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Synthesis and evaluation of pyridine-thiophene clubbed pyrazoline hybrids as potential antimicrobial and antimycobacterial agents: Experimental and computational insights

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In light of the current scarcity of effective antimicrobial and antimycobacterial drugs, often limited by factors such as narrow spectrum, lack of oral formulations, and suboptimal pharmacokinetics, we embarked on synthesizing a series of hybrid pyridine-thiophene clubbed pyrazoline molecules (designated as **8a-j**) to enhance their potency. This has been achieved through a one-pot multicomponent reaction involving substituted benzylideneacetophenone **7a-j** and hydrazine hydrate catalyzed by CH_3COOH in ethanol at reflux temperature. Structure of all the compounds **8a-j** have been confirmed by employing elemental analysis, ESI-mass, ^{1}H NMR and FTIR which supported the suggested structures. Newly synthesized compounds have been screened for antibacterial, antifungal and antimycobacterial activities. Compounds **8a, 8d, 8f, 8g, 8h,** and **8j** have been identified as promising candidates for investigating *in vitro* antimicrobial and antimycobacterial activities in comparison to standard antibiotics. Additionally, studies on molecular docking targeting the functioning site of the KS-AT domains of Mycobacterial Pks13 enzyme has revealed binding affinities ranging from –10.5 to –9.8 kcal/mol. The docking score for the most active compound, **8i** is found to be –10.5 kcal/mol in PYRX Autodock VINA, demonstrating its favorable accommodation within the active site of the PKs enzyme.

Keywords: Pyridine-thiophene clubbed pyrazoline, Antimicrobial activities, Antitubercular activities, Molecular docking, KS-AT domains of Mycobacterial Pks13 enzyme

Suppressing immunity is one of the major threats to human life worldwide due to the rise in infectious diseases such as bacterial, fungal and tubercular infections[1]. Patients who are prone to microbial infections may have their immunity suppressed. Additionally, not only microbial infections but also a variety of factors, including immunosuppressive medications, HIV infection, cancer, aging, surgery, and other medical conditions, may suppress immunity. The situation is made worse by the increasing occurrence of resistant microbes to the great majority of currently recommended antibiotics. Public health is seriously threatened by antimicrobial resistance, leading to the need for the development of new potent antimicrobial and antitubercular drugs to combat drug resistance[2].

A drugs efficacy is influenced by a number of physico-chemical factors, such as toxicity, metabolism, excretion, distribution, absorption and the way the drug interacts with its target cell. A molecular design is necessary for the optimization of these characteristics. The most widely used chemical entities in the field of biology and medical science to generate modified scaffolds with considerably improved and increased attributes are hybrid molecules, which are prepared by combining the structural characteristics of two differentially active segments[3]. Pharmacological potency of a pharmacophore is greater when two or more components are present in a single unit than the each separate component[4]. Biological activity such as antibacterial, tubulin inhibition, antimalarial and anticancer properties are enhanced when two or more heterocyclic rings are hybridized molecularly[5-9].

Many research investigations have looked at the potential use of various sulfur and nitrogen heterocycles, like pyridine, thiophene and thiazole, in the treatment of different diseases. Thiophene-

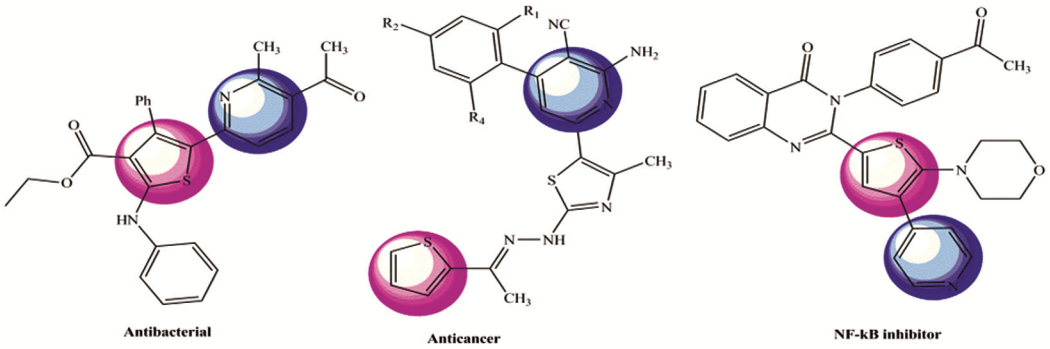


Fig. 1 — Some thiophene-pyridine hybrids used as potent antimicrobial, anticancer, NF-κB inhibitor

pyridine hybrids have been reported in numerous investigations to possess antibacterial, anticancer, and NF-κB inhibitory properties (Fig. 1)[10-12]. According to this perspective, we intended to create a few thiophene-pyridine hybrids involving pyrazoline derivatives (**8a**–**j**) and assess them for use in pharmacological research (Fig. 2).

Heterocycles are unique and extremely important chemical substances[13]. A wide range of heterocyclic compounds have been discovered, particularly N-containing heterocyclic compounds as an essential structure. Numerous natural substances, including hormones, vitamins, alkaloids, antibiotics and many more, contain N heteroatom in large quantities[14-16]. Researchers and pharmacologists have been drawing close attention to pyrazoles, which are five-membered heterocyclic compounds with two nitrogen atoms. This is because of their wide range of biological activities which include antimicrobial[17], anticancer[18], anticonvulsant[19], anti-HIV[20], neurodegenerative disorders[21], antimalarial agents[22], antitubercular[23], *etc*. Famprofazone, Dipyrone, Aminopyrine, Ramifenazone, Phenylbutazone, Celecoxib, Cefoseils are potent analgesic, antipyretic, anti-inflammatory and antibacterial medications that contain pyrazoline as core active ingredient. Fig. 3 illustrates structure of various clinically approved pyrazoline bearing therapeutic agents along with their clinical use.

It has been suggested that the pyrazoline ring N-N bond connection is crucial to their biological actions. In nature, there are fewer compounds containing N-N bonds because N-N bonds are extremely difficult for living things to form[24,25]. In order to develop new hybrid molecules, we decided to synthesize hybrid pyrazoline derivatives and explore their antimicrobial activities, antimycobacterial activities and binding potential with the protein of the *Mycobacterium tuberculosis* bacteria.

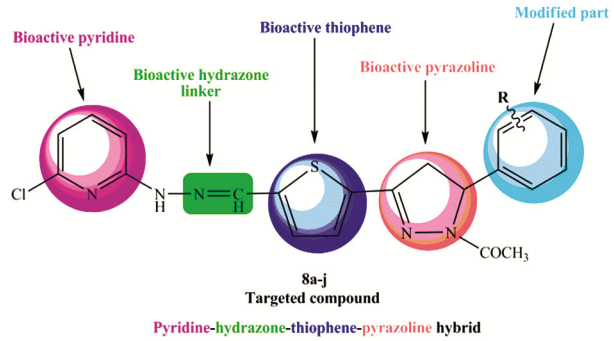


Fig. 2 — Synthesis of some thiaophene-pyridine hybrid involving pyrazoline derivatives as antimicrobial and antimycobacterial

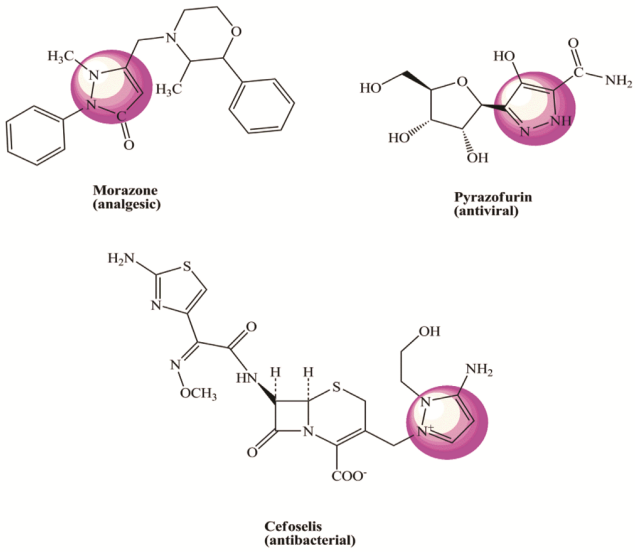


Fig. 3 — Clinically approved drugs containing pyrazoline scaffold

Experimental Section

All the reagents and solvents were of LR grade and commercially available. All the compounds are isolated and purified by recrystallization from ethanol. Elemental analysis and thin layer chromatography (TLC) were used to determine the purity of the

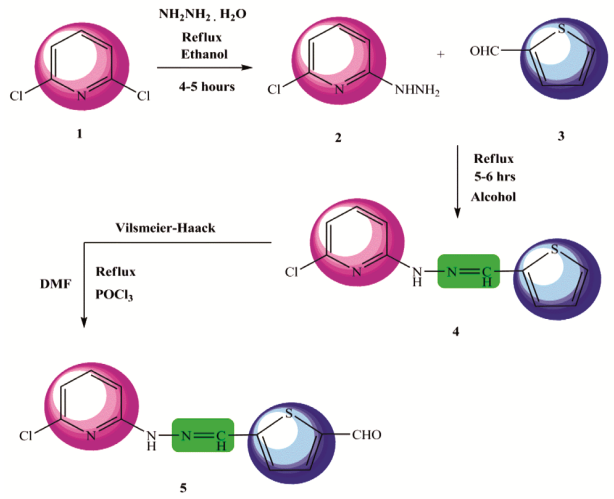
compounds. All melting points were taken on a Veego electronic apparatus VMP-D (Veego instrument corporation, Mumbai, India, digital melting point apparatus) and were uncorrected. FTIR spectra were recorded using the KBr pellet on Shimadzu 8400-S FTIR spectrophotometer using KBr pellets and values are represented in the range of 4000-400 cm^{-1}. The ^1H NMR spectra were recorded on a 500 MHz Bruker Advance in the range of 0.5-16 ppm using $CDCl_3$ solvent. Mass spectra were recorded on Maldi-TOF Synapt XS HD mass spectrometer. The splitting patterns are designated as follows: s, singlet; d, doublet and m, multiplet. The elemental analyses (C, H and N) were performed on a Perkin-Elmer 240 analyzer and the results were found to be within ± 0.4% of each examined element. The reaction progress was monitored on thin-layer chromatography (TLC) using Merck pre-coated silica gel 60 F254 aluminium sheets and visualized using a UV lamp. Antimicrobial and antimycobacterial screening were carried out at Microcare Laboratory Surat, Gujarat, India.

Synthesis of compounds 2, 4, 5 and 7a-j

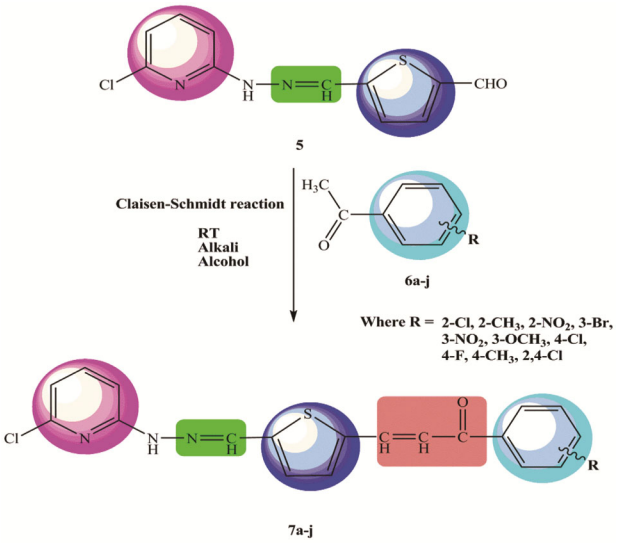
The reaction scheme for the synthesis of compounds **2**, **4**, **5** and **7a-j** are shown in Scheme 1 and Scheme 2. The starting compounds **2, 4, 5** and key intermediate substituted benzylideneace tophenone **7a-j** were prepared according to reported procedure[26-29].

General Synthesis of 1-(5-(substitutedphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono) methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8a-j

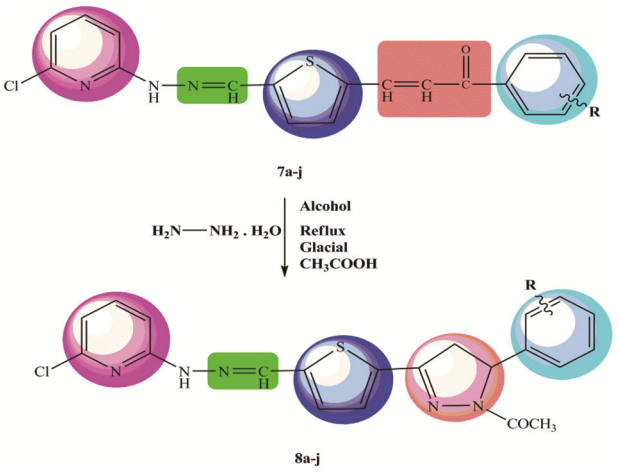
To the (0.01 mol) benzylideneacetophenone **7a-j**, 10 mL glacial acetic acid and hydrazine hydrate (0.05 mol) were added dropwise and then the reaction mixture was refluxed for 4-5 hours. The progress of the reaction was monitored by TLC in toluene and acetone (1:3) as effluent. After the completion of reaction, the warm reaction mixture was cooled, poured into ice cold water and neutralized by a 1 M NaOH solution. The precipitate was filtered off, washed with cold water and dried in desiccator over fused $CaCl_2$ and recrystallized in methanol giving pyrazoline derivatives **8a-j**. The synthetic path of pyrazoline derivatives is shown in Scheme 3. The characteristic data of the entire synthesized compounds **8a-j** are given in the physical and spectral analysis data.



Scheme 1 — Synthetic route for starting compounds **2**, **4** and **5**



Scheme 2 — Synthetic route for preparation of compounds **7a-j**



Scheme 3 — Synthetic route for preparation of compounds **8a-j**

Physical and spectral analysis data

1-(5-(2-Chlorophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8a: Yield 72%. White solid. m.p. 180-182°C. IR (KBr, cm^{-1}): 3326 (-NH str.), 2932 (-C-H str., aromatic), 2815 (-C-H str., alkane), 1719 (-C=O str., ketone), 1542 (-C=N str., pyrazoline), 1509 (-N=CH str.), 1416 (-C=C str., aromatic), 1478 (-C=N str., pyridine), 1431 (-C-N str.), 1388 ($-CH_3$ str.), 1039 (-NH-N str.), 805 (-C-Cl str.), 697 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.3 (3H, s, $-COCH_3$), 3.2 (1H, d, *J*=8.5 Hz, $-CH_X$-CH pyrazoline), 3.6 (1H, d, *J*=8.5 Hz, -CHy-CH pyrazoline), 4.4 (1H, d, -CH-Ar pyrazoline), 7.2 (1H, s, -NH-N), 7.3 - 8.1 (9H, m, Ar-H), 8.2 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}Cl_2N_2OS$: C, 55.03; N, 15.28; H, 3.74. Found: C, 54.65; N, 15.21; H, 3.70%. M.Wt. 458.36. MS: m/z 458, 441, 397, 388, 386, 380, 372, 343, 330, 304, 288, 275, 258, 139, 125, 111, 96, 73, 57, 55.

1-(5-(2-Methylphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8b: Yield 68%. White solid. m.p. 241-243°C. IR (KBr, cm^{-1}): 3389 (-NH str.), 2956 (-C-H str., aromatic), 2826 (-C-H str., alkane), 1710 (-C=O str., ketone), 1610 (-C=N str., pyrazoline), 1605 (-N=CH str.), 1545 (-C=C str., aromatic), 1490 (-C=N str., pyridine), 1412 (-C-N str.), 1325 ($-CH_3$ str.), 1012 (-NH-N str.), 820 (-C-Cl str.), 579 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 1.2 (3H, s, $-CH_3$), 2.4 (3H, s, $-COCH_3$), 3.1 (1H, d, *J*=7.7 Hz, $-CH_X$-CH pyrazoline), 3.9 (1H, d, *J*=7.6 Hz, -CHy-CH pyrazoline), 4.5 (1H, d, -CH-Ar pyrazoline), 6.7 (1H, s, -NH-N), 7.0 – 7.8 (9H, m, Ar-H), 8.4 (singlet, 1H, -N=CH). Anal. Calcd for $C_{22}H_{20}ClN_5OS$: C, 60.34; N, 15.99; H, 4.60. Found: C, 60.31; N, 15.92; H, 4.54%. M.Wt. 437.95. MS: m/z 438, 421, 394, 379, 377, 368, 366, 352, 310, 297, 283, 255, 238, 119, 117, 111, 96, 91, 57, 55.

1-(5-(2-Nitrophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, **8c**: Yield 71%. White solid. m.p. 137-139°C. IR (KBr, cm^{-1}): 3342 (-NH str.), 3091 (-C-H str., aromatic), 2993 (-C-H str., alkane), 1601 (-C=O str., ketone), 1599 (-C=N str., pyrazoline), 1543 (-N=CH str.), 1515 (-N-O str., Nitro), 1459 (-C=C str., aromatic), 1442 (-C=N str., pyridine), 1358 (-C-N str.), 1328 ($-CH_3$ str.), 1091 (-NH-N str.), 766 (-C-Cl str.), 501 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.3 (3H, s, $-COCH_3$), 4.0 (1H, d, *J*=11.05 Hz, $-CH_X$-CH pyrazoline), 4.7 (1H, d, *J*=11.15 Hz, -CHy-CH pyrazoline), 5.5 (1H, d, -CH-Ar pyrazoline), 6.6 (1H, s, -NH-N), 6.9 – 7.6 (9H, m, Ar-H), 7.7 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}ClN_6O_3S$: C, 53.79; N, 17.92; H, 3.65. Found: C, 53.75; N, 17.88; H, 3.59%. M.Wt. 468.92. MS: m/z 469, 422, 410, 396, 380, 361, 358, 314, 312, 286, 285, 271, 257, 256, 150, 123, 111, 107, 96, 55.

1-(5-(3-Bromophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, **8d**: Yield 69%. White solid. m.p. 219-221°C. IR (KBr, cm^{-1}): 3436 (-NH str.), 2925(-C-H str., aromatic), 2892 (-C-H str., alkane), 1670 (-C=O str., ketone), 1589 (-C=N str., pyrazoline), 1568 (-N=CH str.), 1489 (-C=C str., aromatic), 1435 (-C=N str., pyridine),1400 (-C-N str.), 1250 ($-CH_3$ str.), 1012 (-NH-N str.), 833 (-C-Cl str.), 752 (-C-Br str.), 588 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.6 (3H, s, $-COCH_3$), 3.2 (1H, d, *J*=15 Hz, $-CH_X$-CH pyrazoline), 4.4 (1H, d, *J*=15 Hz, -CHy-CH pyrazoline), 5.2 (1H, d, -CH-Ar pyrazoline), 6.9 (1H, s, -NH-N), 7.2 - 7.9 (9H, m, Ar-H), 8.0 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}BrClN_5OS$: C, 50.16; N, 13.93; H, 3.41. Found: C, 50.12; N, 13.87; H, 3.37%. M.Wt. 502.81. MS: m/z 502, 484, 441, 431, 424, 380, 373, 361, 346, 331, 318, 316, 301, 277, 250, 183, 168, 111, 96, 57, 55.

1-(5-(3-Nitrophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8e: Yield 73%. White solid. m.p. 185-187°C. IR (KBr, cm^{-1}): 3389 (-NH str.), 2971 (-C-H str., aromatic), 2903 (-C-H str., alkane), 1689 (-C=O str., ketone), 1625 (-C=N str., pyrazoline), 1609 (-N=CH str.), 1583 (-C=C str., aromatic), 1535 (-N-O str., Nitro), 1506 (-C=N str., pyridine), 1416 (-C-N str.), 1339 ($-CH_3$ str.), 1010 (-NH-N str.), 802 (-C-Cl str.), 600 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.4 (3H, s, $-COCH_3$), 4.3 (1H, d, *J*=12 Hz, $-CH_X$-CH pyrazoline), 4.5 (1H, d, *J*=12 Hz, -CHy-CH pyrazoline), 5.9 (1H, d, -CH-Ar pyrazoline), 6.4 (1H, s, -NH-N), 7.0 - 7.9 (9H, m, Ar-H), 8.3 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}ClN_6O_3S$: C, 53.79; N, 17.92; H, 3.65. Found: C, 53.73; N, 17.86; H, 3.65%. M.Wt. 468.92. MS: m/z 468, 422, 410, 407, 398, 396, 380, 378, 361,

350, 341, 314, 312, 304, 299, 286, 285, 257, 150, 130, 111, 96, 73, 57, 55.

1-(5-(3-Methoxyphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8f: Yield 65%. White solid. m.p. 157-159°C. IR (KBr, cm^{-1}): 3400 (-NH str.), 3054 (-C-H str., aromatic), 2855 (-C-H str., alkane), 1654 (-C=O str., ketone), 1515 (-C=N str., pyrazoline), 1445 (-N=CH str.), 1370 (-C=C str., aromatic), 1323 (-C=N str., pyridine), 1256(-CH_3 str.), 1132 (-C-O-C- str., ether linkage), 1047 (-NH-N str.), 772 (-C-Cl str.), 629 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.4 (3H, s, -$COCH_3$), 3.5 (1H, d, *J*= 9.4 Hz, -CH_X-CH pyrazoline), 3.9 (3H, s, -OCH_3), 4.5 (1H, d, *J*=9.4 Hz, -CHy-CH pyrazoline), 5.3 (1H, d, -CH-Ar pyrazoline), 6.7 (1H, s, -NH-N), 7.0 – 8.0 (9H, m, Ar-H), 8.1 (singlet, 1H, -N=CH). Anal. Calcd for $C_{22}H_{20}ClN_5O_2S$: C, 58.21; N, 15.43; H, 4.44. Found: C, 58.16; N, 15.37; H, 4.38%. M.Wt. 453.94. MS: m/z 454, 410, 396, 380, 376, 368, 304, 299, 277, 271, 250, 138, 135, 129, 123, 114, 93, 91, 65, 57, 55.

1-(5-(4-Chlorophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8g: Yield 69%. White solid. m.p. 160-162°C. IR (KBr, cm^{-1}): 3280 (-NH str.), 2921 (-C-H str., aromatic), 2863 (-C-H str., alkane), 1720 (-C=O str., ketone), 1669 (-C=N str., pyrazoline), 1623 (-N=CH str.), 1552 (-C=C str., aromatic), 1523 (-C=N str., pyridine), 1489 (-C-N str.), 1322 (-CH_3 str.), 1025 (-NH-N str.), 810 (-C-Cl str.), 556 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.8 (3H, s, -$COCH_3$), 3.9 (1H, d, *J*=11.0 Hz, -CH_X-CH pyrazoline), 4.3 (1H, d, *J*=11.5 Hz, -CHy-CH pyrazoline), 5.3 (1H, d, -CH-Ar pyrazoline), 6.3 (1H, s, -NH-N), 7.2 - 8.0 (9H, m, Ar-H), 8.3 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}Cl_2N_5OS$: C, 55.03; N, 15.28; H, 3.74. Found: C, 54.98; N, 15.23; H, 3.69%. M.Wt. 458.36. MS: m/z 458, 441, 399, 397, 388, 384, 380, 355, 350, 330, 317, 303, 288, 275, 273, 260, 258, 239, 125, 111, 96, 73, 57, 55.

1-(5-(4-Fluorophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8h: Yield 64%. White solid. m.p. 183-185°C. IR (KBr, cm^{-1}): 3421 (-NH str.), 2923 (-C-H str., aromatic), 2855 (-C-H str., alkane), 1676 (-C=O str., ketone), 1512 (-C=N str., pyrazoline), 1508 (-N=CH str.), 1475 (-C=C str., aromatic), 1439 (-C=N str., pyridine), 1414 (-C-N str.), 1357 (-CH_3 str.), 1177 (-NH-N str.), 887 (-C-Cl str.), 744 (-C-F str.), 590 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.8 (3H, s, -$COCH_3$), 3.2 (1H, d, *J*=9 Hz, -CH_X-CH pyrazoline), 3.7 (1H, d, *J*=9 Hz, -CHy-CH pyrazoline), 5.0 (1H, d, -CH-Ar pyrazoline), 6.7 (1H, s, -NH-N), 7.0 - 8.0 (9H, m, Ar-H), 8.1 (singlet, 1H, -N=CH). Anal. Calcd for $C_{21}H_{17}ClFN_5OS$: C, 57.08; N, 15.85; H, 3.68. Found: C, 57.05; N, 15.81; H, 3.61%. M.Wt. 441.91. MS: m/z 442, 425, 424, 383, 372, 364, 339, 314, 301, 287, 272, 259, 244, 242, 129, 123, 111, 96, 73, 57, 55.

1-(5-(4-Methylphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8i: Yield 74%. White solid. m.p. 147-149°C. IR (KBr, cm^{-1}): 3402 (-NH str.), 2962 (-C-H str., aromatic), 2855 (-C-H str., alkane), 1670 (-C=O str., ketone), 1582 (-C=N str., pyrazoline), 1513 (-N=CH str.), 1508 (-C=C str., aromatic), 1493 (-C=N str., pyridine), 1439 (-C-N str.), 1359 (-CH_3 str.), 1068 (-NH-N str.), 805 (-C-Cl str.), 630 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 1.2 (3H, s, -CH_3), 2.3 (3H, s, -$COCH_3$), 3.1 (1H, d, *J*=7.7 Hz, -CH_X-CH pyrazoline), 4.0 (1H, d, *J*=7.6 Hz, -CHy-CH pyrazoline), 5.1 (1H, d, -CH-Ar pyrazoline), 6.6 (1H, s, -NH-N), 6.7 – 7.6 (9H, m, Ar-H), 7.8 (singlet, 1H, -N=CH). Anal. Calcd for $C_{22}H_{20}ClN_5OS$: C, 60.34; N, 15.99; H, 4.60. Found: C, 60.28; N, 15.94; H, 4.55%. M.Wt. 437.95. MS: m/z 438, 421, 412, 379, 366, 360, 335, 334, 310, 297, 283, 268, 255, 240, 238, 114, 111, 91, 73, 57, 55.

1-(5-(2,4-Dimethoxyphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone, 8j: Yield 65%. White solid. m.p. 151-153°C. IR (KBr, cm^{-1}): 3401 (-NH str.), 2958 (-C-H str., aromatic), 2853 (-C-H str., alkane), 1674 (-C=O str., ketone), 1572 (-C=N str., pyrazoline), 1531 (-N=CH str.), 1507 (-C=C str., aromatic), 1439 (-C=N str., pyridine), 1407 (-C-N str.), 1380 (-CH_3 str.), 1360 (-OCH_3 str.),1232 (-C-O-C- str., ether linkage), 1068 (-NH-N str.), 835 (-C-Cl str.), 543 (-C-S-C- str.). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.4 (3H, s, -$COCH_3$), 3.1 (1H, d, *J*=10 Hz, -CHx-CH pyrazoline), 3.3 (1H, d, *J*= 10 Hz, -CHy-CH pyrazoline), 3.7 and 3.9 (2×3H, s, -OCH_3), 5.1 (1H, d, -CH-Ar pyrazoline), 6.6 (1H, s, -NH-N), 6.8 -7.9 (8H, m, Ar-H), 8.1 (singlet, 1H, -N=CH). Anal. Calcd for

$C_{23}H_{22}ClN_5O_3S$ (Mol. Wt.483.11): C, 57.08; N, 14.47; H, 4.58. Found: C, 57.03; N, 14.42; H, 4.55%. M.Wt. 483.11. MS: m/z 484, 482, 456, 432, 430, 422, 419, 414, 364, 351, 337, 321, 309, 291, 288, 137, 111, 96, 73, 57, 55.

Antibacterial and antifungal activity

Broth microdilution method by Rattan was used to determine the minimum inhibitory concentrations (MICs) of the synthesized compounds[30]. Antibacterial activities were screened against two Gram +ve (*S. aureus* MTCC 96 and *S. pyogenes* MTCC 443) and two Gram -ve (*E. coli* MTCC 442 and *P. aeruginosa* MTCC 2488) microorganisms. Antifungal activities were screened against three fungal species (*C. albican* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323). The MTCC culture was obtained from CSIR-Institute of Microbial Technology, Chandigarh and examined against the previously listed prescription medications. To get the appropriate drug concentration for testing on common bacterial strains, DMSO was used as a diluent. Serial dilutions were made for both primary and secondary screening. Before being injected, the antibiotic-free control tube was quickly subcultured by uniformly distributing a loopful of medium over a fourth of a plate that was acceptable for the test organisms growth. The plate was then incubated for the entire night at 37°C. To make sure the drug concentrations were accurate, the MIC of the control organisms were assessed. The MIC was defined as the lowest concentration that prevented the organism's development. In the same way as the control tube mentioned above, all of the tubes that did not exhibit any apparent growth were subcultured and incubated for the whole night at 37°C. A comparison was made between the amount of growth from the control tube (which represents the initial inoculum) prior to incubation. Similar number of colonies in subculture suggest bacteriostatic activity; fewer colonies suggest partial or sluggish bactericidal action; and no colonies indicate no growth if the entire inoculum has been destroyed. An organism of known sensitivity must be injected into a second set of the same dilutions for the test to be valid. As a stock solution, each synthetic compound was diluted to a concentration of 2000 µg/mL. 500, 250, and 125 µg/mL quantities of the synthesized compounds were used in the initial screening. The active synthetic compounds discovered in this first screening were examined in a second series of dilution test against every type of microbes. The drug that identified as effective in the first screening were diluted in a similar way to yield concentrations of 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 µg/mL. The maximum dilution that exhibits at least 99% inhibition is designated as the minimum inhibitory concentration or MIC. Table 1 displays the antimicrobial screening data.

Antimycobacterial activity

The preliminary antimycobacterial screening of test compounds were performed against *M. tuberculosis* $H_{37}Rv$ mycobacterial strain using the L. J. (conventional Lowenstein and Jensen) agar dilution method for the measurement of MIC[31]. MIC is defined as the lowest drug concentration that inhibits ≥ 99% of

Table 1 — Antimicrobial activity of the compounds

Compd	*S. a.* MTCC-96	*S. p.* MTCC-442	*E. c.* MTCC-443	*P. a* MTCC-1688	*C.a.* MTCC-227	*A.n.* MTCC-282	*A.c.* MTCC-1323
8a	250	200	100	125	250	500	500
8b	200	100	250	125	>1000	200	>1000
8c	250	200	125	200	500	>1000	>1000
8d	50	125	100	250	100	500	>1000
8e	250	200	250	200	500	500	500
8f	125	100	250	200	500	500	500
8g	100	200	62.5	100	250	500	>1000
8h	250	200	125	50	>1000	500	>1000
8i	200	100	250	200	500	>1000	500
8j	100	125	100	100	500	500	500
Ampicillin	250	100	100	100	–	–	–
Chloramphenicol	50	50	50	50	–	–	–
Ciprofloxacin	50	50	25	25	–	–	–
Greseofulvin	–	–	–	–	500	100	100
Nystatin	–	–	–	–	100	100	100

Key to symbols: – means not tested

the bacterial population present at the beginning of the assay. The liquid L. J. Medium was filled with stock solution of each test substance at dilution of 100, 62.5,50, 25, 12.5, 6.25, 3.12 and 1.56 µg/mL in DMSO. The media were then sterilized using the inspissation procedure. A bijou bottle with 0.85% saline was used to harvest a culture of *M. tuberculosis* $H_{37}Rv$ that was grown on L. J. Medium. After a 24-hour incubation period at 37°C, *M. tuberculosis* $H_{37}Rv$ (5×10^4 bacilli per tube) was streaked into these tubes. Following that, these tubes were incubated at 37°C. Bacilli began to grow after 12 days, 22 days and 28 days of incubation. The compound containing tubes were compared to control tubes that were cultured with *M. tuberculosis* $H_{37}Rv$ using medium alone. MIC of the test substance was determined as the concentration at which <20 colonies or no colony development occurred. The well-known drugs Isoniazid and Rifampicin were used against the reference strain of *M. tuberculosis* $H_{37}Rv$.

Results and Discussion

Spectral discussion of synthesized derivatives

The synthetic route to the target compounds **8a-j** is outlined in Scheme 3. The intermediated compounds **2**, **4**, **5** and **7a-j** synthetic route are shown in Schemes 1 and 2. All the compounds are isolated in satisfactory yields that ranged from 35% to 91% and purified by recrystallization from ethanol. The structures of the newly produced compounds were confirmed by elemental analysis and spectrum data, such as FTIR, ^{1}H NMR and mass. The results aligned with the suggested structures. The FTIR spectrum of compounds **8a-j** observed the presence of pyrazoline -C=N stretching (1680-1500 cm^{-1}), carbonyl functionality -C=O stretching (1720-1600 cm^{-1}) and methyl functionality -C-H stretching (3000-2800 cm^{-1}) which confirmed the cyclisation of **7a-j** into **8a-j**. The additional functionality such as secondary amine -NH-N stretching (3440-3230 cm^{-1}), imine -N=CH stretching (1610-1440 cm^{-1}), pyridine -C=N stretching (1530-1320 cm^{-1}), thiophene -C-S-C- linkage (700-500 cm^{-1}) appeared in FTIR of **8a-j** indicate the successful molecular hybridization. ^{1}H NMR spectrum of **8a-j**, pair of doublets of doublets of CH_x, and CH_y observed at 3.1-34.3 ppm (H_x) and 3.3-4.7 ppm (H_y) which indicate two magnetically non-equivalent protons of the methylene group are present at position 4 of the pyrazoline ring. Additionally -N-H group associated with the pyridine ring proton and imine group proton are observed at δ 6.3 -7.2 ppm and δ 7.7 -8.4 ppm values. The pyridine ring and thiophene ring protons are observed in the range of δ 6.0-8.0 ppm. These indicate the incorporation of multi component species in a single hybrid molecule. Protons from the other group and the aromatic ring are observed to have the anticipated integral values and chemical shift. The mass spectra of pyrazolines showed reasonable instances of molecular ion peaks (M^+). The elemental analyses (C, H and N) were all within ±0.5% of the theoretical values.

Anti microbial activity

The synthetic pyrazoline derivatives were evaluated for their *in vitro* antimicrobial activity, including their antibacterial, antifungal, and antitubercular properties. Any minor alteration in the effectiveness of an antibiotic molecule is frequently utilized to show its therapeutic efficiency through the inhibition of bacteria growth.

Antibacterial and antifungal evaluation

Antibacterial activity analysis reveals that, compound **8d** exhibited strongest action against *Staphylococcus aureus* (MIC-50 µg/mL) as compared to standard drug Ampicillin (MIC-250 µg/mL). Analogs **8b, 8f, 8g, 8i** and **8j** were exerted to be good to moderate active against *Staphylococcus aureus* with MIC values in the range of 100-200 µg/mL. In contrast to Ampicillin and Chloramphenicol, the studied analogs **8b**, **8f** and **8i** (MIC 100 µg/mL) showed comparable potency against *Streptococcus pyogenes*. Comparing **8g** (MIC 62.5 µg/mL) to Ciprofloxacin and Chloramphenicol, it was discovered as the most effective analog against *Escherichia coli* among the studied analogs. The compounds **8a**, **8d** and **8j** demonstrated outstanding action against *Escherichia coli* with MIC 100 µg/mL to those of the standard drugs. Against *Pseudomonas aeruginosa*, compound **8h** (MIC 50 µg/mL) was found to possess excellent activity against Ampicillin and moderate against Chloramphenicol. All other derivatives exhibited moderate to weak antibacterial activity. The remaining substances showed moderate to considerable efficacy against all bacterial species (Table 1).

Based on the antifungal activity data of Table 1, compounds **8a**, **8d** and **8g** demonstrated the strongest antifungal activity against *Candida albican* among the screened compounds with minimum inhibitory concentrations (MICs) of 250, 100 and 250 µg/mL respectively. This activity was found to be superior to that of the standard drug Greseofulvin (MIC-500 µg/mL). Additionally, compounds **8c, 8e, 8i**

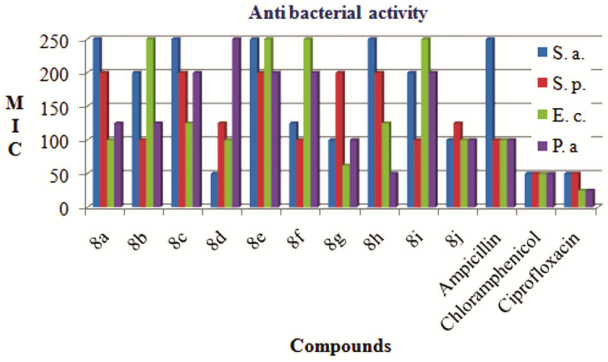


Fig. 4 — Graphical representation of antibacterial activity

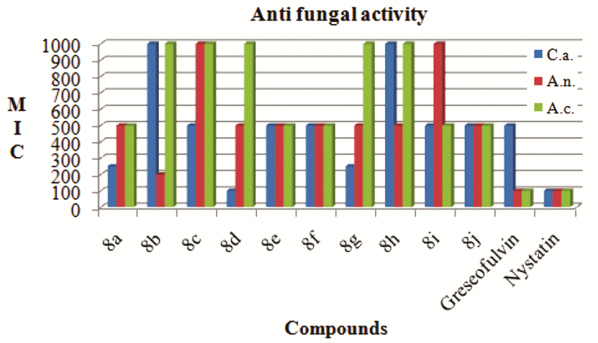


Fig. 5 — Graphical representation of antifungal activity

and **8j** found to be equipotent to Greseofulvin with MIC-500 µg/mL. Compound **8b** (MIC-200 µg/mL) was reported to have mild antifungal efficacy against *Aspergillus niger* compared to the Greseofulvin and Nystatin (MIC-100µg/mL). Rest of the compounds exhibited low to moderate activity against all the fungal strains. The graphical representations of antibacterial and antifungal activities are shown in Fig. 4 and Fig. 5.

Antimycobacterial activity

Based on the promising outcomes of the antimicrobial result, we planned to screen title compounds for mycobacterial tuberculosis screening. Comparing the synthesized compounds to the standard drugs Isoniazid and Rifampicin, *in vitro* antimycobacterial screening revealed that a few compounds exhibit good to exceptional antimycobacterial activity. The results are summarized in Table 2. The results show that the compounds possessed moderate to modest antimycobacterial activity, with the exception of compounds **8b, 8d, 8f, 8h** and **8j** which demonstrated good activity (MIC value 25-50 µg/mL) against *M. tuberculosis* H_{37}RV whereas compounds **8a, 8c** and **8g** showed comparable activity (MIC value 62.5-100 µg/mL). On the other hand, the remaining compounds showed modest to moderate activity (MIC value 125-1000 µg/mL). At a minimum

Table 2 — Antimycobacterial activity of the compounds

Compd	MIC values (µg/mL) of *M. tuberculosis* H_{37}Rv	Inhibition (%)
8a	62.5	96
8b	25	91
8c	100	82
8d	12.5	98
8e	–	76
8f	50	95
8g	62.5	92
8h	25	97
8i	–	69
8j	50	80
Isoniazid	0.20	99
Rifampicin	40.0	98

Key to symbols: – means not tested

inhibitory concentration (MIC) of 250 µg/mL, compounds **8a, 8b, 8c, 8d, 8f, 8g, 8h and 8j** were found to exhibit the highest percentage of inhibition (80 to 99%) respectively. Among all the compounds in the series, **8d** (MIC = 12.5 µg/mL) showed excellent potency against *M. tuberculosis*, showing 98% inhibition as compared to Rifampicin (MIC = 40 µg/mL). Meanwhile, the remaining derivatives showed moderate to good antimycobacterial activity. It is encouraging to observe that compounds **8a**, **8b**, **8d, 8f, 8g, 8h** and **8j** may be enhanced further to improve their potency.

Out of these synthesized compounds, three pyridine-thiophene clubbed pyrazoline hybrids,1-(5-(2-methylphenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone **8b**,1-(5-(3-bromophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone **8d** and 1-(5-(4-fluorophenyl)-3-(5-((2-(6-chloropyridin-2-yl)hydrazono)methyl)thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone **8h** emerged as lead compounds possessing a broad spectrum of antimicrobial and antimycobacterial actions.

Molecular docking analysis

The examination of heterocyclic hybrid compounds **8a-j** and their interactions with KS-AT domains of mycobacterial Pks Protein (PDB id: 8cuz) are done by Molecular Docking analysis using the Autodock Vina PyRx tool[32]. Our data indicates that among the ten compounds studied, three compounds exhibited higher favourable binding energies as shown in Table 3. The calculated binding energies ranged from 10.5 to –9.8 kcal/mol for compounds **8i, 8g** and **8c** respectively.

The KS-AT (Ketosynthase-Acyltransferase) domains are crucial components of the mycobacterial

Table 3 — Docking result of KS-AT domains of mycobacterial Pks 13 with pyridine-thiophene clubbed pyrazoline hybrids having optimal binding energy and H-bond interaction

Compd	Binding Energy (kcal/mol)	H-bond interaction	Distance Å
8a	–9.6	ASN A:659	2.16
		THR A:1061	2.36
		ARG A:207	2.64
		ARG B:367	2.57
8b	–9.1	SER A:204	2.60
8c	–9.8	THR A:1061	2.26
		ASN A:659	2.04,1.81, 2.79
		ARG B:367	3.08, 4.08
		ARG A:207	2.98, 2.86
8d	–8.5	ARG : 1062	3.00
8e	–9.5	ASN B:113	2.92
		ARG B:1065	2.70
8f	–9.1	CYS A:267	3.77
		PHE A:508	3.31
		THR A:405	2.48
8g	–10.2	ASN B:659	2.70
8h	–8.9	ARG A:1.65	2.92
8i	–10.5	ASN B:659	2.64
8j	–9.2	PHE A:508	3.39, 4.42

Pks13 enzyme which is involved in the biosynthesis of mycolic acids, essential components of the mycobacterial cell wall. Mycolic acids play a significant role in the pathogenicity and virulence of *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The biosynthesis of mycolic acids, critical components of the mycobacterial cell wall, involves a complex enzymatic pathway. Among the enzymes involved, Pks13 stands out as a multifunctional megasynthase responsible for the elongation of fatty acids to form the meromycolic chains. Pks13 comprises several domains, including the Ketosynthase (KS) and Acyltransferase (AT) domains which are essential for catalyzing the key steps in the biosynthetic pathway. Understanding the structure and function of this domain is crucial for elucidating the mechanism of mycolic acid biosynthesis and for the development of novel therapeutic interventions against tuberculosis. The 3D and 2D interaction between pyridine-thiophene Clubbed Pyrazoline Hybrids and KS-AT domains of Mycobacterial PKs 13 are shown in Fig. 6 and Fig. 7.

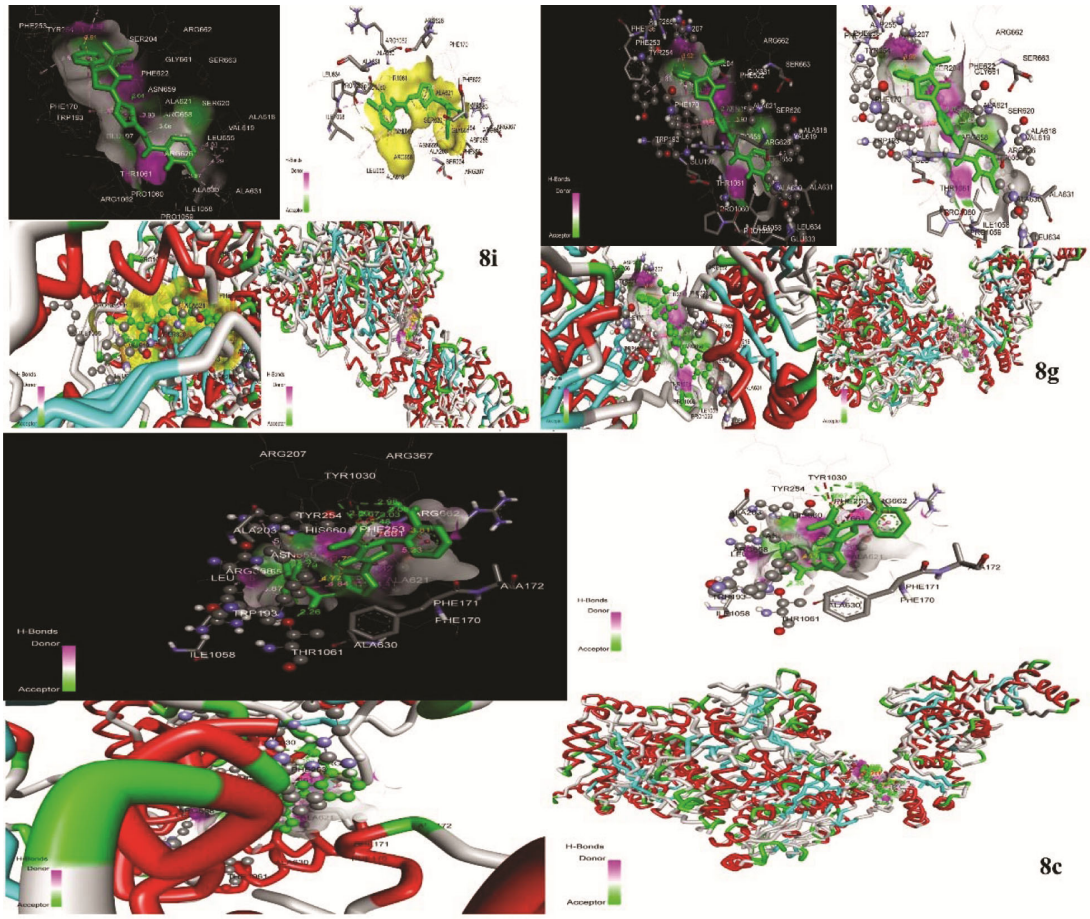


Fig. 6 — 3D interaction between pyridine-thiophene clubbed pyrazoline hybrid and KS-AT domains of mycobacterial PKs 13

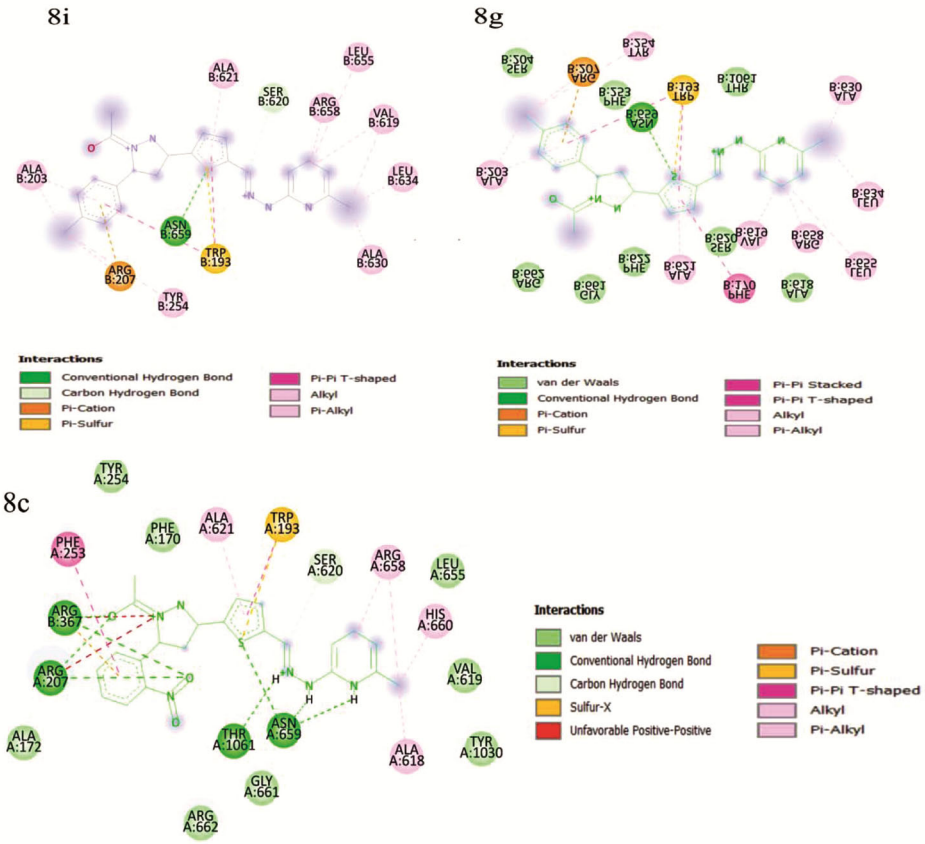


Fig. 7 — 2D interaction between pyridine-thiophene clubbed pyrazoline hybrid and KS-AT domains of mycobacterial PKs 13

Conclusion

Ten new compounds from the class of pyridine-thiophene clubbed pyrazoline hybrids were synthesized and characterized using elemental analytical investigations and spectrum data (IR, 1H NMR, and mass). The antibacterial, antifungal and antitubercular properties of the title compounds were also evaluated against a variety of selected microorganisms. The development of several antimicrobial and antitubercular medicines incorporating pyrazoline and their derivatives were extensively studied. The antibacterial, antifungal and antitubercular properties of the pyrazoline derivatives **8a, 8d, 8f, 8g, 8h,** and **8j** with fluoro, methoxy, bromo and chloro substituents demonstrated good activity according to the analysis of data. A wide range of activities were exerted by all pyridine-thiophene clubbed pyrazoline derivatives; Nevertheless, it was important to highlight that three of these derivatives **8b, 8d** and **8h** displayed even greater antibacterial and antitubercular action than the standard medications. Additionally, studies on molecular docking targeting the functioning site of the KS-AT domains of Mycobacterial Pks13 enzyme revealed well-clustered solutions for the binding modalities of these compounds with binding affinities ranging from –10.5 to –9.8 kcal/mol. The docking score for the most active compound, **8i** was found to be –10.5 kcal/mol in PYRX Autodock VINA, demonstrating its favorable accommodation within the active site of the PKs enzyme, and engagement in a network of bonded and non-bonded interactions. This comprehensive approach sheds light on the potential of these compounds as promising antimicrobial and antimycobacterial agents supported by both experimental and computational evidences. The incorporation of hybrid pyrazoline moieties into the scaffolds has facilitated the development of enhanced antimicrobial and antitubercular efficacy for pharmaceutical chemists. This suggests that they could potentially yield valuable lead molecules for the control of antimicrobial and antitubercular diseases.

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Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/ 1234567 89/58776.

References

1 Danishuddin M, Kaushal L, Hassan B M & Khan A U, *Gen Prot Bioinfo*, 10 (2012) 360.
2 Tuite N L & Lacey K, *Meth Mol Biol*, 968 (2013) 1.
3 Mansour B, El-Sherbeny M A, Al-Omary F A M, Saber S, Ramadan H A, El-Baz A M, Mourad A A E & Abdel-Aziz N I, *ACS Omega,* 8 (2023) 44250.
4 Abbot V, Sharma P, Dhiman S, Noolvi M N, Patel H M & Bhardwaj V, *RSC Adv,* 7 (2017) 28313.
5 Patel A B, Patel P, Patel K & Prajapati K, *AJOMC*, 5 (2020) 227.
6 Salem M E, Abdullah A H, Ibrahim N S, Zaki M E A, Elwahy A H M & Abdelhamid I A, *ACS Omega*, 8 (2023) 44312.
7 Singh H, Singh J V, Gupta M K, Saxena A K, Sharma S, Nepali K & Bedi P M S, *Bioorg Med Chem Lett*, 27 (2017) 3974.
8 Solankee A, Tailor R, *Chem Int*, 2 (2016) 189.
9 Kekeçmuhammed H, Tapera M, Tüzün B, Akkoç S, Zorlu Y & Saripinar E, *Chem Sel*, 7 (2022) e202201502.
10 Mabkhot Y N, Alatibi F, El-Sayed N N E, Kheder N A & Al-Showiman S S, *Molecules,* 21 (2016) 1036.
11 Ashmawy F O, Gomha S M, Abdallah M A, Zaki M E A, Al-Hussain S A & El-Desouky M A, *Molecules*, 28 (2023) 4270.
12 Vasu K K, Digwal C S, Pandya A N, Pandya D H, Sharma J A, Patel S & Agarwal M, *Bioorg Med Chem Lett,* 27 (2017) 5463.
13 Kalaria P N, Karad S C & Raval D K, *Eur J Med Chem*, 158 (2018) 917.
14 Pai G & Chattopadhyay A P, *Tetrahedron Lett*, 57 (2016) 3140.
15 Srivastava M, Singh J, Singh S B, Tiwari K, Pathak K V & Singh J, *Green Chem,* 14 (2012) 901.
16 Zarate-Zarate D, Aguilar R, Hernandez-Benitez R I, Labarrios E M, Delgado F & Tamariz J, *Tetrahedron*, 71 (2015) 6961.
17 Solankee A, Tailor R & Kapadia K, *Indian J Chem,* 55B (2016) 1277.
18 Rana M, Arif R, Khan F I, Maurya V, Singh R, Faizan Md I, Yasmeen S, Dar S H, Alam R, Sahu A, Ahmad T & Rahisuddin, *Bioorg Chem*, 108 (2021) 104665.
19 Beyhan N, Kocyigit-Kaymakcioglu B, Gumru S & Feyza A, *Arab J Chem,* 10 (2017) S2073.
20 Kumar S, Gupta S, Rani V & Sharma P, *Med Chem,* 18 (2022) 831.
21 Ahsan M J, Ali A, Ali A, Thiriveedhi A, Bakht M A, Yusuf M, Salahuddin, Afzal O & Altamimi A S A, *ACS Omega*, 7 (2022) 38207.
22 Ravindar L, Hasbullah S A, Rakesh K P & Hassan N I, *Eur J Pharm Sci*, 183 (2023) 106365.
23 Hazra K & Nandha B, *Indian J Chem*, 59B (2020) 1388.
24 Abunada N M, Hassaneen H M, Kandile N G & Miqdad O A, *Molecules,* 13 (2008) 1011.
25 Shaaban M R, Mayhoub A S & Farag A M, *Expert Opin Ther Pat*, 22 (2012) 253.
26 Dong W L, Xu J Y, Xiong L X & Li Z M, *Molecules*, 17 (2012) 10414.
27 Yousif E, Majeed A & Sammarrae K, *Arab J Chem*, 10 (2013) 1639.
28 Malose J. Mphahlele & Mmonwa M M, *Org Biomol Chem*, 17 (2019) 2204.
29 Solankee A & Tailor R, *Chem Sci Trans*, 4 (2015) 1057.
30 Rattan A, *Antimicrobials in laboratory medicine*, (Churchill Livingstone, New Delhi) (2000) p.85.
31 Anargyros P, Astill D J S & Lim I S L*, J Clin Microbiol,* 28 (1990) 1288.
32 Kim S K, Dickinson M S, Finer-Moore J, Guan Z, Kaake R M, Echeverria I, Chen J, Pulido E H, Sali A, Krogan N J, Rosenberg O S & Stroud R M, *Nat Struct Mol Biol*, 30 (2023) 296.