% yield; IR (cm^{-1}): *v* 3387-3200 (2NH, OH), 3030 (CH aromatic), 2940 (CH aliphatic), 1659(C=O), 1630 (NH bending), 1589-1400 (C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.87-1.89 (m, 4H, 2CH$_2$), 2.75-2.77 (m, 2H, CH$_2$), 2.93-2.95 (m, 2H, CH$_2$), 6.50 (s, 1H, OH), 6.80 (d, *J*= 16.05 Hz, 1H, *CH*=CH-ph), 7.20-8.20 (m, 6H, aromatic protons + CH=*CH*-ph), 8.80 (s, 2H, 2NH); ^{13}C NMR (CDCl$_3$): δ 22.3, 23.0, 25.3, 25.9 (4C of cyclohexene), 111.7 (*CH*=CH-ph), 115.5, 126.4, 128.1, 128.6, 138.4, 138.7, 145.9 (aromatic carbons + thiophene carbons+ CH=*CH*-ph), 151.0 (C=O), 160.5 (C4 of the pyrimidine), 160.9 (C2 of pyrimidine); MS (*m/z*, %): 366 (M^{+}, 3.72), 105 (100); Anal. Calcd. for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29; found: C, 62.32; H, 4.93; N, 15.48.

*4.1.4.3. (E)-3-(6-Amino-10-methylacridin-3-ylamino)-2-styryl-5,6,7,8-tetrahydrobenzo [4,5]thieno [2,3-d]pyrimidine (**6c**)*

Brown crystals, mp >300 ^{o}C, 95% yield; IR (cm^{-1}): *v* 3314-3190 (NH, NH$_2$), 3030 (CH aromatic), 2936 (CH aliphatic), 1639 (NH bending), 1601-1443(C=C); ^{1}H NMR (C*D*Cl$_3$): δ 1.86-1.88 (m, 4H, 2CH$_2$), 2.75-2.77 (m, 2H, CH$_2$), 2.95-2.97 (m, 2H, CH$_2$), 4.10 (s, 3H, CH3), 5.40 (s, 2H, NH$_2$), 6.80 (d, *J*=16.05 Hz, 1H, *CH*=CH-ph), 7.28-8.30 (m, 13H, aromatic protons + CH=*CH*-ph), 9.80 (s, 1H, NH); Anal. Calcd. for $C_{32}H_{28}N_5S^{+}$ (514.66): C, 74.68; H, 5.48; N, 13.61; found: C, 74.66; H, 5.51; N, 13.84.

4.2. Antitumor activity

The potential cytotoxicity of the tested compounds and Doxorubicin as a reference standard was evaluated using the method of Skehan *et al.* [52]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the prepared compounds to allow the attachment of cells to the wall of the plate. The tested compounds were dissolved in dimethylsulfoxide (DMSO) and diluted 1000-fold in the assay. Concentrations 0, 5, 12.5, 25, and 50 μg/ml of the tested compounds were added to the cell monolayer. The monolayer cells were incubated with the compounds for 48 h at 37□C, in atmosphere of 5% CO_2. After 48 h, the cells were fixed, washed and stained with Sulfo-Rhodamine-B stain (SRB). Excess stain was washed with acetic acid. The attached stain was recovered with Tris EDTA buffer. Cell survival and drug activity were determined by measuring color intensity using an ELISA reader. Data are representative of the individual experiment, performed in three replicates for each individual dose and measured by SRB assay. Control values did not exhibit significant changes compared to the DMSO vehicle. The IC_{50} was determined using a program Graph-Pad PRISM version 5. Mean and standard error were determined by SPSS 11 software. The obtained IC_{50} values were illustrated in Table 1.

4.3. Antibacterial activity

Antibacterial activity of the tested compounds was determined using a modified Kirby-Bauer disc diffusion method [53]. Of the many media available, National Committee for Clinical Laboratory Standards NCCLS recommend Mueller-Hinton agar due to its good batch-to-batch reproducibility. Plates were incubated with *Staphylococcus aureus* (ATCC 12600) and *Bacillus subtilis* (ATCC 6051) (as Gram positive bacteria) while *Pseudomonas aeruginosa* (ATCC 10145) and *Escherichia coli* (ATCC 11775) (as Gram negative bacteria) at 35-37□C for 24-48 h, then the diameters of the inhibition zones were measured in millimeters. Standard discs of Ampicillin served as positive control for antibacterial activity and filter discs impregnated with DMSO solvent served as a negative control. Blank paper discs (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with the tested concentration of the stock solutions. The zone

diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS, 1993). The results expressing the inhibition zone in mm were represented in Table 2. The MIC values obtained from the broth microdilution method [54] for the most active compounds **3m** and **3z** were represented in Table 3.

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Figures captions:

Figure 1. Representative anticancer quinazoline and thieno[2,3-d]pyrimidines and our proposed derivatives.

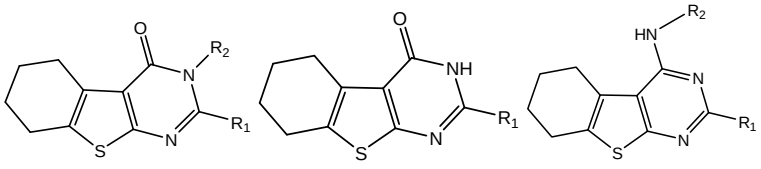
Tables captions:

Table 1. IC_{50} of the tested compounds against human tumor cell lines

Table 2. Inhibition zone (mm) of the tested compounds

Table 3. MIC values (μg/ml) of tested samples against tested microorganisms

Table 1: IC_{50} of the tested compounds against human tumor cell lines



3a-z **4a-d** **6a-c**

Compound	Substitution		Tested human tumor cell lines, IC_{50} μg/ml (μM)	
	R_1	R_2	Breast MCF-7	Liver HEPG-2
Doxorubicin	-	-	2.97 (5.46)	4.00 (7.36)
3a	3,4,5-trimethoxyphenyl	$CSNH_2$	33.50 (77.64)	>100 (>231.75)
3b	3,4,5-trimethoxyphenyl	$CONH_2$	10.90 (26.23)	10.70 (25.75)
3c	3,4,5-trimethoxyphenyl	CONHOH	30.70 (71.15)	6.20 (14.37)
3d	3,4,5-trimethoxyphenyl	4-carboxyphenyl	>100 (>203)	14.60 (29.64)
3e	3,4,5-trimethoxyphenyl	4-sulfamoylphenyl	19.60 (37.15)	>100 (>189.54)
3f	3,4,5-trimethoxyphenyl	1-carboxy-2-phenylethyl	26.10 (50.13)	10.10 (19.40)
3g	3,4,5-trimethoxyphenyl	1-carboxy-2-(1H) indol-3-ylethyl	>100 (>178.60)	17.30 (30.91)
3h	3,4,5-trimethoxyphenyl	3-oxoisoxazolidin-4-yl	25.10 (54.86)	11.40 (24.92)
3i	3,4,5-trimethoxyphenyl	2,4-pyrimidindion-6-yl	40.20 (83.32)	41.70 (86.42)
3j	3,4,5-trimethoxyphenyl	benzo[*d*]thiazol-2-yl	5.80 (11.47)	2.60 (5.14)
3k	3,4,5-trimethoxyphenyl	5-methylbenzo[*d*]thiaz-ol-2-yl	34.10 (65.63)	>100 (>192.46)
3l	3,4,5-trimethoxyphenyl	5-nitrobenzo[*d*]thiazol-2-yl	11.90 (21.61)	20.30 (36.87)
3m	3,4,5-trimethoxyphenyl	6-amino-10-methyl-acridin-3-yl	0.50 (0.86)	3.20 (5.50)
3n	styryl	$CSNH_2$	3.90 (10.61)	6.50 (17.69)
3o	styryl	$CONH_2$	4.70 (13.37)	6.50 (18.50)
3p	styryl	CONHOH	8.80 (23.95)	19.10 (51.98)
3q	styryl	4-carboxyphenyl	19.40 (45.27)	15.80 (36.87)
3r	styryl	4-sulfamoylphenyl	0.20 (0.43)	0.60 (1.29)
3s	styryl	1-carboxy-2-phenylethyl	6.60 (45.27)	14.40 (31.54)
3t	styryl	1-carboxy-2-(1H) indol-3-ylethyl	>100 (>201.78)	14.70 (29.66)
3u	styryl	3-oxoisoxazolidin-4-yl	15.50 (39.39)	2.70 (6.86)
3v	styryl	2,4-pyrimidindion-6-yl	4.70 (11.23)	28.10 (67.15)
3w	styryl	benzo[*d*]thiazol-2-yl	1.90 (4.30)	4.70 (10.64)
3x	styryl	5-methylbenzo[*d*]thiaz-ol-2-yl	7.20 (15.80)	16.70 (36.66)
3y	styryl	5-nitrobenzo[*d*]thiazol-2-yl	0.60 (1.23)	5.00 (10.28)
3z	styryl	6-amino-10-methyl-acridin-3-yl	0.10 (0.19)	3.00 (5.82)
4b	styryl	-----	8.70 (28.24)	3.70 (12.01)
4c	2-nitrostyryl	----	4.20 (11.88)	4.20 (11.88)
4d	1-Phenylprop-1-en-2-yl	----	8.00 (24.81)	10.70 (33.19)
6a	3,4,5-trimethoxyphenyl	CONHOH	6.10 (14.17)	1.00 (2.32)
6b	styryl	CONHOH	2.40 (6.54)	1.20 (3.27)
6c	styryl	6-amino-10-methyl-acridin-3-yl	0.30 (0.58)	0.70 (1.36)

Table 2: Inhibition zone (mm) of the tested compounds

Compound	Inhibition zone diameter (mm)			
	Bacillus subtilis (G^+)	**Staphylococcus aureus (G^+)**	**Escherichia coli (G^-)**	**Pseudomonas aeruginosa (G^-)**
Ampicillin	20	18	22	17
3a	12	12	14	13
3b	16	16	16	17
3c	13	12	14	13
3d	12	11	10	10
3e	14	9	12	13
3f	12	10	13	13
3g	0.0	0.0	0.0	0.0
3h	15	15	17	14
3i	11	11	14	14
3j	13	14	15	15
3k	11	14	12	12
3l	14	15	15	14
3m	23	26	22	25
3n	13	14	13	13
3o	14	16	14	15
3p	12	15	13	14
3q	14	19	16	14
3r	14	14	15	15
3s	13	16	14	14
3t	0.0	0.0	0.0	0.0
3u	15	19	15	16
3v	16	18	15	15
3w	13	16	13	14
3x	15	16	14	14
3y	14	15	14	15
3z	20	24	21	21
4b	0.0	0.0	0.0	0.0
4c	0.0	0.0	10	0.0
4d	0.0	0.0	0.0	0.0
6a	0.0	0.0	0.0	0.0
6b	11	10	10	12
6c	20	21	12	12

Table 3. MIC values (μg/ml) of tested samples against tested microorganisms.

Compound number	MIC (μg/ ml)	
	Staphylococcus aureus **(ATCC 12600)**	***Pseudomonas aeruginosa*** **(ATCC 10145)**
3m	8	11
3z	13	18
Ampicillin	48	56

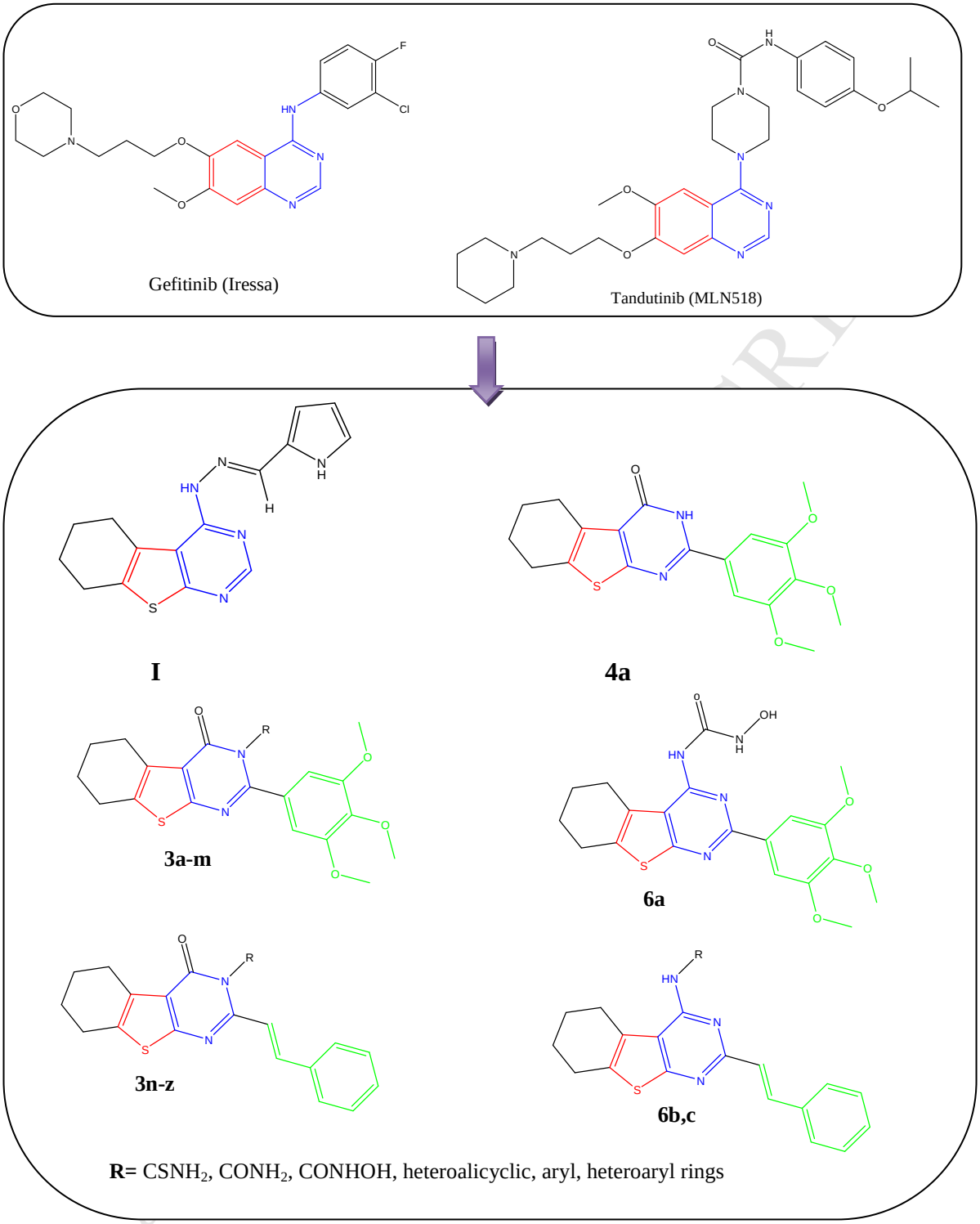
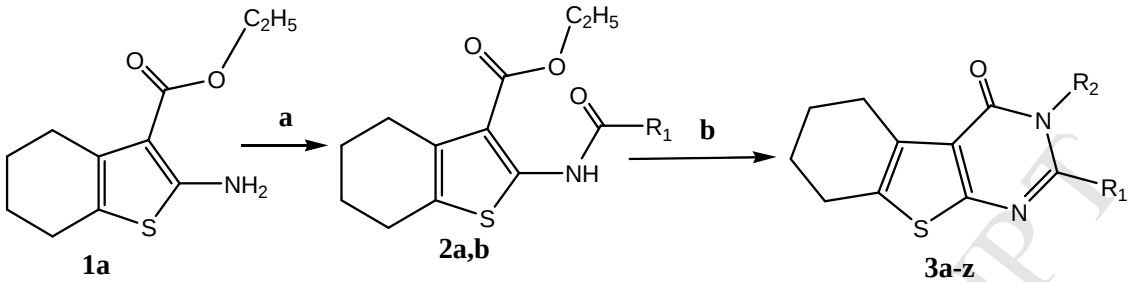

Gefitinib (Iressa)
Tandutinib (MLN518)
I
4a
3a-m
6a
3n-z
6b,c
R= CSNH2, CONH2, CONHOH, heteroalicyclic, aryl, heteroaryl rings

Figure 1



1a **2a,b** **3a-z**

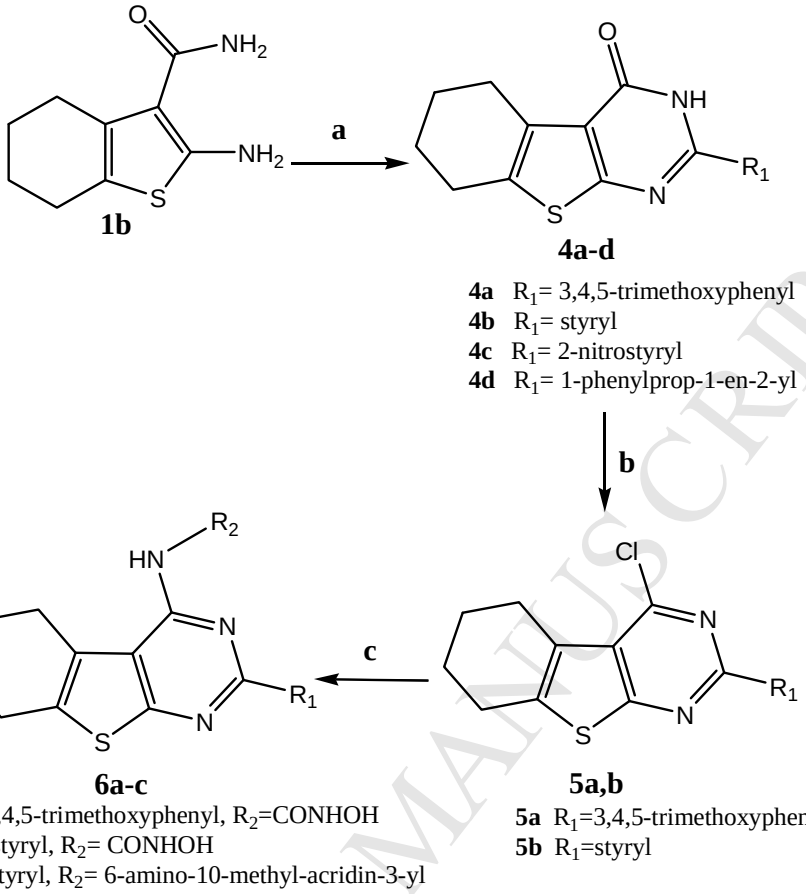
2a R= 3,4,5-trimethoxyphenyl
2b R= styryl

3a-m R_1= 3,4,5-trimethoxyphenyl
3a, **3n** R_2= $CSNH_2$
3b, **3o** R_2= $CONH_2$
3c, **3p** R_2= CONHOH
3d, **3q** R_2= 4-carboxyphenyl
3e, **3r** R_2= 4-sulfamoylphenyl
3f, **3s** R_2= 1-carboxy-2-phenylethyl

3n-z R_1= styryl
3g, **3t** R_2= 1-carboxy-2-(1H) indol-3-ylethyl
3h, **3u** R_2= 3-oxoisoxazolidin-4-yl
3i, **3v** R_2= 2,4-pyrimidindion-6-yl
3j, **3w** R_2= benzo[*d*]thiazol-2-yl
3k, **3x** R_2= 5-methylbenzo[*d*]thiazol-2-yl
3l, **3y** R_2= 5-nitrobenzo[*d*]thiazol-2-yl

3m, **3z** R_2= 6-amino-10-methyl- acridin-3-yl

Scheme 1: reagents and solvents: **a** Appropriate acid chloride, dry benzene; **b** appropriate amine , $POCl_3$, P_2O_5



Scheme 2: reagents and solvents: **a** Appropriate aldehyde, conc.HCl, methanol; **b** $POCl_3$; **c** appropriate amine, microwave 1000W, 45 sec.