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Research Article

SYNTHESIS AND ANTIBACTERIAL SCREENING OF 1-(2-(2-CHLOROPHENYL)-2-(4-(6-FLUOROBENZO[D]ISOXAZOL-3-YL)PIPERIDIN-1-YL) CONTAINING 1,3,4-THIADIAZOLE, 1,2,4-TRIAZOLE AND 1,3,4-OXADIAZOLE DERIVATIVES

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ABSTRACT

A series of novel 1,3,4-thiadiazole, 1,2,4-triazole and 1,3,4-oxadiazole have been synthesized from 1-(2-(2-chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)acetyl)-4-phenyl thiosemicarbazide 6 and their antimicrobial activities were reported. Compounds 6b, 6c, 9a, 9b, 9d and 9e have shown moderate activity towards Bacillus Subtilis and Escherichia Coli bacterial species. The structure of synthesized compounds was confirmed by spectral analysis.

Keywords: Benzo[d]isoxazole, 1,3,4-Thiadiazole, 1,2,4-Triazole and 1,3,4-Oxadiazole.

INTRODUCTION

Benzo[d]isoxazole is one of the oxygen and nitrogen containing heterocyclic compound. In recent years compounds containing benzo[d]isoxazole scaffold attracts many researchers due to their pharmacological activities. Benzo[d]isoxazole and their derivatives are associated with antitubercular[1], antimycobacterial[2], anti-inflammatory[3], antidiabetic[4] and c-Met kinase inhibitor[5] etc activities. Thiosemicarbazide is a versatile intermediate for the synthesis of heterocycles like triazole, thiadiazole, oxadiazole & thiazole. Thiosemicarbazide derivatives are associated with as antioxidant[6], cathepsin L inhibitors[7], metallo-β -lactamase inhibitors[8] etc activities.

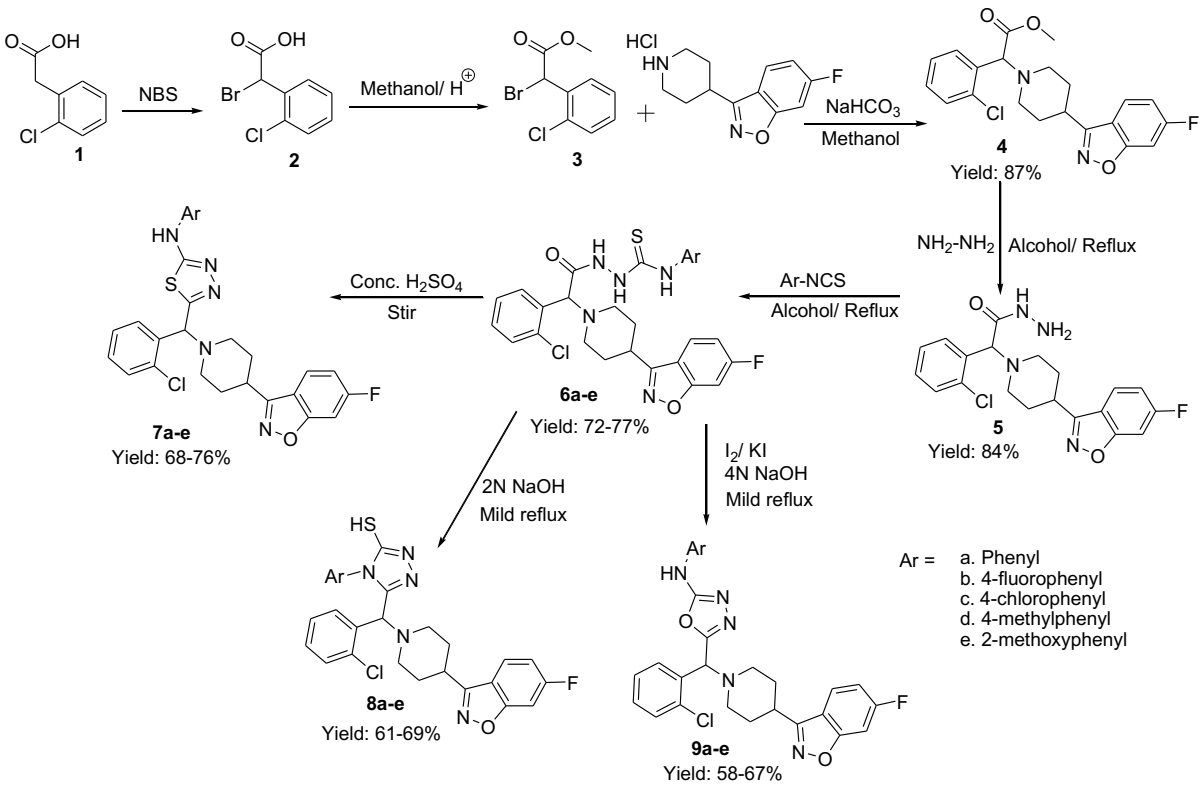
1,3,4-Thiadiazole, 1,2,4-triazole and 1,3,4-oxadiazole are significant class of heterocyclic compound and posses variety of biological activities. 1,3,4-Thiadiazole derivatives have been investigated for antiinflammatory[9], antituberculosis[10], anxiolytic[11], antidepressant[12] and herbicidal[13] activities.. 1,2,4-Triazole and its derivatives exhibit antitumor[14], antioxidant[15], antitubercular[16], antifungal[17,18], antibacterial[17,19], anti-inflammatory[20] and etc activities. 1,2,4-Triazole nucleus found in many drugs such as Letrozole, Vorozole, Voriconazole, Itraconazole, Alprazolam, Etoperidone, etc. 1,3,4-Oxadiazole containing compounds possess various biological activities such as tyrosinase inhibitors[21], anti-inflammatory[22], analgesic[22], anticonvulsant[23], antiproliferative[24] and antitubercular[25].

Activities associated with above heterocycles, it is worthwhile to synthesize 1-(2-(2-chlorophenyl)-2-(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl) bearing various heterocycles such as 1,3,4-thiadiazole, 1,2,4-triazole and 1,3,4-oxadiazole and to evaluate for their antibacterial activities against Bacillus Subtilis and Escherichia Coli bacterial species.

MATERIALS AND METHODS

Substituted 1-(2-(2-chlorophenyl)-2-(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl)acetyl)-4-phenylthiosemicarbazide **6** was synthesized in five steps starting from 2-(2-chlorophenyl)acetic acid **1**. 2-(2-Chlorophenyl)acetic acid **1** was converted into 2-bromo-2-(2-chlorophenyl)acetic acid **2** by using NBS. Methyl ester **3** of compound **2** was treated with 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride in presence of base to afford methyl 2-(2-chlorophenyl)-2-(4-(6-fluorobenzo [d]isoxazol-3-yl)piperidin-1-yl)acetate **4**. 2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)acetohydrazide **5** was prepared by refluxing compound **4** with hydrazine hydrate. Condensation of compound **5** with different aryl isothiocynates in alcohol furnished the corresponding thiosemicarbazides **6**. Thiosemicarbazides undergoes cyclisation to give thiadiazoles in acidic condition whereas to triazoles in basic medium. Oxadiazoles were prepared by treating thiosemicarbazides with iodine and potassium iodide in NaOH. The spectral analysis supports these transformations.

The antibacterial activity of some of the newly synthesized compounds was carried out by agar well diffusion method. Two bacterial species were chosen for the study, one was Gram Positive Bacillus Subtilis and another was Gram Negative Escherichia Coli. Ampicillin was used as a standard drug for this study. Compounds **6b**, **6c**, **9a**, **9b**, **9d** and **9e** have shown moderate activity towards Bacillus Subtilis and Escherichia Coli bacterial species.



SCHEME 1

RESULTS AND DISCUSSION

Melting points were determined in open capillary tubes in liquid paraffin bath and are uncorrected. IR spectra were recorded on Perkin Elmer Spectrophotometer using potassium bromide discs. NMR spectra were recorded on a Varian NMR 400 MHz Spectrometer and chemical shifts are given in δ ppm relative to TMS using deuterated DMSO and deuterated chloroform as a solvents. Mass Spectra were recorded on Water's Acquity Ultra Performance TQ Detector Mass Spectrometer..

Preparation of 1-(2-(2-chlorophenyl)-2-(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl)acetyl)-4-phenylthiosemicarbazide (6a-e).

Equimolar amount (5 mmol) of compound **5** and aryl isothiocyanate was dissolved in 15 mL of ethanol. The reaction mixture was heated under reflux for 2 h. The progress of reaction was monitored by TLC (80% Pet ether + 20% Ethyl acetate). After completion of reaction, contents were cooled and the solid obtained was filtered and recrystallized from ethanol to get the pure product 1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetyl)-4- phenylthiosemicarbazide **6**.

1-(2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)acetyl)-4-phenylthiosemicarbazide (6a): Yield 74%; mp 205-207 °C; IR: 1116, 1459, 1589, 1624, 3110, 3116 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 1.88 (m, 4H, -CH_2, piperidine), 2.10 (m, 1H, -CH, piperidine), 2.42 (m, 1H, -CH, piperidine), 2.90 (m, 1H, -CH, piperidine), 3.01 (m, 1H, -CH, piperidine), 3.20 (m, 1H, -CH, piperidine), 4.90 (s, 1H, -CH), 7.10 (t, J = 8.8 Hz, 2H, Ar-H), 7.25 (m, J = 7.2 & 8.8 Hz, 1H, Ar-H), 7.21-7.35 (m, 6H, Ar), 7.62 (m, J = 7.2 Hz, 2H, Ar-H), 8.01 (m, 1H, Ar-H), 9.45 (bs, 1H, -NH), 9.70 (bs, 1H, -NH), 10.27 (bs, 1H, -NH), MS: m/z = 537.14.

1-(2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)acetyl)-4-(4-fluorophenyl)thiosemicarbazide (6b): Yield 77%; mp 204-206 °C; IR: 1111, 1456, 1577, 1620, 3089, 3116 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 1.98 (m, 4H, -CH_2, piperidine), 2.13 (m, 1H, -CH, piperidine), 2.40 (m, 1H, -CH, piperidine), 2.91 (m, 1H, -CH, piperidine), 3.06 (m, 1H, -CH, piperidine), 3.22 (m, 1H, -CH, piperidine), 4.91 (s, 1H, -CH), 7.15 (t, J = 8.8 Hz, 2H, Ar), 7.27 (m, J = 7.2 & 8.8 Hz, 1H, Ar), 7.31-7.41 (m, 5H, Ar), 7.65 (m, J = 7.2 Hz, 2H, Ar), 8.03 (m, 1H, Ar), 9.5 (bs, 1H, -NH), 9.71 (bs, 1H, -NH), 10.29 (bs, 1H, -NH), MS: m/z = 556.13 (M+1).

1-(2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)acetyl)-4-(4-chlorophenyl) thiosemicarbazide (6c): Yield 71%; mp 203-205 °C; IR: 1110,1458, 1590, 1621, 3093, 3120 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 1.89 (m, 4H, -CH_2, piperidine), 2.10 (m, 1H, -CH, piperidine), 2.42 (m, 1H, -CH, piperidine), 2.93 (m, 1H, -CH, piperidine), 3.03 (m, 1H, -CH, piperidine), 3.20 (m, 1H, -CH, piperidine), 4.90 (s, 1H, -CH), 7.18 (t, J = 8.8 Hz, 2H, Ar-H), 7.37 (m, J = 7.2 & 8.8 Hz, 1H, Ar-H), 7.28 - 7.39 (m, 5H, Ar-H), 7.63 (m, J = 7.2 Hz, 2H, Ar-H), 8.07 (m, 1H, Ar-H), 9.45 (bs, 1H, -NH), 9.74 (bs, 1H, -NH), 10.30 (bs, 1H, -NH), MS: m/z = 572.4 (M+1).

1-(2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)acetyl)-4-p-tolylthiosemicarbazide (6d): Yield 78%; mp 192-194 °C; IR: 1112,1457, 1575, 1625, 3085, 3117 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 1.96 (m, 4H, -CH_2, piperidine), 2.16 (m, 1H, -CH, piperidine), 2.30 (s, 3H, -CH_3), 2.41 (m, 1H, -CH, piperidine), 2.89 (d, 1H, -CH, piperidine), 2.91 (m, 1H, -CH, piperidine), 3.24 (m, 1H, -CH, piperidine), 4.87 (s, 1H, -CH), 7.11 (d, J = 8.4 Hz, 2H, Ar-H), 7.25 (m, 4H, Ar-H), 7.48 (m, 1H, Ar-H), 7.59 (m, 2H, Ar-H), 7.65 (dd, 1H, Ar-H), 8.03 (m, J = 5.6 & 10.4 Hz, 1H, Ar-H), 9.25 (bs, 1H, -NH), 9.71 (bs, 1H, -NH), 10.19 (bs, 1H, -NH), MS: m/z = 552.3(M+1).

1-(2-(2-Chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidin-1-yl)acetyl)-4-(2-methoxyphenyl) thiosemicarbazide (6e): Yield 71%; mp 192-194 °C; IR: 1115,1458, 1589, 1625, 3105, 3120 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 1.95-1.99 (m, 4H, -CH_2, piperidine), 2.18 (m, 1H, -CH, piperidine), 2.48 (m, 1H,-CH, piperidine), 2.85 (m, 1H, -CH, piperidine), 3.12 (m, 2H, -CH_2, piperidine), 3.74 (s, 3H, -OCH_3), 4.74 (s, 1H, -CH), 6.89 (t, J = 7.6 Hz, 1H, Ar-H), 7.02 (d, J = 8.0 Hz, 1H, Ar-H), 7.10 (m, 2H, Ar-H), 7.26 (m, 1H, Ar-H), 7.35 (m, J = 8.0 Hz, 2H, Ar-H), 7.48 (d, J = 7.2 Hz, 1H, Ar-H), 7.66 (d, J = 7.2 Hz, 2H, Ar-H), 8.02 (m, 1H, Ar-H), 9.25 (bs, 1H, -NH), 9.95 (bs, 1H, -NH), 10.51 (bs, 1H, -NH). MS: m/z = 568.06 (M+1).

Preparation of 5-((2-chlorophenyl)(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine (7).

Thiosemicarbazide **6** (1 mmol) was dissolved in 4 mL of conc. H_2SO_4 in a 100 mL RBF. The reaction mixture was stirred at room temperature for 3 h. After completion of reaction 20 g of crushed ice was added in it. The solid obtained was separated by filtration and recrystallized from ethanol to afford thiadiazoles **7**.

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine (7a): Yield 66%; mp 171-173 °C; IR: 1113, 1460, 1580, 1625 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 2.22 (m, 4H, CH_2, piperidine), 3.01 (m, 1H, CH, piperidine), 3.20 (m, 2H, CH_2, piperidine), 3.52 (m, 2H, CH_2, piperidine), 5.45 (s, 1H, CH), 7.10 (t, J = 8.8 & 9.2 Hz, 2H, Ar-H), 7.30 (t, J = 8.8 & 9.2 Hz, 2H, Ar-H), 7.37(m, J = 4.8 & 8.4 Hz, 1H, Ar-H), 7.44 (m, 2H, Ar-H), 7.67 (m, J = 7.6 Hz, 2H, Ar-H), 7.75 (m, 1H, Ar-H), 8.00 (m, J = 4.8 Hz, 1H, Ar), 9.95 (s, 1H, -NH), MS: m/z = 520.10 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (7b): Yield 69%; mp 167-169 °C; IR: 1114, 1460, 1582, 1625 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 2.26 (m, 4H, CH_2, piperidine), 3.04 (m, 1H, CH, piperidine), 3.19 (m, 2H, CH_2, piperidine), 3.53 (m, 2H, CH_2, piperidine), 5.45 (s, 1H, CH), 7.15 (t, J = 8.8 & 9.2 Hz, 2H, Ar-H), 7.35 (m, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 7.65 (m, J = 7.6 Hz, 2H, Ar-H), 7.74 (m, 1H, Ar-H), 7.99 (m, J = 4.8 Hz, 1H, Ar), 9.90 (s, 1H, -NH), MS: m/z = 538.2 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (7c): Yield 64%; mp 170-172 °C; IR: 1110, 1465, 1580, 1626 cm^{-1},^1H NMR (DMSO- d_6), δ, ppm: 2.26 (m, 4H, CH_2, piperidine), 3.04 (m, 1H, CH, piperidine), 3.19 (m, 2H, CH_2, piperidine), 3.53 (m, 2H, CH_2, piperidine), 5.45 (s, 1H, CH), 7.15 (t, J = 8.8 & 9.2 Hz, 2H, Ar-H), 7.29 (t, J = 8.8 & 9.2 Hz, 2H, Ar-H), 7.35 (m, J = 4.8 & 8.4 Hz, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 7.65 (m, J =7.6 Hz, 2H, Ar-H), 7.74 (m, 1H, Ar-H), 7.99 (m, J = 4.8 Hz, 1H, Ar-H), 9.85 (s, 1H, -NH), MS: m/z = 554.2 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-p-tolyl-1,3,4-thiadiazol-2-amine (7d): Yield 68%; mp 166-168 °C; IR: 1112, 1460, 1585, 1625 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 2.25 (s, 3H, -CH_3), 2.29 (m, 4H, CH_2, piperidine), 3.05 (m, 2H, CH_2, piperidine), 3.23 (m, 1H, CH, piperidine), 3.41 (m, 2H, CH, piperidine), 5.51 (s, 1H, CH), 7.12 (d, J = 8.4 Hz, 2H, Ar-H), 7.24 (d, J = 7.6 Hz, 2H, Ar-H), 7.31 (d, J = 9.2 Hz, 2H, Ar-H), 7.63 (m, J = 7.2 Hz, 2H, Ar-H), 7.65 (m, J = 7.6 Hz, 2H, Ar-H), 8.02 (m, J =7.6 & 5.6 Hz, 1H, Ar-H), 9.90 (s, 1H, -NH), MS: m/z = 534.7 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(2-methoxy phenyl)-1,3,4-thiadiazol-2-amine (7e): Yield 62%; mp 169-171 °C; IR: 1110, 1461, 1585, 1622 cm^{-1}, ^1H NMR (DMSO-d_6), δ, ppm: 2.19 - 2.30 (m, 4H, CH_2, piperidine), 2.82 (m, 1H, CH, piperidine), 3.21-3.39 (m, 4H, CH_2, piperidine), 3.73 (s, 3H, -OCH_3), 5.53 (s, 1H, CH), 6.98 (d, J = 8.8 Hz, 1H, Ar-H), 7.30 (t, J = 8.0 & 8.8 Hz, 1H, Ar-H), 7.43 (d, J = 8.0 Hz, 1H, Ar-H), 7.52 (m, 3H, Ar-H), 7.65 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 8.01 (m, 1H, Ar-H), 10.5 (s, 1H, -NH), MS: m/z = 550.3 (M+1).

Preparation of 5-((2-chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (8).

Thiosemicarbazide **6** (1mmol) was dissolved in 10 mL of 2N NaOH. The reaction mixture was heated under mild reflux. The progress of reaction was monitored by TLC (80% Pet ether + 20% Ethyl acetate). After completion of reaction, contents were cooled and poured into crushed ice. Then it was acidified with glacial acetic acid. The product was separated by filtration and recrystallized from ethanol to get corresponding triazoles **8**.

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (8a): Yield 61%; mp 223-225 °C; IR: 1114, 1461, 1584, 1627, 2357 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 2.08 (m, 4H, -CH_2, piperidine), 2.34 (m, 1H, -CH, piperidine), 2.49 (m, 1H, -CH, piperidine), 2.97 (m, 2H, -CH, piperidine), 3.13 (d, 1H, -CH, piperidine), 5.18 (s, 1H, -CH), 7.04 (m, J = 7.2 & 8.8 Hz, 3H, Ar-H), 7.12 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.25 (m, 1H, Ar-H), 7.30 (m, 2H, Ar-H), 7.49 (m, J = 4.8 Hz, 1H, Ar-H), 7.60 (m, J = 4.8 Hz, 1H, Ar-H), 11.08 (bs, 1H, -SH), MS: m/z = 520.05 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol (8b): Yield 65%; mp 223-225 °C; IR: 1110, 1458, 1580, 1628, 2359 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 2.02 (m, 4H, -CH_2, piperidine), 2.28-2.49 (m, 4H, -CH_2, piperidine), 3.10 (m, 1H, CH, piperidine), 5.15 (s, 1H, -CH), 7.07 (m, J = 7.2 & 8.8 Hz, 2H, Ar-H), 7.21-7.30 (m, 5H, Ar-H), 7.49 (m, J = 4.8 Hz, 1H, Ar-H), 7.60 (m, J = 4.8 Hz, 1H, Ar-H), 11.06 (bs, 1H, -SH), MS: m/z = 538.36 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (8c): Yield 67%; mp 225-227 °C; IR: 1113, 1452, 1587, 1624, 2364 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 2.02 (m, 4H, CH_2, piperidine), 2.32 (m, 1H, CH, piperidine), 2.47 (m, 1H, CH, piperidine), 2.99 (m, 2H, CH, piperidine), 3.10 (d, 1H, CH, piperidine), 5.15 (s, 1H, CH), 7.07 (m, J = 7.2 & 8.8 Hz, 2H, Ar-H), 7.15 (m, 2H, Ar-H), 7.24 (m, 2H, Ar-H), 7.29 (m, 1H, Ar-H), 7.31 (m, 2H, Ar-H), 7.49 (m, J = 4.8 Hz, 1H, Ar-H), 7.60 (m, J = 4.8 Hz, 1H, Ar-H), 11.06 (bs, 1H, -SH), MS: m/z = 554.40 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-p-tolyl-4H-1,2,4-triazole-3-thiol (8d): Yield 64%; mp 228-230 °C; IR: 1111, 1458, 1581, 1620, 2360 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.99 (m, 4H, CH_2, piperidine), 2.30 (m, 1H, CH, piperidine), 2.43 (s, 3H, CH_3), 2.97 (m, 2H, CH_2, piperidine), 3.08 (m, 2H, CH_2, piperidine), 5.15 (s, 1H, CH), 7.03 (m, J = 1.6 & 8.9 Hz, 1H, Ar-H), 7.26 (m, 8H, Ar-H), 7.55 (m, 1H, Ar-H), 7.61 (m, 1H, Ar-H), 10.80 (bs, 1H, -SH), MS: m/z = 534.3 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-4-(2-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (8e): Yield 61%; mp 227-229 °C; IR: 1113, 1455, 1582, 1622, 2366 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.95 (m, 4H, CH_2, piperidine), 2.26 (m, 2H, CH_2, piperidine), 3.0 (m, 2H, -

CH_2, piperidine), 3.30 (m, 1H, CH, piperidine), 3.84 (s, 3H, -OCH_3), 5.15 (s, 1H, CH), 6.88 (m, 1H, Ar-H), 6.87-7.05 (m, 3H, Ar-H), 7.20-7.22 (m, 3H, Ar-H), 7.31-7.51 (m, 2H, Ar-H), 7.64 (m, 2H, Ar-H), 10.82 (bs, 1H, -SH), MS: m/z = 550.05 (M+1).

Preparation of 5-((2-chlorophenyl)(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl)methyl)-N-phenyl-1,3,4-oxadiazol-2-amine (9).

The mixture thiosemicarbazide **6** (1 mmol), potassium iodide (2 mmol), iodine (2 mmol) was dissolved in 4N sodium hydroxide (10mL). The reaction mixture was heated under mild reflux for 5h. The progress of reaction was monitored by TLC (80% Pet ether + 20% Ethyl acetate). After completion of reaction, contents were poured over crushed ice and were extracted with ethyl acetate. The crude product isolated after evaporation of ethyl acetate was recrystallized from ethanol to get pure product **9**.

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-phenyl-1,3,4-oxadiazol-2-amine (9a): Yield 69 %; mp 200-202 °C; IR: 1040, 1445, 1560, 1625, 3250 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.56-1.64 (m, 1H, CH, piperidine), 2.05-2.10 (m, 2H, CH_2, piperidine), 2.08-2.10 (m, 2H, CH_2, piperidine), 2.42-2.46 (m, 2H, CH_2, piperidine), 3.03-3.10 (m, 2H, CH_2, piperidine), 5.42 (s,1H, CH), 7.01 (m, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 7.10 (d, 2H, Ar-H), 7.24 (m, 1H, Ar-H), 7.30 (m, 3H, Ar-H), 7.42 (d, 1H, Ar-H), 7.62 (m, 1H, Ar-H), 7.80 (dd, 1H, Ar-H), 9.80 (bs, 1H, N-H), MS: m/z = 504.04 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (9b): Yield 63%; mp 195-197 °C; IR: 1042, 1449, 1598, 1625, 3245 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.58-1.67 (m, 1H, CH, piperidine), 2.0-2.02 (m, 2H, CH_2, piperidine), 2.07-2.11 (m, 2H, CH_2, piperidine), 2.40-2.48 (m, 2H, CH_2, piperidine), 3.01-3.09 (m, 2H, CH_2, piperidine), 5.40 (s,1H,CH), 7.03 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.14 (d, 2H, Ar-H), 7.22 (m, 1H, Ar-H), 7.31 (m, 3H, Ar-H), 7.40 (d, 1H, Ar-H), 7.64 (m, 1H, Ar-H), 7.83 (dd, 1H, Ar-H), 9.75 (bs, 1H, N-H), MS: m/z = 522.04 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (9c): Yield 60%; mp 185- 187 °C; IR: 1042, 1447, 1597, 1625, 3255 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.55-1.62 (m, 1H, CH, piperidine), 2.02-2.05 (m, 2H, CH_2, piperidine), 2.08-2.10 (m, 2H, CH_2, piperidine), 2.42-2.50 (m, 2H, CH_2, piperidine), 3.04-3.11 (m, 2H, CH_2, piperidine), 5.38 (s, 1H, CH), 7.05 (m, 1H, Ar-H), 7.08 (m, 1H, Ar-H), 7.12 (d, 2H, Ar-H), 7.24 (m, 1H, Ar-H), 7.33 (m, 3H, Ar-H), 7.42 (d, 1H, Ar-H), 7.66 (m, 1H, Ar-H), 7.85 (dd, 1H, Ar-H), 10.15 (bs, 1H, N-H), MS: m/z = 538.52 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-p-tolyl-1,3,4-oxadiazol-2-amine (9d): Yield 67%; mp 182-184 °C; IR: 1046, 1449, 1602, 1628, 3240 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 1.58-1.67 (m, 1H, -CH, piperidine), 2.0-2.02 (m, 2H, CH_2, piperidine), 2.07-2.11 (m, 2H, CH_2, piperidine), 2.27 (s, 3H, -CH_3), 2.40-2.48 (m, 2H, CH_2, piperidine), 3.01-3.09 (m, 2H, CH_2, piperidine), 5.40 (s, 1H, CH), 7.03 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.14 (d, 2H, Ar-H), 7.22 (m, 1H, Ar-H), 7.31 (m, 3H, Ar-H), 7.40 (d, 1H, Ar-H), 7.64 (m,1H, Ar-H), 7.83 (dd,1H, Ar-H), 9.95 (bs, 1H, N-H), MS: m/z = 518.3 (M+1).

5-((2-Chlorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)-N-(2-methoxyphenyl)-1,3,4-oxadiazol-2-amine (9e): Yield 66%; mp 200-202 °C; IR: 1045, 1448, 1627, 3249 cm^{-1}, ^1H NMR ($CDCl_3$), δ, ppm: 2.08 (m, 2H, CH_2, piperidine), 2.40-2.50 (m, 2H, CH_2 piperidine), 3.04 (m, 1H, CH, piperidine), 3.20 (m, 4H, CH_2, piperidine), 3.90 (s, 3H, -CH_3), 5.42 (s, 1H, CH), 6.8-6.90 (m, 1H, Ar-H), 7.0-7.08 (m, 3H, Ar-H), 7.33-7.37 (m, 2H, Ar-H), 7.41 (d, J = 8.0 Hz, 1H, Ar-H), 7.66 (m, 1H, Ar-H), 7.86 (m,1H, Ar-H), 8.13 (dd, J = 4.8 & 6.8Hz, 1H, Ar-H), 10.20 (bs, 1H, N-H), MS: m/z = 534.3 (M+1).

ANTIBACTERIAL SCREENING

Bacillus Subtilis and Escherichia Coli species were chosen for the study of antibacterial activity. The activity was tested by agar well diffusion method. The bacteria were cultured on nutrient agar. The concentration of the compounds taken was 10mg/mL of which 0.1mL was used in this assay. Ampicillin was used as a standard drug and its final concentration used was 1mg. The zone inhibition of antibacterial activity was measured in mm and the results were produced as an average of three repeated assays. The result of this assay is given in **Table 1**. It was found that the compounds **6b**, **6c**, **9a**, **9b**, **9d** and **9e** have shown moderate activity towards both bacterial species.

Table 1: Antibacterial Screening of synthesized compounds

Compound	E. Coli	B. Subtilis	Compound	E. Coli	B. Subtilis
6a	11	12	**8a**	13	13
6b	**15**	**16**	**8b**	14	12
6c	**14**	**17**	**8c**	15	11
6d	13	12	**8d**	13	14
6e	12	16	**8e**	13	15
7a	9	14	**9a**	**15**	**15**
7b	8	14	**9b**	**14**	**16**
7c	9	15	**9c**	15	12
7d	9	13	**9d**	**13**	**16**
7e	11	15	**9e**	**15**	**13**
Standard drug: Ampicillin				16	17

CONCLUSION

A series of novel 1,3,4-thiadiazole, 1,2,4-triazole and 1,3,4-oxadiazole have been synthesized from 1-(2-(2-chlorophenyl)-2-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)acetyl)-4-phenyl thiosemicarbazide **6** and their antimicrobial activities were reported. Compounds **6b**, **6c**, **9a**, **9b**, **9d** and **9e** have shown moderate activity towards Bacillus Subtilis and Escherichia Coli bacterial species but none of them was as active as standard ampicillin.

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