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SYNTHESIS OF *N*-(ARYL SUBSTITUTED)-2-((6-OXO-5,6-DIHYDROIMIDAZO[2,1-*b*][1,3,4] THIADIAZOL-2-YL)THIO)ACETAMIDE DERIVATIVES TOWARDS ANTIMICROBIAL ACTIVITIY

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ABSTRACT

A *series of novel N-(aryl substituted)-2-((6-oxo-5,6-dihydroimidazo[2,1-b] [1,3,4]thiadiazol-2-yl)thio)acetamide conjugates* ***(4a-m)*** *have been synthesized by intermolecular cyclisation of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-N-(aryl substituted)acetamides with ethyl chloro acetate in glacial acetic acid and the obtained products were elucidated by spectrochemical techniques (IR, ^{1}H-NMR, MS and ^{13}C-NMR) and elemental analysis. These conjugates were evaluated for their antibacterial activity against* Staphylococcus aureus, *Bascillus Subtilis, Escherichia coli and* Pseudomonas aeruginosa *strains. Antifungal activity against Aspergillus fumigatus, Candida albicans, Aspergillus niger and Aspergillus clavatus strains. Among the tested compounds,* ***4b*** *and* ***4c*** *with the substituents 4-chloro and 3-chloro-4-fluoro showed appreciable pharmacological activity profile compared to the standard drug streptomycin against the bacterial strain P.aerugenosa.* ***4a***, ***4f*** *and* ***4i*** *with the substituents 4-fluoro, diphenyl and 4-methyltrifluoro with the phenyl ring exhibit significant activity against the fungal strains Aspergillus fumigatus, Aspergillus niger and Aspergillus clavatus.*

Keywords: *5-amino-1,3,4-thiadiazole, Imidazothiadiazole, Antibacterial, Antifungal activity.*

INTRODUCTION

Imidazo[2,1-*b*][1,3,4]thiadiazole containing compounds were known from the early 1950s and they have been extensively investigated towards their versatile broad spectrum and eminent biological importance with significant development in their chemistry and biology [1]. Many of them have shown interesting pharmacological activity profile by emerging as a potent antibacterial [2-4], antifungal [5-7], anticancer [8, 9], antiproliferativeactivity [10], antitumor [11], antituberculosis [12, 13], antihypertensive [14, 15] and anticonvulsant [16, 17] activities.

The pathogenic microorganisms have been resistant to these antimicrobial agents by continuous use and widespread. Hence, there is need for newer class of safer and efficient drugs [18]. The structural modifications of these chemical scaffolds of imidazo[2,1-*b*][1,3,4]thiadiazole ring system have consistently resulted in compounds with diverse pharmacological activities and the heterocyclic scaffolds having thioacetamide system linked to the [1,3,4] thiadiazole have not been reported earlier. Hence it is a challenging task to synthesize the heterocyclic moieties having the fused heterocyclic ring with thioacetamide linkage in a specific manner. By knowing the above facts from the literature in this paper, a feasible and scalable etiquette for the synthesis of *N*-(aryl substituted)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide derivatives with enhanced pharmacological potential can be illustrated.

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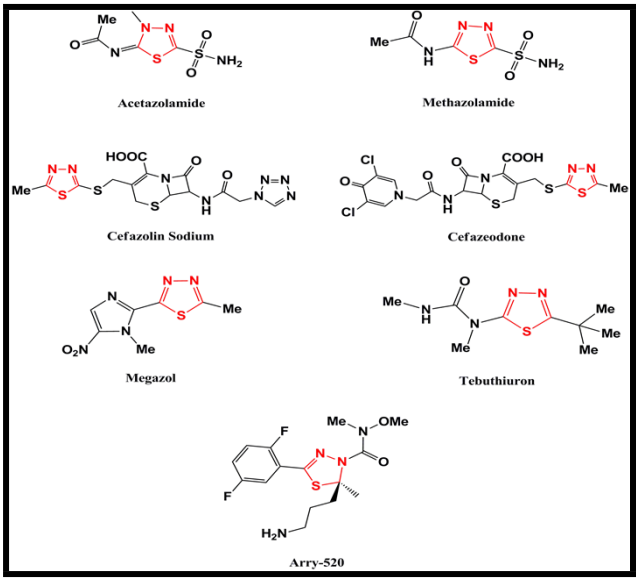


Fig. 1: Chemical Class of drugs which contain 1,3,4-thiadiazole motify

Pharmacology:

Antibacterial activity:

In the antibacterial activity study, the newly synthesized compounds were screened for their *in-vitro* antibacterial activity against two gram positive bacterial strains such as *Staphylococcus aureus* and *Bascillus Subtilis*, and two gram negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa,* by employing the serial plate dilution method [19, 20]. Serial dilutions of the drug in the Muller Hinton broth were taken in tubes and their pH was adjusted to 5.0 using the phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37 ^{o}C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Into each petri dish 20mL of agar media. Excess of suspension

was decanted and the plates were dried by placing them in an incubator at 37 °C for an hour. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labelled well. A control was also prepared for the plates in the same way using the DMSO as a solvent. The petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 days. Any antibacterial activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with streptomycin as the standard [21, 22]. The results are summarized in (**Table 2)**.

The MIC values were evaluated at a concentration range of 3.12-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm in the parentheses.

Antifungal activity:

The synthesized compounds were also evaluated for their antifungal activity against *Aspergillus fumigatus, Candida albicans, Aspergillus niger* and *Aspergillus clavatus* in DMSO by serial plate dilution method [23, 24]. Sabourauds agar media was prepared by dissolving peptone (1g). D-glucose (4g) and agar (2g) in distilled water (100mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the sore of the fungal strains for lawning. A loopful of a particular fungal strain was transferred to 3mL saline to get a suspension of the corresponding species. Twenty milliliters of the agar media was poured into each petri dish. The excess of suspension was decanted and the plates were dried by placing them in an incubator at 37 °C for 1h. Using a punch, wells were made on these seeded agar plates. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labelled well. A control was also prepared for the plates in the same way using the solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Any antifungal activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with Itraconazole as the standard. The results are summarized in (**Table 3)**.

The MIC values were evaluated at a concentration range of3.12-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm in parentheses.

EXPERIMENTAL

The melting points were determined by an open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FTIR 157 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on 400MHz spectrometer using the TMS as an internal standard. The mass spectra were recorded in Agilent Technology LC-mass spectrometer. The elemental analyses (CHNS) were performed on the CHNS ElementarVario EL III. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates.

General procedure for the preparation of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(substitutedphenyl)acetamide (3a-m):

A mixture of **2** [27] (0.005mol), anhydrous potassium carbonate (0.005mol) and 5-amino-1,3,4-thiadiazole-4-thiol (0.005mol) in dry acetone (20 mL) was heated under reflux for 14hrs, and then allowed to cool. The reaction mixture was filtered and the obtained precipitate was washed with H_2O, dried and purified by dissolving in glacial acetic acid and re-precipitation by dil. NH_4OH.

3a- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3358 (NH), 3255 (NH_2), 3099 (Ar C-H), 2933 (C-H), 1649(C=O),1544 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, δ ppm): 1.91 (s, 2H, CH_2), 2.45 (s, 2H, NH_2), 6.972-7.532 (m, 4H, 4-fluorophenyl), 9.64 (s, 1H, NH). LC-MS: *m/z* = 285(M^+).

General procedure for the synthesis of 2-((6-(aryl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide (4a-m):

An equimolar ratio of compound **(3a-m)** (0.005mol) and ethyl chloroacetate (0.005 mol) in glacial acetic acid (20mL) was heated under reflux for 17h. The reaction mixture was poured into ice cold water. The precipitated solid was filtered, dried and recrystallized from ethanol.

4b- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3354 cm^{-1} (NH), 3060 cm^{-1} (Ar C-H), 1614 (C=O), 1203 cm^{-1} (C-S), 1588 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.05 (s, 2H, imidazole), 4.18 (s, 2H, CH_2), 7.13-7.18(m, 2H, 4-fluorophenyl), 7.56-7.59 (m, 2H, 4-fluorophenyl), 10.37 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 145.79, 142.14, 121.36, 138.89 (imidazo[2,1-*b*][1,3,4]thiadiazole), 112.84, 115.73, 164.83, 139.84 (4-fluorophenyl), 128.52, 124.61, 126.72, 136.96 (4-methoxyphenyl), 37.64 (CH_2); LC-MS: [M^++1], (m/z) : 325.

4c- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3353 cm^{-1} (NH), 3064cm^{-1} (Ar C-H), 1629 (C=O), 1209 cm^{-1} (C-S), 1600 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.32 (s, 2H, imidazole), 4.23 (s, 2H, CH_2), 7.61-7.63(d, 2H, *J* = 8Hz, 4-chlorooorophenyl), 7.86-7.88 (d, 2H, *J* = 8Hz, 4-chlorophenyl), 10.82 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.68 (C=O adjacent to amide) , 167.40 (C=O,imidazole) 160.62, 157.07 (2C atoms,Thiadiazole moiety), 146.98, 145.89, 126.46 and 120.55 (6C atoms, 4-chlorophenyl), 63.07 (CH_2, imidazole), 29.76 (CH_2, aliphatic), 37.64 (CH_2); LC-MS: [M^++1], (m/z) : 341.05/343.04.

4d- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3277 cm^{-1} (NH), 3069cm^{-1} (Ar C-H), 1630 (C=O), 1212 cm^{-1} (C-S), 1605 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.17 (s, 2H, imidazole), 4.28 (s, 2H, CH_2), 7.92(s, 1H, 2,4,5-trichlorophenyl), 8.10 (s, 1H, 2,4,5-trichlorophenyl), 10.09 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 165.34 (C=O adjacent to amide) , 165.25(C=O, imidazole) 160.89, 158.90 (2C atoms, thiadiazole moiety), 135.01, 131.91, 129.97 and 127.50 (6C atoms, 3-chloro-4-fluorophenyl), 63.65 (CH_2, imidazole), 30.49 (CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 410.28/412.96/414.95.

4e - FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3352 cm^{-1} (NH), 3068cm^{-1} (Ar C-H), 1631 (C=O), 1202 cm^{-1} (C-S), 1603 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.34 (s, 2H, imidazole), 4.21 (s, 2H, CH_2), 7.63-7.65(d, 2H, *J* = 8.2Hz, 4-bromophenyl), 7.87-7.89(d, 2H, *J* = 8Hz, 4-bromophenyl), 10.76 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.62 (C=O adjacent to amide) , 167.37 (C=O, imidazole) 160.71, 157.11(2C atoms, thiadiazole moiety), 146.41, 145.82, 126.42 and 120.56 (6C atoms, 4-chlorophenyl), 63.11 (CH_2, imidazole), 29.63 (CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 385.21/387.41.

4f- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3073cm^{-1} (Ar C-H), 1639 (C=O), 1204 cm^{-1} (C-S), 1611 cm^{-1} (C=N); ^{1}H-NMR (400MHz, DMSO, δ ppm): 3.39 (s, 2H, imidazole), 4.28 (s, 2H, CH_2), 7.71-7.78(m, 5H, diphenyl), 7.81-7.86(m, 5H, diphenyl); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.64 (C=O adjacent to amide) , 167.41 (C=O, imidazole) 161.82, 156.23(2C atoms, thiadiazole moiety), 134.21, 132.29, 129.60, 128.32, 126.42 and 120.56 (12C atoms, diphenyl), 63.11 (CH_2, imidazole), 30.13 (CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 383.06.

4g- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3242 cm^{-1} (NH), 3068cm^{-1} (Ar C-H), 1634 (C=O), 1221 cm^{-1} (C-S), 1529 and 1367 (-NO_2 asymmetric and symmetric stretch); ^{1}H-NMR (400MHz, DMSO, δ ppm): 2.15 (s,1H, CH_3),3.36 (s, 2H, imidazole), 4.23 (s, 2H, CH_2), 7.94-7.87(dd, 2H, *J* = 8.2Hz, 4-bromophenyl), 7.63-7.63(d, 2H, *J* = 3.2Hz, 4-methyl-3-nitrophenyl), 10.28 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.77 (C=O adjacent to amide) , 167.43 (C=O,imidazole) 160.58, 156.19(2C atoms, thiadiazole moiety), 149.40,137.76, 133.47, 129.12, 127.38 and 114.46 (6C atoms, 4-methyl-3-nitrophenyl), 62.33 (CH_2, imidazole), 30.63 (CH_2, aliphatic), 20.26 (methyl); LC-MS: [M^++1], (m/z) : 366.06.

4h- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3349 cm^{-1} (NH), 3070cm^{-1} (Ar C-H), 1635 (C=O), 1205 cm^{-1} (C-S), 1609 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.36 (s, 2H, imidazole), 4.18 (s, 2H, CH_2), 7.71-7.76(d, 2H, *J* = 8Hz, 4-nitrophenyl), 7.81-7.85(d, 2H, *J* = 8.2Hz, 4-nitrophenyl), 10.81 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.68 (C=O adjacent to amide) , 165.87 (C=O, imidazole) 161.24, 158.22(2C atoms, thiadiazole moiety), 147.38, 144.76, 125.24 and 121.42 (6C atoms, 4-nitrophenyl), 63.23 (CH_2,imidazole), 30.21 (CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 352.32.

4i- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3351 cm^{-1} (NH), 3068cm^{-1} (Ar C-H), 1625 (C=O), 1211 cm^{-1} (C-S), 1591 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.11 (s, 2H, imidazole), 4.23 (s, 2H, CH_2), 7.13-7.18(m, 3H, 3-trifluorophenyl), 7.56-7.59 (m, 1H, 3-trifluorophenyl), 10.24 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.69 (C=O adjacent to amide) , 165.81 (C=O, imidazole) 162.02, 158.24(2C atoms, thiadiazole moiety), 146.37, 144.74, 125.31 and 121.39 (6C atoms, 4-trifluorophenyl), 63.27 (CH_2,imidazole), 30.02 (CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 375.27.

4j- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3349 cm^{-1} (NH), 3078cm^{-1} (Ar C-H), 1629 (C=O), 1209 cm^{-1} (C-S), 1593 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.14 (s, 2H, imidazole), 3.21(s,1H, methyl), 4.31 (s, 2H, CH_2), 7.19-7.23(d, 2H, *J* = 8Hz , 4-methylphenyl), 7.46-7.51 (d, 2H, *J* = 8.4Hz, 4-methylphenyl), 10.23 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.54 (C=O adjacent to amide) , 165.69(C=O, imidazole) 162.21, 158.33(2C atoms, thiadiazole moiety), 146.29, 144.61, 125.32 and 121.29 (6C atoms, 4-methylphenyl), 63.24 (CH_2,imidazole), 30.32(CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 321.24.

4k- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3349 cm^{-1} (NH), 3075cm^{-1} (Ar C-H), 1632 (C=O), 1207 cm^{-1} (C-S), 1589 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 2.29 (m, 2H, *J* = 8.4Hz , methyne), 3.14 (s, 2H, imidazole), 4.07 (q, 3H, *J* = 7.2Hz ester methylene) 4.283 (s, 2H, CH_2), 7.13-7.18(m, 3H, 3-trifluorophenyl), 7.56-7.59 (m, 1H, 3-trifluorophenyl), 10.24 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.69 (C=O adjacent to amide) , 165.81 (C=O, imidazole) 162.02, 158.24(2C atoms, thiadiazole moiety), 146.37, 144.74, 125.31 and 121.39 (6C atoms, 4-ethoxyphenyl), 63.27 (CH_2,imidazole), 30.43(CH_2,ester), 30.02 (CH_2, aliphatic), 20.24 (methyl); LC-MS: [M^++1], (m/z) : 351.26.

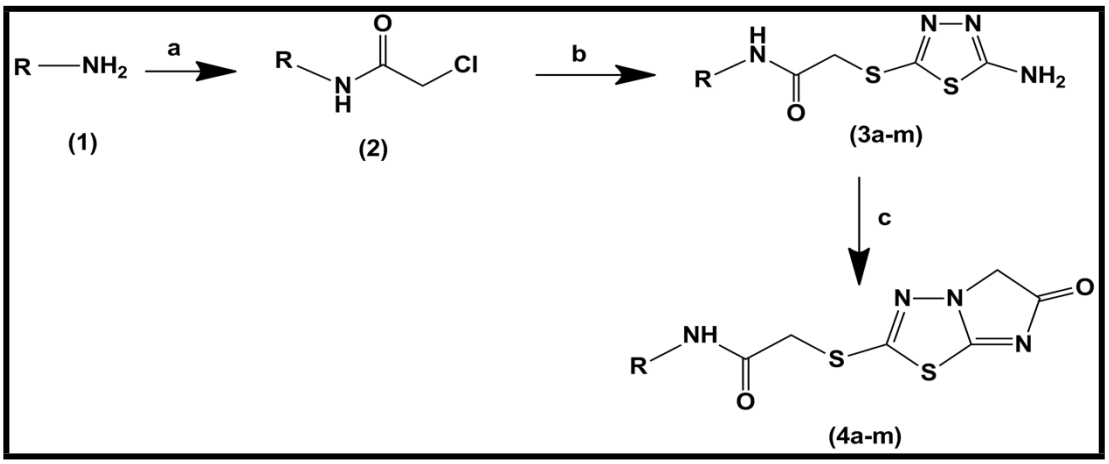
4l- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3343 cm^{-1} (NH), 3071cm^{-1} (Ar C-H), 1632 (C=O), 1207 cm^{-1} (C-S), 1604 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.29 (s, 2H, imidazole), 4.26 (s, 2H, CH_2), 7.61-7.63(d, 2H, *J* = 8.2Hz, pyrazine), 7.86-7.88 (s, 1H, pyrazine), 10.79 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.58 (C=O adjacent to amide) , 167.46 (C=O, imidazole) 160.71, 157.72 (2C atoms, thiadiazole moiety), 145.71, 144.74, 126.22 and 119.59 (4C atoms, pyrazine), 63.07 (CH_2,imidazole), 29.71 (CH_2, aliphatic), 37.62(CH_2); LC-MS: [M^++1], (m/z) : 310.04.

4m- FT-IR (KBr, $\gamma_{max,}$ cm^{-1}): 3345 cm^{-1} (NH), 3071cm^{-1} (Ar C-H), 1626 (C=O), 1210 cm^{-1} (C-S), 1593 cm^{-1} (C=N); ^{1}H-NMR (400 MHz, DMSO, δ ppm): 3.12 (s, 2H, imidazole), 3.79 (s,6H, 3,4-dimethoxy) 4.34 (s, 2H, CH_2), 7.18-7.23(d, 2H, *J* = 8Hz , 3,4-dimethoxyphenyl), 7.46-7.51 (s, 1H, 3,4-dimethoxyphenyl), 10.29 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm: 167.48 (C=O adjacent to amide) , 165.52 (C=O, imidazole), 162.29, 158.38(2C atoms, thiadiazole moiety), 149.32, 146.31, 144.73, 139.78, 125.32 and 121.29 (6C atoms, 3,4-dimethoxyphenyl), 63.28 (CH_2,imidazole), 51.26 and 51.22 (2C, dimethoxy), 30.32(CH_2, aliphatic); LC-MS: [M^++1], (m/z) : 367.18.

RESULTS AND DISCUSSIONS

Chemistry:

The reaction conditions adopted for the synthesis of *N*-(aryl substituted)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4] thiadiazol-2-yl)thio)acetamide **(4a-m)** derivatives are depicted in **Scheme 1**. The representatives 2-chloro-N-(substituted) acetamide **(2),** 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(substituted) acetamide (**3a-m)** were synthesized according to the convenient and efficient synthetic procedure [25]. The target compounds **(4a-m)** were synthesized by treating 3(a-m) with ethylchloro acetate by following the literature [26].



Scheme-1: Reagents and Conditions: (a) ethanol, $ClCOCH_2Cl$, stirring; (b) ethanol, 5-amino-1,3,4-thiadiazole-2-thiol, reflux 14h; (c) ethylchloroacetate, reflux 8h.

The Chemical structure of the compound **(3a)** and their corresponding imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **4c**, **4e**, **4f** and **4k** were finally ascertained by physicochemical and spectral analysis. The formation of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide **(3a)** was confirmed by FT-IR spectrum, with the appearance of the characteristic bands at 3255cm^{-1} and 3201cm^{-1} for NH_2 absorption. The C-H, C=O and C=N stretching vibrations were observed at 3099cm^{-1},1649cm^{-1}, and 1544cm^{-1}. In the 400MHz ^{1}H- NMR spectrum of compound **(3a)** revealed two protons for amine NH_2 at δ 3.55 and a prominent singlet signal for the amide proton at δ 9.64ppm, while the aromatic protons appeared as doublets/multiplet signals between δ 6.97-7.53ppm. Further, singlet at δ 4.9ppm indicated two methylene protons. The mass spectrum of **(3a)** showed the protonated molecular ion peak at m/z 285.04.

In the next step, 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide **(3a)** was subjected to condensation reaction to afford *N*-(substituted)-2-((6-oxo-5,6-dihydroimidazo [2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide **(4a-m)** and their structures were confirmed by spectral analysis. The FT-IR spectrum of compound (**4a)** showed characteristic absorption bands at 3255cm^{-1} (NH), 3024cm^{-1} (ArC-H), 1614 (C=O), 1263cm^{-1} (C-S) and 1588cm^{-1}, which could be attributed to (C=N). The 400MHz ^{1}H-NMR spectrum of compound **(4c)**, showed a singlet for the amide NH proton at δ 10.37ppm. The signal corresponding to the two protons of NH_2 group in the 2-aminothiadiazole ring disappeared and a new sharp singlet for imidazole protons was appeared at δ 3.05ppm. A singlet resonating at δ 4.18 ppm integrated with the two protons of CH_2, which is adjacent to the carbonyl group. The four protons of 4-fluorophenyl ring resonated as multiplets between δ 7.13ppm-7.59ppm. The ^{13}C-NMR ($CHCl_3$/DMSO-d_6) spectrum of compound **(4c)** showed signals at δ 29.76, 63.070, 120.55, 126.462, 145.89, 146.98, 157.071, 160.62, 167.40 and 167.68. Also, its mass spectrum showed a protonated molecular ion peak at m/z 325, which was consistent with the molecular formula $C_{12}H_9FN_4O_2S$**.** The characterization data of *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide **(4a-m)** is depicted in **Table 1**.

Table No. 1: Chraractization data of *N*-(aryl substituted)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*] [1,3,4]thiadiazol-2-yl) thio)acetamide conjugates (4a-m).

Compounds	R	Mol.Formula (Mol. Wt)	Yield (%)	M.P. (°C)	% Composition, Found(Calcd)		
					C	H	N
4a	4-F-phenyl	$C_{12}H_9FN_4O_2S_2$ (324)	67	162-165	44.44(44.12)	2.80(2.78)	17.27(17.25)
4b	4-Cl- phenyl	$C_{12}H_9ClN_4O_2S_2$ (341)	59	184-187	42.29(42.28)	2.66(2.67)	16.44(16.41)

4c	3-Cl-4-F-phenyl	$C_{12}H_8ClFN4O_2S_2$ (361)	72	171-173	40.17(40.13)	2.25(2.24)	15.62(19.64)
4d	2,4,5-trichloro-phenyl	$C_{12}H_7Cl_3N_4O_2S_2$ (410)	63	150-153	36.65(36.67)	1.79(1.84)	14.25(14.19)
4e	4-Br-phenyl	$C_{12}H_9BrN_4O_2S_2$ (385)	58	143-145	37.99(37.94)	2.35(2.38)	14.53(14.57)
4f	N,N-diphenyl	$C_{18}H_{14}N_4O_2S_2$ (382)	54	191-194	56.53(56.56)	3.69(3.71)	14.65(14.69)
4g	4-CH_3 -3-NO_2-phenyl	$C_{13}H_{11}N_5O_4S_2$ (365)	62	207-210	42.73(42.69)	3.03(2.97)	19.17(19.21)
4h	4-NO_2-phenyl	$C_{12}H_9N_5O_4S_2$ (351)	55	195-198	41.02(41.06)	2.58(2.55)	19.93(19.86)
4i	4-CF_3-phenyl	$C_{13}H_9F_3N_4O_2S_2$ (374)	50	179-182	41.71(41.76)	2.42(2.39)	14.97(14.91)
4j	4-CH_3-phenyl	$C_{13}H_{12}N_4O_2S_2$ (320)	73	164-167	48.73(48.76)	3.78(3.81)	17.49(17.44)
4k	4-OC_2H_5-phenyl	$C_{14}H_{14}N_4O_3S_2$ (350)	68	198-201	38.95(38.91)	2.62(2.65)	27.26(27.21)
4l	Pyrazine	$C_{10}H_8N_6O_2S_2$ (308)	51	176-179	45.89(45.86)	3.85(3.81)	15.29(15.26)
4m	3,4-dimethoxy phenyl	$C_{25}H_{25}N_5O_2S$ (366)	76	184-187	45.89(45.91)	3.85(3.79)	15.29(15.26)

Pharmocological Screening:

All the synthesized compounds *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide **(4a-m)** were screened for their in vitro antimicrobial activity against *Staphylococcus aureus*, *Bascillus Subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* with the standard drug streptomycin and *Aspergillus fumigatus, Candida albicans, Aspergillus niger* and *Aspergillus clavatus* for their antifungal activity with itraconazole as the standard drug by using the zone inhibition technique.

Among the synthesized compounds, the antibacterial activity results revealed that the compounds **4a**, **4c**, **4d,** and **4e** with the electron withdrawing 4-fluoro, 4-chloro, 3-chloro-4-fluoro and 2,4,5-trichloro substituents on the phenyl ring showed significant activity compared to the standard streptomycin by showing the zone of inhibition at 36 - 40 mm,11-15 mm and 30-35 mm against *Staphylococcus aureus*, *Bascillus Subtilis* strains, whereas compounds **4b** and **4c** with chloro and fluoro substituents at positions **4** and **3** showed enhanced activity against *Pseudomonas aeruginosa* with the zone of inhibition less than 10 mm at 25 (µg/mL). **4d** and **4e** having electron withdrawing groups exhibited better activity against the bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa* by showing the zone inhibition of 11-15mm at 12.5µg/mL and 36-40mm at 6.25µg/mL compared to standard, whereas, compound **4f** bearing two phenyl groups attached to the nitrogen atom showed almost equal to the standard against the bacterial strain *Staphylococcus aureus* with the zone of inhibition of 30-35mm at 3.125 µg/mL (**Fig 2, Table 2**).

Table No. 2: Antibacterial Activity (Zone of Inhibition) of *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-m) and Streptomycin (Ref. Standard).

MIC (in µg/mL) and zone of inhibition (mm) in parentheses				
Compounds	***S.aureus***	***B. subtilis***	***E. coli***	***P.aerugenosa***
4a	6.25(36-40)	6.25(36-40)	12.5(11-15)	12.5(11-15)
4b	0	0	0	25(<10)
4c	12.5(11-15)	12.5(11-15)	0	25(<10)
4d	12.5(11-15)	12.5(11-15)	6.25(36-40)	6.25(36-40)
4e	3.125 (30-35)	6.25(36-40)	12.5(11-15)	6.25(36-40)
4f	3.125 (30-35)	0	0	0
4g	0	0	0	0
4h	0	0	0	0
4i	0	0	0	0
4j	0	0	0	0
4k	0	0	0	0
4l	0	0	0	0
4m	0	0	0	0
Streptomycin	3.125 (30-35)	6.25(36-40)	1.625(20-30)	3.125 (30-35)

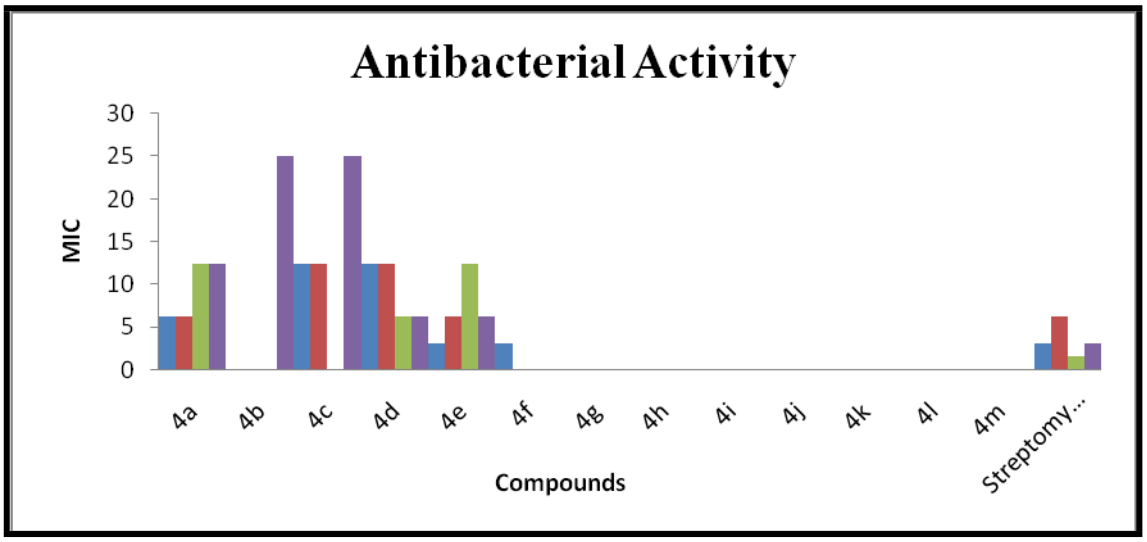


Fig. 2: Antifungal Activity (Zone of Inhibition) profile of *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-m) and Streptomycine (Ref. Standard).

The investigation of antifungal activity showed that compound **4a** with fluoro substituent exhibited excellent activity against the fungal strain *Aspergillus fumigatus* and having zone of inhibition less than 10mm at the concentration of 25µg/mL and better activity for the remaining strains *Candida albicans, Aspergillus niger* and *Aspergillus clavatus* have zone of inhibition 11-15mm at 12.5 µg/mL. The compounds **4b**, **4d**, **4g**, **4i** and **4j** showed moderate to significant activity against *Aspergillus fumigatus*. Whereas, compounds **4f** and **4i** showed significant activity against *Aspergillus niger* and *Aspergillus clavatus* by having zone of inhibition less than 10mm at the conc 25 µg/mL and 11-15mm at 12.5 µg/mL. the rest of the compounds **4c**, **4e**, **4h**, **4k**, and **4m** with 3-chloro-4-fluoro, 4-bromo,4-nitro,4-ethoxy,2-aminopyrazine and 3,4-dimethoxy substituents were completely inactive (**Fig 3, Table 3**).

Table No. 3: Antifungal Activity (Zone of Inhibition) of *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-m) and Itraconazole (Ref. Standard).

MIC (in µg/mL) and zone of inhibition (mm) in parentheses				
Compounds	***A.fumigatus***	***C. albicans***	***A.niger***	***A.clavatus***
4a	25(<10)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4b	12.5(11-15)	6.25(36-40)	0	0
4c	0	0	0	0
4d	3.125 (30-35)	6.25(36-40)	0	0
4e	0	0	0	0
4f	0	0	12.5(11-15)	25(<10)
4g	12.5(11-15)	0	0	0
4h	0	0	0	0
4i	6.25(36-40)	0	25(<10)	12.5(11-15)
4j	6.25(36-40)	0	0	0
4k	0	0	0	0
4l	0	0	0	0
4m	0	0	0	0
Itraconazole	6.25(36-40)	3.125 (30-35)	6.25(36-40)	6.25(36-40)

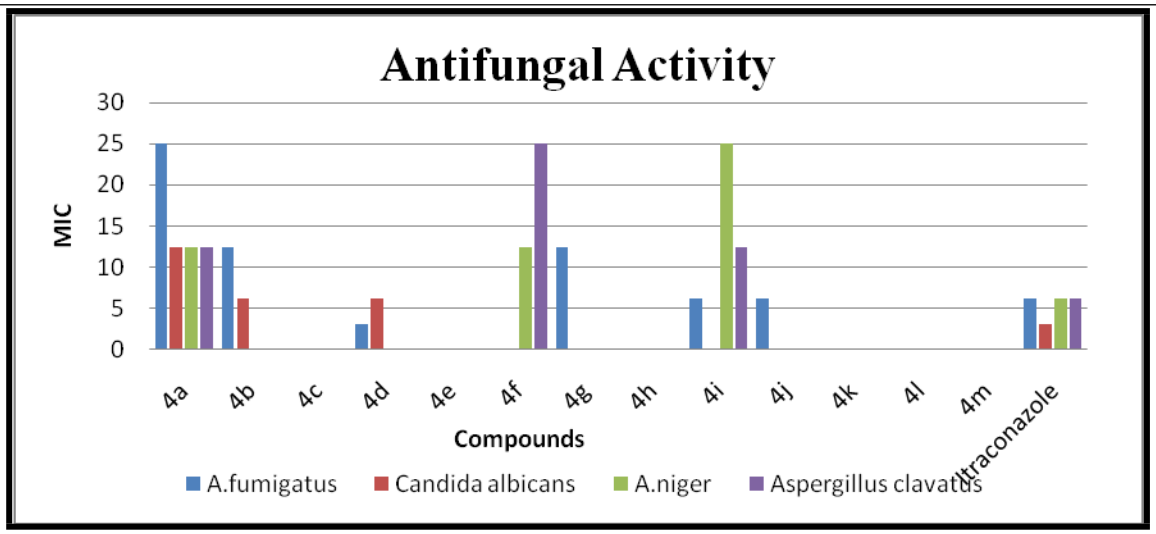


Fig. 3: Antifungal Activity (Zone of Inhibition) profile of *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-m) and Itraconazole (Ref. Standard).

CONCLUSION

The objective of the present study is to synthesize novel *N*-(substitutedphenyl)-2-((6-oxo-5,6-dihydroimidazo[2,1-*b*][1,3,4] thiadiazol-2-yl)thio)acetamide**(4a-m)** derivatives and investigate their antimicrobial activity profile. Compared to the reference standard streptomycin, the antibacterial activity results revealed that the compounds **4a**, **4b**,**4c**, **4d** and **4e** bearing halide groups such as 4-fluoro, 4-chloro, 3-chloro-4-fluoro, 2,4,5-trichloro and 4-bromo substituents exhibited excellent antibacterial activity at different positions on the phenyl ring against the *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bascillus subtilis*, and *Escherichia coli*. Similarly the antifungal activity results showed that the compounds **4a**, **4i** having halide groups such as fluoro and trifluoromethyl groups on the phenyl ring and compound **4f** having N,N-diphenyl groups exhibited highest activity against the fungal strains *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus clavatus*. Among the synthesized compounds, only compound **4a** with electron withdrawing fluoro group at the *p*-position was found to exhibit significant activity against all the bacterial and fungal strains which deserves as a better candidate for further investigation in clinical trials.

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REFERENCES:

1. Alegaon SG, Alagawadi KR, Sonkusare PV, Chaudhary SM, Dadwe DH and Shah AS. *Bioorg. Med. Chem. Lett.,* **2012;** 22: 1917-1921.
2. Foroumadi A, Emami S, Hassanzadeh A, Rajaee M, Sokhanvar K, Moshafi MH and Shafiee A. *Bioorg. Med. Chem. Lett.,* **2005;** 15(20): 4488-4492.
3. Banday MR and Rauf A. *Chin. Chem. Lett.,* **2008;** 19: 1427-1430.
4. Talath S and Gadad AK. *Eur. J. Med. Chem.,* **2006;** 41(8): 918-924.
5. Lamani RS, Shetty NS, Kamble RR and Khazi IAM. *Eur. J. Med. Chem.,* **2009;** 44: 2828-2833.
6. Kritsanida M, Mouroutsou A, Marakos P, Pouli N, Garoufalias SP, Pannecouque C, Witvrouw M and Clercq ED. *Il Farmaco.,* **2002;** 57(3): 253-257.

7. Guzeldemirci NU and Kucukbasmaci O. *Eur. J. Med. Chem.,* **2010** 45: 63-68.
8. Karki SS, Panjamurthy K, Kumar S, Nambiar M, Ramareddy SA, Chiruvella KK and Raghavan SC. *Eur. Med. Chem.,* **2011;** 46: 2109-2116.
9. Sunil D, Isloor AM, Shetty P, Satyamoorthy K and Prasad ASB. Arabian J. Chem., **2010;** 3(4): 211-217.
10. Matysik. *Chem. Pharm. Bull.,* **2006;** 54: 988-991.
11. A. Andreani A, D. Bonazzi D and Rambaldi M. *Arch. Pharm.,* **1982;** 315: 451-456.
12. Forumadi A, Soltani F, Jabini R, Moshafi M and Rasnani MF. *Arch. Pharm. Res.,* **2004;** 27(5): 502-506.
13. Kolavi G, Hegde V, Khan I and Gadad P. *Bioorg. Med. Chem.,* **2006;** 14: 3069-3080.
14. Turner S, Myers M, Gadie B, Nelson AJ, Pape R, Saville JF, Doxey JC and Berridge TL. *J. Med. Chem.,* **1988;** 31: 902-906.
15. Turner S, Myers M, Gadie B, Hale SA, Horsley A, Nelson AJ, Pape R, Saville JF, Doxey JC and Berridge TL. *J. Med. Chem.,* **1988;** 31: 907-913.
16. Chapleo CB, Myers M, Myers PL, Saville JF, Smith ACB, Stillings MR, Terlloch IF, Walter DS and Welbourn APJ. *Med. Chem.,* **1986;** 29: 2273-2280.
17. Chapleo CB, Myers PL, Smith AC, Stillings MR, Tulloch IF and Walter DS. *J. Med. Chem.,* **1988;** 31: 7-11.
18. Menozzi G, Merello L, Fossa P, Schenone S, Ranise A, Mosti L, Bondavalli F, Loddo R, Murgioni C, Mascia V, Colla PL and Tamburini E. *Bioorg. Med. Chem.,* **2004;** 20: 5465-5483.
19. Maclowry DJ, Jaqqu MJ and Selepak ST. *Appl. Microbial.,* **1970;** 20(1): 46-53.
20. Khan ZK. *Proceeding Int. Workshop UNIDO-CDRI.,* **1997;** 210-211.
21. Fenlon CH and Cynamon MH. *Antimicrob. Agents Chemother.,* **1986;** 29: 386-388.
22. Davis R, Marham A and Balfour JA. *Drugs.,* **1986;** 51(6): 1019-1074.
23. Arthington-Skaggs BA, Motley M, Warnock DW and Morrison CJ. *J. Clin. Microbiol.,***2000;** 38: 2254-2260.
24. Verma RS, Khan ZK, Singh AP, Antifungal Agents: Present and Future Prospects, National Academy of Chemistry and Biology. Lucknow, India: **1986;** 55.
25. Rostom SAF, El-Ashmawy IM, El Razik HAA, Badr MH and Ashour HMA. Bioorg. Med. Chem., **2009;** 17: 882-895.
26. El-Gohary NS and Shaaban MI. Eur. J. Med. Chem., **2013;** 63: 185-195.
27. Gadad AK, Khazi IM and Mahajanshetti CS. Indian J. Hetrocycl. Chem., **1992;** 2: 125-128.

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