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Synthesis of Tetrahydrobenzothieno[2,3-*d*]pyrimidine and Tetrahydrobenzothieno[3,2-*e*]-[1,2,4]triazolo[4,3-*c*]pyrimidine Derivatives as Potential Antimicrobial Agents

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Abstract

Five series of tetrahydrobenzothieno[2,3-*d*]pyrimidine and tetrahydrobenzothienotriazolopyrimidine derivatives have been synthesized namely: 4-(substituted amino)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines **4a–d**, 4-substituted (methylidenehydrazino)-5,6,7,8-tetrahydro[1]benzothieno-[2,3-*d*]pyrimidines **6a–c**, 4-(3,5-disubstituted pyrazol-1-yl)-5,6,7,8-tetrahydro-[1]benzothieno[2,3-*d*]pyrimidines **7a,b**, 3-substituted-8,9,10,11-tetrahydro-[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidines **8a,b**, *N*-(phenyl or 4-substituted phenyl)-2-(8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]-triazolo[4,3-*c*]pyrimidin-3-ylsulfanyl)acetamides **10a–c**. Preliminary antimicrobial testing revealed that compounds **4a** and **10b** were the most active among the tested compounds against *C. albicans* showing IZ = 22 mm and MIC = MBC = 31.25 µg/ml, with no significant antibacterial activity. Compounds **6b** and **6c** showed the highest antibacterial activity against *S. aureus* (IZ = 21 mm, MIC = 62.5 µg/ml, MBC = 125 µg/ml for **6b**; IZ = 21 mm, MIC = MBC = 125 µg/ml for **6c**). Compounds **4c** and **6c** showed the highest antibacterial potency against *P. aeruginosa* among the tested compounds (IZ = 19–20 mm, MIC = MBC = 62.5 µg/ml). None of the tested compounds showed significant antibacterial activity against E. *coli*.

Keywords

Tetrahydrobenzothieno[2,3-*d*]pyrimidine • Triazolotetrahydrobenzothienopyrimidine • Pyrazole • Synthesis • Antimicrobial activity

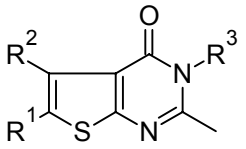
Introduction

Thienopyrimidines are potential bioactive molecules as they are structural analogs of biogenic purines and can be considered as potential nucleic acid antimetabolites. They are characterized by a broad spectrum of biological activities. Thienopyrimidine derivative **Ia** showed significant antibacterial and antimycobacterial activity [1, 2] (Fig.1) while compound **Ib** was proved to possess anti-inflammatory activity [3]. Moreover, compound **II** displayed IC_{50} of 0.019 and 0.83 μg/ml against cyclin dependant kinases Cdk4 and Cdk2, respectively. Thus it might be useful for treatment of hyperproliferative diseases such as cancer [4].

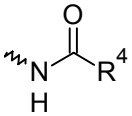
From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. The appearance of qualitatively new properties of an annelated molecule, enlargement of the possibility of varying pharmacophore groups in different positions of the molecule and the ability of the latter to interact with a wider spectrum of receptors adopting various conformations are apparently of crucial importance [5].

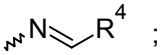
Several 2,3-disubstituted tetrahydrobenzo[*b*]thienopyrimidinone derivatives exhibited both antibacterial and antifungal activities [6]. Compound **IIIa** (Fig. 1), as an example**,** showed comparable potency to Nystatin [6]. On the other hand, compound **IIIb** exhibited more potent analgesic and anti-inflammatory activities with lower ulcerogenic index than the reference standard Diclofenac sodium [7]. In addition, introduction of a pyrazolyl or pyrazolinyl moiety at 4-position of tetrahydrobenzothienopyrimidine nucleus gave compounds **IVa,b** that showed broad spectrum of antimicrobial activity [8, 9].

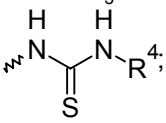
Furthermore, inclusion of thienopyrimidine nucleus within a tetracyclic system afforded large number of bioactive derivatives (Fig.1). Among such compounds, dihydro-naphthothienopyrimidine derivatives **IVc** showed antimicrobial activities against *B. subtilis*, *E. coli*., *Aspergillus niger* and *C. albicans* [10]. Their ester-containing derivatives demonstrated more antimicrobial activities than the corresponding cyano-containing analogs. Tetrahydrobenzothieno[3,2-*e*]imidazo[1,2-*c*]pyrimidine **V** was found to exhibit antibacterial activity comparable to that of Ampicillin against *B. cereus* and *S. typhi* and higher activity than Nystatin against *A. alternata* and *C. corchori* [8]. While octahydrobenzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidine **VI** exhibited a potent blood platelet aggregation inhibition activity *in vivo* [11]. The tetrahydrobenzothieno[3,2-*e*]-triazolo[4,3-*c*]pyrimidine **VII** was almost as potent as Nystatin against *C. albicans* exhibiting MBC of 15.62 μg/mL [12]. On the other hand, tetrahydrobenzothieno[3,2-e]-[1,2,4]triazolo[4,3-a]pyrimidin-5(4*H*)-one **VIII** exhibited significant analgesic activity comparable to that of morphine [13].

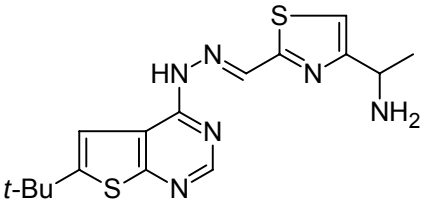


I

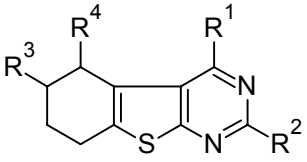
Ia :
R^1 = H; R^2 =  ; X = S or O; R^3 = 

or  ; R^4 = 4-$NO_2C_6H_4$, 2,4-diClC_6H_3, 2,4-diFC_6H_3, 2-F,4-$CF_3C_6H_3$

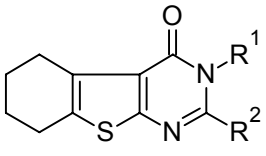
Ib :
$R^1 = R^2 = CH_3$;
R^3 =  ; R^4 = 4-$CH_3C_6H_4$.



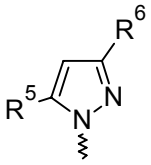
II

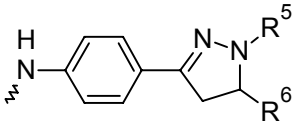


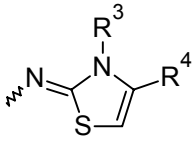
IV

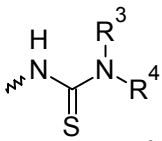


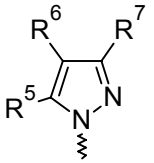
III

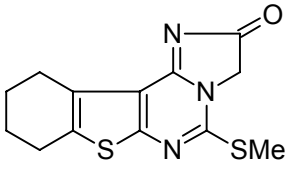
IVa
R^1 =  ; $R^2 = R^3 = R^4$ = H; $R^5 = R^6 = CH_3$

IVb
R^1 =  ; $R^2 = CH_3$; $R^3 = R^4$ = H;
R^5 = 2,4-$(NO_2)_2C_6H_3$; R^6 = 4-$NO_2C_6H_5$.

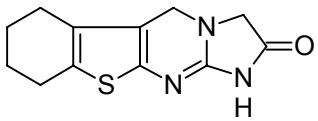
IIIa
R^1 =  ; $R^2 = CH_3$;
$R^3 = C_6H_5$, 4-ClC_6H_4;
$R^4 = C_6H_5$, 4-$NO_2C_6H_4$

IIIb
R^1 =  ; R^2 = SCH_3;
$R^3 = CH_3$, C_2H_5;
$R^4 = CH_3$, C_2H_5; $R^3R^4 = C_4H_8$ (pyrrolidinyl)

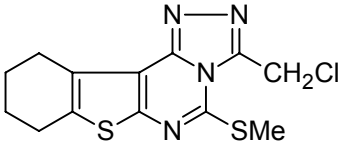
IVc
R^1 =  ; R^2 = H; $R^3R^4 = C_4H_4$; $R^5 = NH_2$;
R^6 = COOEt, CN; R^7 = H, CN



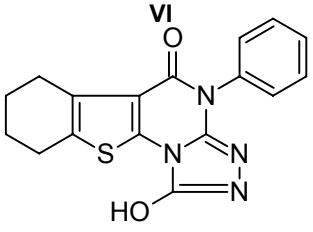
V



VI



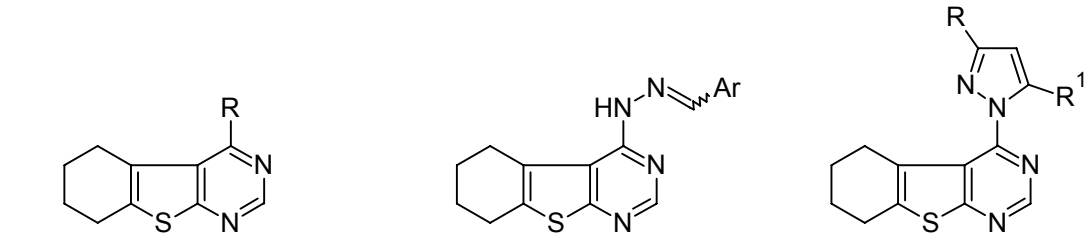
VII



VIII

Fig. 1. Some selected models of thienopyrimidine derivatives possessing various pharmacological activities.

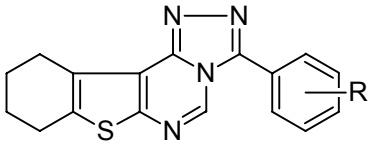
In view of the above and in continuation of our research program concerned with structural modification of certain biologically active heterocyclic nuclei with the purpose of enhancing their biological activity [14–19]. We reported herein the synthesis of several novel analogs of the tetrahydrobenzothienopyrimidine system containing 4-substituted amino (**4a–d**) or 4-substituted methylidenehydrazino (**6a–c**) functions (Fig. 2) in an attempt to improve the antimicrobial profile of compounds containing the tetrahydrobenzothienopyrimidine ring system. Furthermore, the wide range of bioactivities displayed by pyrazoles which include antimicrobial [20–22], antiviral [23, 24] and anticancer [25–27] activities encouraged us to synthesize hybrid analogs that incorporate a 3,5-disubstituted pyrazolyl ring with tetrahydrobenzothienopyrimidine in one framework (**7a,b**). In addition, we prepared two series of the tetracyclic [1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidines substituted at 3-position with substituted phenyl (**8a,b**) or 4-substituted phenylcarbamoylmethylthio (**10a–c**) (Fig.2) moieties to obtain compounds of better antimicrobial activity. The products were *in vitro* screened for their antimicrobial activity.



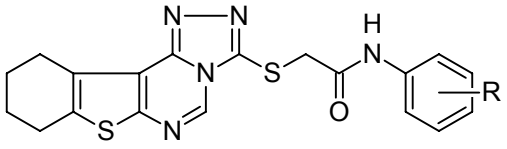
4a–d
R = cyclic secondary amine

6a–c
Ar =
1,3-diphenylpyrazol-5-yl,
4-pyridyl, 3-thienyl

7a,b
R = CH_3, COOEt
R^1 = Ph



8a,b
R = 3-Cl, 4-NO_2



10a–c
R = H, CH_3, Cl

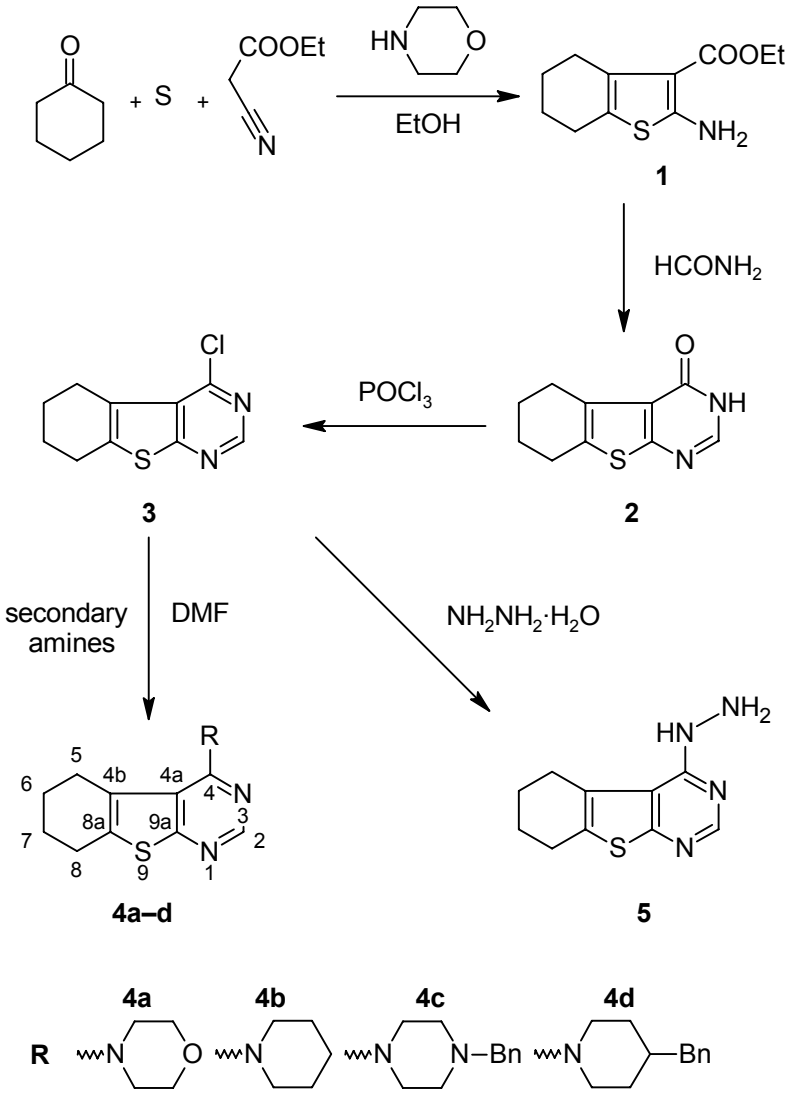
Fig. 2. The proposed design of the newly synthesized thienopyrimidine derivatives **4**, **6–8** and **10**.

Results and Discussion

Chemistry

The synthetic routes of the proposed compounds are outlined in Schemes 1 and 2. 4-(Substituted amino)- and 4-hydrazino-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine derivatives **4a–d** and **5** were synthesized starting from ethyl 2-amino-4,5,6,7-tetrahydro-benzothiophene-3-carboxylate (**1**) (Scheme 1). Compound **1** was readily available through Gewald reaction [28] using cyclohexanone, ethyl cyanoacetate, sulfur and a secondary amine as starting materials. Heating **1** with excess formamide gave 5,6,7,8-tetrahydro-[1]benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one (**2**) [29, 30] which was treated with phosphorous oxychloride to afford the corresponding chloro derivative **3** [31]. Reacting **3** with the

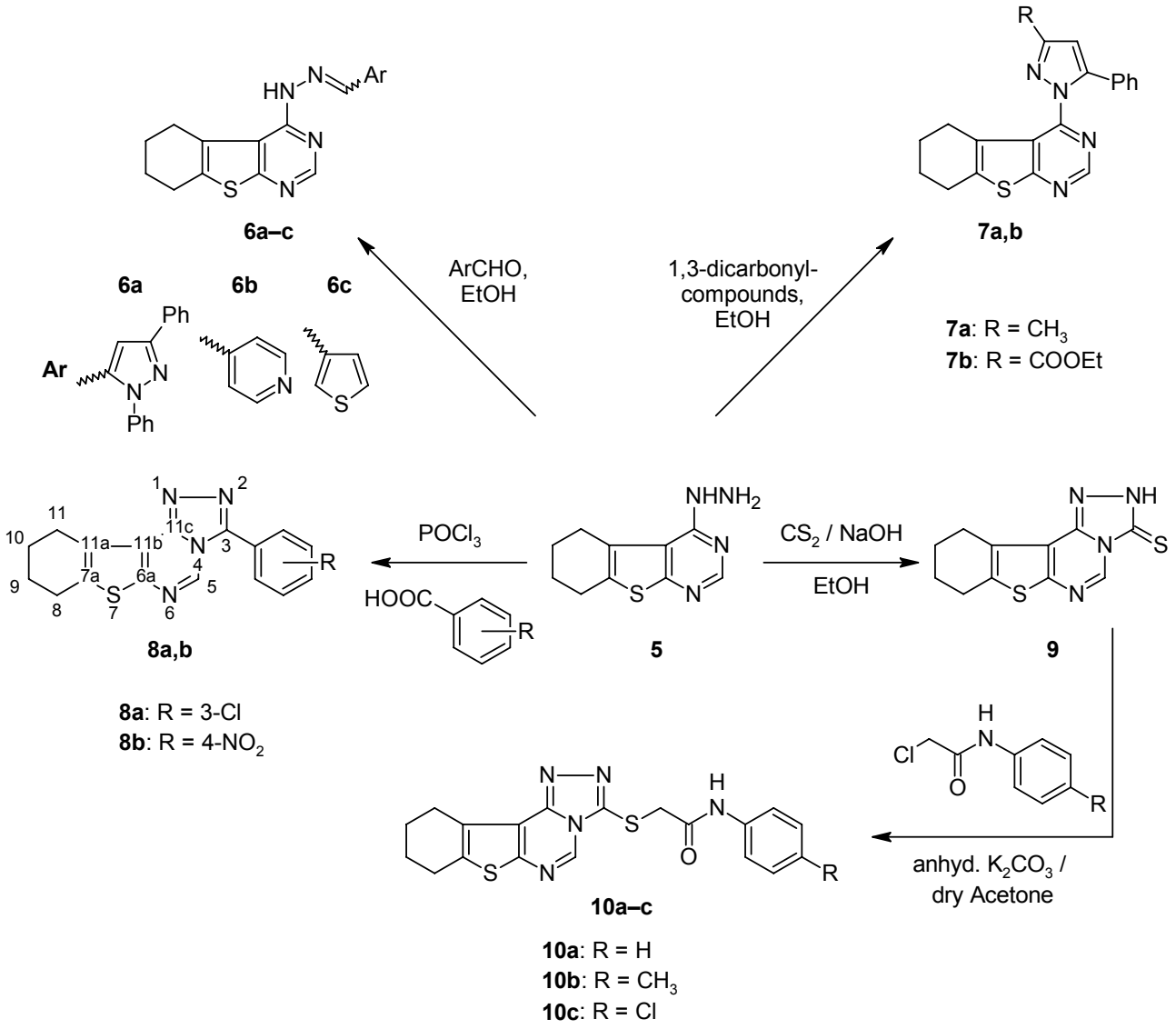
appropriate amine in DMF at 80°C or heating under reflux with hydrazine hydrate [32] led to the target compounds **4a–d** and **5**, respectively.



Sch. 1.

The IR spectra of **4a–d** showed the presence of C-N bands at 1112–1138 cm^{-1} which was not present in the precursor. The ^{1}H NMR spectra of **4a–d** showed signals for the protons of the substituted amino function at the 4-position in addition to the signals for $C_{2, 5–8}$ -H of the tetrahydrobenzothienopyrimidine nucleus at their expected chemical shifts. ^{13}C NMR spectrum of **4a** showed six highly shielded signals due to 8 methylene carbons, Four signals at 22.87, 23.06, 25.89 and 26.85 ppm due to the four cyclohexenyl carbons and two signals at 51.15 and 66.72 ppm due to the other four morpholine carbons indicating the equivalency between morpholine C_3 & C_5 and morpholine C_2 & C_6. The spectrum also showed five highly deshielded signals at 121.38, 127.06, 135.88, 162.03 and 167.75 ppm corresponding to the five quaternary carbons C_{4a}, C_{4b}, C_{8a}, C_{9a} and C_4, respectively. In addition, it showed a signal for the methine carbon at the 2- position at 151.09 ppm. The peaks due to quaternary carbon atoms of the structure disappeared on DEPT

experimentation. The structure of **4a** was confirmed by its ^{13}C NMR coupled spectrum which showed the quaternary carbon C_{4a} as a singlet at 121.38 ppm, while the quaternary carbon C_{4b} appeared as a triplet at 127.06 ppm indicating the presence of a neighboring CH_2. In addition, each of the signals at 162.03 and 167.75 ppm corresponding to quaternary carbons C_{9a} and C_4, respectively appeared as doublet due to meta coupling with C_2-H. ^{13}C NMR spectrum of **4c** showed the signals corresponding to the tetrahydrobenzothienopyrimidine carbons present almost at the same chemical shift as in compound **4a**. In addition, the spectrum showed three highly shielded signals due to the equivalent piperazine C_2 & C_6 and piperazine C_3 & C_5 at 50.12 and 52.60 ppm, respectively and the benzyl-CH_2 at 62.98 ppm. It also showed three signals due to the five methine carbons in the aromatic region indicating the equivalency between the two ortho and the two meta carbons of the phenyl moiety and a deshielded signal for the phenyl C_1 quaternary carbon.



Sch. 2.

The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation. The IR spectrum of **5** showed three NH absorption bands at 3306, 3234 and 3156 cm^{-1}. Heating the hydrazine derivative **5** under reflux with the appropriate aldehydes e.g. 1,3-diphenylpyrazole-5-carbaldehyde, thiophene-3-carbaldehyde or pyridine-4-carbaldehyde in absolute ethanol afforded 4-substituted (methylidene-hydrazino)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine derivatives **6a–c** in good yields (Scheme 2). IR spectra of compounds **6a–c** showed absorption bands for NH group at 3288-3108 cm^{-1}, beside the absorption bands due to C=N, C=C and C−S−C functions. ^{1}H NMR spectra of **6a**, **6b** and **6c** showed a singlet at 9.08, 8.51 and 7.55 ppm, respectively due to N=CH. The spectra of **6a**, **6b** and **6c** also showed a deuterium-exchangeable signal for the NH proton at 11.68, 10.48 and 8.10 ppm, respectively in addition to the other signals at their expected chemical shifts. ^{13}C NMR spectrum of **6b** showed three signals for five methine carbons at 124.54, 135.42 and 148.84 ppm due to pyridine $C_{3,5}$, N=CH and pyridine $C_{2,6}$, respectively and one signal at 145.03 ppm corresponding to the pyridine C_4 quaternary carbon. In addition, the spectrum showed the signals due to tetrahydrobenzothienopyrimidine carbons at their expected chemical shifts. The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation. Condensation of **5** with 1,3-dicarbonyl compounds such as benzoylacetone and ethyl benzoylpyruvate in accordance with the reported procedures [8, 12] afforded 4-(3,5-disubstituted pyrazol-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine derivatives **7a,b**. The IR spectra of compounds **7a,b** lacked the stretching NH bands present in the precursor. The ^{1}H NMR spectra of **7a** and **7b** showed a singlet at 6.40 and 7.35 ppm, respectively due to pyrazolyl-C_4-H in addition to the other signals due to aromatic protons and tetrahydrobenzothienopyrimidine nucleus at their expected chemical shifts.

The ^{13}C NMR spectrum of **7b** showed two high field signals for the methyl and methylene carbons of the O-ethyl group at 14.35 and 44.17 ppm, respectively. It also showed six signals due to the methine carbons of the pyrazolyl C_4 and phenyl C_4, C_2, C_5, C_3 and C_6 at 98.35, 128.29, 128.64, 128.77, 128.88 and 133.81 ppm, respectively and the signals characteristic for the four deshielded quaternary carbons, phenyl C_1, pyrazolyl C_5, pyrazolyl C_3 and C=O at 136.07, 142.42, 154.56 and 196.52 ppm, respectively. In addition, the spectrum showed the signals due to tetrahydrobenzothienopyrimidine carbons at their expected chemical shifts. The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation. The preparation of 3-substituted-8,9,10,11-tetrahydro-[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **8a,b**, in the present study, was achieved via one pot reaction of the 4-hydrazino derivative **5** with the appropriate carboxylic acid in presence of phosphorous oxychloride. The IR spectra of compounds **8a,b** lacked the absorption bands due to NH functions present in the precursor. The ^{1}H NMR spectra of compound **8a,b** showed signals due to aromatic protons in addition to the other signals for the tetrahydrobenzothienotriazolopyrimidine-$C_{5,\ 8–11}$-protons at their expected chemical shifts. ^{13}C NMR spectrum of **8a** showed four signals for the highly shielded methylene carbons C_{10}, C_9, C_{11} and C_8 at 22.30, 23.10, 25.41 and 25.67 ppm, respectively. The five methine carbons of the 3-chlorophenyl C_6, C_2, C_4, C_5 and the tetrahydrobenzothienotriazolopyrimidine C_5 resonated as five signals at 125.78, 127.85, 130.14, 130.62 and 135.39 ppm, respectively. In addition to eight signals due to the eight quaternary carbons C_{11b}, C_{11a}, phenyl C_1, phenyl C_3, C_{7a}, C_{6a}, C_3 and C_{11c} at 120.56, 129.40, 132.16, 134.87, 139.29, 149.70, 153.65 and 163.91 ppm, respectively.

The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation. Furthermore, the hydrazine derivative **5** was reacted with carbon disulfide and NaOH in ethanol to afford 8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]-triazolo[4,3-*c*]pyrimidine-3(2*H*)-thione (**9**) [34], which was alkylated by stirring with the appropriate 4-substituted chloroacetanilide and anhydrous K_2CO_3 in dry acetone at room temperature to give *N*-(phenyl or 4-substituted phenyl)-2-(8,9,10,11-tetrahydro-[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3-ylsulfanyl)acetamides derivatives **10a–c**. The IR spectra of compounds **10a–c** showed absorption bands due to NH function at 3383-3114 and C=O moiety at 1676–1685 cm^{-1}. The ^1H NMR spectra of compounds **10a**, **10b** and **10c** showed a singlet at 4.04, 4.03 and 4.13 ppm, respectively for SCH_2 protons. The spectra of **10a**, **10b** and **10c** also showed a deuterium-exchangeable singlet at 10.19, 10.11 and 10.18 ppm, respectively for NH protons. In addition to the other signals at their expected chemical shifts. ^{13}C NMR spectrum of **10b** showed in addition to the signals due to the tetrahydrobenzothienotriazolopyrimidine carbons at their expected chemical shifts, two shielded signals due to CH_3 and SCH_2 at 20.98 and 39.20 ppm, respectively and two signals at 119.66 and 129.67 ppm due to four methine carbons of the 4-tolyl moiety indicating the equivalency between the two ortho and two meta carbons. The spectrum also showed two signals corresponding to the quaternary C_4 and C_1 of the phenyl group at 133.12 and 136.55 ppm respectively while the most deshielded signal at 166.10 ppm was characterized for C=O. The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation.

Antimicrobial activity

Tab. 1. The inhibition zones (IZ) in mm diameter.

Compound No.	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	–	15	16	22
4b	18	15.5	17	20
4c	–	16	19	16
4d	–	16	16	16
6a	–	13	–	18
6b	21	16	–	20
6c	21	17	20	20
7a	–	15	16	15
7b	–	15	16	18
8b	–	16	–	20
10a	–	16	–	17
10b	–	17	–	22
10c	–	17	–	20
Ampicillin	25	28	32	–
Clotrimazole	–	–	–	35

(–) no inhibition zone

Most of the prepared compounds were *in vitro* evaluated for their antimicrobial activity using the cup diffusion technique [35] against *Staphylococcus aureus* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria in

addition to *Candida albicans* as fungi. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the active compounds, having inhibition zones (IZ) ≥ 18mm, were studied and compared with Ampicillin and Clotrimazole as reference antibiotics. The compounds displayed variable activities against the four tested microorganisms (Table 1, 2).

The data indicate that, compounds **4a** and **10b** were the most active among the tested compounds against *C. albicans* showing IZ = 22 mm and MIC = MBC = 31.25 µg/ml, or about one-sixth the activity of Clotrimazole with no significant antibacterial activity. In addition, compounds **4b**, **6b**, **6c**, **8b** and **10c** showed MIC = MBC = 31.25 µg/ml against *C. albicans* but their IZ = 20 mm. Replacement of the morpholino moiety at 4-position of the tetrahydrobenzothienopyrimidine nucleus in **4a** by a piperidine moiety gave compound **4b** that showed nearly the same activity of **4a** against *C. albicans* (IZ = 20 mm, MIC = MBC = 31.25 µg/ ml). In addition, compound **4b** showed activity against *S. aureus* (IZ = 18 mm, MIC = MBC = 62.5 µg/ml). On the other hand, replacement of the morpholino moiety in **4a** by 4-benzylpiperazine in **4c** led to increase the antibacterial potency of **4c** against *P. aeruginosa* showing IZ = 19 mm, MIC = MBC = 62.5 µg/ml and reduced the antifungal activity.

Tab. 2. MIC and MBC in µg/ml of the most active compounds.

Compound No.	*S. aureus*		*E. coli*		*P.aeruginosa*		*C. albicans*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
4a							31.25	31.25
4b	62.5	62.5					31.25	31.25
4c					62.5	62.5		
6a							125	125
6b	62.5	125					31.25	31.25
6c	125	125			62.5	62.5	31.25	31.25
7b							125	125
8b							31.25	31.25
10b							31.25	31.25
10c							31.25	31.25
Ampicillin	5		10		25			
Clotrimazole							5	

Introduction of a methylidenehydrazino linkage at the 4-position of the tetrahydrobenzothienopyrimidine nucleus as in **6b** enhanced the antibacterial activity against *S. aureus* (IZ = 21 mm, MIC = 62.5 µg/ml and MBC = 125 µg/ml) and retained the same antifungal potency as compound **4b** (IZ = 20 mm, MIC = MBC = 31.25 µg/ml). Replacement of the pyridinyl function present in **6b** by a thiophene moiety led to compound **6c** which was equipotent with **6b** against *C. albicans* (IZ = 20 mm, MIC = MBC = 31.25 µg/ml), while the potency of **6c** against *S. aureus* as compaired with **6b** was diminished (IZ = 21 mm, MIC = MBC = 125 µg/ml). However, **4c** and **6c** showed the highest antibacterial potency against *P. aeruginosa* among the tested compounds with IZ = 19 and 20 mm, respectively and MIC = MBC = 62.5 µg/ml or nearly one-third the activity of Ampicillin.

Experimental

Chemistry

Melting points were determined in open-glass capillaries on a Gallen–Kamp melting point apparatus and are uncorrected. The IR spectra (KBr) were recorded on a Perkin-Elmer 1430 spectrophotometer. The ^{1}H NMR spectra were determined on a JNM-LA 400 FT NMR system (400 MHz), Faculty of Science, Assuit University and on Jeol (500 MHz), Faculty of Science, Alexandria Univesity, using TMS as internal standard. The chemical shifts are given in ppm δ values (s, singlet; d, doublet; t, triplet and m, multiplet). ^{13}C NMR spectra were determined on Jeol (125 MHz), Faculty of Science, Alexandria Univesity, using TMS as internal standard. Mass spectra were run on a Finnigan mass spectrometer model S SQ/7000 (70 ev), Faculty of Science, Cairo University. Microanalysis were performed at the Microanalytical Unit, Faculty of Science, Cairo University and The Microanalytical Unit, Faculty of Science, Assuit University, A. R. Egypt. The results of the microanalysis were within ±0.4% of the calculated values. Follow up the reactions and checking the homogeneity of the compounds were made by ascending TLC run on silica gel G (Merck 60) coated glass plates. The spots were visualized, by exposure to iodine vapour or UV-Lamp at λ 254 nm for few seconds.

Compounds **2** [29, 30], **3** [31], and **9** [34] were prepared according to the published procedures. Compound **8a** has been previously synthesized in a two step procedure [32, 33] through condensation of the 4-hydrazino derivative **5** [32] with 3-chloro-benzaldehyde and further ring closure of the produced Schiff 's base using bromine in acetic acid at 45°C.

General procedure for the preparation of 4-(substituted amino)-5,6,7,8-tetrahydro-[1]benzothieno[2,3-d]pyrimidines (4a–d)

A mixture of **3** (0.67 gm, 3 mmol) and the appropriate amine (**a–d**) (6 mmol) in 5 ml DMF was stirred at (60–80°C) for 6 h, cooled, added to ice cold water and the obtained product was filtered, washed with water and crystallized from the appropriate solvent to afford **4a–d** in 59–70% yields.

*4-(Morpholin-4-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**4a**)*

Pale brown crystals (59%, EtOH); mp: 98–99°C; IR (KBr) ν (cm^{-1}): 1554, 1532, 1501 (C=N, C=C), 1264, 1069 (C-S-C), 1251, 1026 (C-O-C), 1112 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.76–1.79 (m, 2H, tetrahydrobenzothienopyrimidine C_6-H), 1.89–1.91 (m, 2H, tetrahydrobenzothienopyrimidine C_7-H), 2.85 (t, *J* = 5.6 Hz, 2H, tetrahydrobenzothienopyrimidine C_5-H), 2.88 (t, *J* = 5.6 Hz, 2H, tetrahydrobenzothienopyrimidine C_8-H), 3.36 (t, *J* = 5.0 Hz, 4H, morpholino $C_{3,5}$-H), 3.83 (t, *J* = 5.0 Hz, 4H, morpholino $C_{2,6}$-H), 8.49 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H), ^{13}C NMR (normal/DEPT-135)(125 MHz, $CDCl_3$) δ (ppm): 22.87 (-ve, C_6), 23.06 (-ve, C_7), 25.89 (-ve, C_5), 26.85 (-ve, C_8), 51.15 (-ve, morphlino $C_{3,5}$), 66.72 (-ve, morpholino $C_{2,6}$), 121.38 (ab, C_{4a}), 127.06 (ab, C_{4b}), 135.88 (ab, C_{8a}), 151.09 (+ve, C_2), 162.03 (ab, C_{9a}), 167.75 (ab, C_4).

*4-(Piperidin-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**4b**)*

Off-white powder (68%, $CHCl_3$/EtOH); mp: 79–80°C; IR (KBr) ν (cm^{-1}): 1554, 1529, 1500 (C=N, C=C), 1274, 1057 (C-S-C), 1130 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.62–

1.81 (m, 6H, piperidine $C_{3,4,5}$-H), 1.86–1.95 (m, 4H, tetrahydrobenzothienopyrimidine $C_{6,7}$-H), 2.86 (t, *J* = 6.1 Hz, 2H, tetrahydrobenzothienopyrimidine C_5-H), 2.90 (t, *J* = 6.1 Hz, 2H, tetrahydrobenzothienopyrimidine C_8-H), 3.36–3.40 (m, 4H, piperidine $C_{2,6}$-H), 8.48 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H).

*4-(4-Benzylpiperazin-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**4c**)*

Brown clusters of needles (70%, EtOH/H_2O); mp: 134–135°C; IR (KBr) ν (cm^{-1}): 1552, 1528, 1498 (C=N, C=C), 1263, 1071 (C-S-C), 1138 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.76-1.80 (m, 2H, tetrahydrobenzothienopyrimidine C_6-H), 1.89–1.93 (m, 2H, tetrahydrobenzothienopyrimidine C_7-H), 2.57–2.69 (m, 4H, tetrahydrobenzothienopyrimidine $C_{5,8}$-H), 2.84–2.88 (m, 4H, piperazine $C_{3,5}$-H), 3.41–3.49 (m, 4H, piperazine $C_{2,6}$-H), 3.59 (s, 2H, CH_2), 7.27 (t, *J* = 6.9 Hz, 1H, C_6H_5-C_4-H), 7.32 (d, *J* = 6.9 Hz, 2H, C_6H_5-$C_{2,6}$-H), 7.36 (t, *J* = 6.9 Hz, 2H, C_6H_5-$C_{3,5}$-H), 8.50 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H), ^{13}C NMR (normal/DEPT-135)(125 MHz, $CDCl_3$) δ (ppm): 22.93 (-ve, C_6), 23.09 (-ve, C_7), 25.91 (-ve, C_5), 26.90 (-ve, C_8), 50.12 (-ve, piperazine $C_{2,6}$), 52.60 (-ve, piperazine $C_{3,5}$), 62.98 (-ve, phenyl-CH_2), 121.37 (ab, C_{4a}), 127.29 (+ve, C_6H_5-C_4), 127.70 (ab, C_{4b}), 128.56 (+ve, C_6H_5-$C_{2,6}$), 129.53 (+ve, C_6H_5-$C_{3,5}$), 135.22 (ab, C_{8a}), 135.30 (ab, C_6H_5-C_1), 151.54 (+ve, C_2), 162.00 (ab, C_{9a}), 168.41 (ab, C_4).

*4-(4-Benzylpiperidin-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**4d**)*

Brown crystals (65%, EtOH); mp: 142–143°C; IR (KBr) ν (cm^{-1}): 1559, 1525, 1502 (C=N, C=C), 1246, 1048 (C-S-C), 1125 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.40–1.48 (m, 4H, piperidine $C_{3,5}$-H), 1.75–1.81 (m, 4H, tetrahydrobenzothienopyrimidine $C_{6,7}$-H), 1.89–1.94 (m, 1H, piperidine C_4-H), 2.60–2.61 (d, *J* = 6.9 Hz, 2H, CH_2), 2.84–2.91 (m, 4H, tetrahydrobenzothienopyrimidine $C_{5,8}$-H). 3.79-3.82 (m, 4H, piperidine $C_{2,6}$-H), 7.17 (d, *J* = 7.6 Hz, 2H, C_6H_5-$C_{2,6}$-H), 7.20 (t, *J* = 7.6 Hz, 1H, C_6H_5-C_4-H), 7.29 (t, *J* = 7.6 Hz, 2H, C_6H_5-$C_{3,5}$-H), 8.49 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H).

4-Hydrazino-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (5)

Compound **5** was prepared from 4-chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]-pyrimidine (**3**) [31] according to the previously described method [32]. Yield (79%); mp: 175°C (reported 180–181°C) [32]. IR (cm^{-1}): 3306, 3234, 3156 (NH), 1627 (C=N), 1566 (δ NH), 1502 (C=C), 1297, 1069 (C-S-C).

General procedure for the preparation of 4-substituted (methylidenehydrazino)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines (6a–c)

To a solution of **5** (0.44 g, 2 mmol) in 10 ml absolute ethanol the appropriate aldehyde (2 mmol) was added and the mixture was refluxed for 2 h. The solid obtained, was filtered off and crystallized from the appropriate solvent to afford **6a–c** in 68–75% yields.

*4-{2-[(1,3-Diphenyl-1*H*-pyrazol-5-yl)methylidene]hydrazino}-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**6a**)*

Yellow crystals (75%, EtOH); mp: 220°C; IR (KBr) ν (cm^{-1}): 3110 (NH), 1625 (C=N), 1597, 1504 (C=C), 1566 (δ NH), 1242, 1060 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.71-1.77 (m, 4H, tetrahydrobenzothienopyrimidine $C_{6,7}$-H), 2.67–2.99 (m, 4H, tetrahydrobenzothienopyrimidine $C_{5,8}$-H), 7.36 (t, *J* = 7.6 Hz, 1H, N-C_6H_5-C_4-H), 7.44 (t, *J* = 7.6 Hz, 1H, C_6H_5-C_4-H), 7.51 (t, *J* = 7.6 Hz, 2H, C_6H_5-$C_{3,5}$-H), 7.55 (t, *J* = 7.6 Hz, 2H, N-C_6H_5-$C_{3,5}$-

H), 7.67 (d, *J* = 7.6 Hz, 2H, N-C_6H_5-$C_{2,6}$-H), 7.78 (s, 1H, pyrazolyl C_4-H), 7.88 (d, *J* = 7.6 Hz, 2H, C_6H_5-$C_{2,6}$-H), 8.31 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H), 9.08 (s, 1H, N=CH), 11.68 (s, 1H, NH, D_2O exchangeable).

*4-[2-(Pyridin-4-ylmethylidene)hydrazino]-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**6b**)*

Yellow needles (68%, EtOH); mp: 190–192°C; IR (KBr) ν (cm^{-1}): 3288, 3115 (NH), 1618 (C=N), 1577, 1527 (C=C), 1550 (δ NH), 1248, 1052 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.80–1.81 (m, 4H, tetrahydrobenzothienopyrimidine $C_{6,7}$-H), 2.71–2.73 (m, 2H, tetrahydrobenzothienopyrimidine C_5-H), 2.97–2.99 (m, 2H, tetrahydrobenzothienopyrimidine C_8-H), 7.50–8.40 (m, 2H, pyridine $C_{3,5}$-H), 8.51 (s, 1H, N=CH), 8.73 (d, 2H, pyridine $C_{2,6}$-H), 8.82 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H), 10.48 (s, 1H, NH, D_2O exchangeable), ^{13}C NMR (normal/DEPT-135)(125 MHz, $CDCl_3$) δ (ppm): 22.50 (-ve, C_6), 22.86 (-ve, C_7), 23.24 (-ve, C_5), 27.03 (-ve, C_8), 118.98 (ab, C_{4a}), 124.54 (+ve, pyridine $C_{3,5}$), 130.96 (ab, C_{4b}), 133.19 (ab, C_{8a}), 135.42 (+ve, N=CH), 145.03 (ab, pyridine C_4), 148.84 (+ve, pyridine $C_{2,6}$), 149.51 (ab, C_{9a}), 149.87 (+ve, C_2), 150.63 (ab, C_4).

*4-[2-(Thiophen-3-ylmethylidene)hydrazino]-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**6c**)*

Yellow clusters of needles (70%, dioxane/H_2O); mp: 193–194°C; IR (KBr) ν (cm^{-1}): 3170, 3108 (NH), 1620 (C=N), 1566, 1511 (C=C), 1539 (δ NH), 1249, 1081 (C-S-C); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.84–1.93 (m, 4H, tetrahydrobenzothienopyrimidine $C_{6,7}$-H), 2.77–2.83 (m, 2H, tetrahydrobenzothienopyrimidine C_5-H), 3.03–3.08 (m, 2H, tetrahydrobenzothienopyrimidine C_8-H), 7.25 (s, 1H, thiophene C_2-H), 7.28–7.29 (m, 1H, thiophene C_5-H), 7.55 (s, 1H, N=CH), 7.59 (d, *J* = 4.5 Hz, 1H, thiophene C_4-H), 8.10 (s, 1H, NH, D_2O exchangeable), 8.33 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H).

General procedure for the preparation of 4-(3,5-disubstituted-pyrazol-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidines (7a,b)

A mixture of **5** (0.44 g, 2 mmol) and the appropriate 1,3-dicarbonyl compounds (2 mmol) was refluxed in 10 ml ethanol for 2 h and then cooled. The obtained product was filtered, dried and crystallized from the appropriate solvent to give **7a,b** in 65-66% yields.

*4-(3-Methyl-5-phenyl-1*H*-pyrazol-1-yl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (**7a**)*

Yellow needles (66%, $CHCl_3$/EtOH); mp: 150–152°C; IR (KBr) ν (cm^{-1}): 1566, 1499 (C=N, C=C), 1265, 1071 (C-S-C), 1138 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.68–1.73 (m, 2H, tetrahydrobenzothienopyrimidine, C_6-H), 1.74–1.80 (m, 2H, tetrahydrobenzothienopyrimidine C_7-H), 2.37 (t, *J* = 6.0 Hz, 2H, tetrahydrobenzothienopyrimidine C_5-H), 2.50 (s, 3H, CH_3), 2.88 (t, *J* = 6.0 Hz, 2H, tetrahydrobenzothienopyrimidine C_8-H), 6.40 (s, 1H, pyrazolyl C_4-H), 7.20-7.26 (m, 5H, Ar-H), 8.80 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H).

*Ethyl 5-phenyl-1-(5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-3-carboxylate (**7b**)*

Orange fine needles (65%, EtOH); mp 191–192°C; IR (KBr) ν (cm^{-1}): 1683 (C=O), 1619 (C=N), 1578, 1533 (C=C), 1279, 1079 (C-S-C), 1250, 1025 (C-O-C), 1124 (C-N); ^{1}H NMR

(500 MHz, $CDCl_3$) δ (ppm): 1.23 (t, *J* = 6.9 Hz, 3H, CH_2-*CH_3*), 1.92–1.95 (m, 2H, tetrahydrobenzothienopyrimidine C_6-H), 1.99-2.03 (m, 2H, tetrahydrobenzothienopyrimidine C_7-H), 2.86–2.88 (m, 2H, tetrahydrobenzothienopyrimidine C_5-H), 3.13–3.15 (m, 2H, tetrahydrobenzothienopyrimidine C_8-H), 3.82–3.89 (q, *J* = 6.9 Hz, 2H, *CH_2*-CH_3), 7.35 (s, 1H, pyrazolyl C_4-H), 7.49 (t, *J* = 7.65 Hz, 1H, C_6H_5-C_4-H), 7.55–7.61 (m, 2H, C_6H_5-$C_{3,5}$-H), 7.97 (d, *J* = 7.65 Hz, 2H, C_6H_5-$C_{2,6}$-H), 8.70 (s, 1H, tetrahydrobenzothienopyrimidine C_2-H), ^{13}C NMR (normal/DEPT-135)(125 MHz, $CDCl_3$) δ (ppm): 14.35 (+ve, CH_3), 22.33 (-ve, C_6), 22.49 (-ve, C_7), 25.85 (-ve, C_5), 25.92 (-ve, C_8), 44.17 (-ve, O-CH_2), 98.35 (+ve, pyrazolyl C_4), 116.19 (ab, C_{4a}), 125.59 (ab, C_{4b}), 128.29 (+ve, C_6H_5-C_4), 128.64 (+ve, C_6H_5-C_2), 128.77 (+ve, C_6H_5-C_5), 128.88 (+ve, C_6H_5-C_3), 133.81 (+ve, C_6H_5-C_6), 136.07 (ab, C_6H_5-C_1), 137.99 (ab, C_{8a}), 142.42 (ab, pyrazolyl C_5), 149.36 (+ve, C_2), 154.56 (ab, pyrazolyl C_3), 162.31 (ab, C_{9a}), 163.13 (ab, C_4), 196.52 (ab, C=O).

General procedure for the preparation of 3-substituted-8,9,10,11-tetrahydro-[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidines (8a,b)

A mixture of **5** (0.44 g, 2 mmol) and the appropriate carboxylic acid (2 mmol) in 5 ml phosphorous oxychloride was refluxed for 5 h and left to cool. The product was poured into crushed ice while stirring, filtered washed with sodium bicarbonate solution then with water, left to dry and crystallized from the appropriate solvent to afford **8a,b** in 70–85% yields.

*3-(3-Chlorophenyl)-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]-pyrimidine (**8a**)*

Off-white needles (70%, dioxane/H_2O); mp: 198–199°C (Ref. [33] 216–217°C); IR (KBr) ν (cm^{-1}): 1614 (C=N), 1571, 1508, 1494 (C=C), 1241, 1077 (C−S−C), 894 (C−Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.99–2.03 (m, 4H, tetrahydrobenzothienotriazolopyrimidine-$C_{9,10}$-H), 2.96 (t, *J* = 6.0 Hz, 2H, tetrahydrobenzothienotriazolopyrimidine-C_{11}-H), 3.27 (t, *J* = 6.0 Hz, 2H, tetrahydrobenzothienotriazolopyrimidine-C_8-H), 7.45–7.50 (m, 2H, 3- Cl-C_6H_4-$C_{5,6}$-H), 8.23 (dt, *J* = 6.0, 2.1 Hz, 1H, 3-Cl-C_6H_4-C_4-H), 8.34 (s, 1H, 3-Cl-C_6H_4-C_2-H), 9.15 (s, 1H, tetrahydrobenzothienotriazolopyrimidine-C_5-H), ^{13}C NMR (normal/DEPT-135)(125 MHz, $CDCl_3$) δ (ppm): 22.30 (-ve, C_{10}), 23.10 (-ve, C_9), 25.41 (-ve, C_{11}), 25.67 (-ve, C_8), 120.56 (ab, C_{11b}), 125.78 (+ve, 3-Cl-C_6H_4-C_6), 127.85 (+ve, 3-Cl-C_6H_4-C_2), 129.40 (ab, C_{11a}), 130.14 (+ve, 3-Cl-C_6H_4-C_4), 130.62 (+ve, 3-Cl-C_6H_4-C_5), 132.16 (ab, 3-Cl-C_6H_4-C_1), 134.87 (ab, 3-Cl-C_6H_4-C_3), 135.39 (+ve, C_5), 139.29 (ab, C_{7a}), 149.70 (ab, C_{6a}), 153.65 (ab, C_3), 163.91 (ab, C_{11c}), MS m/z (%): 342 (42)(M^++2), 340 (100)(M^+), 314 (12)(M^++2 − C_2H_4),312 (25)(M^+ − C_2H_4), 175 (7)(M^+ − 4-ClC_6H_4, − C_4H_8, + 2H).

*3-(4-Nitrophenyl)-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine (**8b**)*

Yellowish crystals (85%, dioxane); mp: 258–260°C; IR (KBr) ν (cm^{-1}): 1613 (CN), 1518, 1459 (C=C), 1550, 1335 (NO_2), 1240, 1011 (C−S−C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40–2.50 (m, 4H, tetrahydrobenzothienotriazolopyrimidine $C_{9,10}$-H), 3.66 (t, *J* = 4.5 Hz, 2H, tetrahydrobenzothienotriazolopyrimidine C_{11}-H), 3.86 (t, *J* = 4.5 Hz, 2H, tetrahydrobenzothienotriazolopyrimidine C_8-H), 8.60 (s, 1H, tetrahydrotriazolobenzothieno-pyrimidine C_5-H), 8.84 (d, *J* = 8.8 Hz, 2H, 4-NO_2-C_6H_4, $C_{2,6}$-H), 8.98 (d, *J* = 8.8 Hz, 2H, 4-NO_2-C_6H_4, $C_{3,5}$-H).

General procedure for the preparation of N-*(phenyl or 4-substituted phenyl)-2-(8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3-ylsulfanyl)acetamides (10a–c)*

To a mixture of **9** (0.52 g, 2 mmol) and anhydrous K_2CO_3 (0.28 g, 2 mmol) in 20 ml dry acetone, the appropriate 4-substituted chloroacetanilide (2 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was poured into crushed ice and the product was filtered, washed with water, dried and crystallized from the appropriate solvent to give **10a–c** in 68–74% yields.

N-*Phenyl-2-(8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3-ylsulfanyl)acetamide (**10a**)*

Yellow crystals (74%, dioxane/H_2O); mp: 190–191°C; IR (KBr) ν (cm^{-1}):= 3261, 3199, 3137 (NH), 1685 (C=O), 1607, 1551, 1518, 1496 (δ NH, C=N, C=C), 1242, 1075 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.82-1.88 (m, 4H, benzothienotriazolopyrimidine $C_{9,10}$-H), 2.84-2.89 (m, 2H, benzothienotriazolopyrimidine C_{11}-H), 2.99-3.10 (m, 2H, benzothienotriazolopyrimidine C_8-H), 4.04 (s, 2H, CH_2), 7.00 (t, *J* = 7.6 Hz, 1H, C_6H_5-C_4-H), 7.23 (t, *J* = 7.6 Hz, 2H, C_6H_5-$C_{3,5}$-H), 7.42 (d, *J* = 7.6 Hz, 2H, C_6H_5-$C_{2,6}$-H), 9.15 (s, 1H, benzothienotriazolopyrimidine C_5-H), 10.19 (s, 1H, NH, D_2O exchangeable).

N-*(4-Methylphenyl)-2-(8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]-pyrimidin-3-ylsulfanyl)acetamide (**10b**)*

Off-white crystals (70%, EtOH); mp: 229–230°C; IR (KBr) ν (cm^{-1}): 3383, 3260, 3123 (NH), 1676 (C=O), 1645 (C=N), 1606, 1511 (C=C), 1548 (δ NH), 1244, 1040 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.77–1.87 (m, 4H, benzothienotriazolopyrimidine $C_{9,10}$-H), 2.21 (s, 3H, CH_3), 2.85–2.95, (m, 2H, benzothienotriazolopyrimidine C_{11}-H), 2.96–3.10 (m, 2H, benzothienotriazolopyrimidine C_8-H), 4.03 (s, 2H, CH_2), 7.05 (d, *J* = 8.3 Hz, 2H, 4-CH_3-C_6H_4-$C_{3,5}$-H), 7.31 (d, *J* = 8.3 Hz, 2H, 4-CH_3-C_6H_4-$C_{2,6}$-H), 9.16 (s, 1H, triazolo-benzothienopyrimidine C_5-H), 10.11 (s, 1H, NH, D_2O exchangeable), ^{13}C NMR (normal/DEPT-135)(125 MHz, DMSO-d_6) δ (ppm): 20.98 (+ve, CH_3), 22.24 (-ve, C_{10}), 23.20 (-ve, C_9), 25.34 (-ve, C_{11}), 25.62 (-ve, C_8), 39.20 (-ve, S-CH_2), 118.16 (ab, C_{11b}), 119.66 (+ve, 4-CH_3-C_6H_4-$C_{2,6}$), 129.60 (ab, C_{11a}), 129.67 (+ve, 4-CH_3-C_6H_4-$C_{3,5}$), 133.12 (ab, 4-CH_3-C_6H_4-C_4), 134.97 (+ve, C_5), 136.55 (ab, 4-CH_3-C_6H_4-C_1), 138.77 (ab, C_{7a}), 140.94 (ab, C_{6a}), 147.59 (ab, C_3), 150.05 (ab, C_{11c}), 166.10 (ab, C=O).

N-*(4-Chlorophenyl)-2-(8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]-pyrimidin-3-ylsulfanyl)acetamide (**10c**)*

Yellow crystals (68%, EtOH); mp: 256–257°C; IR (KBr) ν (cm^{-1}): 3260, 3190, 3114 (NH), 1679 (C=O), 1645 (C=N), 1608, 1518 (C=C), 1548 (δ NH), 1243, 1074 (C-S-C); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.73–1.98 (m, 4H, benzothienotriazolopyrimidine $C_{9,10}$-H), 2.92–3.01 (m, 2H, benzothienotriazolopyrimidine C_{11}-H), 3.20–3.30 (m, 2H, triazolobenzo-thienopyrimidine C_8-H), 4.13 (s, 2H, CH_2), 7.25 (d, *J* = 8.4 Hz, 2H, 4-Cl-C_6H_4-$C_{2,6}$-H), 7.57 (d, *J* = 8.4 Hz, 2H, 4-Cl-C_6H_4-$C_{3,5}$-H), 8.71 (s, 1H, benzothienotriazolopyrimidine C_5-H), 10.18 (s, 1H, NH, D_2O exchangeable). MS m/z (%) : 431 (20)(M^++2), 429 (38)(M^+), 272 (100)(M^+ − 4-ClC_6H_4NH, − C=O, − 3H).

In vitro antimicrobial screening

Inhibition zone measurement

Using the cup diffusion technique [35], the products as 1 mg/ml solution in DMF were *in vitro* evaluated for antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8735) and *Pseudomonas aeruginosa* (ATCC 9027) and for antifungal activity against *Candida albicans* (ATCC 10231).

The activities were estimated as zones of inhibition in mm diameter (Table 1). A 5 µg/ml solution of Ampicillin and a solution containing 0.01% of Clotrimazole in DMF were used as reference standards. Each cup (8 mm in diameter) received 0.1 ml of the test compound or reference standard solution. DMF did not show any inhibition zones.

Minimal inhibitory concentration (MIC) measurement

The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 18 mm) was then evaluated using the two fold serial dilution technique [36]. Two fold serial dilutions of the test compounds and reference drugs solutions were prepared using the proper nutrient broth. The final concentration of the solutions varied between 500 and 7.81 µg/ml with the concentration of DMF not exceeding 2.5%. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 hours for bacteria and 48 hours for fungi (about 1×10^6 cells/ml), each 5 ml received 0.1 ml of the above inoculum and were incubated at 37 °C for 48 hours. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC) (Table 2).

Minimal bacteriostatic concentration (MBC) measurement

To determine the minimum bactericidal concentration (MBC), a loopful from the tube not showing visible growth in the MIC experiment was spread over a quarter of Muller-Hinton agar plate. After incubation for 18 hours, the plates were examined for growth. The tube containing the lowest concentration of the test compound that prevented growth on subculture plates were judged to contain the MBC of that compound for the respective test organism (Table 2).

Conclusions

The overall results indicated that, most of the tested compounds (**4a**, **4b**, **6b**, **6c**, **8b**, **10b** and **10c**) showed antifungal activity against *C. albicans*, few compounds exhibited anti-bacterial activity against *S. aureus* (**4b**, **6b** and **6c**) and *P. aeruginosa* (**4c** and **6c**) while none of the tested compounds showed significant antibacterial activity against *E. coli.*

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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