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Synthesis of some new 5-amino-3-(substituted-amino)-6-(fluoro/nitro)aryl-1,2,4-triazine derivatives as lamotrigine analogs and their evaluation *in vitro* as antibacterial agents

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Abstract: Some new fluorine-substituted 3,5-disubstituted amino-1,2,4-triazines have been obtained from aryl-amination of 2,2,2-trifluoro-*N*-[2-(5-hydroxy-3-thioxo-2,3-dihydro-1,2,4-triazin-6-yl)-4-nitrophenyl] acetamide followed by ammonolysis to produce *N*-(2-(5-amino-3-(arylamino)-1,2,4-triazin-6-yl)-4-nitrophenyl)-2,2,2-trifluoroacetamides which reacted with *N*-phenylthiourea. The structures of products were deduced from their elemental analysis and spectral measurements. The new lamotrigine analogs were evaluated *in vitro* as antibacterial. Interestingly, some compounds showed interesting activity against the *Bacillus subtilis*, *Streptococcus faecalis*, *Micrococcus luteus*, and *Staphylococcus aureus* bacteria.

Keywords: Fluoro/nitroaryl; Lamotrigine analogs; Thiourea; Antibacterial activity; 1,2,4-Triazines; Arylamination.

Introduction

In recent years, there has been increasing interest in the design of new drugs as to inhibition of the resistance of microbial towards the drugs were used. Among these drugs, Lamotrigine drug (3,5-diamino-6-(2`,4`-dichlorophenyl)-1,2,4-triazine) (Fig. 1) this is used as antiepileptic, bipolar disorder [1-4], anticonvulsant, neurological lesions and act as a tranquilizer as well as behaves an effective mood stabilizer [5,6]. Also, Lamotrigine and its ammonium salt complexes used as antimicrobial activity [7].

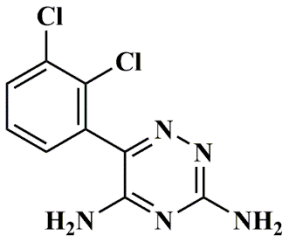

Figure 1. Lamotrigine drug

Recently, Makki *et al.* [8,9] reported a simple route to synthesize Lamotrigine analogs as an anti-inflammatory and antioxidant agent. 1,2,4-triazine nucleus is scaffold of various biological activities, also have a significant variety of pharmaceutical properties, such as antitumor [10], anti-HIV, antimicrobial [11]. Also, isoxazole, 1,2,4-triazole, and benzo[*d*]imidazole cores have a significant role in towads bacteria [12-14].

Moreover, thiourea moieties play a role of biological activity involving antibacterial [15], antifungal [15,16], antitubercular [17], antithyroid [18], and insecticidal agents [19].

Therefore, there is a need to develop an improved process for producing lamotrigine analogs that reduce the manufacturing cost and batch cycle time [20]. Thus, the present work describes a short route to obtain some new lamotrigine analogs as fluorine, arylamines, and *N*-phenylthioureas substituents given their antibacterial activity.

Results and Discussion

In the present work in the search for new highly bioactive drugs, fluorine substituted 3,5-disubstituted amino-1,2,4-triazines **11-14** as a lamotrigine analogs have been synthesized and evaluated as antibacterial probes. The starting material 2,2,2-trifluoro-*N*-[2-(5-hydroxy-3-thioxo-2,3-dihydro-1,2,4-triazin-6-yl)-4-nitrophenyl] aceta- mide **2** obtained from refluxing of 6-(2-amino-5-nitrophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5 (2 *H*)-one **1** with ethyl 2,2,2-trifluoroacetate in THF. Compound **1** also preparing[21] by refluxing 5-nitroisatin with thiosemicarbazide in *aq*. NaOH Scheme 1.

A simple primary arylamines such as 3,4-dimethylisoxazol-5-amine, 4*H*-1,2,4-triazol-3-

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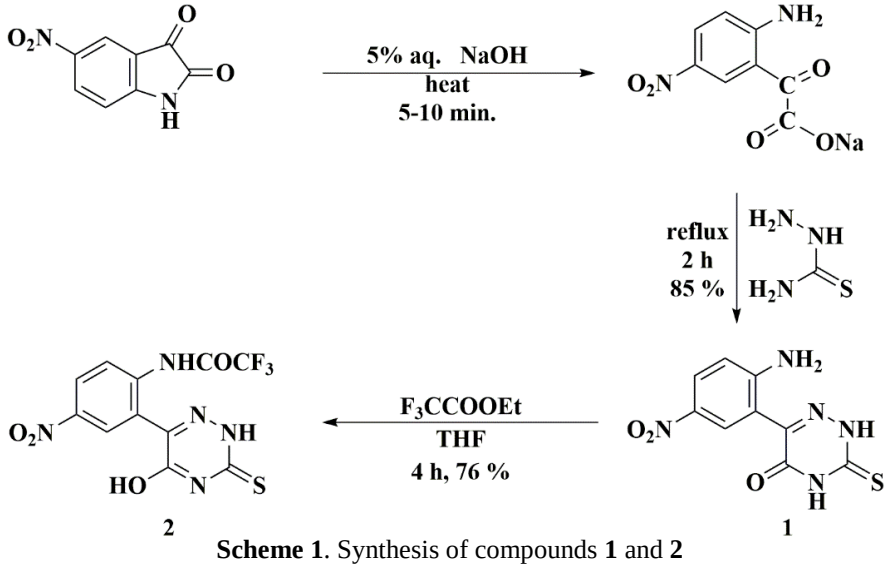
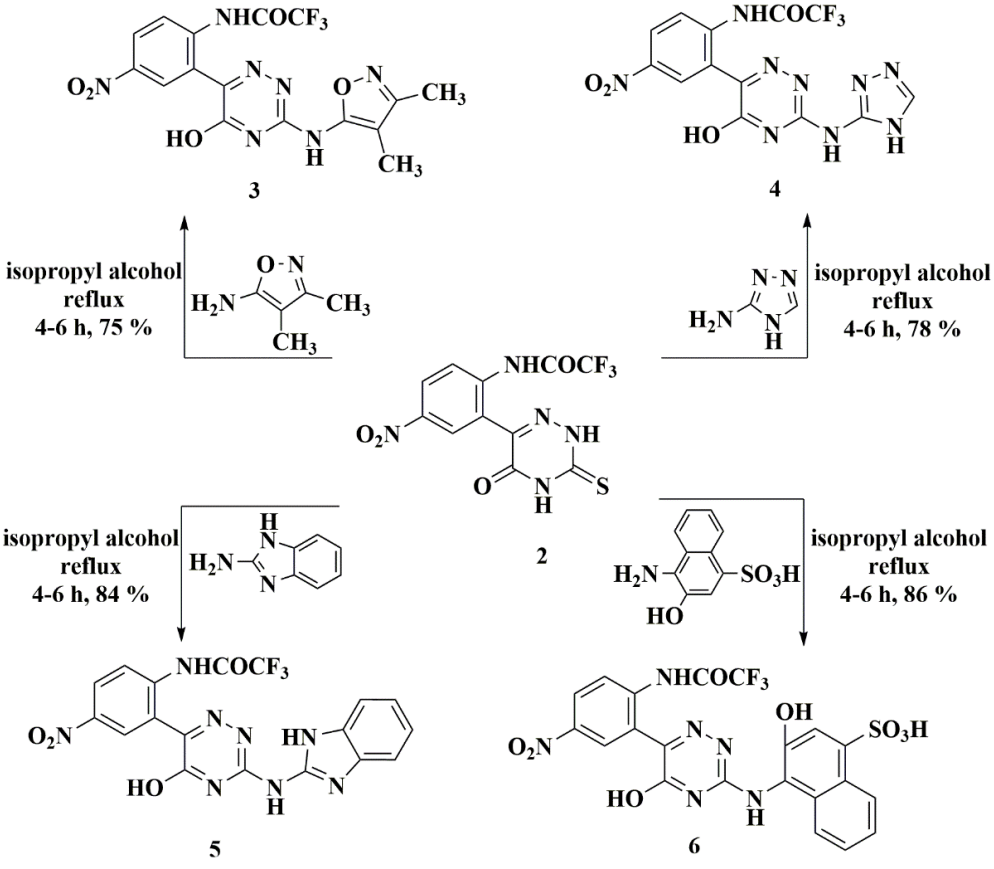
amine, 1*H*-benzo[d]imidazol-2-amine, and 4-amino-3-hydroxynaphthalene-1-sulfonic acid towards 2,2, 2-trifluoro-*N*-[2-(5-hydroxy-3-thioxo-2,3-dihydro-1,2,4-triazin-6-yl)-4-nitrophenyl]acetamide **2** in refluxing isopropyl alcohol led to the formation 3-(substituted-amino)-5-hydroxy-6-aryl-1,2,4-triazines **3-6** Scheme 2.

On the other hand, aminolysis of compounds **3-6** by refluxing with NH_3/EtOH afforded the 5-amino-3-(substituted-amino)-6-aryl-1,2,4-triazines **7-10** as lamotrigine analogs Scheme 3.

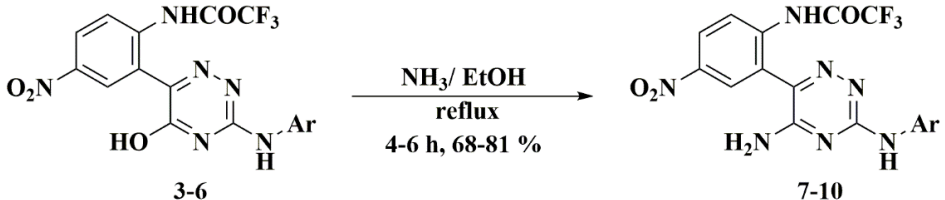
Finally, reaction of compounds 7-**10** with *N*- phenylthiourea in DMF furnished 2,2,2-trifluoro-*N*-[2-(3-(substituted-amino)-5-(3-phenylthioureido)-1,2,4-triazin-6-yl)-4-nitrophenyl] acetamides **11-14** as lamotrigine analogs Scheme 4. Formation of compounds **11-14** may be as shown in (Fig. 2).



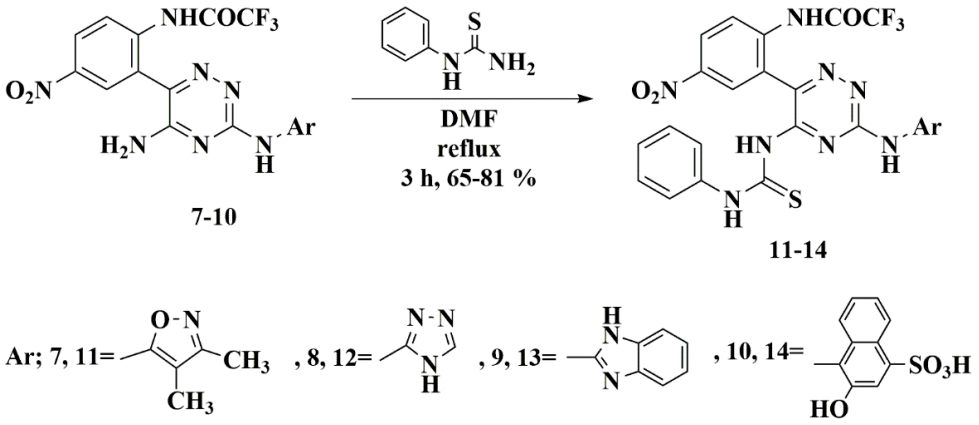
Scheme 1. Synthesis of compounds **1** and **2**



Scheme 2. Synthesis of compounds **3-6**



Scheme 3. Synthesis of compounds **7-10**



Scheme 4. Synthesis of compounds **11-14**

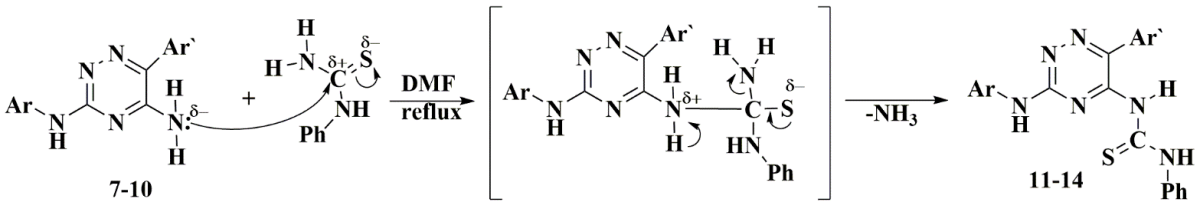


Figure 2. Formation of compounds **11-14**

Structure of the new fluorinated Lamotrigine analogs deduced from their correct elemental analysis and spectral data. FT-IR spectra of the compounds **3**, **7**, and **11** showed ῡ 2930 ~2905, and 2885 ~2883 cm^{-1} for CH_3 of isoxazole moiety. Besides, ^1H NMR of **3**, **7**, and **11** exhibited two signals at δ 2.29 ~1.98, and 2.16 ~1.97 ppm for the two CH_3 of isoxazole moiety.

Interestingly, the ^{13}C NMR of **3**, **7**, and **11** showed δ at 28.8 ~27.0 and 11.9 ~10.07 ppm for two CH_3. ^1H NMR of compounds **4**, **8**, and **12** showed δ 14.30 ~14.35 ppm attribute to the NH of 1,2,4-triazole. Similarly, ^1H NMR of compounds **5**, **9**, and **13** showed δ 14.39 ~14.38 ppm attribute to the NH of benzimidazole.

^1H NMR for compounds **1-14** exhibited δ at 13.60~13.50 ppm referring to $NHCOCF_3$ beside δ at 8.95 ~ 6.70 ppm for aromatic protons. FT-IR spectra indicated the presence of C=O at ῡ 1709 ~1696 cm^{-1} and 1553 ~1513, 1387 ~1370 cm^{-1} for asymmetrical and symmetrical for NO_2 respectively for compounds **1-14**.

Furthermore, FT-IR spectra of the compounds **3-6** showed a new exo-NH at ῡ 3350 ~3190 cm^{-1}.

Moreover, the ^1H NMR spectrum of **3-6** exhibited δ at 8.59 ~ 8.33 ppm for exo NH. ^{13}C NMR of compounds **3-6** indicated that disappeared C=S, all of these shreds of evidence confirmed that structures of **3-6**, and the arylamination of compound **2** occurred.

Structures of lamotrigine analogs **7-10** established from the presence of the vibration bands at 3441 ~3412 and 3342 ~3300 cm^{-1} (stretching of NH_2) with lacks of OH group. Also, ^1H NMR of **7-10** showed resonated signals at δ 4.14-4.13 ppm attribute to NH_2. Exhibition of signals at δ 178 ~ 176 ppm in ^{13}C NMR of compounds **11-14** indicated that the presence of C=S of thiourea and confirmed the reaction of compounds **7-10** with *N*-phenylthiourea

happened. ^{19}F NMR spectra of all compounds **1-14** showed δ -78 ~ -75 ppm attribute to CF_3.

Finally, the newly synthesized lamotrigine analogs (**7-14**) were screened for their *in vitro* antibacterial activity against four bacterial isolated *Bacillus subtilis*, *Streptococcus faecalis*, *Micrococcus luteus*, and *Staphylococcus aureus*. The results of the study revealed that the compounds **7-14** showed significant antibacterial potency.

As expected, compounds **12** and **13** were showed excellent activity against *B. subtilis* and *S. faecalis* bacteria, due to the presence of 1,2,4-triazine, CF_3, thiourea, and 1,2,4-triazole and/ or benzo[*d*]imidazole moieties. On the other hand, 3-hydroxynaphthalene moiety in compounds **10** and **14** may be reduced their biological activity against the tested bacteria, because of these compounds had the lowest activities among the other synthesized compounds. The result is shown in Table 1.

Table 1. In vitro antibacterial activity.

Compound No.	Concenteration (μg/mL)	Inhibition zone of Bacteria (mm)			
		B. subtilis	***S. faecalis***	***M. luteus***	***S. aureus***
7	5	9	11	8	14
8	5	12	15	10	18
9	5	10	13	12	15
10	5	8	9	14	13
11	5	8	9	10	7
12	5	19	18	17	20
13	5	18	21	20	25
14	5	7	8	11	9
Ampicillin	200 μg/mL	22	17	45	40

Conclusion

5-Amino-3-(substituted-amino)-6-(fluoro/nitro) aryl-1,2,4-triazine and their derivatives were synthesized and evaluated against four isolated bacteria (*B. subtilis*, *S. faecalis*, *M. luteus*, and *S. aureus*). Some compounds exhibited a good activity towards the tested bacteria.

Experimental

All chemicals purchased from Merck and Fluka and used as received without any further purification. The melting points recorded by Stuart scientific SMP30 (Bibby, UK) melting point apparatus and reported as uncorrected. A Perkin Elmer model RXI-FT-IR 55,529 cm^{-1} used for recording the IR spectra. A Brucker advance DPX 400 MHz using TMS as an internal standard used for recording the ^{1}H,^{13}C, and ^{19}F NMR spectra at (400 MHz), (100 MHz), and (84.25 MHz) respectively in DMSO-d_6 (δ in ppm) as a solvent. Elemental microanalysis was performed on a Perkin-Elmer CHN-2400 analyzer. TLC analyses were performed on Merck silica gel 60 F_{254} aluminum plates with hexane/ethyl acetate mixtures. Compounds **1** and **2** were obtained according to the reported method [9].

Synthesis of 3-(Substituted-amino)-5-hydroxy-6-aryl-1,2,4-triazines 3-6

In a round-bottom flask, an equimolar amount of compound **2** and 3,4-dimethylisoxazol-5-amine, 4*H*-1,2,4-triazol-3-amine, 1*H*-benzo[*d*]imidazol-2-Amine, and 4-amino-3-hydroxynaphthalene-1-sulfonic acid were dissolved in isopropyl alcohol (70 mL) then heated under reflux for 6 h. The progress of the reactions was monitored by TLC. After completion of the reactions, colled at room temperature. The resulting solid was filtered off in a Buchner funnel, then washed with small amounts of cooled water and dried. Finally, the products crystallized from suitable solvents to give compounds **3-6**.

N-[2-(3-((3,4-Dimethylisoxazol-5-yl)amino)-5-hydroxy-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 3:

Orange crystals (EtOH), yield 3.621g, 75%, M.p: 217-219° C.
FT-IR (ATR, $\bar{\upsilon}$, cm^{-1}): 3430(OH), 3190 (NH), 2930, 2885(CH_3), 1697(C=O), 1629 (C=C), 1520, 1383 (asym., sym. NO_2), 1263.97(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.56 (s, 1H, $NHCOCF_3$), 13.16(s, 1H, OH), 8.59(s, 1H, NH), 8.31(s, 1H, aromatic proton), 6.98, 6.76(d,d, 2H, aromatic protons), 1.98, 1.97(s,s, 6H, $2CH_3$).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.46(C=O), 153.59(C=N), 145.98(C-F), 135.11(C=N), 128.03-126.48(aromatic carbons), 114.42, 113.79(C5, C6 of 1,2,4-triazine), 27, 11.9($2CH_3$).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75(J_{C-F}=259 Hz, CF_3).
Calculated: $C_{16}H_{12}F_3N_7O_5$ (M^+ 439): C, 43.74; H, 2.75; N, 22.32%. Found: C, 43.52; H, 2.68; N, 22.07%.

N-[2-(3-((4H-1,2,4-Triazol-3-yl)amino)-5-hydroxy-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoro-acetamide 4:

Orange crystals (EtOH), yield 3.526g, 78%, M.p: 254-256°C.

FT-IR (ATR, ῡ, cm^{-1}): 3415(OH), 3320(NH), 1696(C=O), 1616(C=C), 1522, 1377 (asym., sym. NO_2), 1239(C-F).
^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.30 (s, 1H, NH-triazole), 13.56(s, 1H, $NHCOCF_3$), 13.17(s, 1H, OH), 8.58(s, 1H, NH), 8.32(s, 1H, aromatic proton), 6.99, 6.76(d,d, 2H, aromatic proton), 6.55(s, 1H, C5- triazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.41(C=O), 153.59, 152.75(C=N), 145.89 (C-F), 135.11(C=N), 128.03-126.48 (aromatic carbons) 114.42, 113.79(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{13}H_8F_3N_9O_4$ (M^+ 411): C, 37.97; H, 1.96; N, 30.65%. Found: C, 37.75; H, 1.81; N, 30.53%.

N-[2-(3-((1H-Benzo[d]imidazol-2-yl)amino)-5-hydroxy-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 5:

Brown crystals (EtOH), yield 3.797g, 84%, M.p: 210-212°C.
FT-IR (ATR, ῡ, cm^{-1}): 3442(OH), 3350(NH), 1699(C=O), 1616(C=C), 1513, 1338 (asym., sym. NO_2), 1258.74(C-F).
^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.39 (s, 1H, NH-benzoimidazole), 13.54(s, 1H, $NHCOCF_3$), 12.50(s, 1H, OH), 8.33(s, 1H, NH), 8.94-6.74(m, 8H, aromatic proton).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.41(C=O), 168.13-152.75(C=N), 145.89(C-F), 135.12(C=N), 128.03-121.35 (aromatic carbons) 118.51, 109.44(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{18}H_{11}F_3N_8O_4$ (M^+460): C, 46.97; H, 2.41; N, 24.34%. Found: C, 46.84; H, 2.36; N, 24.19%.

3-Hydroxy-4-((5-hydroxy-6-(5-nitro-2-(2,2,2-trifluoroacetamido)phenyl)-1,2,4-triazin-3-yl) amino)naphthalene-1-sulfonic acid 6:

Black crystals (EtOH), yield 5.354g, 86%, M.p: 248-250°C.
FT-IR (ATR, ῡ, cm^{-1}): 3446(OH), 3300 (OH), 3210(NH), 1699(C=O), 1613(C=C), 1553, 1370(asym., sym. NO_2), 1237(C-F).
^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.60 (s, 1H, $NHCOCF_3$), 12.45(s, 1H, OH), 8.60, 8.59 (s,s, 2H, OH), 8.33(s, 1H, NH), 8.95-8.00, 7.99-6.74(m, 9H, aromatic proton).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.44(C=O), 167.51-152.80(C=N), 145.85(C-F), 138.86, 135.11(C=N), 128.03-121.28(aromatic carbons), 114.44, 112.39(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{21}H_{13}F_3N_6O_8S$ (M^+566): C, 44.53; H, 2.31; N, 14.84%. Found: C, 44.14; H, 2.23; N, 14.79%.

Synthesis of 5-Amino-3-(substituted-amino)-6-aryl-1,2,4-triazines 7-10

In a round-bottom flask, a mixture of compounds **3-6** (6.5 mmol) and ammonia (37%, 40 ml) in ethanol (50 ml) heated under reflux for 4-6 h. Progress of the reactions was monitored by TLC. After completion of the reactions, cooled at room temperature then poured onto ice-drops AcOH. The yielded solids filtered off in a Buchner funnel, then washed with a small amount of cooled water, dried. Finally, the products crystallized from proper solvents, to give compounds **7-10** respectively.

N-(2-(5-Amino-3-((3,4-dimethylisoxazol-5-yl) amino)-1,2,4-triazin-6-yl)-4-nitrophenyl)-2,2,2-trifluoroacetamide 7:

Black crystals (THF), yield 2.047g, 72%, M.p: 224-226°C.
FT-IR (ATR, ῡ, cm^{-1}): 3412.5, 3300 (NH_2), 3197(NH), 3152(NH), 2909, 2883.7 (aliphatic CH_3), 1699.75(C=O), 1614.9(C=C), 1519, 1470(asym., sym. NO_2), 1268.7(C-F).
^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.57 (s, 1H, $NHCOCF_3$), 8.58(s, 1H, NH), 8.33(s, 1H, aromatic proton), 6.93, 6.74(d,d, 2H, aromatic proton), 4.14(s, 2H, NH_2), 2.29, 2.16(s,s, 6H, $2CH_3$).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.40(C=O), 162.9(C=N), 145.88(C-F), 135.11(C=N), 128.03-126.48(aromatic carbons), 116.09, 113.69(C5, C6 of 1,2,4-triazine), 28.8, 10.73($2CH_3$).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{16}H_{13}F_3N_8O_4$ (M^+438): C, 43.84; H, 2.99; N, 25.56%. Found: C, 43.81; H, 2.90; N, 25.37%.

N-[2-(3-((4H-1,2,4-Triazol-3-yl)amino)-5-amino-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoro-acetamide 8:

Black crystals (THF), yield 1.812g, 68%, M.p: 251-253°C.
FT-IR (ATR, ῡ, cm^{-1}): 3425, 3325 (NH_2), 3197(NH), 3150(NH), 1699.92(C=O), 1614.86(C=C), 1522, 1481 (asym., sym. NO_2), 1267.4(C-F).
^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.35 (s, 1H, NH-triazole), 13.57(s, 1H, $NHCOCF_3$), 8.32(s, 1H, NH), 8.00(s, 1H, aromatic proton), 7.08, 6.76(d,d, 2H, aromatic proton), 6.74(s, 1H, C5- triazole). 4.13(s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 173.41(C=O), 153.59, 152.75(C=N), 145.88(C-F), 135.12(C=N), 128.03-126.48 (aromatic carbons) 114.44, 113.70(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{13}H_9F_3N_{10}O_3$ (M^+410): C, 38.06; H, 2.21; N, 34.14%. Found: C, 37.87; H, 2.05; N, 34.00%.

N-[2-(3-((1H-Benzo[d]imidazol-2-yl)amino)-5-amino-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 9:

Black crystals (THF), yield 2.267g, 76%, M.p: 242-244°C.
FT-IR (ATR, ῡ, cm^{-1}): 3441, 3329(NH_2), 3195.7(NH), 3165(NH), 1699.7(C=O), 1614.74(C=C), 1521, 1481(asym., sym. NO_2), 1266.77(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.39 (s, 1H, NH-benzoimidazole), 13.56(s, 1H, $NHCOCF_3$), 8.32(s, 1H, NH), 8.94-6.74(m, 8H, aromatic proton).
^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 173.14(C=O), 162.11-153.75(C=N), 145.89(C-F), 135.12(C=N), 128.03-121.36(aromatic carbons) 121.36, 113.73(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{18}H_{12}F_3N_9O_3$ (M^+459): C, 47.07; H, 2.63; N, 27.44%. Found: C, 46.86; H, 2.46; N, 27.25%.

4-[(5-Amino-6-(5-nitro-2-(2,2,2-trifluoroacetamido)phenyl)-1,2,4-triazin-3-yl)amino]-3-hydroxy naphthalene-1-sulfonic acid 10:

Black crystals (THF), yield 2.974g, 81%, M.p: 230-232°C.
FT-IR (ATR, ῡ, cm^{-1}): 3436, 3342(NH_2), 3300(OH), 3186(NH), 3165(NH), 1699(C=O), 1614(C=C), 1521, 1380(asym., sym. NO_2), 1254(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.56 (s, 1H, $NHCOCF_3$), 8.58, 8.47(s,s, 2H, OH), 8.32 (s, 1H, NH), 8.94-8.01, 7.98-6.74(m, 9H, aromatic proton), 4.13(s, 2H, NH_2).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.45(C=O), 164.02-152.81(C=N), 145.87(C-F), 138.86, 135.11(C=N), 128.03-121.21 (aromatic carbons), 114.43, 112.35(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{21}H_{14}F_3N_7O_7S$ (M^+565): C, 44.61; H, 2.50; N, 17.34%. Found: C, 44.56; H, 2.47; N, 17.08%.

Synthesis of 2,2,2-Trifluoro-N-(2-(3-(substituted-amino)-5-(3-phenylthioureido)-1,2,4-triazin-6-yl)-4-nitrophenyl)acetamides 11-14

In a round-bottom flask, equimolar amounts of compounds **7-10** and *N*-phenylthiourea in DMF (40 ml) heated under reflux for 3 h. Progress of the reactions was monitored by TLC. After completion of the reactions, colled at room temperature then poured onto ice. The resulting solids filtered off in a Buchner funnel, then washed with small amounts of cooled water, dried. Finally, the products crystallized from suitable solvents to give compounds **11-14**, respectively.

N-[2-(3-((3,4-Dimethylisoxazol-5-yl)amino)-5-(3-phenylthioureido)-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 11:

Black crystals (Dioxane), yield 0.827g, 81%, M.p: 273-275°C.
FT-IR (ATR, ῡ, cm^{-1}): 3450-3125(NH, NH, NH), 2905, 2884(aliphatic CH_3), 1709.01(C=O), 1625(C=C), 1523.37, 1482(asym., sym. NO_2), 1250(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.60 (s, 1H, $NHCOCF_3$), 9.65, 9.52(s,s, 2H, NH, NH), 8.69(s, 1H, NH), 8.33-6.78(m, 8H, aromatic proton), 2.29, 1.98(s,s, 6H, $2CH_3$).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 177.6(C=S), 163(C=N), 145.25(C-F), 135.42(C=N), 128-126 (aromatic carbons), 115.69, 112.45(C5, C6 of 1,2,4-triazine), 27.39, 10.78($2CH_3$).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{23}H_{18}F_3N_9O_4S$ (M^+573): C, 48.17; H, 3.16; N, 21.98%. Found: C, 47.94; H, 3.02; N, 21.74%.

N-[2-(3-((4H-1,2,4-Triazol-3-yl)amino)-5-(3-phenylthioureido)-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 12:

Black crystals (Dioxane), yield 0.786g, 76%, M.p: 265-268°C.
FT-IR (ATR, ῡ, cm^{-1}): 3447-3153(NH, NH, NH), 1702.21 (C=O), 1615(C=C), 1521.79, 1484(asym., sym. NO_2), 1262.5(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.32 (s, 1H, NH-triazole), 13.58(s, 1H, $NHCOCF_3$), 9.69, 9.67(s,s, 2H, NH, NH), 8.61(s, 1H, NH), 8.73-7.08 (m, 8H, aromatic proton), 6.70(s, 1H, C5-triazole).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 178(C=S), 173(C=O), 153.42, 152.71(C=N), 145.89 (C-F), 135.12(C=N), 127.93-125.27(aromatic carbons), 115.42, 113.29(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{20}H_{14}F_3N_{11}O_3S$ (M^+545): C, 44.04; H, 2.59; N, 28.25%. Found: C, 43.89; H, 2.37; N, 28.12%.

N-[2-(3-((1H-Benzo[d]imidazol-2-yl)amino)-5-(3-phenylthioureido)-1,2,4-triazin-6-yl)-4-nitrophenyl]-2,2,2-trifluoroacetamide 13:

Black crystals (Dioxane), yield 0.733g, 65%, M.p: 146-148°C.
FT-IR (ATR, ῡ, cm^{-1}): 3453-3179(NH, NH, NH), 1709(C=O), 1624(C=C), 1523.37, 1487(asym., sym. NO_2), 1262(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.38 (s,1H,NH-benzoimidazole), 13.57(s, 1H, $NHCOCF_3$), 9.70, 9.69(s,s, 2H, NH, NH), 8.34 (s, 1H, NH), 8.93-6.76(m, 13H, aromatic proton).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 177(C=S), 173(C=O), 163-153(C=N), 145(C-F), 135(C=N), 127-121(aromatic carbons), 114, 112(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).

Calculated: $C_{25}H_{17}F_3N_{10}O_3S$ (M^+594): C, 50.51; H, 2.88; N, 23.56%. Found: C, 50.36; H, 2.69; N, 23.46%.

3-Hydroxy-4-[(6-(5-nitro-2-(2,2,2-trifluoroacetamido)phenyl)-5-(3-phenylthioureido)-1,2,4-triazin-3-yl)amino])naphthalene-1-sulfonic acid 14:

Black crystals (Dioxane), yield 0.944g, 71%, M.p: 228-230°C.
FT-IR (ATR, ῡ, cm^{-1}): 3530-3186.64(4NH, OH), 1707(C=O), 1615(C=C), 1522, 1381(asym., sym. NO_2), 1271(C-F).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.50 (s, 1H, $NHCOCF_3$), 9.69, 9.67(s,s, 2H, NH, NH), 8.68, 8.57(s,s, 2H, OH), 8.34(s, 1H, NH), 8.90-7.20(m, 14H, aromatic proton).
^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 176(C=S), 173(C=O), 163-153 (C=N), 145(C-F), 137, 134(C=N), 127-121(aromatic carbons), 114, 112(C5, C6 of 1,2,4-triazine).
^{19}F NMR (84.25 MHz, DMSO-d_6) δ (ppm): -78 ~ -75 (J_{C-F}=259 Hz, CF_3).
Calculated: $C_{28}H_{19}F_3N_8O_7S_2$ (M^+700): C, 48.00; H, 2.73; N, 15.99%. Found: C, 47.85; H, 2.64; N, 15.74%.

The antibacterial activity

The *in vitro* antimicrobial activity of lamotrigine analogs **7**-**14** was screened against some selected bacterial (*Bacillus subtilis*, *Streptococcus faecalis*, *Micrococcus luteus*, *and Staphylococcus aureus*) by the reported method. The suspension of each microorganism rubbed onto the surface of solidified nutrient agar already set into Petri dishes with swap stick.

The stock solution suitably diluted to get dilution of 5 µg/mL concentration (DMSO) of the tested compounds **7**-**14**. Wells (6 mm in diameter) dug in the agar media with the help of a sterile metallic borer. Ampicillin 200 µg/mL used as controls. The wells incubated immediately at 37° C for 48 h. The activity determined by measuring the diameter of zones indicating complete inhibition (mm) and comparing the values with the standard.[22]

Conflict of interest

The authors declare no conflict of interest.

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