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NEW MERCAPTOACETAMIDE DERIVATIVES: SYNTHESIS AND ASSESSMENT AS ANTIMICROBIAL AND ANTIMYCOBACTERIAL AGENTS

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During last few years, the frightening elevation of bacterial resistance was accompanied by dramatic decline in recent treatments of infectious diseases, which became a point of anxiety for healthcare industries. MDR and XDR strains of *Mycobacterium tuberculosis* (*Mtb*) result in the tuberculosis. In this regard, herein, a series of new mercaptoacetamide derivatives were synthesized via multipot synthetic pathway and the rationale was the appraisal of bioactivity in compact heteronuclei and their assessment as potential antimicrobial and antimycobacterial agents against virulent strain of *Mtb,* $H_{37}Ra$ for structure–activity relationship (SAR) studies. The inhibition zones of compounds **4c** and **4e** were found to be nearest to that of standard drug Ciprofloxacin, while compounds **4h** and **4j** were mild to moderately active against Gram positive bacteria (*Staphylococcus aureus, Streptococus pneumonia*) and Gram negative bacteria (*Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli*). MIC_{90} assays indicated that new mercaptoacetamides did not exhibit *in vitro* activity against *Mtb* in contrast to Rifampicin and Streptomycin, first-line antimycobacterial chemotherapeutic agents. According to the present study, it was concluded that mercaptoacetamides of the new series succeeded as antimicrobial agents but could not develop as potential lead compounds against *Mtb* when tested in concentrations of 50, 25, 12.5 and 6.25 μg/mL.

Keywords: *Mycobacterium tuberculosis*; mercaptoacetamides; anti-tuberculosis activity; antimicrobial activity

1. INTRODUCTION

In the present milieu, tuberculosis (TB) is wild on grounds of the exposure of new cases, negative sequels of first-line anti-TB drugs rifampicin (RIF) and isoniazid (INH), emergence of MDR and XDR strains of causative pathogenic *Mycobacterium tuberculosis* (*Mtb*) and comorbidity with HIV infections [1 – 3]**.** These grounds gather speed by the collapsed anti-TB drug discovery efforts. Current reports designate the prompt inefficacy of Directly Observed Treatment Short-Course (DOTS) in the areas covering high prevalence of MDR-TB [4 – 7]. In this set of conditions, the sole option in prescription for *Mtb* is a combination of second line drugs with DOTS but this combination therapy is inadequate for riddance of XDR *Mtb* [8]. Consequently, necessity for the evolution of novel anti-TB drugs possessing boosted outcome such as eradication of disease quickly, diminished toxicity, elevated activity against MDR, prompt mechanism of action against *Mtb*, shortened treatment duration and host cell perforation potential is instantly required.

Various acetamides were synthesized to increase the molecular array in the series of antimicrobial agents and were subsequently shown to exhibit significant antimycobacterial activity. A series of 2-(3-fluoro-4-nitrophenoxy)-N-phenylacetamide derivatives (Fig. 1a) were synthesized and screened for anti-TB activity. It was found that all the new derivatives exerted potent or moderate activity against *M. tuberculosis* H37Rv, with MIC values ranging from 4 to 64 μg/mL. The presence of nitro group at position 2 of *N*-phenylacetamide nucleus resulted in most potent activity with an identical MIC value of 4 μg/mL for both *M. tubercu-*

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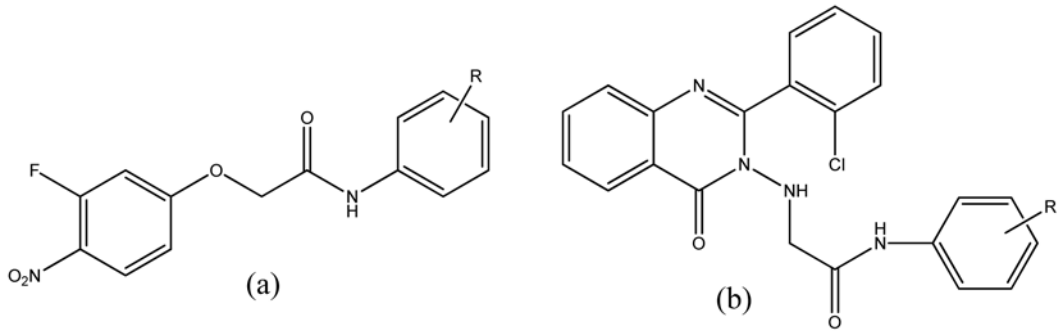

Fig. 1. Commonly used structures for antimycobacterial activity: (a) 2-phenoxy-*N*-phenylacetamide); (b) *N*-(4-oxo-2-phenyl-3,4-dihydroquinolin-3-yl)acetamide).

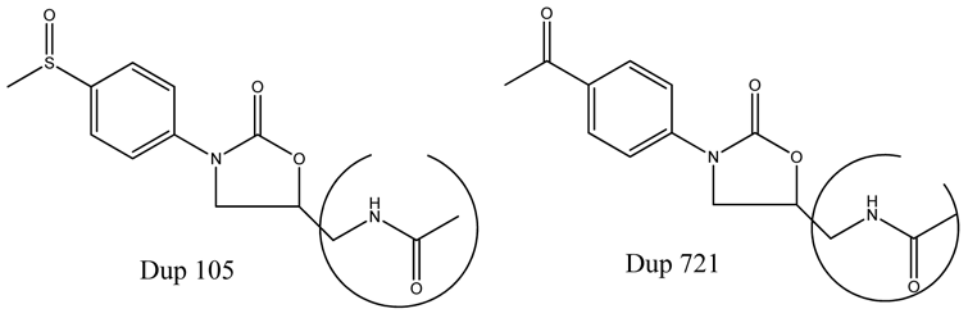

Fig. 2. Antibacterial compounds with acetamide group.

losis H37Rv and rifampin-resistant *M. tuberculosis* 261 [9 – 11]. When 2-{[2-(2-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]amino}-*N*-(substituted phenyl)acetamide derivatives (Fig. 1b) were synthesized and evaluated against *Bacillus subtilis* (NCIM 2697), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2065) and *Klebsiella pneumonia* (NCIM 5082) strains, it was found that compounds having 2,6-dichloro and 2-chloro phenyl substituents showed the highest degree of activity against *Bacillus subtilis* and *Staphylococcus aureus* while compounds having 4-methoxy, 2,6-dichloro and 2-chloro phenyl substituents showed highest degree of activity against *Escherichia coli* and *Klebsiella pneumonia* [12]. Dupont have reported DUP 105 and DUP 721 (Fig. 2) with an acetamide substitution and have proved that the group imparts improved antibacterial activity. Moreover, compounds bearing acetanilide nucleus occupy an illustrious place in medicinal chemistry because of its consequential pharmacological properties such as anticancer [13 – 15], anti-inflammatory**,** antipyretic, analgesic [16, 17] and antioxidant [18 – 20]**.** Some acetamides have also been considered as chemotherapeutic agents for inflammation associated cancers [21, 22].

In our prolonged endeavour to develop new anti-TB agents, a new class of mercaptoacetamides were synthesized and characterized. Compounds were evaluated for their antimicrobial activity and antimycobacterial activity where Ciprofloxacin, Rifampicin and Streptomycin were used as reference drugs for collation.

2. EXPERIMENTAL

2.1. General Considerations

Organic solvents and chemicals were purchased from Sigma-Aldrich and CDH (Central Drug House P. Ltd., New Delhi, India) [23]. The purity of compounds and completion of reactions were monitored by thin layer chromatography (TLC) using silica gel G as the stationary phase and mobile phases of n-hexane and ethyl acetate in different ratios. Visualization was accomplished by exposure to either iodine vapor or UV light. Chemical nomenclature was generated using Chem Draw Ultra (version 12.0, Cambridgesoft). Melting points were determined in open capillaries on Thomas Hoover apparatus and remained uncorrected. The IR spectra were recorded on Shimadzu IR-435 spectrophotometer (IR Prestige-21, Shimadzu Corporation, Kyoto, Japan) using KBr pellets; ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker Corporation, Massachusetts, USA); mass spectra (MS) were recorded on Micromass Q-TOF Micro (Waters Corporation Massachusetts, USA). Elemental analyses were recorded using Vario EL III ele-

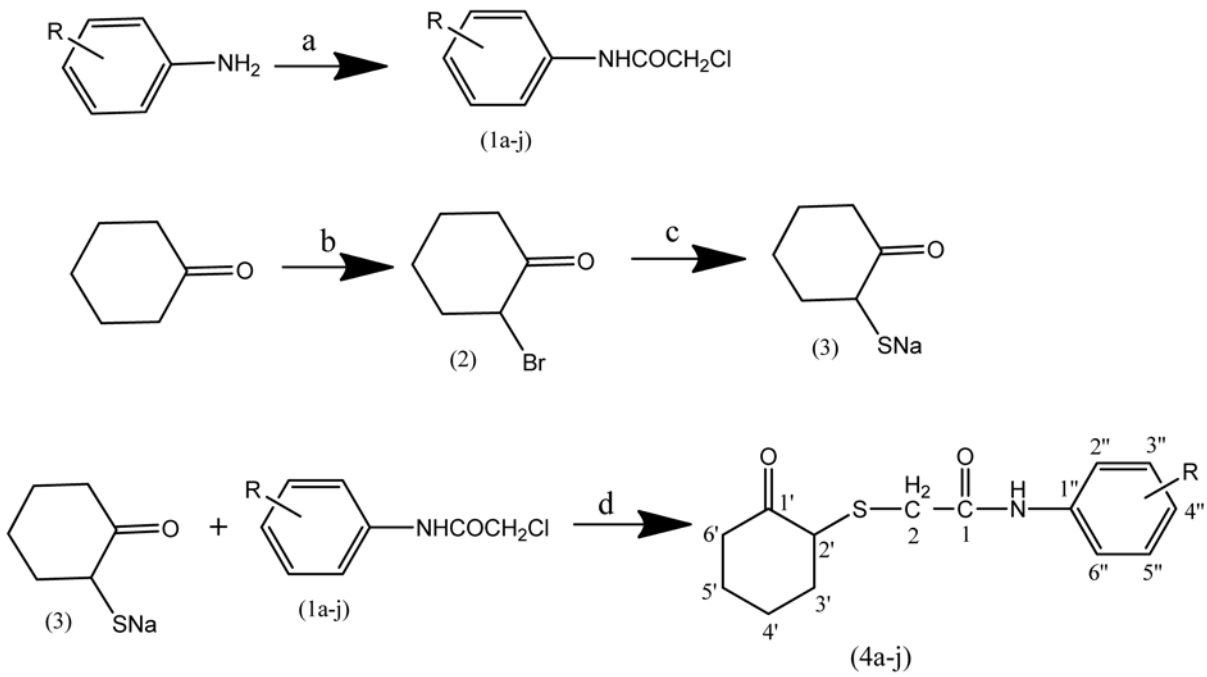

Fig. 3. Synthesis of 2-((2-oxocyclohexyl)thio)-N-(substituted phenyl)acetamide derivatives (**4a**–4j). Reagents and conditions: (a) Chloroacetyl chloride, 10% sodium hydroxide, ice bath, pH 9 – 10. (b) Water, bromine, 1h, 5°C, stirring > room temp, 1h, stirring > extraction, diethyl ether > washing, water, saturated NaCl solution > distillation, vacuo. (c) Sodium sulphide, DMF, 100 – 120°C, 1h. (d) DMF, 1 – 2 h, 100 – 120°C.

mental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

2.2. Synthesis

Synthesis of 2-((2-oxocyclohexyl)thio)-N-(substituted phenyl)acetamide derivatives (Fig. 3) is divided into four steps:

1. Chloroacetylation of amines to form initial chloro compounds (**1a**–**1j**);

2. Synthesis of 2-bromocyclohexanone (**2**) from cyclohexanone;

3. Synthesis of sodium2-oxocyclohexanethiolate (**3**) from 2-bromocyclohexanone and sodium sulphide;

4. Synthesis of 2-((2-oxocyclohexyl)thio)-N-(substituted phenyl)acetamide derivatives (**4a**–**4j**) from sodium 2-oxocyclohexanethiolate **3** and compounds **1a**–**1j**.

2-((2-Oxocyclohexyl)thio)-N-(p-tolyl)acetamide (**4a**): IR (KBr, cm^{-1}): 3210, 3016, 2830, 1700, 1682, 1480, 705. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.64 (t, J = 7.5 Hz, 2H, H-2″, H-6″), 7.34 (s, 1H, NH), 7.20 (t, J = 8.2 Hz, 2H, H-3″, H-5″), 4.21 (s, 2H, CH_2-2), 3.85 (t, J = 7.0 Hz, 1H, H-2'), 3.63 (s, 3H, CH_3-4″), 3.50 – 3.42 (m, 2H, CH_2-3′), 3.12 – 2.83 (m, 2H, CH_2-6′), 2.51 – 2.45 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 204.1 (C=O, C-1′), 161.4 (C=O, C-1), 139.6 (C, C-4″), 138.1 (C, C-1″), 131.6 (CH, C-3″, 5″), 120.3 (CH, C-2″, 6″), 60.8 (CH, C-2′), 41.3 (CH_2, C-6′), 40.8 (CH_2, C-2), 31.9 (CH_2, C-3′), 27.3 (CH_2, C-5′), 21.3 (CH_2, C-4′), 20.6 (CH_3, CH_3-4″). MS (m/z): 277 (M+), 278 (M+1, 17.4 %), 279 (M+2, 6.7 %). Anal. Calc. for $C_{15}H_{19}NO_2S$: C 64.95, H 6.90, N 5.05. Found: C 64.91, H 6.85, N 5.10.

N-(4-Methoxyphenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4b**): IR (KBr, cm^{-1}): 3232, 3018, 2815, 1655, 1610, 1490, 675. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.76 (d, J = 7.5 Hz, 2H, H-2″, H-6″), 7.51 (s, 1H, NH), 7.13 (d, J = 7.9 Hz, 2H, H-3″, H-5″), 4.64 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2-2), 3.56 (t, J = 7.0 Hz, 1H, H-2′), 3.13 – 2.78 (m, 2H, CH_2-3′), 2.56 – 2.41 (m, 2H, CH_2-6′), 2.36 – 2.22 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 201.5 (C=O, C-1′), 170.4 (C=O, C-1), 163.6 (C, C-4″), 143.5 (C, C-1″), 133.9 (CH, C-2″, 6″), 128.1 (CH, C-3″, 5″), 73.8 (CH_3, OCH_3), 61.1 (CH, C-2′), 43.6 (CH_2, C-6′), 42.2 (CH_2, C-2), 39.6 (CH_2, C-3′), 33.8 (CH_2, C-5′), 31.5 (CH_2, C-4′). MS (m/z): 293(M+), 294 (M+1, 17.3%), 295 (M+2, 6.8%). Anal. Calc. for $C_{15}H_{19}NO_3S$: C 61.41, H 6.53, N 4.77. Found: C 61.46, H 6.52, N 4.80.

4-(2-((2-Oxocyclohexyl)thio)acetamido)benzoic acid (**4c**): IR (KBr, cm^{-1}): 3305, 3212, 2865, 1710, 1635, 1522, 620. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.81 (s, 1H, OH), 8.15 (d, J = 7.9 Hz, 2H, H-3″, H-5″), 7.83 (d, J = 7.5 Hz, 2H, H-2″, H-6″), 7.68 (s, 1H, NH), 4.12 (s, 2H, CH_2-2), 3.48 (t, J = 7.2 Hz, 1H, H-2′), 3.78 – 3.61 (m, 2H, CH_2-3′), 3.50 – 3.38 (m, 2H, CH_2-6′), 3.21 – 2.94 (m, 4H,

CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 205.1 (C=O, C-1′), 173.4 (C=O, COOH), 170.6 (C=O, C-1), 149.3 (C, C-1″), 141.6 (CH, C-3″, 5″), 130.7 (C, C-4″), 123.9 (CH, C-2″, 6″), 64.1 (CH, C-2′), 51.7 (CH_2, C-6′), 50.4 (CH_2, C-2), 48.3 (CH_2, C-3′), 33.6 (CH_2, C-5′), 29.4 (CH_2, C-4′). MS (m/z): 307 (M+), 308 (M+1, 17.3%), 309 (M+2, 6.2%). Anal. Calc. for $C_{15}H_{17}NO_4S$: C 58.61, H 5.57, N 4.56. Found: C 58.63, H 5.60, N 4.55.

N-(4-Chlorophenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4d**): IR (KBr, cm^{-1}): 3185, 2992, 2840, 1695, 1680, 1506, 710. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.12 (d, *J* = 7.5 Hz, 2H, H-2″, H-6″), 7.86 (d, *J* = 8.0 Hz, 2H, H-3″, H-5″), 7.57 (s, 1H, NH), 4.83 (s, 2H, CH_2-2), 4.25 (t, *J* = 7.2 Hz, 1H, H-2′), 3.81 – 3.65 (m, 2H, CH_2-3′), 3.72 – 3.53 (m, 2H, CH_2-6′), 2.91 – 2.78 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 205.1 (C=O, C-1′), 173.8 (C=O, C-1), 153.4 (C, C-1″), 151.7 (C, C-4″), 137.7 (CH, C-3″, 5″), 131.6 (CH, C-2″, 6″), 61.8 (CH, C-2′), 58.9 (CH_2, C-6′), 57.6 (CH_2, C-2), 44.8 (CH_2, C-3′), 41.7 (CH_2, C-5′), 38.1 (CH_2, C-4′). MS (m/z): 297 (M+), 298 (M+1, 16.4 %), 299 (M+2, 38.5 %). Anal. Calc. for $C_{14}H_{16}ClNO_2S$: C 56.46, H 5.42, N 4.70. Found: C 56.41, H 5.47, N 4.72.

N-(4-Nitrophenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4e**): IR (KBr, cm^{-1}): 3310, 3186, 2820, 1705, 1610, 1495, 712. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.07 (d, *J* = 8.0 Hz, 2H, H-3″, H-5″), 7.92 (d, *J* = 7.5 Hz, 2H, H-2″, H-6″), 7.64 (s, 1H, NH), 5.12 (s, 2H, CH_2-2), 4.63 (t, *J* = 7.2 Hz, 1H, H-2′), 3.91 – 3.77 (m, 2H, CH_2-3′), 3.08 – 2.94 (m, 2H, CH_2-6′), 2.81 – 2.62 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 204.6 (C=O, C-1′), 172.9 (C=O, C-1), 154.5 (C, C-1″), 152.8 (C, C-4″), 138.1 (CH, C-3″, 5″), 130.9 (CH, C-2″, 6″), 62.4 (CH, C-2′), 57.3 (CH_2, C-6′), 54.4 (CH_2, C-2), 45.7 (CH_2, C-3′), 38.8 (CH_2, C-5′), 29.7 (CH_2, C-4′). MS (m/z): 308 (M+), 309 (M+1, 17.3 %), 310 (M+2, 6.5 %). Anal. Calc. for $C_{14}H_{16}N_2O_4S$: C 54.53, H 5.23, N 9.08. Found: C 54.56, H 5.20, N 9.10.

2-((2-Oxocyclohexyl)thio)-N-(o-tolyl)acetamide (**4f**): IR (KBr, cm^{-1}): 3186, 3120, 2885, 1685, 1626, 1476, 648. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.10 (d, *J* = 8.2 Hz, 1H, H-3″), 7.75 – 7.62 (m, 3H, NH, H-5″, H-6″), 7.51 (t, *J* = 7.6 Hz, 1H, H-4″), 3.64 (s, 2H, CH_2-2), 3.58 (t, *J* = 7.2 Hz, 1H, H-2′), 3.47 – 3.31 (m, 2H, CH_2-3′), 3.11 (s, 3H, CH_3_2″), 2.74 – 2.68 (m, 2H, CH_2-6′), 2.51 – 2.33 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 208.1 (C=O, C-1′), 173.3 (C=O, C-1), 144.9 (C, C-1″), 140.6 (C, C-2″), 139.4 (CH, C-3″), 135.1 (CH, C-4″), 132.4 (CH, C-5″), 129.0 (CH, C-6″), 69.4 (CH, C-2′), 54.1 (CH_2, C-6′), 53.8 (CH_2, C-2), 48.6 (CH_2, C-3′), 29.4 (CH_2, C-5′), 28.6 (CH_2, C-4′), 23.3 (CH_3, C-2″). MS (m/z): 277 (M+), 278 (M+1, 17.5 %), 279 (M+2, 6.1 %). Anal. Calc. for $C_{15}H_{19}NO_2S$: C 64.95, H 6.90, N 5.05. Found: C 64.94, H 6.93, N 5.02.

N-(2,6-Dimethylphenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4g**): IR (KBr, cm^{-1}): 3220, 3135, 2795, 1690, 1675, 1526, 676. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.81 (s, 1H, NH), 7.65 (t, *J* = 7.6 Hz, 1H, H-4″), 7.32 (d, *J* = 8.0 Hz, 2H, H-3″, H-5″), 5.22 (s, 2H, CH_2-2), 4.87 (t, *J* = 7.2 Hz, 1H, H-2′), 4.53 – 4.37 (m, 2H, CH_2-3′), 3.82 (s, 6H, CH_3_2″, CH_3_6″), 3.71 – 3.63 (m, 2H, CH_2-6′), 3.37 – 2.83 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 207.4 (C=O, C-1′), 170.3 (C=O, C-1), 156.9 (C, C-1″), 145.4 (C, C-2″, 6″), 133.4 (C, C-3″, 5″), 125.6 (CH, C-4″), 94.6 (CH, C-2′), 73.1 (CH_2, C-6′), 71.6 (CH_2, C-2), 70.2 (CH_2, C-3′), 56.4 (CH_2, C-5′), 54.1 (CH_2, C-4′), 36.3 (CH_3, C-2″, 6″). MS (m/z): 291 (M+), 292 (M+1, 18.6 %), 293 (M+2, 6.9 %). Anal. Calc. for $C_{16}H_{21}NO_2S$: C 65.95, H 7.26, N 4.81. Found: C 65.90, H 7.28, N 4.84.

N-(4-Ethoxyphenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4h**): IR (KBr, cm^{-1}): 3236, 3140, 2810, 1700, 1636, 1456, 692. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.15 (t, *J* = 7.2 Hz, 2H, H-2″, H-6″), 7.92 (s, 1H, NH), 7.55 (t, *J* = 7.5 Hz, 2H, H-3″, H-5″), 6.73 (q, 2H, CH_2, OCH_2CH_3), 5.18 (s, 2H, CH_2-2), 4.45 (t, *J* = 7.2 Hz, 1H, H-2′), 4.13 – 3.65 (m, 2H, CH_2-3′), 3.41 – 3.17 (m, 2H, CH_2-6′), 2.94 – 2.71 (m, 4H, CH_2-4′, 5′), 2.36 (t, *J* = 8.0 Hz, 3H, CH_3, OCH_2CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 209.4 (C=O, C-1′), 193.6 (C=O, C-1), 181.7 (C, C-4″), 169.1 (C, C-1″), 133.8 (CH, C-2″, 6″), 127.9 (CH, C-3″, 5″), 111.4 (CH_2, OCH_2CH_3), 96.8 (CH, C-2′), 64.6 (CH_2, C-6′), 63.1 (CH_2, C-2), 59.3 (CH_2, C-3′), 57.6 (CH_2, C-5′), 38.4 (CH_2, C-4′), 27.1 (CH_3, OCH_2CH_3). MS (m/z): 307 (M+), 308 (M+1, 18.6 %), 309 (M+2, 6.5 %). Anal. Calc. for $C_{16}H_{21}NO_3S$: C 62.51, H 6.89, N 4.56. Found: C 62.50, H 6.85, N 4.58.

N-(4-Methoxy-3,5-dimethylphenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4i**): IR (KBr, cm^{-1}): 3305, 3110, 2815, 1720, 1676, 1486, 682. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.72 (s, 2H, H-2″, H-6″), 8.56 (s, 1H, NH), 6.42 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2-2), 4.53 (t, *J* = 7.2 Hz, 1H, H-2′), 4.48 – 4.12 (m, 2H, CH_2-3′), 3.92 (s, 6H, CH_3_2″, CH_3_6″), 3.78 – 3.52 (m, 2H, CH_2-6′), 3.48 – 3.17 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 207.1 (C=O, C-1′), 190.8 (C=O, C-1), 184.7 (C, C-4″), 165.5 (C, C-1″), 139.4 (C, C-3″, 5″), 126.6 (CH, C-2″, 6″), 110.5 (CH_3, OCH_3), 98.7 (CH, C-2′), 68.1 (CH_2, C-6′), 63.9 (CH_2, C-2), 51.3 (CH_2, C-3′), 52.2 (CH_2, C-5′), 37.1 (CH_2, C-4′), 21.6 (CH_3, C-3″, 5″). MS (m/z): 321 (M+), 322 (M+1, 19.7 %), 323 (M+2, 7.3 %). Anal. Calc. for $C_{17}H_{23}NO_3S$: C 63.52, H 7.21, N 4.36. Found: C 63.50, H 7.26, N 4.39.

N-(2-Fluoro-4-methoxyphenyl)-2-((2-oxocyclohexyl)thio)acetamide (**4j**): IR (KBr, cm^{-1}): 3292, 3236, 2905, 1640, 1606, 1472, 675. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.79 (q, 1H, H-6″), 8.53 (s, 1H, NH), 8.21 (dd, *J* = 8.0, 1.5 Hz, 1H, H-3″), 7.95 (dd, *J* = 7.5, 1.5 Hz, 1H, H-5″), 7.83 (s, 3H, OCH_3), 5.68 (s, 2H, CH_2-2), 5.17 (t, *J* = 7.2 Hz, 1H,

TABLE 1. Physicochemical Data for 2-((2-Qxocyclohexyl)thio)-N-(substituted phenyl)acetamide Derivatives (**4a–4j**).

Compound	R	Yield (%)	Melting point (°C)	R_f	Molecular formula
4a	4-CH_3	67.3	165 – 166	0.48	$C_{15}H_{19}NO_2S$
4b	4-OCH_3	58.1	161 – 162	0.39	$C_{15}H_{19}NO_3S$
4c	4-COOH	60.4	158 – 159	0.32	$C_{15}H_{17}NO_4S$
4d	4-Cl	71.6	133 – 134	0.57	$C_{14}H_{16}ClNO_2S$
4e	4-NO_2	53.1	147 – 148	0.65	$C_{14}H_{16}N_2O_4S$
4f	2-CH_3	75.5	172 – 173	0.39	$C_{15}H_{19}NO_2S$
4g	2,6-CH_3	68.7	139 – 140	0.43	$C_{16}H_{21}NO_2S$
4h	4-OCH_2CH_3	73.9	166 – 167	0.48	$C_{16}H_{21}NO_3S$
4i	4-OCH_3 , 3,5-CH_3	70.5	171 – 172	0.56	$C_{17}H_{23}NO_3S$
4j	2-Fl, 4-OCH_3	68.2	159 – 160	0.62	$C_{15}H_{18}FNO_3S$

TABLE 1a. Analysis of Variance for Rf values in Table 1

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	9	0.320253	0.035584	150.35	0.000
Error	20	0.004733	0.000237	-	-
Total	29	0.324987	-	-	-

H-2′), 4.83 – 4.56 (m, 2H, CH_2-3′), 4.43 – 4.17 (m, 2H, CH_2-6′), 3.73 – 3.56 (m, 4H, CH_2-4′, 5′). ^{13}C NMR (DMSO-d_6, δ, ppm): 209.4 (C=O, C-1′), 193.3 (C=O, C-1), 187.6 (C, C-2″), 163.1 (C, C-4″), 141.8 (CH, C-6″), 127.3 (C, C-1″), 119.5 (CH, C-5″), 112.5 (CH, C-3″), 94.6 (CH_3, OCH_3), 71.1 (CH, C-2′), 54.3 (CH_2, C-6′), 51.9 (CH_2, C-2), 43.4 (CH_2, C-3′), 29.8 (CH_2, C-5′), 27.7 (CH_2, C-4′). MS (m/z): 311 (M+), 312 (M+1, 17.5 %), 313 (M+2, 6.9 %). Anal. Calc. for $C_{15}H_{18}FNO_3S$: C 57.86, H 5.83, N 4.50. Found: C 57.83, H 5.87, N 4.52.

Physical parameters of synthesized target compounds **4a–4j** are given in Table 1. Analysis of Variance of the Rf values, model summary and Rf means are presented in Tables 1a, 1b and 1c, respectively. The interval plot of C2 vs. C1 and residual plots for C2 are presented in Figs. 4 and 5, respectively.

TABLE 1b. Model Summary of Table 1

S	R-sq	R-sq(adj)	R-sq(pred)
0.0153840	98.54%	97.89%	96.72%

TABLE 1c. Rf Means of Table 1

C1	N	Mean	StDev	95% CI
a	3	0.48333	0.00577	(0.46481, 0.50186)
b	3	0.38667	0.00577	(0.36814, 0.40519)
c	3	0.3400	0.0346	(0.3215, 0.3585)
d	3	0.56667	0.00577	(0.54814, 0.58519)
e	3	0.6600	0.0173	(0.6415, 0.6785)
f	3	0.39333	0.00577	(0.37481, 0.41186)
g	3	0.42333	0.01155	(0.40481, 0.44186)
h	3	0.48333	0.00577	(0.46481, 0.50186)
i	3	0.55667	0.00577	(0.53814, 0.57519)
j	3	0.6333	0.0231	(0.6148, 0.6519)

Pooled StDev = 0.0153840.

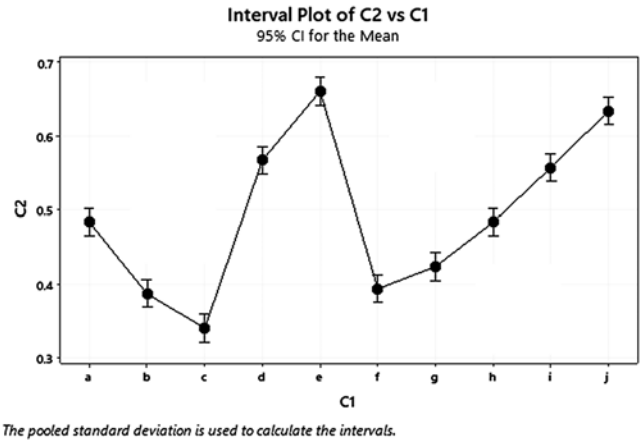

Fig. 4. Interval plot of C2 vs. C1 for Table 1.

TABLE 2. Results of Antimicrobial Activity Assay for Compounds **4a–4j**

Compound	Microorganism inhibition zone size (mm))				
	Gram positive bacteria		Gram negative bacteria		
	Sa	Sp	Pa	St	Ec
4a	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA
4c	19.6	18.5	19.7	**19.6**	20.7
4d	NA	NA	NA	NA	NA
4e	18.5	**19.3**	19.9	18.4	19.8
4f	NA	NA	NA	NA	NA
4g	NA	NA	NA	NA	NA
4h	17.8	17.7	17.6	18.1	17.3
4i	NA	NA	NA	NA	NA
4j	17.2	16.5	15.2	14.6	14.9
CF	20.1	20.5	19.4	19.6	20.4

Gram positive bacteria: *Staphylococcus aureus* (Sa), *Streptococus pneumonia* (Sp); Gram negative bacteria: *Pseudomonas aeruginosa* (Pa), *Salmonella typhimurium* (St) and *Escherichia coli* (Ec); CF: Ciprofloxacin; Comp: Compound; NA: No activity. Results represented by bold letters indicate higher antimicrobial activity of compounds as compared to others

TABLE 2a. Analysis of Variance for Inhibition Zones in Table 2

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	1.134	0.2834	0.08	0.988
Error	20	72.244	3.6122	-	-
Total	24	73.378	-	-	-

TABLE 2b. Model Summary of Table 2

S	R-sq	R-sq(adj)	R-sq(pred)
1.90058	1.54%	0.00%	0.00%

TABLE 2c. Means of Inhibition Zones in Table 2

Factor	N	Mean	StDev	95% CI
Sa	5	18.640	1.210	(16.867, 20.413)
Sp	5	18.500	1.523	(16.727, 20.273)
Pa	5	18.360	1.988	(16.587, 20.133)
St	5	18.060	2.051	(16.287, 19.833)
Ec	5	18.62	2.47	(16.85, 20.39)

Pooled StDev = 1.90058

2.3 Details of Biological Assay for Antimicrobial Activity

The agar well diffusion method [24] was used for antibacterial screening of all the synthesized compounds against gram positive and gram negative bacterial strains. With the aid of 1-mL pipette, 0.2 mL of the broth culture was added to 18 mL sterile molten diagnostic sensitivity test agar. This was mixed properly and poured into previously labelled sterilized petri dishes. Holes were digger into medium using sterile cork borer in sterile nutrient agar plates. The wells were filled up by Pasteur pipettes with the solution of compound in DMSO. The plates were allowed to stand in a cooled incubator at 4°C for 1 hour for proper diffusion of the agents into the medium followed by incubated at 37°C for tested bacteria for 24 h. Ciprofloxacin was used as antibacterial positive control. After the triplicate experiment, average zone of inhibition was calculated.

2.4 Details of Biological Assay for Antimycobacterial Activity

Microplate Alamar Blue Assay (MABA):

(i) Compounds were dissolved in DMSO (Stock con. 10 mg/ml);

(ii) Compounds were seeded in MB7H9 media (100 µl volume) enriched with ADC (10% v/v) with decreasing double dilutions starting with 50 µg/ml (for 250 µl volume) in 96 well plate;

(iii) 150 µL of culture (*Mycobacterium tuberculosis* H37Ra, 10^6 CFU/mL) was added to each well except blank (negative control);

(iv) Culture control, Blanks (media alone), Rifampin and streptomycin were taken as test control.

(v) Plate was incubated for 5 days at 37°C incubator;

(vi) On 6th day, 25 µL Resazurin (0.01% w/v, stock conc.) was added and plate was incubated further for 24 h at 37°C;

(vii) The color change from purple to pink was assessed visually and fluorescence was measured at 530 ± 25 nm and 590 ± 25 nm for excitation and emission, respectively, in Synergy Biotek plate reader. Inhibitory concentrations were calculated by plotting fluorescence values using Microsoft Excel template.

2.5 In Vitro Cytotoxicity

The most active and moderately active compounds **4c, 4e, 4h** and **4j** were assessed against human lung cancer cell line (A549) by using Sulforhodamine B assay and Doxorubicin as the reference drug. Table 5 presents the IC_{50} values of selected compounds. Tested compounds do not exhibit any significant cytotoxicity against A549 cell line, hence, providing a high therapeutic index.

3. RESULTS AND DISCUSSION

The IR spectra of target compounds (**4a–4j**) showed characteristic absorption bands in the intervals of

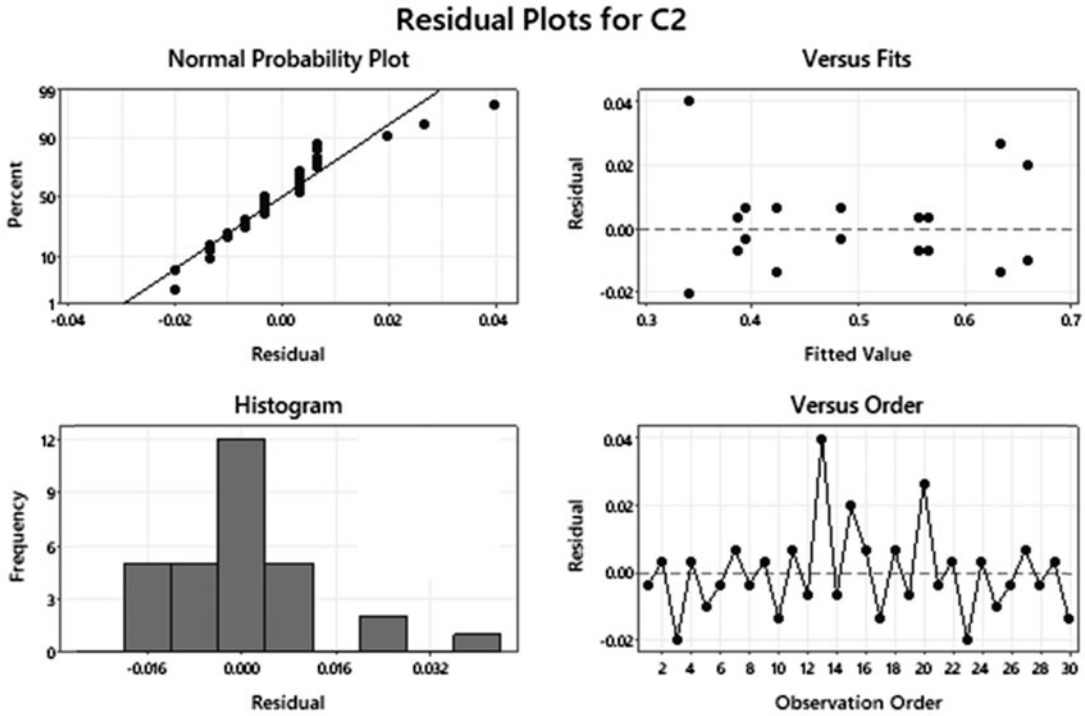

Fig. 5. Residual plots for C2.

3185 – 3310 accounting for N-H, 2992 – 3236 attributed to C-H str. aromatic, 2795 – 2905 for C-H str. aliphatic, 1640 – 1720 for C=O of cyclohexanone, 1606 – 1682 for C=O of NHCO, 1456 – 1526 for C-N str. and 620 – 712 for C-S-C linkage. The structures of compounds were further supported by ^{1}H NMR, ^{13}C NMR, mass spectroscopy data and elemental analyses.

The results of antimicrobial evaluation are presented in Table 2. Analysis of Variance of inhibition zones, Model summary and means of inhibition zones are presented in Tables 2a, 2b and 2c, respectively. Interval plot of factor Sa, Sp, Pa, St and Ec is presented in Fig. 6. Figure 7 shows residual plots of these factors.

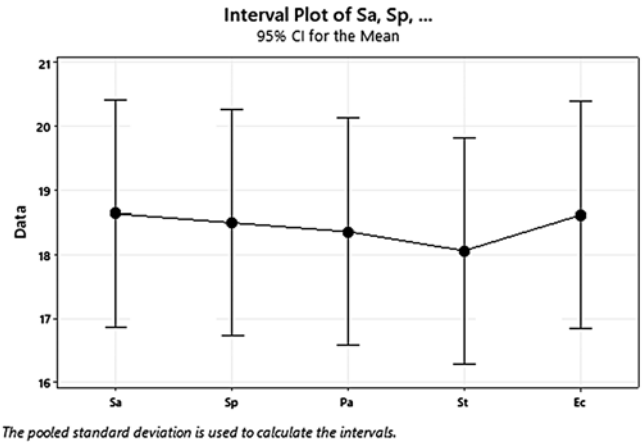

Fig. 6. Interval plot of Sa, Sp, Pa, St, and Ec.

Results of antimycobacterial evaluation as average and % survival and MIC_{90} values of compounds **4a–4j** are presented in Tables 3, 4 and 5. According to the data obtained, it is concluded that compounds **4c** and **4e** are the most active against Gram positive bacterial strains (*Staphylococcus aureus, Streptococcus pneumonia*) and Gram negative bacterial strains (*Pseudomonas aeruginosa, Salmonella typhimu-*

TABLE 3. Results of Antimycobacterial Activity as Average and % Survival of Compounds **4a–4j**

(μg/ml)	50	25	12.50	6.25
Compounds	Control 4477 (100% survival)	Rifampin(2.0) 405 (8.6%)	Streptomycin (2.5) 428 (9%)	Blank 395
4a	4201 (93.8%)	4253 (95%)	4380 (97.8%)	4231 (94.5%)
4b	4231 (94.5%)	4243 (94.7%)	4279 (95.6%)	4306 (96%)
4c	4170 (93%)	4193 (93.6%)	4197 (93.7%)	4253 (95%)
4d	4237 (94.6%)	4188 (93.5%)	4197 (93.7%)	4380 (97.8%)
4e	4135 (92.3%)	4168 (93%)	4186 (93.5%)	4256 (95%)
4f	4273 (95%)	4272 (95%)	4322 (96%)	4364 (97.5%)
4g	4192 (93.5%)	4188 (93.5%)	4201 (93.8%)	4221 (94.2%)
4h	4241 (95%)	4257 (95%)	4365 (97.5%)	4335 (96.8%)
4i	4237 (94.6%)	4284 (95.6%)	4306 (96.1%)	4343 (97%)
4j	4228 (94.4%)	4286 (95.7%)	4367 (97.5%)	4380 (97%)

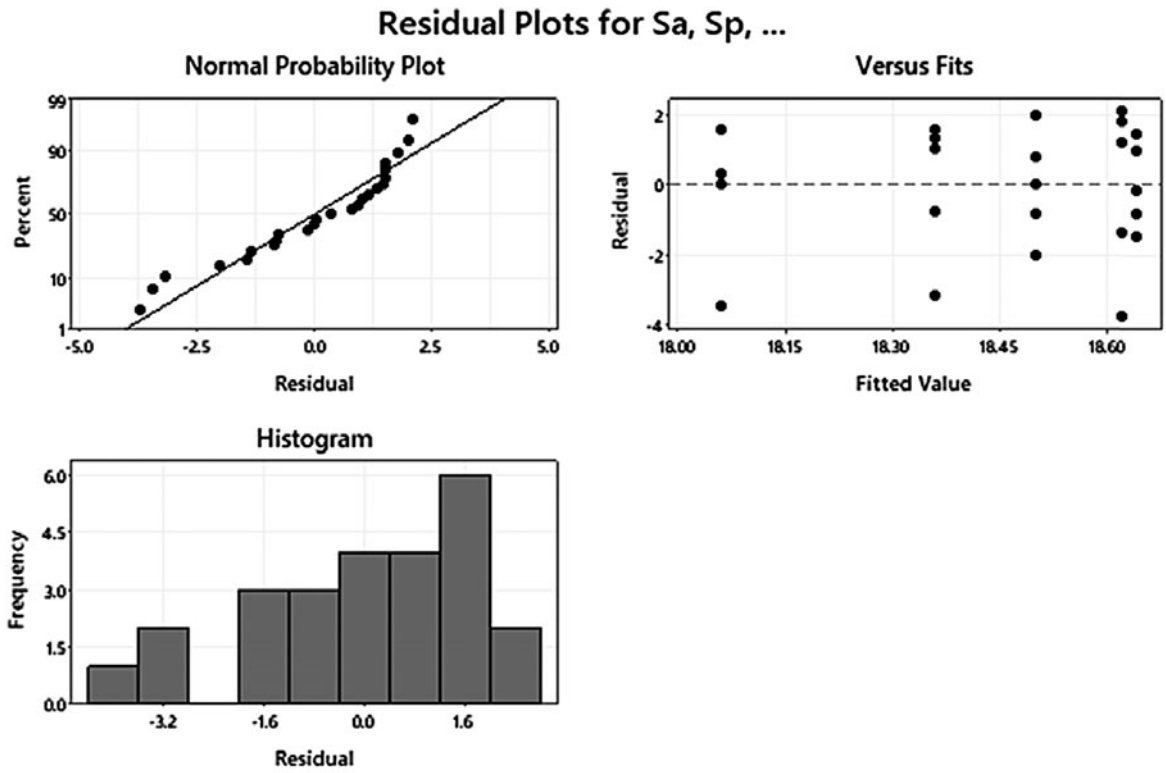

Fig. 7. Residual plots of Sa, Sp, Pa, St, and Ec.

rium and Escherichia coli). Compounds **4h** and **4j** showed only slight activity and the rest of compounds were completely inactive on the basis of comparison with reference drug Ciprofloxacin, and this series of mercaptoacetamides did not show any significant effect on mycobacterial strains.

In concluding, we brought into light the synthesis of various novel mercaptoacetamides. These were screened for antimicrobial activity against numerous Gram positive and Gram negative bacterial strains and antimycobacterial activity against H37Ra. Two analogs **4c** and **4e** (probably due to the presence of electron withdrawing groups –COOH and $–NO_2$, respectively, on the phenyl nucleus of the mercaptoacetamide concatenation) emerged as influential antimicrobial agents; on the other hand, compounds **4h** and **4j** exhibited mild to moderate potency. All analogs demonstrated total absence of antimycobacterial activity in the serial dilutions tested. We did not find even mild improvement in antimycobacterial potency as represented by MIC_{90} values and observed no activity among all analogs with the reference drugs Rifampin and Streptomycin supporting that this new chemotype can only be explored further as a good lead for subsequent optimization in search of new and advantageous antimicrobial agents.

TABLE 4. MIC_{90} Values for Anti-TB Activity of Compounds **4a–4j**

S. N.	Compound	MIC90 (μg/mL)
1	**4a**	> 50
2	**4b**	> 50
3	**4c**	> 50
4	**4d**	> 50
5	**4e**	> 50
6	**4f**	> 50
7	**4g**	> 50
8	**4h**	> 50
9	**4i**	> 50
10	**4j**	> 50

TABLE 5. *In Vitro* Cytotoxicity of Potent and Moderately Active Compounds

Compound	IC_{50} (μM)
4c	100
4e	75
4h	100
4j	100
Doxorubicin	2.4

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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