Research Article

Synthesis, Evaluation and Molecular Docking Study of Some New 2-[(2,5-Disubstitutedanilino) Phenyl] Acetic Acid Derivatives

Shashidhar Kerur[1]*, Kallanagouda Alagawadi[2], Hailiang Zhu[3] and Fakkirappa Manvi[2]

[1]Deparment of Pharmaceutical Chemistry, Karnataka Lingayath Education University College of Pharmacy, Hubli-580031, INDIA
[2]Deparment of Pharmaceutical Chemistry, Karnataka Lingayath Education University College of Pharmacy, Belgaum-580010, INDIA.
[3]State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing-210093, CHINA.

ABSTRACT

In the present study synthesis and antimicrobial activity of some new 2-[(2,5-disubstitutedanilino)phenyl]acetic acid derivatives **5a-f** are described. The structures of the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. All compounds were screened for antitubercular and antimicrobial activity. Molecular modeling studies were performed to dock compounds into the ecKAS III binding site, which suggested probable inhibition mechanism. The results revealed that most of the compounds showed high to moderate biological activity against tested microorganisms.

Key words: 1,3,4-Thiadiazole, Phenacyl bromide, Molecular Docking, Antitubercular activity, Antimicrobial activity, Mycobacterium tuberculosis.

INTRODUCTION

Despite the alarming spread of tuberculosis to the public, no sufficiently effective and promising antituberculosis agents have been launched into the pharmaceutical market over the past many years.[1] Till date Isoniazid (INH), Pyrazinamide, Ethambutol and Rifampicin are considered as promising treatment drugs against the MTB infection. Many published reports[2,3] on the resistance of these agents against the virulence strains of MTB incite there to develop the antitubcular agents on the priority basis.[4]

During recent years, there has been intense investigations on thiadiazole i.e 2,5-disubstituted -1,3,4-thiadiazole compounds, many of which are known to possess interesting biological properties such as antimicrobial,[5] anti-inflammatory,[6] antifungal,[7] anticonvulsant,[8] anti-tumor[9] activities. Some members of the 2,5-disubstituted -1,3,4-thiadiazole family displayed good activity against *M. tuberculosis* H_{37}Rv strain.[10]

Type II fatty acid synthesis (FAS II) pathway has been recently reported as an attractive targeting for their efficacy against infections caused by mutiresistant Gram-positive bacteria.[11] There are plenty of fatty acids available to the bacteria inside of the host.[12] However, FAS II it's proven to be a good target for Gram-negative bacteria. Notably, KAS III, regulates the fatty acid biosynthesis rate via an initiation pathway and its substrate specificity is a key factor in membrane fatty acid composition and this protein represents a promising target for the antimicrobial drugs design.[13]

Inspired from these observations, we planned to synthesize some novel 1,3,4-thiadiazole derivatives (Scheme 1) and get them evaluated for their antitubercular and antimicrobial activity.

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Correspondence:
Shashidhar S. Kerur, M.Pharm, Ph.D
Assistant Professor, Department of Pharmaceutical Chemistry, Karnataka Lingayath Education University College of Pharmacy, Hubli-580031, Karnataka, India.
Tel: +91.836.2373174
Mob:9964317316
Fax: +91.836.2371674
E-mail: shashi_kerur2002@yahoo.com

www.ijper.org

Experimental

Instrumentation

All chemicals and reagents used in current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Nanjing University, Nanjing, China). The FT-IR spectra were recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison WI, USA) by using KBr pellets. ^{1}H NMR spectra were collected on a Bruker DPX400 or DPX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). ^{13}C NMR spectra were recorded (in $CDCl_3$/ DMSO-d_6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. ESI mass spectra were obtained on a Mariner system 5304 mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument.

Synthesis

Synthesis of 2-(2,5-diisusbtituted phenylamino)acetic acid (1)

Equimolar quantities of substituted aniline (0.1 N), chloroacetic acid and sodium acetate trihydrate were added in presence of ethanol (50 mL) and were refluxed in an oil bath at 125°C for 5 h. The reaction mixture was poured into ice-cold water (200 mL), the precipitated solid was filtered, washed with cold water, dried and recrystallized using ethanol.

2-(2,5-dichlorophenylamino)acetic acid (**1a**): Yield: 78%. M.p.: 123-124°C. IR (KBr, ν, cm^{-1}): 3375 (NH str.), 3115 (-OH), 2953 (CH_2), 1680 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 11.0 (s, 1H, OH), 7.77-7.74 (d, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 4.08 (s, 1H, -NH), 4.08 (s, 1H, D_2O Exchange exp.), 2.82(s, 2H, CH_2).

2-(2,5-difluorophenylamino)acetic acid (**1b**): Yield: 66%. M.p.: 142-144 °C. IR (KBr, ν, cm^{-1}): 3415 (NH str.), 3010 (OH), 2874 (CH_2), 1687 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 11.3 (s, 1H, OH), 7.65 (s, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 4.01 (s, 1H, -NH), 4.01 (s, 1H, D_2O Exchange exp.), 2.99(s, 2H, CH_2).

Synthesis of ethyl-2-(2,5-disubstitutedphenylamino) acetate (2)

1 gram of 2-(2,5-trisusbtituted phenylamino)acetic acid compounds were dissolved in 10-15 mL of ethanol, few drops of conc. sulphuric acid were poured along the sides of the container and refluxed for 8-12 h. During synthesis TLC of the sample were taken for every one hour. TLC solvent ratio: ethanol: chloroform; 7:3.

Ethyl-2-(2,5-dichlorophenylamino) acetate (**2a**): Yield: 75%. M.p.: 103-104°C. IR (KBr, ν, cm^{-1}): 3475 (NH str.), 2933 (CH_2), 1722 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 7.70 (s, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 3.99 (s, 1H, -NH), 3.73 (s, 2H, CH_2), 2.95 (s, 2H, CH_2), 1.15 (s, 3H, CH_3).

Ethyl-2-(2,5-difluorophenylamino)acetate (**2b**): Yield: 61%. M.p.: 137-139°C.

IR (KBr, ν, cm^{-1}): 3401 (NH str.), 2988 (CH_2), 1698 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 7.89 (s, 1H, Ar-H), 7.70(s, 1H, Ar-H), 7.54(s, 1H, Ar-H), 4.02 (s, 1H, -NH), 3.81 (s, 2H, CH_2), 2.85 (s, 2H, CH_2), 1.34 (s, 3H, CH_3).

Synthesis of 2-(substituted phenylamino) acetohydrazide (3)

To the prepared solution of an ester in absolute ethanol (50 mL) were added with hydrazine hydrate 99% in equimolar quantity. The resulting mixture were refluxed on a steam bath for 8 h, the excess ethanol were removed under reduced pressure. The resulting residue was poured into ice cold water (200 mL). The solid hydrazides thus obtained were recrystallized using ethanol.

2-(2,5-dichlorophenylamino) acetohydrazide (**3a**): Yield: 70%. M.p.: 110-112 °C. IR (KBr, ν, cm^{-1}): 3450 (NH_2), 3265 (NH str.), 2953 (CH_2), 1680 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.22 (s, 1H, NH_2), 7.98 (s, 1H, NH), 7.70 (s, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 4.08 (s, 1H, NH), 2.98 (s, 2H, CH_2).

2-(2,5-difluorophenylamino)acetohydrazide (**3b**): Yield: 58%. M.p.: 140-142 °C. IR (KBr, ν, cm^{-1}): 3398 (NH_2), 3235 (NH str.), 2935 (CH_2), 1678 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.20 (s, 1H, NH_2), 7.76 (s, 1H, NH), 7.70 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 4.01 (s, 1H, NH), 2.89 (s, 2H, CH_2).

General procedure: Synthesis of 5-((2,5-disubstituted phenylamino)methyl)-1,3,4-thiadiazole-2-thiol (4)

Equimolar quantity of 2-(2,5-disubstituted phenylamino) acetohydrazide, carbon disulphide and few drops of conc. sulphuric acid are added in presence of 15 mL ethanol and refluxed for 2 h, after reflux cooled to room temperature, which is then poured into crushed ice and neutralized with dilute acetic acid. The resulting solid was filtered, washed with cold water, dried and recrystallized using ethanol.

5-((2,5-dichlorophenylamino)methyl)-1,3,4-thiadiazole-2-thiol (**4a**): Yield: 68%. M.p.: 125-126 °C. IR (KBr, ν, cm^{-1}): 3377 (NH str.), 3117 (CH_2), 784 (Cl str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 12.87 (s, 1H, -SH), 7.86 (s, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 4.01 (s, 1H, NH), 2.88 (s, 2H, CH_2).

5-((2,5-difluorophenylamino)methyl)-1,3,4-thiadiazole-2-thiol (**4b**): Yield: 57%. M.p.: 191-193 °C. IR (KBr, ν, cm^{-1}): 3437 (NH str.), 3271 (CH_2), 760 (Cl str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 12.78 (s, 1H, -SH), 7.87 (s, 1H, Ar-H), 7.67-7.64 (d, 2H, Ar-H), 4.07 (s, 1H, NH), 2.65 (s, 2H, CH_2).

Preparation of derivatives of 1-(2,5-disubstitutedphenyl)-2-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone

Equimolar quantity of 5-((2,5-disubstitutedphenylamino) methyl)-1,3,4-thiadiazole-2-thiol compounds (0.005 mole) were treated with *p*-substituted phenacyl bromide (0.005 mole) in the presence of ethanol (50 mL). Refluxed for 1 h on oil bath to give the different derivatives of 1,3,4-thiadiazole at the C_2 positions respectively.

2-(5-((2,5-dichlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-phenyl ethanone (**5a**):

Yield: 62%. M.p.: 232-234 °C. IR(KBr, ν, cm^{-1}): 3375 (NH str.), 3046 (CH_2), 1722 (C=O), 717 (Cl str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.10 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.64-7.62 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.33-7.31 (d, *J* = 7.6 Hz, 2H, Ar-H), 6.93(s, 1H, Ar-H), 4.03 (s, 1H, NH), 3.87 (s, 2H, -S-CH_2-CO), 2.50 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 193.52 (C=O), 160.07 (C2-triazolic ring), 157.06 (C5-triazolic ring), 142.11, 140.03, 131.32, 130.15, 124.99, 121.50, 119.61, 112.97 (Aromatic ring), 49.23 (CH_2), 39.02 (-S-CH_2-CO).

MS (ESI-QqTOF, *m/z*): 409.09 $[M+H]^+$. Anal. calcd. for $C_{17}H_{13}Cl_2N_3OS_2$: C, 49.71; H, 3.05; N, 10.31. Found: C, 49.68; H, 3.03; N, 10.30%.

2-(5-((2,5-difluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-phenylethanone (**5b**):

Yield: 51%.

M.p.: 245-247 °C. IR(KBr, ν, cm^{-1}): 3348 (NH str.), 3037 (CH_2), 1738 (C=O), 714 (F str.).

^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 7.91 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.57-7.54 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.24-7.21 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.02 (s, 1H, Ar-H), 4.06 (s, 1H, NH), 3.54 (s, 2H, -S-CH_2-CO), 2.07 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 187.33(C=O), 158.27 (C2-triazolic ring), 150.12 (C5-triazolic ring), 141.17, 140.09, 134.76, 130.57, 127.19, 120.77, 118.78, 112.07 (Aromatic ring), 50.21 (CH_2), 38.22 (-S-CH_2-CO).

MS (ESI-QqTOF, *m/z*): 377.41 $[M+H]^+$. Anal. calcd. for $C_{17}H_{13}F_2N_3OS_2$: C, 54.10; H, 3.45; N,11.12. Found: C, 54.08; H, 3.42; N, 11.08%.

2-(5-((2,5-dichlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-chloro phenyl)ethanone (**5c**):

Yield: 45%. M.p.: 240-241 °C. IR(KBr, ν, cm^{-1}): 3375 (NH str.), 3048 (CH_2), 1723 (C=O), 725 (Cl str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.61 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 7.77-7.75 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.25-7.24 (d, *J* = 7.6 Hz, 2H, Ar-H), 3.99 (s, 1H, NH), 3.01 (s, 2H, -S-CH_2-CO), 2.62 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 194.78 (C=O), 164.96 (C2-triazolic ring), 135.75 (C5-triazolic ring), 133.88, 131.63,130.57, 130.01, 129.03, 127.16, 114.72, 110.17 (Aromatic ring), 50.89 (CH_2), 38.87 (-S-CH_2-CO). MS (ESI-QqTOF, *m/z*): 444.81 $[M+H]^+$. Anal. calcd. for $C_{17}H_{12}Cl_3N_3OS_2$: C, 45.91; H, 2.71; N, 9.47. Found: C, 45.90; H, 2.70; N, 9.45%.

2-(5-((2,5-difluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-chloro phenyl)ethanone (**5d**): Yield: 54%. M.p.: 215-216 °C. IR(KBr, ν, cm^{-1}): 3441 (NH str.), 3377 (CH_2), 1715 (C=O), 730 (F str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 7.83 (s, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.12-7.09 (d, *J* = 7.8 Hz, 2H, Ar-H), 6.98-6.96 (d, *J* = 7.2 Hz, 2H, Ar-H), 4.01 (s, 1H, NH), 3.85 (s, 2H, -S-CH_2-CO), 2.46 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 192.71 (C=O), 167.69(C2-triazolic ring), 136.76(C5- triazolic ring), 131.18, 130.63,130.07, 129.88, 129.03, 126.16; 115.72, 111.17(aromatic ring), 49.89(CH_2), 39.97(-S-CH_2-CO).

MS (ESI-QqTOF, *m/z*): 411.1 $[M+H]^+$. Anal. calcd. for $C_{17}H_{12}ClF_2N_3OS_2$: C, 49.56; H, 2.93; N, 9.23. Found: C, 49.54; H, 2.92; N, 9.24%.

2-(5-((2,5-dichlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-fluoro phenyl)ethanone (**5e**): Yield: 58%. M.p.: 212-215 °C. IR(KBr, ν, cm^{-1}): 3375 (NH str.), 3046 (CH_2), 1722 (C=O), 717 (Cl str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.12 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.35-7.32 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.05-7.02 (d, *J* = 7.4 Hz, 2H, Ar-H), 4.07 (s, 1H, NH), 3.81 (s, 2H, -S-CH_2-CO), 2.42 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 193.20 (C=O), 162.50 (C2-triazolic ring), 152.72 (C5-triazolic ring), 141.30, 139.89, 134.38, 132.37, 130.06, 129.09, 127.88, 127.33, 126.61 (Aromatic ring), 49.27 (CH_2), 39.63 (-S-CH_2-CO). MS (ESI-QqTOF, *m/z*): 428.32 $[M+H]^+$.

Anal. calcd. for $C_{17}H_{12}Cl_3FN_3OS_2$: C, 47.67; H, 2.80; N, 16.55. Found: C, 47.66; H, 2.79; N, 16.54%.

2-(5-((2,5-difluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-fluoro phenyl)ethanone (**5f**): Yield: 54%. M.p.: 240-241 °C. IR(KBr, ν, cm^{-1}): 3348 (NH str.), 3037 (CH_2), 1738 (C=O), 717 (F str.). ^{1}H NMR (400 MHz, DMSO-*d6*, δ, ppm): 8.12 (s, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.52 -7.50 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.60-6.58 (d, *J* = 6.6.Hz, 2H, Ar-H), 4.09 (s, 1H, NH), 3.83 (s, 2H, -S-CH_2-CO), 2.52 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 193.1(C=O), 162.77(C2-triazolic ring), 152.10(C5-triazolic ring), 142.90, 140.71, 133.68, 130.91, 130.20, 127.60, 123.9, 123.7, 115.18 (Aromatic

ring), 49.21 (CH_2), 39.17 (-S-CH_2-CO). MS (ESI-Qq-TOF, *m/z*): 495.41 $[M+H]^+$.

Anal. calcd. for $C_{17}H_{12}F_3N_3OS_2$: C, 51.64; H, 3.06; N, 10.63. Found: C, 51.62; H, 3.06; N, 10.62%.

Biological evaluation

In vitro evaluation of antimicrobial activity

The MIC determination of the tested compounds were carried out in comparison with *Norfloxacin* for their antibacterial activity against two micro-organisms viz. *E. coli* (NCTC 10418) and *S. aureus* (NCTC 6571) by Cup-plate agar diffusion method using Mueller-Hinton agar. The MIC determinations of the tested compounds were carried out by comparison with *Griseofulvin* for their antifungal activity against *C. albicans* (ATCC 10231) and *A. niger* (ATCC 16404) by Cup-plate agar diffusion method using Sabouraud-Dextrose agar. Drugs (10mg) were dissolved in Dimethylsulfoxide (DMSO, 1 mL). The tubes were inoculated with 10^5 cfu/mL (colony forming unit/mL) and incubated at 37°C for 18 h. The MIC was the lowest concentration of the tested compound that yields no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with DMSO at the same dilutions as used in the experiments and it was observed that DMSO with 2% had no effect on the microorganisms in the concentrations studied.

In vitro evaluation of antitubercular activity

The antimycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA). The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL and incubated at 37°C for five days. After this 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. Against the standard drug Isoniazid.

Experimental protocol of molecular docking studies

The synthesized molecules were subjected for molecular docking by calculating the minimum energy to inhibit the target protein involved in the catalysis of complex reaction. The ligands were drawn in Chemdraw Ultra 11.0 assigned with proper 2D orientation (Chemoffice package) and the structure of each ligand was analysed by using Chem-3D Ultra 11.0 (Chem Office package) and was checked for the connection error in bond order. ADMET property was achieved through Pre ADMET server a web-based application for predicting ADMET data and building drug-like library using *in silico* method. Energy of the molecules was minimized by using MOPAC with 100 interactions and minimum RMS. Then the file was opened in Accelrys, DS visualizer 2.0 [Accelrys Inc., San Diego, CA (2007)] and to determine their binding orientations, molecular modeling, and evaluation of the hydrogen bonds. Active pockets were identified and ligplot of PDBSum provided in the External links of PDB for the proteins was downloaded from PDB. CASTp (Computed Atlas of Surface Topography of proteins) server was used to crosscheck the active pockets on target protein molecules. Autodock V4.0 was used to perform molecular docking. The docking results for ligand molecules against ecKAS III synthase [PDB CODE: 1HNJ], showed minimum docking energy, binding energy, number of binding sites with 0.0 RMS as documented in Table 2.

RESULT AND DISCUSSION

The synthetic route of the compounds (5a-f) is outlined in Scheme 1. The 2-(substituted phenylamino)acetic acid (1) was prepared by the reaction of equimolar quantities of chloroacetic acid and substituted aniline according to the established procedures.[13] Ethyl-2-(substitutedphenylamino)acetate (2) was obtained by refluxing 2-(susbtituted phenylamino)acetic acid (1) and Concentrated sulphuric acid in presence of dry ethanol to form esters.[14] 2-(substituted phenylamino)acetohydrazide (3) was prepared by hydrozinolysis of the esters. Synthesis of 5-((substitutedphenylamino)methyl)-1,3,4-thiadiazole-2-thiol (4) was achieved by adopting a simple one pot procedure that involves reacting hydazides with carbon disulfide and conc. sulphuric acid.[15] The alkylation of 1,3,4-thiadiazoles (4) with substituted phenycyl bromide, in presence of dimethyl sulfoxide afford a new series of 1-(2,5-disubstitutedphenyl)-2-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone (5a-f).[16]

The formation of 2-substituted phenylamino acetic acid (1) was confirmed by IR spectra, which showed the presence of amine(-NH) bands at around 3415 to 3375 and ^{1}H NMR D_2O exchange experiment around δ4.08 ppm. Ethyl-2-(substituted phenylamino) acetate (2) showed around 1722 to 1698 (C=O). 2-(Substituted phenylamino) acetohydrazide (3) was confirmed by 3450 to 3398 (NH_2) and the signal was observed around δ 8.22 in the ^{1}H NMR. A new signal for SH group was appeared as singlet at δ 12.87-12.78 ppm. In the ^{1}H NMR spectra of 1-(2,5-disubstitutedphenyl)-

Table 1: Antimicrobial and anti-tubercular screening results of compound (MIC values μg/mL)

Compounds	Anti-microbial activity				Anti-tubercular activity
	E. coli	*S. aureus*	*A. niger*	*C. albicans*	*M. tubercular* $H_{37}Rv$
5a	16	8	16	8	0.8
5b	31.25	62.5	31.25	125	3.125
5c	125	500	62.5	250	25
5d	16	8	16	125	0.8
5e	62.5	31.25	8	16	6.25
5f	16	125	16	125	3.125
Norfloxacin	<1	<5	NT	NT	NT
Griseofulvin	NT	NT	<1	<5	NT
Isoniazid	NT	NT	NT	NT	<0.2

NT: not tested.

Table 2: Molecular docking simulation results with ecKAS III synthase

Molecule No	Binding energy	Docking energy	No of binding sites
5a	-5.49	-5.70	04
5b	-6.79	-6.92	03
5c	-5.79	-5.92	02
5d	-6.53	-6.81	04
5e	-6.99	-7.22	03
5f	-5.19	-5.24	02

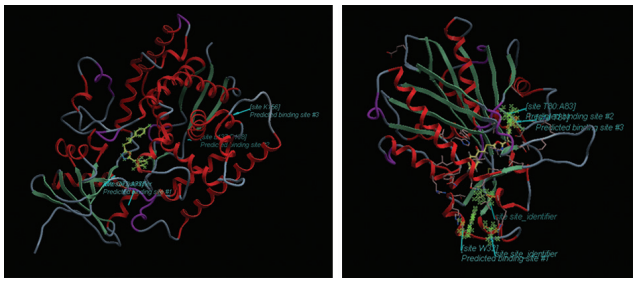
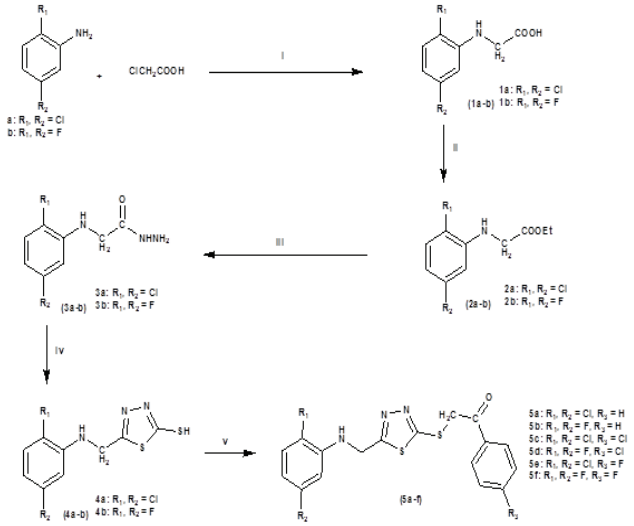


Figure 1: Compound 1b is bound into ecKAS III receptor site via hydrophobic interactions and hydrophilic binding by hydrogen bond between its O and H-S of Asn 274 (S-H…02: 2.03 Å, 137.8°) and H-S of Ala 109 (S-H…02: 2.08 Å, 148.7°) and extending into the mouth of the substrate tunnel (2d(ii)).



Scheme 1: Reagents used: i) Ethanol, 5 h, 125 °C; ii) Conc. sulphuric acid, dry ethanol, 8 h, 90 °C; iii) Hydrazine hydrate, ethanol, 8 h, 85 °C; iv) Carbon disulphide, conc. sulphuric acid, 2 h, 85 °C; v) p-substituted phenacyl bromide, 1 h, 80°C.

2-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-ylthio)ethanone (5a-f) were confirmed by absence of SH peak, while the signal of methylene proton (2.62-2.07ppm) from compounds were appeared. The above facts were further evidenced by ^{13}C NMR data which displayed C=O signals at δ 194.78- δ187.77 ppm, the heterocyclic carbons resonated at δ 167.69 to δ 110.17 ppm and CH_2 group resonated at 39.97 to 38.22 ppm respectively. The mass spectrum of compounds showed molecular ion peaks at *m/z* 475.1 to 395.0 corresponding to molecular formula and elemental analysis of these compounds further confirmed the assigned structures.

Biological evaluation

In vitro antimicrobial studies

The investigation of antimicrobial screening revealed that some of compounds showed moderate to good bacterial and fungal inhibition. Particularly compounds 5a and 5d showed good activity against *E. Coli* and *S. aureus* with MIC values between 8 to 16 µg/mL. All the remaining compounds 5b, 5e and 5f showed moderate activity, where as 5c shown less activity. The investigation of antifungal screening revealed that compounds 5a and 5e showed good activity against *A. niger and C. albicans* with MIC values between 8 to 16 µg/mL. All the remaining compounds 5b, 5c and 5f showed moderate activity, where as 5d shown less activity. The MIC values of tested compounds are given in Table 1.[17]

In vitro antitubercular studies

The antitubercular screening revealed that some of the tested compounds showed moderate to good inhibition against standard drug Isoniazid. Particularly compounds 5a and 5d**,** have shown good activity with MIC values between 0.4 to 1.6 µg/mL. All the remaining compounds 5b, 5e and 5f showed moderate activity, where as 5c has shown less activity.[18]

Molecular docking studies

With *in vitro* antimicrobial results in hand, it is thought worth-while to do *in silico* studies to support the *in vitro* activity. Automated docking was used to determine the orientation of inhibitors bound in the active site of ecKAS III synthase. A Lamarckian genetic algorithm method was employed. The docking of ligand molecules with ecKAS III synthase reveals that all the inhibitor compounds are exhibited the bonding with one or other amino acids in the active pockets which is showed in Figure 1. The protein structure file (PDB ID: 1hnj) taken from PDB (www.rcsb.org/pdb) was edited by removing the hetero atoms, adding C-terminal oxygen. Figure 1 also shows the *in silico* active pocket prediction of amino acids of protein ecKAS III synthase involved in binding with the ligands obtained from PDB sum. Theoretically all the molecules showed very good binding energy and docking energy ranging from -5.19 to -6.99 kJ/mol and -5.24 to -7.22 kJ/mol, respectively. Among the 6 molecules, docking of ecKAS III synthase with 5b and 5f revealed that its docking energy and binding energy were -6.79, -6.92, -6.99, and -7.22 kJ/mol, respectively, and it may be considered as good inhibitor of ecKAS III synthase. In *in-vitro* also 5b and 5f has emerged as active against all tested microorganisms, so it can be predicted as the activity may be due to inhibition of enzyme ecKAS III synthase.[19,20]

CONCLUSION

We have synthesized series of novel S-substituted phenacyl-1,3,4-thiadiazole-thiol derivatives (5a-f). The results of antimicrobial screening revealed the discovery of new compounds as one of the promising agents. This observation may promote a further development of this group of 1,3,4-thiadiazole-thiol may lead to compounds with better pharmacological profile than standard antimicrobial drugs. Molecular docking studies also revealed that **5b** and **5f** has minimum binding and docking energy and may be considered as a good inhibitor of ecKAS III. Hence this study has widened the scope of developing these derivatives as promising antitubercular, antibacterial and antifungal agents.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest for this article.

ABBREVIATION USED

MTB: Mycobacterium Tuberculosis; **FAS:** Fatty Acid Synthesis; **KAS:** Beta-ketoacyl-ACP synthase; **PDB:** Protein Data Bank; **MIC:** Minimum Inhibitory Concentration.

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SUMMARY

- MTB has in the emergence of drug-resistant tuberculosis.
- There has been intense investigations on thiadiazole i.e 2,5-disubsti¬tuted -1,3,4-thiadiazole compounds, which are known to possess interesting biological properties.
- The results of antimicrobial screening revealed the discovery of new compounds as one of the promising agents.
- Molecular Docking study also reveals promising results for synthesized compounds.
- This study has widened the scope of developing these derivatives as promising antitubercular, antibacterial and antifungal agents.