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DESIGN, SYNTHESIS AND CHARACTERIZATION OF NOVEL S-SUBSTITUTED PHENACYL-1,3,4-TRIAZOLE-THIOL DERIVATIVES FOR ANTIMICROBIAL STUDIES

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ABSTRACT
*In the present study synthesis and antimicrobial activity of 2,5-disubstituted triazole derivatives **4a-i** and **5a-l** 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 antibacterial activity. The results revealed that most of the compounds showed high or moderate biological activity against tested microorganisms.*

Key words: *Antitubercular activity, Antimicrobial activity, Mycobacterium tuberculosis; 1,3,4-Triazole and phenacyl bromide.*

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INTRODUCTION

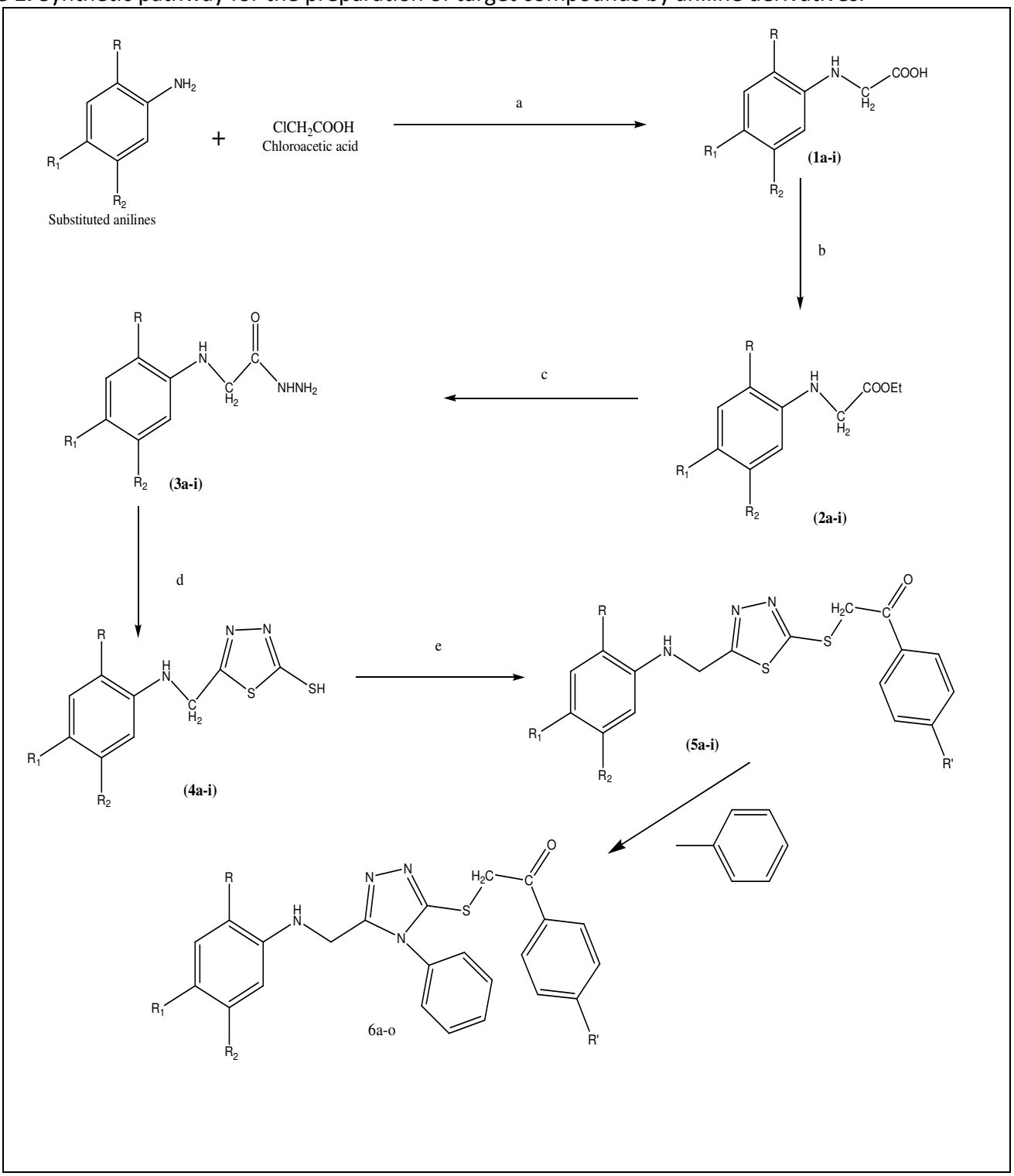
Tuberculosis (TB) is the leading infectious cause of death in the world today, with approximately three million patients deceasing every year. Nearly one-third of the world's population is infected with *Mycobacterium tuberculosis* and the World Health Organization (WHO) estimates that about 30 million people will be infected within the next 20 years. During recent years, *M. tuberculosis* and microorganisms increased resistance against drugs[1].

As resistance to antimicrobial drugs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent and less toxic antimicrobial agents[2]. The situation is becoming alarming with the recent emergence of multi-drug resistant (MDR) strains and its synergy with global human immunodeficiency virus (HIV) [3,4].

During recent years, there have been intense investigations on triazole i.e 2,5-disubstituted -1,3,4-triazole compounds, many of which are known to possess interesting biological properties such as antimicrobial[5], anti-inflammatory[6], antifungal[7], anticonvulsant[8], antitumour[9], antitubercular[10], diuretic[11], analgesic[12] activities. Some members of the 2,5-disubstituted -1,3,4-triazole family displayed good activity against *M. tuberculosis* $H_{37}Rv$.

The purpose of the present work was to explore and develop the novel molecules with improved potential for treating tuberculosis. In this paper, we report the synthesis antimycobacterial and antimicrobial evaluation of various 2,5-disubtituted-1,3,4-triazole derivatives(scheme 1).

Scheme 1. Synthetic pathway for the preparation of target compounds by aniline derivatives.



Reagents used: a) Ethanol, 6h, 125^{o}C; b) Thionyl chloride, Dry ethanol, 8h, 90^{o}C; c) Hydrazine hydrate, Ethanol, 5h, 85^{o}C;d) Carbon disulphide, Conc. Sulphuric acid, 5h, 85^{o}C; e) P-substituted phenacyl bromide, 1h, 80^{o}C,.

2.EXPERIMENTAL

2.1 Materials and reagents

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). ^{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, and were within ±0.4% of the theoretical values.

2.2 Synthesis

2.2.1 Synthesis of 2-(susbtituted phenylamino)acetic acid (1)

Equimolar quantities of substituted aniline (0.1 N), chloroacetic acid and sodium acetate trihydrate are added in presence of ethanol (50 ml) and were refluxed in an oil bath at 125^{o}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. Yield 78%; m.p; 123-124˚C.

IR (KBr) cm^{-1}; 3375.53 (NH stretch), 2953.50(CH_2), 1740.66(C=O), 3115.10(O-H); **^{1}H NMR (DMSO-d_6) δ in ppm:** 11.0 (s, 1H, OH), 7.77(d, 2H, Ar-H), 6.65(d, 2H, Ar-H), 4.08 (s, H, -NH); ^{1}H NMR (DMSO-d_6) D_2O Exchange experiment δ in ppm: 4.10 (s, 1H).

2.2.2 Synthesis of ethyl-2-(substitutedphenylamino)acetate (2)

1 gram of synthesized compound was dissolved in 10-15 ml of ethanol, few drops(2-3drops) of thiosemicarbazide was poured along the sides of the container and refluxed for 8-12 h. Simultaneous in between TLC of the sample was taken. i.e. for every one hour. TLC solvent ratio; Ethanol: Chloroform; 7:3. Yield 75%; m.p; 103-104˚C.

IR (KBr) cm^{-1}; 3475.53 (NH stretch), 2053.50(CH_2) and 1722.99(C=O); **^{1}H NMR (DMSO-d_6) δ in ppm:** 7.70(d, 2H, Ar-H), 6.56(d, 2H, Ar-H), 3.80 (s, H, -NH), 3.28 (m, C=O).

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

To a prepared solution of an ester in absolute ethanol (50 ml) was added with hydrazine hydrate 99% in equimolar quantity. The resulting mixture was refluxed on a steam bath for 8 h, the excess ethanol was removed under reduced pressure. The resulting residue was poured into ice cold water (200 ml). The solid hydrazide thus obtained was recrystallized using ethanol. Yield 70%; m.p; 110-112˚C.

IR (KBr) cm^{-1}; 3450.66 (NH_2), 3265.30 (NH stretch), 2953.50(CH_2) and 1740.66(C=O); **^{1}H NMR (DMSO-d_6) δ in ppm:** 8.20 (s, 1H, NH), 7.70(d, 2H, Ar-H), 6.56(d, 2H, Ar-H), 3.80 (s, H, -NH), 2.28 (s, 2H, NH_2).

2.2.4. General procedure: synthesis of 5-((substitutedphenylamino)methyl)-1,3,4-triazole-2-thiol (4)

To the obtained 2-(substituted phenylamino)acetohydrazide, add equimolar quantity of carbon disulphide and few drops (2 - 3drops) of H_2SO_4 in presence of ethanol until the evolution of hydrogen sulphide gas ceased, then cooled to room temperature and poured into crushed ice and neutralized with sodium bicarbonate solution. The resulting solid was filtered, washed with cold water, dried and then recrystallized from ethanol.

2.2.4.1.5-((2-Chlorophenylamino)methyl)-1,3,4-triazole-2-thiol ***(4a).*** Yield 78%; m.p; 156-158˚C; **IR(KBr, υ, cm^{-1}):**3378.29(-N-H str); 782.89(-Cl str); **^{1}H NMR (300 mHz. DMSO-d_6. δ ppm) :** 13.17 (s, 1H, -SH); 7.26-6.50, (m, 4H, Ar-H); 4.28(s, 2H-CH2); 3.80 (s, 1H. -NH). ESI-MS: 257.9 C9 H8 Cl N3 S2 + , [M+H] +).

2.2.4.2. 5-((2-Fluorophenylamino)methyl)-1,3,4-triazole-2-thiol ***(4b).*** Yield 62%; m.p; 215-216˚C; **IR(KBr, υ, cm^{-1}):** 3378.34(-N-H str); 718.88(-F str). **^{1}H NMR (300 mHz. DMSO-d_6. δppm) :** 8.32 (s, 1H, -SH); 7.28-6.42(m, 4H, Ar-H); 4.01(s, 2H-CH2); 3.34 (s, 1H. -NH).

2.2.4.3. 5-((2-Methoxyphenylamino)methyl)-1,3,4-triazole-2-thiol ***(4c).*** Yield 65%; m.p; 190-192˚C; **IR(KBr, υ, cm^{-1}):** 3412.17(-N-H str), 3214.12(Ar-CH); 1642.79(O-CH_3); **^{1}H NMR (300 mHz. DMSO-d_6. δppm) :** ^{1}H NMR (300 mHz. DMSO-d_6. δppm) : 8.32 (s, 1H, -SH); 7.28-6.42 (m, 4H, Ar-H); 4.01(s, 2H-CH2); 3.34 (s, 1H. -NH).

2.2.4.4.5-((2,4-Dichlorophenylamino)methyl)-1,3,4-triazole-2-thiol ***(4d).***Yield 75%; m.p; 142-144˚C; **IR(KBr, υ, cm^{-1}):** 3312.32(-N-H str); 733.77(-F str); **^{1}H NMR 3c:** 1H NMR (300 mHz. CDCl3. δppm) : ^{1}H NMR (300 mHz. DMSO-d_6. δppm) : 10.45 (s, 1H, -SH); 7.31-6.41, (m, 3H, Ar-H); 4.11 (s, 1H. -NH); 3.52(s, 2H-CH2).

*2.2.4.5. 5-((2,4-Difluorophenylamino)methyl)-1,3,4-triazole-2-thiol****(4e).*** Yield 60%; m.p; 232-234˚C; **IR(KBr, υ, cm^{-1}):** 3342.61(-N-H str); 731.71(-F str); **^{1}H NMR (300 mHz. DMSO-*d*6. δppm) :** 11.98 (s, 1H, -SH); 7.31-6.57(m, 3H, Ar-H); 4.51 (s, 1H. -NH); 3.83(s, 2H-CH2).

2.2.4.6. 5-((2,4-Dimethoxyphenylamino)methyl)-1,3,4-triazole-2-thiol ***(4f).*** Yield 62%; m.p; 180-182˚C; **IR(KBr, υ, cm^{-1}):** 3411.56(-N-H str); 3074.23(Ar-CH); 1711.10(O-CH3 str); **^{1}H NMR (300 mHz. DMSO-*d*6. δppm) :** 11.01 (s, 1H, -SH); 7.25-6.98 (m, 3H, Ar-H); 4.12 (s, 1H. -NH); 3.54(s, 2H-CH2).

*2.2.4.7. 5-((2,4,5-Trichlorophenylamino)methyl)-1,3,4-triazole-2-thiol****(4g).*** Yield 64%; m.p; 175-176˚C; **IR(KBr, υ, cm^{-1}):** 3457.69(-N-H str); 736.98(-Cl str); **^{1}H NMR (300 mHz. DMSO-d_6. δppm) :** 12.58 (s, 1H, -SH); 7.32-6.85(m, 2H, Ar-H); 4.10 (s, 1H. -NH); 2.17(s, 2H-CH2).

2.2.4.8. 5-((2,4,5-Trifluorophenylamino)methyl)-1,3,4-triazole-2-thiol ***(4h).*** Yield 55%; m.p; 224-226˚C; **IR(KBr, υ, cm^{-1}):** 3369.34(-N-H str); 788.27(-F str); **^{1}H NMR (300 mHz. DMSO-d_6. δppm) :** 13.15 (s, 1H, -SH); 6.34-5.54 (m, 2H, Ar-H); 3.30 (s, 1H. -NH); 2.51 (s, 2H-CH2).

2.2.4.9.5-((2,4,5-Trimethoxyphenylamino)methyl)-1,3,4-triazole-2-thiol ***(4i).*** Yield 60%; m.p; 200-202˚C; **IR(KBr, υ, cm^{-1}):** 3417.34(-N-H str); 3153.18(Ar-CH); 1671.21(1650.22); **^{1}H NMR (300 mHz. DMSO-d_6. δppm) :** 12.17 (s, 1H, -SH); 7.85-7.54(m, 2H, Ar-H); 4.07(s, 1H. -NH); 3.02 (s, 2H-CH2).

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

These obtained 5-((substitutedphenylamino)methyl)-1,3,4-triazole-2-thiol compound (0.005mole) were treated with equimolar quantity of p-substituted phenacyl bromide(0.005mole in the presence of ethanol(50ml). Refluxed for 8 h on oil bath to give the different derivatives of 1,3,4-thidiazole at the C_2 positions respectively.

*2.2.5.1. 2-(5-((2-chlorophenylamino)methyl)-1,3,4-thidiazole-2-ylthio)-1-phenylethanone***(5a).** Yield 67%; m.p; 163-164˚C; **IR(KBr, υ, cm^{-1}):** 3370.09 (NH); 3000.43(aromatic C-H); 1722.21 (C=O); 733.28 (C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.21-8.20(d, 2H, Ar-H); 7.91-7.89(m, 5H, Ar-H); 6.98-6.96(d, 2H, Ar-H); 5.02(d, 2H, -S-CH_2-CO); 4.72(d, 2H, CH_2); 4.06(s, 1H, NH). MS(ESI-QqTOF) m/z: 359.0$[M+H]^+$.

*2.2.5.2. 2-(5-((2-fluorophenylamino)methyl)-1,3,4-thidiazole-2-ylthio)-1-phenylethanone***(5b).** Yield 58%; m.p; 240-242˚C; **IR(KBr, υ, cm^{-1}):** 3470.34 (NH); 3076.95(aromatic C-H); 1682.17 (C=O); 715.14 (C-F); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.02-7.99(m, 5H, Ar-H);7.32-7.31(d, 2H, Ar-H); 6.50-6.51(d, 2H, Ar-H); 4.77(d, 2H, -S-CH_2-CO); 4.49(d, 2H, CH_2); 4.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 194.01(C=O); 165.22(C2-triazolic ring); 157.99(C5- triazolic ring); 150.40, 134.89,133.07, 130,51, 128.64, 125.11, 121.83, 115.12(aromatic ring); 50.71(CH_2); 38.99(-S-CH_2-CO).MS(ESI-QqTOF) m/z: 343.01$[M+H]^+$.

2.2.5.3.2-(5-((2-methoxyphenylamino)methyl)-1,3,4-thidiazole-2-ylthio)-1-phenylethanone **(5c).** Yield 60%; m.p; 180-182˚C; **IR(KBr, υ, cm^{-1}):** 3456.51 (NH); 3120.13(aromatic C-H); 1622.10 (C=O); **^{1}H NMR (DMSO-d_6, δ ppm):** 7.83-7.77(m, 7H, Ar-H); 6.51(s, 1H, Ar-H); 4.97(d, 2H, -S-CH_2-CO); 4.49(d, 2H, CH_2); 4.10(s, 1H, NH);

2.2.5.4.1-(4-chlorophenyl)-2-(5-((2-chlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio) ethanone **(5d).** Yield 70%; m.p; 166-168; **IR(KBr, υ, cm^{-1}):** 3481.19 (NH); 1633.73(C=O); 747.60 (C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.42 (s,

1H, Ar-H); 7.82-6.99(m, 7H, Ar-H); 5.12 (d, 2H, -S-CH_2-CO); 4.67(d, 2H, CH_2); 3.89(s, 1H, NH); ^{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: 409.00$[M+H]^+$.

2.2.5.5. 1-(4-fluorophenyl)-2-(5-((2-fluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio) ethanone **(5e).** Yield 52%; m.p; 232-234; **IR(KBr, υ, cm^{-1}):** 3375.92 (NH); 3046.32 (aromatic C-H); 1722.85 (C=O); 717.60 (C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.11-8.10(d, 2H, Ar-H);7.64-7.62(m, 4H, Ar-H); 6.59-6.58(d, 2H, Ar-H); 4.87 (d, 2H, -S-CH_2-CO); 4.50(d, 2H, CH_2); 4.03(s, 1H, NH).

2.2.5.6. 1-(4-methoxyphenyl)-2-(5-((2-methoxyphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone **(5f).** Yield 62%; m.p; 174-176˚C; **IR(KBr, υ, cm^{-1}):** 3348.94 (NH); 3037.57(aromatic C-H); 1738.68 (C=O); **^{1}H NMR (DMSO-d_6, δ ppm):** 7.91 -7.40(m, 8H, Ar-H); 5.54(s, 2H, -S-CH_2-CO); 5.07(d, 2H, CH_2); 4.36(s, 1H, NH); MS(ESI-QqTOF) m/z: 401.0$[M+H]^+$.

*2.2.5.7. 1-(4-chlorophenyl)-2-(5-((2,4-dichlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone***(5g).** Yield 70%; m.p; 165-166˚C; **IR(KBr, υ, cm^{-1}):** 3145.68 (NH); 3048.60(aromatic C-H); 1690.43 (C=O); 713.82 (C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.14 -7.80(m, 8H, Ar-H); 5.02(s, 2H, -S-CH_2-CO); 4.80(d, 2H, CH_2); 3.79(s, 1H, NH); **^{13}C NMR (DMSO-d_6, δ ppm):** 193.18(C=O); 160.71(C2-triazolic ring); 157.25(C5- triazolic ring); 142.01, 140.05,139.89, 134,38, 132.61, 130.06; 129.09, 127.88, 126.66(aromatic ring); 47.62(CH_2); 39.65(-S-CH_2-CO). **MS(ESI-QqTOF) m/z:** 444.1$[M+H]^+$.

*2.2.5.8. 2-(5-((2,4-difluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-fluoro phenyl)ethanone***(5h).** Yield 54%; m.p; 214-216˚C; **IR(KBr, υ, cm^{-1}):** 3370.46 (NH); 2923.01(aromatic C-H); 1720.07 (C=O); 732.28 (C-F);**^{1}H NMR (DMSO-d_6, δ ppm):** 8.64 -8.63(d, 2H, Ar-H); 7.99-7.97(d, 2H, Ar-H); 7.32-7.29(t, 3H, Ar-H); 6.98(s, 1H, Ar-H); 5.02(s, 2H, -S-CH_2-CO); 4.71(d, 2H, CH_2); 4.10(s, 1H, NH); **^{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:** 395.1$[M+H]^+$.

2.2.5.9. 2-(5-((2,4-dimethoxyphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)-1-(4-methoxy phenyl)ethanone **(5i).** Yield 58%; m.p; 174-176˚C; **IR(KBr, υ, cm^{-1}):** 3327.33 (NH); 3289.33(aromatic C-H); 1684.77 (C=O); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.52 (s, 1H, Ar-H); 7.31-7.01(m, 5H, Ar-H);6.49-6.50(d, 2H, Ar-H); 5.47(s, 2H, -S-CH_2-CO); 4.70(d, 2H, CH_2); 4.03(s, 1H, NH).

*2.2.5.10. 1-(4-chlorophenyl)-2-(5-((2,4,5-trichlorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone***(5j).** Yield 69%; m.p; 126-128˚C; **IR(KBr, υ, cm^{-1}):** 3424.55 (NH); 2921.36(aromatic C-H); 1729.71 (C=O); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.76(s,1H, Ar-H); 8.54-8.52(s,2H, Ar-H); 8.42 -8.39(d, 2H, Ar-H);7.92(s, 1H, Ar-H); 4.81(s, 2H, -S-CH_2-CO); 4.53(d, 2H, CH_2); 4.23(s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 192.49 (C=O); 163.76(C2-triazolic ring); 152.08(C5- triazolic ring); 141.18, 140.10,135.92, 129,32, 128.92, 128.23; 126.30, 126.09(aromatic ring);49.47(CH_2); 38.70(-S-CH_2-CO).

*2.2.5.11. 1-(4-fluorophenyl)-2-(5-((2,4,5-trifluorophenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone***(5k).** Yield 52%; m.p; 222-224˚C; **IR(KBr, υ, cm^{-1}):** 3375.53 (NH); 3048.22(aromatic C-H); 1723.23 (C=O); 725.44 (C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 8.61(d, 2H, Ar-H); 7.77-7.52(s, 4H, Ar-H); 5.01(s, 2H, -S-CH_2-CO); 4.62d, 2H, CH_2); 3.99(s, 1H, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 193.24 (C=O); 160.21(C2-triazolic ring); 152.72(C5- triazolic ring); 141.35, 139.35,139.31, 135,69, 128.10, 127.38; 126.34, 126.39(aromatic ring);50.27(CH_2); 38.17(-S-CH_2-CO).

*2.2.5.12. 1-(4-methoxyphenyl)-2-(5-((2,4,5-trimethoxyphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone***(5l).** Yield 58%; m.p; 178-180˚C; **IR(KBr, υ, cm^{-1}):** 3441.83 (NH); 3377.12(aromatic C-H); 1715.39 (C=O); 730.22(C-Cl); **^{1}H NMR (DMSO-d_6, δ ppm):** 7.83(s,1H, Ar-H); 7.12-7,09(m,4H, Ar-H); 6.98(s, 1H, Ar-H); 4.85(s, 1H,Ar-H); 4.85(s, 2H, -S-CH_2-CO); 4.47(d, 2H, CH_2); 4.01(s, 1H, NH);

3.BIOLOGICAL EVALUATION

3.1. In vitro evaluation of Antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* $H_{37}Rv$ strain, the MIC of each drug was determined by broth dilution assay and is defined as the lowest concentration of drug, which inhibits ≥99% of bacterial population present at the beginning of the assay [13]. A frozen culture in Middlebrook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 10^5 cfu ml^{-1} dilutions. Each test compound was dissolved in DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 ml of standardized culture and then incubated at 37^0C for 21 days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard Isoniazid [14]. The MIC values of tested compounds are given in Table 2.

Table 1. Anti-tubercular screening results of compounds

Compounds	**Antitubercular activity of compounds (MIC values µg/ml)**
	M. tubercular H_{37} Rv
4a	250
4b	8
4c	250
4d	500
4e	125
4f	250
4g	125
4h	31.25
4i	62.5
5a	16
5b	125
5c	62.5
5d	8
5e	250
5f	62.5
5g	500
5h	16
5i	16
5j	8
5k	250
5l	62.5
Isoniazid	0.25

Table 2. Antimicrobial screening results of compounds

Compounds	Antibacterial activity of compounds (MIC values µg/ml)			
	E. Coli	*S. Aureus*	*A. Niger*	*C.Albicans*
4a	250	250	250	125
4b	250	250	125	250
4c	125	250	250	62.5
4d	250	500	250	500
4e	8	62.5	8	16
4f	62.5	250	250	31.25
4g	125	250	62.5	125
4h	8	16	16	125
4i	62.5	125	125	125
5a	16	8	16	8
5b	125	62.5	125	125
5c	62.5	125	62.5	125
5d	16	8	16	125
5e	62.5	125	8	16
5f	62.5	125	16	8
5g	500	250	125	500
5h	62.5	62.5	62.5	125
5i	62.5	62.5	62.5	125
5j	8	8	8	16
5k	500	125	250	62.5
5l	62.5	125	62.5	125
Norfloxacin	<1	<5	NT	NT
Griseofulvin	NT	NT	≤1	≤5

NT, not tested

3.2. In vitro evaluation of Antibacterial activity

The MIC determination of the tested compounds were carried out in side-by-side 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 determination 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^{-1} (colony forming unit/ml) and incubated at 37^0C for 18 h. The MIC was the lowest concentration of the tested compound that yields no visible growth on the plate [15]. 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 [16]. The MIC values of tested compounds are given in Table 3.

Table 3: Physical Datas of Compounds.

COMP CODE	Molecular formula	R	R_1	R_2	R'	Elemental analysis found % (Calcd)		
						C	H	N
4a	$C_9 H_8 Cl N_3 S_2$	Cl	H	H	-	41.74 41.70	3.13 3.15	16.30 16.26
4b	$C_9 H_8 F N_3 S_2$	F	H	H	-	44.80 44.77	3.34 3.32	17.47 17.45
4c	$C_{10} H_{11} N_3 O S_2$	OCH3	H	H	-	47.41 47.40	4.38 4.36	16.59 16.56
4d	$C_9 H_8 Cl_2 N_3 S_2$	Cl	Cl	H	-	36.99 36.99	2.41 2.40	14.38 14.37
4e	$C_9 H_7 F_2 N_3 S_2$	F	F	H	-	41.65 41.62	2.72 2.70	16.20 16.21
4f	$C_{11} H_{13} N_3 O_2 S_2$	OCH3	OCH3	H	-	46.62 46.62	4.62 4.60	14.83 14.80
4g	$C_9 H_8 Cl_3 N_3 S_2$	Cl	Cl	Cl	-	33.08 33.08	1.85 1.83	12.83 12.82
4h	$C_9 H_6 F_3 N_3 S_2$	F	F	F	-	38.98 38.95	2.20 2.18	15.15 15.11
4i	$C_{12} H_{15} N_3 O_3 S_2$	OCH3	OCH3	OCH3	-	45.99 45.98	4.52 4.50	13.40 13.40
5a	$C_{17} H_{14} Cl N_3 O_2 S$	Cl	H	H	H	54.32 54.32	3.74 3.70	11.18 11.17
5b	$C_{17} H_{14} F N_3 O_2 S$	F	H	H	H	56.81 56.82	3.93 3.88	11.19 11.17
5c	$C_{18} H_{17} N_3 O_3 S$	OCH3	H	H	H	58.20 58.20	4.62 4.61	11.31 11.30
5d	$C_{17} H_{13} Cl_2 N_3 O_2 S$	Cl	H	H	Cl	49.76 49.77	3.19 3.18	10.24 10.22
5e	$C_{17} H_{13} F_2 N_3 O_2 S$	F	H	H	F	54.10 54.09	3.47 3.45	11.13 11.12
5f	$C_{19} H_{19} N_3 O_4 S$	OCH3	H	H	OCH3	56.84 56.79	4.77 4.75	10.47 10.46
5g	$C_{17} H_{12} Cl_3 N_3 O_2 S$	Cl	Cl	H	Cl	45.90 45.90	2.72 2.70	9.45 9.44
5h	$C_{17} H_{12} F_3 N_3 O_2 S$	F	F	H	F	51.62 51.60	3.06 3.05	10.63 10.62
5i	$C_{20} H_{21} N_3 O_5 S$	OCH3	OCH3	H	OCH3	55.67 55.65	4.90 4.88	9.74 9.72
5j	$C_{17} H_{11} Cl_4 N_3 O_2 S$	Cl	Cl	Cl	Cl	42.61 42.57	2.32 2.30	8.77 8.75
5k	$C_{17} H_{11} F_4 N_3 O_2 S$	F	F	F	F	49.38 49.37	2.69 2.68	10.16 10.15
5l	$C_{21} H_{23} N_3 O_6 S$	OCH3	OCH3	OCH3	OCH3	54.65 54.62	5.02 5.01	9.10 9.11

4.RESULT AND DISCUSSION

4.1. Chemistry

The synthetic route of the compounds (**4a-i** and **5a-l**) is outlined in Scheme 1 respectively. The 2-(susbtituted phenylamino)acetic acid was

prepared by cyclisation of equimolar quantities of chloroacetic acid and substituted aniline by published procedures [17]. Ethyl-2-(substitutedphenylamino)acetate(**2)** was obtained by refluxing 2-(susbtituted phenylamino)acetic acid and thiosemicarbazide in presence of dry ethanol to form esters[18]. 2-(substituted phenylamino)acetohydrazide was prepared by hydrozinolysis of the esters. Synthesis of 5-((substitutedphenylamino)methyl)-1,3,4-triazole-2-thiol was achived by adopting a simple one pot procedure that involves reacting hydazides with carbon disulfide under strong acidic conditions with Sulphuric acid (**4a-i**) [19]. The alkylation of 1,3,4-triazoles **4** with phenacyl bromide or p-substituted phenycyl bromide, in presence of dimethyl sulfoxide afford a new series of 1-(4-substitutedphenyl)-2-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-ylthio)ethanone (**5a-l**)[20].

The formation of 2-substituted phenylamino acetic acid(**1**) was confirmed by **IR** spectra, which showed the presence of amine(-NH) band and ^{1}H NMR D_2O exchange experiment around δ 4.10 ppm. Ethyl-2-(substitutedphenylamino)acetate (**2**) showed around 1740.66(C=O). 2-(substituted phenylamino)acetohydrazide(**3**) was confirmed by 3450.66(NH_2) and the signal was observed around δ 8.22 in the ^{1}H NMR. The formation of 5-((substitutedphenylamino)methyl)-1,3,4-triazole-2-thiol (**4a-i**) showed a strong band at 1680-1740cm^{-1}. A new signal for SH group was appeared as singlet at δ10.12-12.87ppm. In the ^{1}H NMR spectra of 1-(4-substitutedphenyl)-2-(5-((substitutedphenylamino) methyl)-1,3,4-thiadiazol-2-ylthio)ethanone(**5a-l**) were confirmed by absence of SH peak while the signal of methylene proton (4.81-4.95ppm) from compounds were appeared. The ^{13}C NMR spectrum of (**5a-l**) displayed (C=O) signals around 192.18-193.18ppm.

The mass spectra and elemental analysis of these compounds further confirmed the assigned structures. The physical data of the synthesized compounds are given in Table 3.

2.2. Biological evaluation

2.2.1. In vitro antitubercular studies

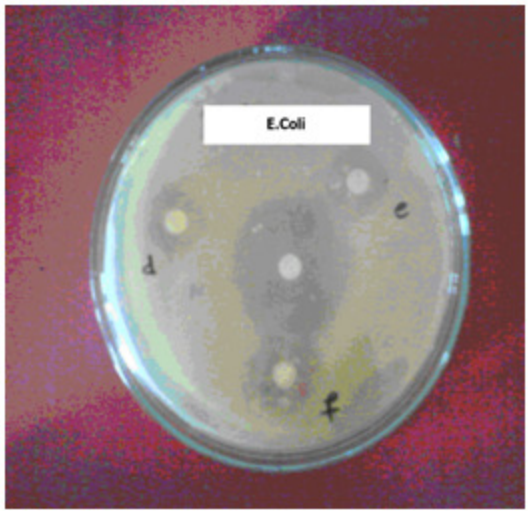
The antitubercular screening was carried out by Lowenstain–Jensen egg medium (L. J. Medium) as described by Watt against *H_{37}Rv.* Strain. L. J. Medium containing standard drug as well as control, Then L. J. Medium was also inoculated with mycobacterium tuberculosis of H_{37}Rv Strain. The medium inoculated was incubated for 37°C for 6 weeks. At the end of 6 weeks readings were taken [21].

The antitubercular screening revealed that some of the tested compounds showed moderate to good inhibition against standard drug Isoniazid. Particularly compounds **4b, 5a, 5d, 5h** and **5j** have shown good activity with MIC values between 8 to 32.5µg/ml. All the remaining compounds **4a, 4c, 4e, 4f, 4g, 4h, 4i, 5b, 5c, 5e, 5f, 5i, 5k** and **5l** showed moderate activity, where as **4d** and **5g** shown less activity.

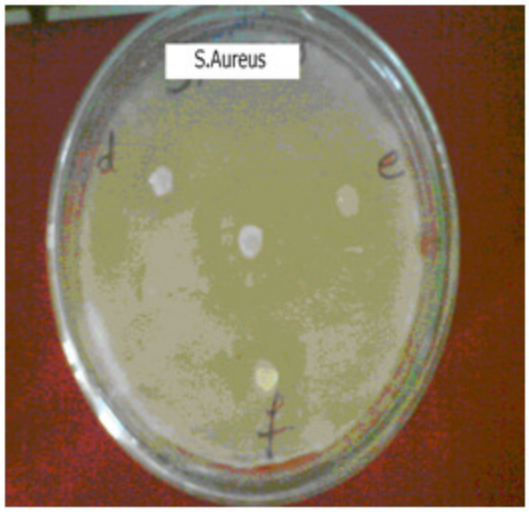
2.2.2. In vitro antimicrobial studies

The compounds were tested *in-vitro* 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 against standard drug Norfloxacin. The compounds were tested for *in-vitro* for their antifungal activity against *C. albicans* (ATCC 10231) and *A. niger* (ATCC 16404) by Cup-plate agar diffusion method using Sabouraud-Dextrose agar against standard drug Greseofulvin[22]. Figure 1. Shows the antibacterial MIC zone.

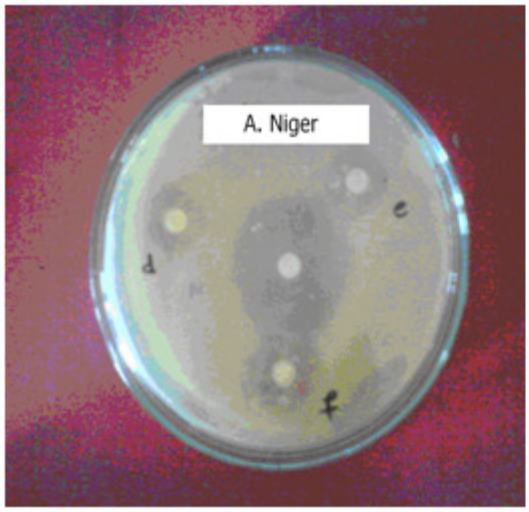
Figure 1. Antibacterial and antifungal screening of synthesized compounds.



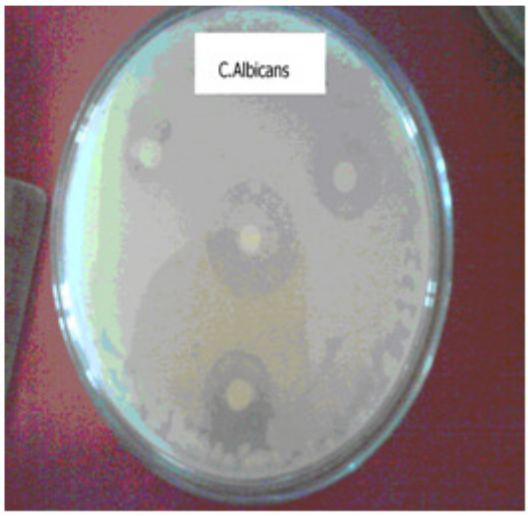
E. Coli



S. Aureus



A.Niger



C. Albicans

The investigation of antimicrobial screening revealed that some of compounds showed moderate to good bacterial and fungal inhibition. Particularly compounds **4e, 4h, 5a, 5d** and **5j** showed good activity against *E.Coli* and *S.aureus* with MIC values between 8 to 32.5µg/ml. All the remaining compounds **4a, 4b, 4c, 4f, 4g, 4i, 5b, 5c, 5e, 5f, 5h**, **5i, 5k** and **5l** showed moderate activity, where as **4d** and **5g** shown less activity. The investigation of antifungal screening revealed that compounds **4e, 5a, 5d** and **5j** showed good activity against *A.Niger and C.Albicans* with MIC values between 8 to 32.5µg/ml. All the remaining compounds **4a, 4b, 4c, 4h, 4f, 4g, 4i, 5b, 5c, 5e, 5f, 5h, 5i, 5k** and **5l** showed moderate activity, where as **4d** and **5g** shown less activity.

3. CONCLUSION

We have synthesized series of novel S-substituted phenacyl-1,3,4-triazole-thiol derivatives. The results of antimicrobial screening revealed the discovery of new compounds as one of the promising agents. The mode of action of these compounds was unknown. This observation may promote a further development of this group of 1,3,4-triazole-thiol may lead to compounds with better pharmacological profile than standard antimicrobial drugs.

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Compound name	IR	^{1}H NMR	^{13}C NMR	MASS	
4a	3378.29(-N-H str) 782.89(-Cl str)	13.17 (s, 1H, -SH); 7.26-6.50 (m, 4H, Ar-H); 4.28(s, 2H-CH2);3.80 (s, 1H. -NH)		--	257.9
4b	3378.34(-N-H str) 718.88(-F str)	8.32 (s, 1H, -SH); 7.28-6.42 (m, 4H, Ar-H);4.01(s, 2H-CH2); 3.34 (s, 1H. -NH)			
4c	3412.17(-N-H str) 3214.12(Ar-CH); 1642.79(O-CH3);	8.32 (s, 1H, -SH); 7.28-6.42 (m, 4H, Ar-H); 4.01(s, 2H-CH2); 3.34 (s, 1H. -NH).			
4d	3312.32(-N-H str); 733.77(-Cl str);	10.45 (s, 1H, -SH); 7.31-6.41, (m, 3H, Ar-H); 4.11 (s, 1H. -NH); 3.52(s, 2H-CH2).			
4e	3342.61(-N-H str); 731.71(-F str);	11.98 (s, 1H, -SH); 7.31-6.57(m, 3H, Ar-H); 4.51 (s, 1H. -NH); 3.83(s, 2H-CH2).			
4f	3411.56(-N-H str); 3074.23(Ar-CH); 1711.10(O-CH3 str)	11.01 (s, 1H, -SH); 7.25-6.98 (m, 3H, Ar-H); 4.12 (s, 1H. -NH); 3.54(s, 2H-CH2).			
4g	3457.69(-N-H str); 736.98(-Cl str);	12.58 (s, 1H, -SH); 7.32-6.85(m, 2H, Ar-H); 4.10 (s, 1H. -NH); 2.17(s, 2H-CH2).			
4h	3369.34(-N-H str); 788.27(-F str);	13.15 (s, 1H, -SH); 6.34-5.54 (m, 2H, Ar-H); 3.30 (s, 1H. -NH); 2.51 (s, 2H-CH2).			
4i	3417.34(-N-H str); 3153.18(Ar-CH); 1671.21(1650.22);	12.17 (s, 1H, -SH); 7.85-7.54(m, 2H, Ar-H); 4.07(s, 1H. -NH); 3.02 (s, 2H-CH2).			

Compound	IR	^{1}H NMR	^{13}H NMR	Mass
5a	3194.40 (NH); 2839.52(aromatic C-H); 1713.71 (C=O); 724.28 (C-Cl)	8.61-8.59(d, 2H, Ar-H); 7.77-7.67(m, 7H, Ar-H); 7.45-7.41(m, 4H, Ar-H); 6.98-6.96(s, 1H, Ar-H); 4.76(d, 2H, -S-CH_2-CO); 4.42(d, 2H, CH_2); 4.12(s, 1H, NH)	--	434.10
5b	3470.34 (NH); 3076.95(aromatic C-H); 1682.17 (C=O); 715.14 (C-F)	8.02-7.99(m, 5H, Ar-H);7.32-7.31(m, 4H, Ar-H); 6.50-6.46(m, 5H, Ar-H); 4.97(d, 2H, -S-CH_2-CO); 4.39(d, 2H, CH_2); 4.01 (s, 1H, NH)	194.01(C=O); 165.22(C2-triazolic ring); 157.99(C5-triazolic ring); 150.40, 134.89,133.07, 130,51, 128.64, 125.11, 121.83, 115.12(aromatic ring); 50.71(CH_2); 38.99(-S-CH_2-CO)	359.01
5c	3375.92 (NH); 3100.65 (aromatic C-H); 1725.76 (C=O);	8.34-8.27(m, 7H, Ar-H); 7.77-7.72(m, 4H, Ar-H); 7.21-7.18(t, 3H, Ar-H); 5.57(d, 2H, -S-CH_2-CO); 4.90(d, 2H, CH_2); 4.11(s, 1H, NH)	--	--
5d	3375.53 (NH); 3048.22(aromatic C-H); 1723.23 (C=O); 725.43 (C-Cl)	8.42-8.37(d, 2H, Ar-H); 7.82-7.79(m, 5H, Ar-H); 7.82-6.99(m, 6H, Ar-H); 5.12 (d, 2H, -S-CH_2-CO); 4.67(d, 2H, CH_2); 3.89(s, 1H, NH)	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)	409.01
5e	3482.60 (NH); 3105.16(aromatic C-H); 1650.51 (C=O); 764.29 (C-F)	8.11-8.10(d, 2H, Ar-H);7.64-7.62(m, 5H, Ar-H); 6.59-6.58(d, 6H, Ar-H); 4.87 (d, 2H, -S-CH_2-CO); 4.50(d, 2H, CH_2); 4.03(s, 1H, NH)	--	
5f	3383.45 (NH); 3106.47(aromatic C-H); 1650.71 (C=O)	8.52 -8.40(m, 5H, Ar-H); 7.91 -7.40(m, 8H, Ar-H); 5.54(s, 2H, -S-CH_2-CO); 5.07(d, 2H, CH_2); 4.36(s, 1H, NH)	--	401.0

5g	3345.68 (NH); 3048.60(aromatic C-H); 1690.43 (C=O); 713.82 (C-Cl)	8.44 -8.32(m, 5H, Ar-H); 7.94 -7.80(m, 7H, Ar-H); 5.02(s, 2H, -S-CH_2-CO); 4.80(d, 2H, CH_2); 3.79(s, 1H, NH)	193.18(C=O); 160.71(C2-triazolic ring); 157.25(C5-triazolic ring); 142.01, 140.05,139.89, 134,38, 132.61, 130.06; 129.09, 127.88, 126.66(aromatic ring); 47.62(CH_2); 39.65(-S-CH_2-CO)	429.0
5h	3461.06 (NH); 3086.01(aromatic C-H); 1720.07 (C=O); 732.28 (C-F)	8.64 -8.62(t, 3H, Ar-H); 7.99-7.95(m, 5H, Ar-H); 7.32-7.29(t, 3H, Ar-H); 6.98(s, 1H, Ar-H); 5.02(s, 2H, -S-CH_2-CO); 4.71(d, 2H, CH_2); 4.10(s, 1H, NH)	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)	479.1
5i	3392.10 (NH); 3046.68(aromatic C-H); 1739.15 (C=O)	8.52 (s, 1H, Ar-H); 7.81-7.75(m, 6H, Ar-H);6.49-6.50(m, 5H, Ar-H); 5.47(s, 2H, -S-CH_2-CO); 4.70(d, 2H, CH_2); 4.03(s, 1H, NH)	--	--
5j	3424.55 (NH); 3077.12(aromatic C-H); 1719.71 (C=O); 714.22(C-Cl)	8.76(d,2H, Ar-H); 8.54-8.47(m,6H, Ar-H); 7.42 -7.39(d, 2H, Ar-H);6.92(s, 1H, Ar-H); 4.81(s, 2H, -S-CH_2-CO); 4.53(d, 2H, CH_2); 4.23(s, 1H, NH)	192.49 (C=O); 163.76(C2-triazolic ring); 152.08(C5-triazolic ring); 141.18, 140.10,135.92, 129,32, 128.92, 128.23; 126.30, 126.09(aromatic ring);49.47(CH_2); 38.70(-S-CH_2-CO)	
5k	3275.13 (NH); 3028.27(aromatic C-H); 1703.23 (C=O); 715.44 (C-F)	8.61(d, 2H, Ar-H); 7.77-7.52(m, 4H, Ar-H); 6.97-6.92(m, 5H, Ar-H); 5.01(s, 2H, -S-CH_2-CO); 4.62d, 2H, CH_2); 3.99(s, 1H, NH)	193.24 (C=O); 160.21(C2-triazolic ring); 152.72(C5-triazolic ring); 141.35, 139.35,139.31, 135,69, 128.10, 127.38; 126.34, 126.39(aromatic ring);50.27(CH_2); 38.17(-S-CH_2-CO)	

5l	3347.03 (NH); 3016.62(aromatic C-H); 1715.33 (C=O)	3347.03 (NH); 3016.62(aromatic C-H); 1715.33 (C=O)	--	

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