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Synthesis and antimicrobial activity of some new tetrahydrothienopyridopyrimidine derivatives

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ABSTRACT

A series of novel 3-amino-7-(phenylmethyl)-2-phenylamino-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno[2,3 d]pyrimidine were prepared by simple, appropriate and shortest synthetic route in which piperidine , ethyl cyanoacetate , sulfur, morpholine were treated with substituted anilines in the presence of dry pyridine. Structures of the newly synthesized compounds were assigned on the basis of FTIR, [1]HNMR and Mass spectral studies. The newly synthesized compounds were tested for their in vitro antibacterial and antifungal activity against a variety of microorganism.

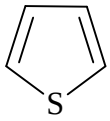
Keywords: Tetrahydrothienopyridopyrimidine, OECD, Hinsberg synthesis, Gewald Reaction, Antimicrobial activity.

INTRODUCTION

Thienopyridine moiety consists of fused heterocycles with a lot of promising pharmacological activities like antiplatelet[1], analgesic and anti-inflammatory[2-4], antiarrhythmic[5], antibacterial[6-7],antifungal[8-10], COT inhibitor[11], Inhibitor of phenylethanolamin N-Methyltransferase [12], antidiabetic [13], antioxidant[14], antidepressant[15] activities etc. These observations led to the conception that title compound would possess potential antimicrobial properties.

Chemistry of Thiophenes
The simple thiophenes are stable liquids, which closely resemble the corresponding benzene compounds in boiling point and even in smell. They occur in coal tar distillates the discovery of thiophene in coal tar benzene provides one of the classic anecdotes of organic chemistry. In the early days, color reactions were of great values in diagnosis: an important one for benzene involved the production of a blue color on heating with isatin and concentrated sulfuric acid.

In 1882, during a lecture demonstration by ***Viktor Meyer*** before an undergraduate audience, this test failed, no doubt the delight of everybody except the professor, and especially the professor's lecture assistant. An enquiry revealed that the lecture assistant ran out of commercial benzene and had provided a sample of benzene, which he has prepared by decarboxylation of pure benzoic acid. It was thus clear that commercial benzene contained an impurity and that it was this, not benzene, which was responsible for the color reaction. In subsequent investigations, Meyer isolated the impurity via its sulfonic acid derivative and showed it to be the first representative of a the new ring system, which was named thiophene from *theion*, the Greek word for sulfur, and another Greek word *phaino* which means shinning.

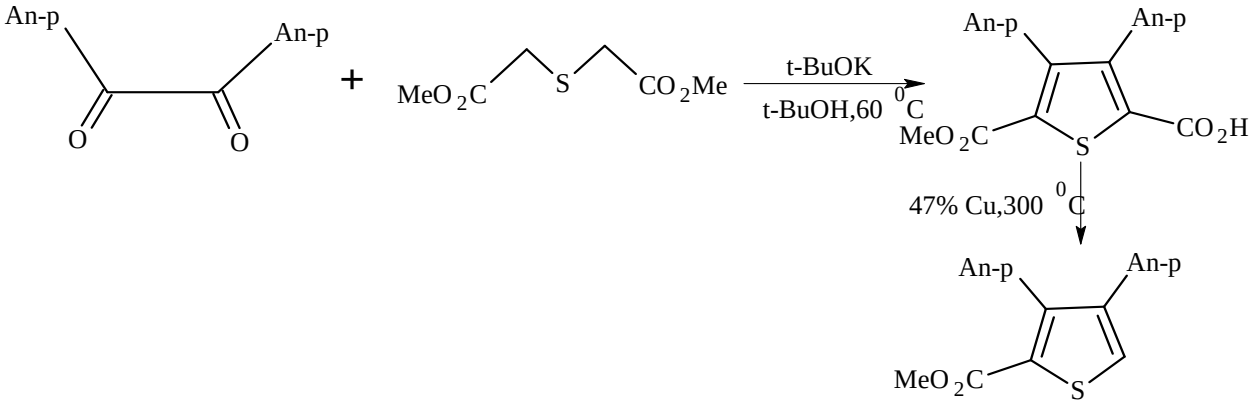


Thiophene

Synthesis of Thiophenes

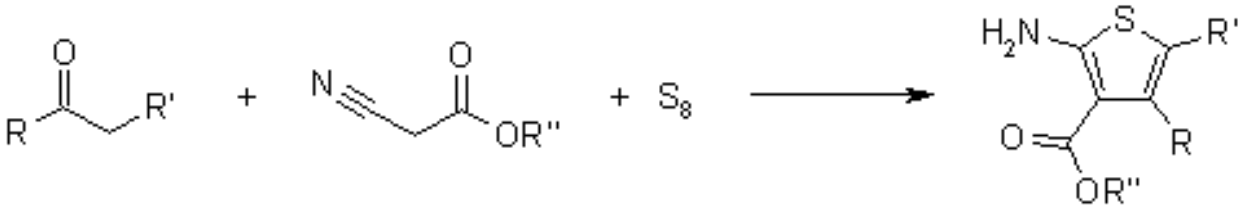
The Hinsberg synthesis

Two consecutive aldol condensations between 1, 2-dicarbonyl compound and diethyl thiodiacetate give thiophenes. The immediate product is an ester acid, produced by a Stobbe-type mechanism, but the reaction is often worked up via hydrolysis to afford an isolated diacid.



Gewald Reaction

Gewald synthesis is the usual route to 2-aminothiophenes. It consists of the base-catalyzed condensation of a ketone having CH_2 group with α -ketonitrile to form an olefin, followed by cyclisation with elemental sulfur.



MATERIALS AND METHODS

Melting points of all synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra were recorded by dispersing the compounds in KBr pellets on a Shimadzu FT-IR8400 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker ACF-300 MHz spectrometer using tetramethyl silane as an internal standard and all the chemical shift values were reported as δ. Mass spectra were recorded using SHIMADZU GCMS-2010 mass spectrometer.

CHEMICALS

The chemicals used in the present work were AR grade and LR grade, purchased from Loba, Merck and Fisher scientific fine chemicals.

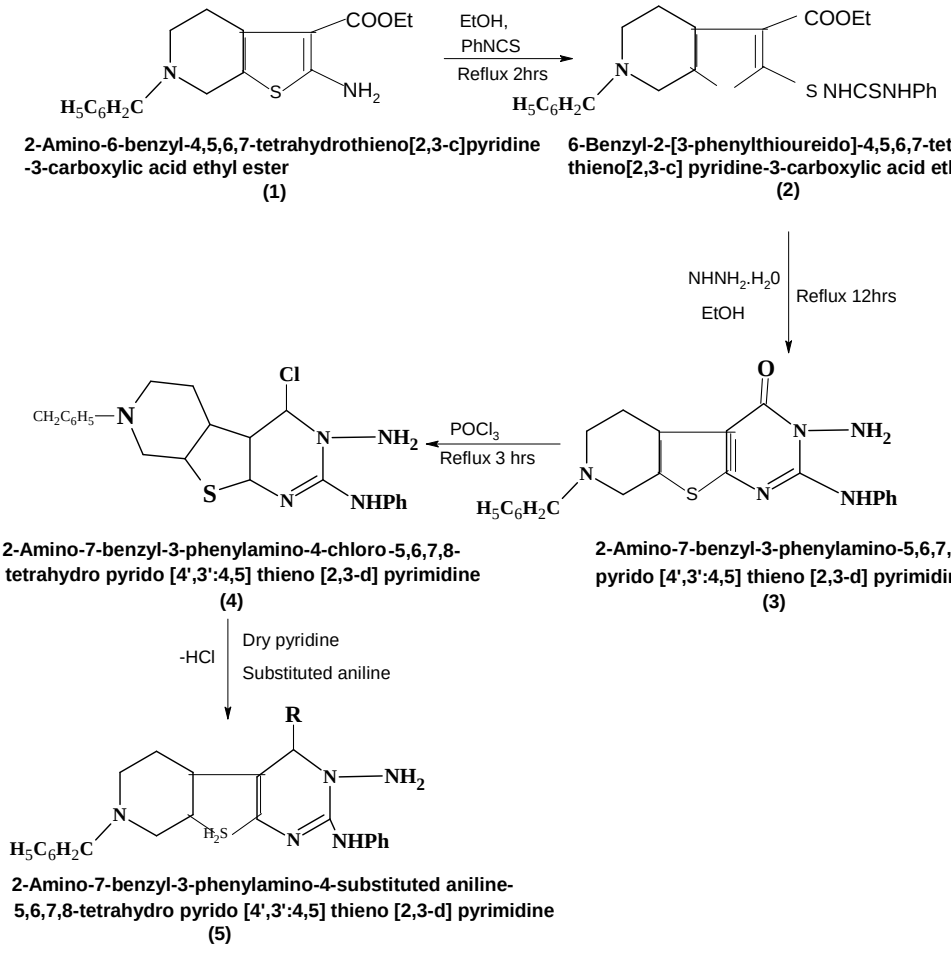
Antibacterial susceptibility test (AST)

The assays were performed according to the National Committee for Clinical Laboratory Standards (NCCLS), in Mueller–Hinton medium as described elsewhere. Briefly, the 14 strains were grown at 37 _C in Mueller– Hinton medium, and 1 lL of the stock solution (5 mg/mL) of each derivative in dimethyl sulfoxide (DMSO) was placed in Whatman disks (5 mm diameter). The disks were put on exponentially growing plated cultures with appropriate dilution to 1.0 x 107 colony forming unit (CFU/mL), which were then incubated for 24 h at 37 ^{o}C. The inocula used in growth method were those where turbidity was equal to 0.5 McFarland Standard. The results were verified by measuring the inhibitory zones surrounding the disk. Ciprofloxacin and Pyrimithanil were used as positive controls, and the halo >15 mm was considered the minimum value for positive antibacterial activity as it generally leads to a minimal inhibitory concentration (MIC) near that observed for the newest antibiotics which are currently present in the market (MIC = 1– 40 mg/mL) using these assays. Pyrimithanil and ciprofloxacin presented halos 15–17 and 23–25 mm, respectively, in the strains tested herein ($p < 0.005$).

Minimal inhibitory concentration assays (MIC)

MIC was determined only for active compounds on the AST by using the macro-dilution broth method. All MIC were performed in triplicate as described previously. Briefly, after 5 h of the bacterial growth, the culture was diluted to obtain 1.0 x 105 colony forming unit (CFU/mL). Then each compound was added to reach a final concentration from 0.5 to 1024 lg/mL, and was incubated at 37°C for 24 h. MIC was defined as the lowest compound concentration preventing visible bacterial growth. All strains were tested at least in duplicate in four separate experiments, and a reference antibiotic (Pyrimithanil) was used as a positive control (MIC = 2 mg / mL).

In the present study a novel series of 3-amino-7-benzyl-2-phenylamino-4-substituted aniline-5,6,7,8-tetrahydro-pyrido[4',3':4,5]thieno[2,3 d]pyrimidin were synthesized. Our synthetic strategy for the synthesis of some new tetrahydrothienopyridopyrimidine derivatives is illustrated in **Scheme 1**. The synthesis starts with refluxing mixture of 4-oxo-1-(phenylmethyl)-3-piperidine ,ethyl cyanoacetate , sulfur and morpholine (**1**) which is Subsequently; Ethyl-6-methyl-2-(3-phenylthioureido)-4,5,6,7- tetrahydrothieno [2,3-c]pyridine-3-carboxylate has been prepared (**2**). In step three 3-amino-7-methyl-2-phenylamino-5,6,7,8-tetrahydro-3*H*-pyrido[4',3':4,5]thieno[2,3 d]pyrimidin-4-one has been prepared. In fourth step 3-amino-4-chloro-7-(phenylmethyl)-2-phenylamino-5,6,7,8-tetrahydro-3*H*-pyrido[4',3':4,5] thieno[2,3 d] pyrimidine has been prepared. In fifth step 4-substitutedaniline-3-amino-7-(phenylmethyl)-2-phenylamino-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno[2,3 d]pyrimidine has been prepared (**3**) 60-85% yield. Formation of all synthesized compounds was confirmed by spectral and analytical data.



2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine -3-carboxylic acid ethyl ester (1)

6-Benzyl-2-[3-phenylthioureido]-4,5,6,7-tetrahydro-thieno[2,3-c] pyridine-3-carboxylic acid ethyl ester (2)

2-Amino-7-benzyl-3-phenylamino-4-chloro-5,6,7,8-tetrahydro pyrido [4',3':4,5] thieno [2,3-d] pyrimidine (4)

2-Amino-7-benzyl-3-phenylamino-5,6,7,8-tetrahydro-pyrido [4',3':4,5] thieno [2,3-d] pyrimidin-4-one (3)

2-Amino-7-benzyl-3-phenylamino-4-substituted aniline-5,6,7,8-tetrahydro pyrido [4',3':4,5] thieno [2,3-d] pyrimidine (5)

Scheme 1

General method for the preparation of 3-amino-7-benzyl-2-phenylamino-4-substituted aniline-5,6,7,8-tetrahydro-pyrido[4',3':4,5]thieno[2,3 d]pyrimidine (D-1to D-20)

A mixture of 3-amino-7-benzyl-4-chloro-2-phenylamino-5,6,7,8-tetrahydro-pyrido[4',3':4,5]thien [2,3 d]pyrimidine (**4**) (4.2 g, 0.01 mol) and substituted aniline (0.01 mol) were refluxed for 4 hours in presence of dry pyridine (10.0 ml) with constant stirring and maintaining the temperature below 10°C throughout the reaction. After completion pour the reaction mixture in beaker containing mixture of cold water (50 ml) and concentrated HCl (2 ml) with constant stirring precipitate was obtained. Collect the precipitated, washed with water and recrystalized from ethanol. Using above method twenty different derivatives (D-1 to D-20) was synthesized.

*3-Amino-7-benzyl-4-(3-chlorophenyl)amino-2-phenylamino-5,6,7,8 tetrahydropyrido [4',3':4,5] thieno [2,3d]pyrimidine**(D-1)***
Yield 65%; m.p. 242-243°C; IR: (KBr: γ/cm−1) NH_2 3419 , -NH 1534, Ar C=C 1554, -CH2 2975, Monosubstituted benzene 692, C-S stretching 505 , C-Cl 744, C-H 2914; ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O DART–MS (m/z): 515 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(2-chlorophenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno[2,3d] pyrimidine**(D-2)***
Yield 63.4%, m.p. 242-243^{o}C, IR: (KBr: γ/cm−1)-NH_2 3415 , -NH 1533, Ar C=C 1552, -CH_2 2977, Monosubstituted benzene 696, C-S 507 , C-Cl 748, C-H 2918, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O) , DART–MS (m/z): 515 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-ethylphenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-3)***
Yield 61.4%, m.p. 245-246 ^{o}C, IR: (KBr: γ/cm−1-NH_2 3420, -NH 1536, Ar C=C ,1555, -CH_2 2974, monosubstituted benzene 688, C-S 502, C-H 2951, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z): 508.6 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-Nitrophenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno[2,3d] pyrimidine**(D-4)***
Yield 59.6%, m.p. 243-244^{o}C, IR: (KBr: γ/cm−1) NH_2 3411, -NH 1530, Ar C=C 1559, -CH_2 2971, monosubstituted benzene 699, C-S 509, C-NO_2 1535, C-H 2916, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 525.6[M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-bromophenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-5)***
Yield 66.2%, m.p. 246-247^{o}C, IR: (KBr: γ/cm−1) NH_2 3412, -NH 1530, Ar C=C 1556, -CH_2 2972, monosubstituted benzene 691, C-S 504, C-Br 742, C-H 2917, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O) , DART–MS (m/z):), 559.5 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-methoxyphenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-6)***
Yield 64.7%, m.p. 236-237^{o}C, IR: (KBr: γ/cm−1) NH_2 3418, -NH 1530, Ar C=C 1556, -CH_2 2978, monosubstituted benzene 690, C-S 505, C-O 1454, C-H 2916, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O) DART–MS (m/z):), 510.6[M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-chlorophenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-7)***
Yield 67.8%, m.p. 242-243^{o}C, IR: (KBr: γ/cm−1NH_2 3415, -NH 1538, Ar C=C 1550, -CH_2 2971, monosubstituted benzene 698, C-S 504, C-Cl 746, C-H 2919, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 515 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(2,4-dinitrophenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-8)***
Yield 70.5%, m.p. 243-244^{o}C, IR: (KBr: γ/cm−1) NH_2 stretching 3422, -NH deformation 1540, Ar C=C stretching 1551, -CH_2 stretching 2971, monosubstituted benzene 698, C-S stretching 507, C-NO_2 stretching 1620, C-H aromatic stretching 2915, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 570.6 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(3-methylphenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine**(D-9)***
Yield 66.6%, m.p. 239-240^{o}C, IR: (KBr: γ/cm−1) -S- stretch 2630, -CN stretch 2220, -N-H stretch1665, -C =C- aromatic stretch 3100, -C-Br stretch 600, -C-Cl stretch 763, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q,

2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 494.6 [M+] (11), base peak at 252 [M+-$COCH_3$].

3-Amino-7-benzyl-4-(2-chloro-4-nitrophenyl)-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno [2,3-d] pyrimidine ***(D-10)***
Yield 65.7%, m.p. 239-240^oC, IR: (KBr: γ/cm−1) NH_2 stretching 3421, -NH deformation 1541, Ar C=C stretching 1556, -CH_2 stretching 2978, monosubstituted benzene 699, C-S stretching 507, C-Cl stretching 755, C-H aromatic stretching2916,^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, 2CH_3), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D2O), DART–MS (m/z):), 604.5 [M+] (11), base peak at 252 [M+-$COCH_3$].

3-Amino-7-benzyl-4-(2-chloro-3-nitrophenyl)-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno [2,3-d] pyrimidine ***(D-11)***
Yield 68.5%, m.p. 240-242^oC, IR: (KBr: γ/cm^{-1}) NH_2 stretching 3425, -NH deformation 1539, Ar C=C stretching 1555, -CH_2 stretching 2977, monosubstituted benzene 670, C-S stretching 509, C-Cl stretching 757, C-H aromatic stretching2917,^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, 2CH_3), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D2O), DART–MS (m/z):), 605.5 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(3-ethylphenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine****(D-12)***
Yield 65.8%, m.p. 235-237^oC, IR: (KBr: γ/cm^{-1}) -S- stretch 2630, -CN stretch 2222, -N-H stretch1666, -C =C- aromatic stretch 3100, -C-Br stretch 601, -C-Cl stretch 765, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, 2CH_3), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 496.8 [M+] (11), base peak at 252 [M+-$COCH_3$].

3-Amino-7-benzyl-4-(4-chloro-2-nitrophenyl)-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno [2,3-d] pyrimidine ***(D-13)***
Yield 66.2%, m.p. 234-235^oC, IR: (KBr: γ/cm−1) NH_2 stretching 3423, -NH deformation 1544, Ar C=C stretching 1552, -CH_2 stretching 2969, monosubstituted benzene 689, C-S stretching 505, C-Cl stretching 758, C-H aromatic stretching2915, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, 2CH_3), 2.40 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 605.5 [M+] (11), base peak at 252 [M+-$COCH_3$].

3-Amino-7-benzyl-4-(2-bromo-4-nitrophenyl)-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno [2,3-d] pyrimidine ***(D-14)***
Yield 66.6%, m.p. 231-232^oC, IR: (KBr: γ/cm^{-1}) -S- stretch 2620, N-H stretch1665, -C =C- aromatic stretch 3110, C-Br stretching 765, ^{1}H NMR((271 MHz,δ ppm, DMSO-d6)): 1.10 and 1.40 (2t, 6H, 2CH_3), 2.41 (s, 3H, $COCH_3$), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 495.8 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(4-propylphenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine****(D-15)***
Yield 64.6%, m.p. 241-243 ^{o}C, IR: (KBr: γ/cm^{-1}-NH_2 3421, -NH 1536, Ar C=C ,1555, -CH_2 2974, C-S 502, C-H 2951, ^{1}H NMR((270 MHz,*δ* ppm, DMSO-d6)): 2.42 (s, 3H, $COCH_3$), 2.71 (m, 2H, CH_2N), 2.78-2.96 (m, 4H, CH_2CH_2), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z): 505.2 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(2-propylphenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine****(D-16)***
Yield 63.6%, m.p. 243-245^oC, IR: (KBr: γ/cm^{-1}, NH_2 3415, -NH 1525, Ar C=C ,1555, -CH_2 2945, C-S 501, C-H 2953, ^{1}H NMR((270 MHz,*δ* ppm, DMSO-d6)): 2.42 (s, 3H, $COCH_3$), 2.75 (m, 2H, CH_2N), 3.52 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z): 502.5 [M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(3-propylphenyl)amino-2-phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine****(D-17)***
Yield 65.6%, m.p. 245-246^oC, IR: (KBr: γ/cm^{-1}-NH_2 3415, -NH 1525, Ar C=C ,1555, -CH_2 2945, C-S 501, C-H 2953, ^{1}H NMR((270 MHz,*δ* ppm, DMSO-d6)): 2.41 (s, 3H, $COCH_3$), 2.78 (m, 2H, CH_2N), 3.50 (s, 2H, CH_2), 4.54 (q, 2H, CH_2), 11.55 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z): 506.1[M+] (11), base peak at 252 [M+-$COCH_3$].

*3-Amino-7-benzyl-4-(2-methoxyphenyl)amino-2- phenylamino-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno [2,3 d]pyrimidine****(D-18)***
Yield 62.5%, m.p. 232-233^oC, IR: (KBr: γ/cm^{-1}) NH_2 3335, -NH 1532, Ar C=C 1545, -CH_2 2975, monosubstituted benzene 685, C-S 502, C-O 1450, C-H 2911, ^{1}H NMR((270 MHz,δ ppm, DMSO-d6)): 1.12 and 1.36 (2t, 6H, 2CH_3), 2.41 (s, 3H, $COCH_3$), 2.70 (m, 2H, CH_2N), 2.85-2.94 (m, 4H, CH_2CH_2), 3.51 (s, 2H, CH_2), 4.50 (q, 2H,

CH_2), 11.51 (bs, 1H, NH, exchangeable with D_2O) ,DART–MS (m/z):), 511.5[M+] (11), base peak at 252 [M+-$COCH_3$].

3-Amino-7-benzyl-4-(2-hydroxyphenyl)amino-2-phenylamino-5,6,7,8 tetrahydropyrido [4',3':4,5] thieno [2,3d]pyrimidine ***(D-19)***
Yield 61.5%; m.p. 246-247^oC ; IR: (KBr: γ/cm^{-1}) NH_2 3330 , -NH 1531, Ar C=C 1555, -CH_2 2965, Monosubstituted benzene 652, C-S stretching 502 , C-OH 788, C-H 2914; ^1H NMR((270 MHz,δ ppm, DMSO-d6)): 1.21 and 1.36 (2t, 6H, $2CH_3$), 2.42 (s, 3H, $COCH_3$), 2.85 (m, 2H, CH_2OH), 2.74-2.63(m, 4H, CH_2CH_2), 3.55 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.50 (bs, 1H, NH, exchangeable with D_2O DART–MS (m/z): 514 [M+] (11), base peak at 255 [M+-COOH].

3-Amino-7-benzyl-4-(2-bromo-4-nitrophenyl)-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno [2,3-d] pyrimidine ***(D-20)***
Yield 55.5%, m.p. 275-277^oC, IR: (KBr: γ/cm^{-1}) NH_2 stretching 3454, -, Ar C=C stretching 1558, -CH_2 stretching 2974, monosubstituted benzene 654, C-Br stretching 750, C-H aromatic stretching 2912,^1H NMR((270 MHz,δ ppm, DMSO-d6)): 1.12 and 1.45 (2t, 6H, $2CH_3$), 2.40 (s, 3H, $COCH_3$), 2.65 (m, 2H, CH_2Br), 2.71-2.92(m, 4H, CH_2CH_2), 3.53 (s, 2H, CH_2), 4.50 (q, 2H, CH_2), 11.36 (bs, 1H, NH, exchangeable with D_2O), DART–MS (m/z):), 645.6 [M+] (11), base peak at 252 [M+-$COCH_3$].

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activity was determined using disc diffusion method by measuring zone of inhibition in mm [16]. All the synthesized compounds; D-1to D-20 were screened in-vitro at a concentration of 10 µg/disc for their antibacterial activity against two Gram-positive strains (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative strains (Escherichia coli and Pseudomonas aeruginosa). Antifungal evaluation was also carried out against Candida albicans and Aspergillus niger at a concentration of 10 µg/disc. Standard antibacterial drug ciprofloxacin (10µg/disc) and antifungal drug pyrimethanil (10µg/disc) were also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken. The antibacterial activity was classified as highly active (≥25 mm), moderately active (11-24 mm) and least active (<11 mm). The results of antibacterial and antifungal activities are expressed in terms of zone of inhibition and presented in Table 1.

Table 1 Antimicrobial activity data of the synthesized compounds

COMPOUNDS	ZONE OF INHIBITION IN MM					
	ANTIBACTERIAL ACTIVITY				ANTIFUNGAL ACTIVITY	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
D-1	**15**	**16**	**15**	**16**	**18**	**19**
D-2	**16**	**18**	**14**	**13**	**15**	**17**
D-3	**17**	**18**	**14**	**13**	**12**	**11**
D-4	**18**	**19**	**18**	**18**	**16**	**15**
D-5	**13**	**12**	**12**	**11**	**12**	**13**
D-6	**14**	**18**	**16**	**17**	**18**	**19**
D-7	**15**	**14**	**15**	**16**	**18**	**17**
D-8	**15**	**15**	**15**	**14**	**14**	**17**
D-9	**16**	**19**	**18**	**20**	**21**	**19**
D-10	**20**	**20**	**21**	**22**	**22**	**23**
D-11	**15**	**16**	**15**	**16**	**18**	**19**
D-12	**16**	**18**	**14**	**13**	**15**	**17**
D-13	**17**	**18**	**14**	**13**	**12**	**11**
D-14	**18**	**19**	**18**	**18**	**16**	**15**
D-15	**13**	**12**	**12**	**11**	**12**	**13**
D-16	**15**	**16**	**15**	**16**	**18**	**19**
D-17	**15**	**15**	**15**	**14**	**14**	**17**
D-18	**16**	**19**	**18**	**20**	**21**	**19**
D-19	**14**	**18**	**16**	**17**	**18**	**19**
D-20	**15**	**14**	**15**	**16**	**18**	**17**
Ciprofloxacin	**25**	**26**	**27**	**28**	-	-
Pyrimithanil	-	-	-	-	**25**	**26**

Note: Zone of Inhibition diameter in mm, (-) indicates no activity.

The newly synthesized compounds were tested to evaluate their antibacterial and antifungal activity. All these compounds were found to exhibit moderate antibacterial and antifungal activity against different species of bacteria and fungi. From the above mentioned activity data (Table 1) it was observed that among all the compounds tested, compound D-10and D-14 showed good activity against all the tested bacteria and fungi. Among the other compounds showed moderate to least activity against all pathogens.

CONCLUSION

In conclusion, we have synthesized a series of novel 3-amino-7-(phenylmethyl)-2-phenylamino-5,6,7,8-tetrahydropyrido [4',3':4,5] thieno[2,3 d]pyrimidine and screened them for antibacterial and antifungal activities. The results of biological activity studies showed that compound D-10and D-14 having electron withdrawing $–NO_2$ group attached to a phenyl ring at ortho and para position showed very good activity against all tested bacteria and fungi as good as standard itself.

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