Synthesis of focused library of novel aryloxyacids and pyrazoline derivatives: Molecular docking studies and antimicrobial investigation

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Abstract: Infectious diseases are on the rise due to development of multidrug-resistant strains, and this renders the search for newer antimicrobials. Hybrid compounds of different scaffolds are expected to enhance the bioactivity by improved affinity to target proteins while retaining the biological efficacy of each of the components. In view of this, a series of pyrazolines with aryloxy acid in the side chain are synthesized and evaluated for their antimicrobial potential. Pyrazoline-substituted aryloxy acids were synthesized in very good yields, starting from chalcones. Synthetic method is adopted in such a way that the use of any solvents is avoided. The structures of these compounds were confirmed using FTIR, NMR, and Mass spectrometry. The potential of these molecules as antimicrobial agents was predicted using molecular docking studies. The activities were also assessed using zone of inhibition and minimum inhibitory concentration (MIC) measurement against tuberculosis variant bacteria, *Mycobacterium smegmatis*; Gram-positive pathogen, *Staphylococcus aureus*; Gram-negative *Escherichia coli*; and fungi, *Candida albicans*. The positional isomers with the electron-withdrawing group farthest from the acid function showed the best activity in both chalcone acids as well as pyrazoline acids. Many of the compounds exhibited zones of inhibition comparable with the standard drugs, ciprofloxacin and fluconazole, considered for the study. Although many compounds exhibited significant zones of inhibition, their minimum
inhibitory concentrations established by broth assay were higher, suggesting these molecules are not potent at lower concentrations.

Subjects: Chemistry; Medicinal & Pharmaceutical Chemistry; Organic Chemistry

Keywords: pyrazoline; aryloxyacetic acid; chalcone; molecular docking; antimicrobial

1. Background

There is an increasing demand for antimicrobial agents with novel structural characteristics, owing to the emergence of newer microbes with enhanced resistance (Tunçbilek, Kiper, & Altanlar, 2009). It was reported that mortality rate of infectious diseases across the globe is higher than mortality rate due to cancer (Armstrong, Conn, & Pinner, 1999). It was also reported that infectious diseases increase risk factor for developing cancer and at least 25% of such malignancies could be related to various infections by microbes (Kalaria, Satasia, & Raval, 2014). Synthesis of novel molecules based on hybrid pharmacophores is expected to achieve pluripotent pharmacological activities, and contribute in developing new drugs against such infectious diseases (Brogden, 1986).

Pyrazolines are an important class of compounds that have varied biological significance, being closely related in structure to the pyrazolone family of drugs (Ioffe & Burmanova, 1971). Among the three isomers of pyrazolines, 1,2-pyrazolines are the most thermodynamically stable and thus are explored exhaustively for a spectrum of biological applications (Baer, Michaelson, McKinstry, & Beyer, 1964; Léavai, 2002). Some of the selected marketed drugs containing pyrazoline scaffold are represented in Figure 1.

Aryloxyacetic acid scaffolds are extensively studied for diuretic action as one of the important and widely used loop diuretic drug ethacrynic acid belongs to this class of compounds (Abdel-Wahab, Khidre, & Awad, 2012; Kitagawa et al., 1991). Recently, there was shift in this interest toward preparing and studying analogs of these scaffolds for their antimicrobial action (Gołębiowski et al., 2014). Moreover, aryloxyacetic acids could be seen as bioisosteric analog of phenylpropionic acid or dihydromannic acid. Some phenylpropionic acids have shown potent antifungal activities after their isolation from fly species (Singh & Bhat, 2011). Some derivatives of phenylpropionic acids have shown potent antimicrobial action as per recent reports (Furniss, Hannaford, Smith, & Tatchell, 2005).

Figure 1. Drugs with pyrazoline moiety.
In this article, we report the synthesis, characterization, and antimicrobial screening of chalcone-based aryloxyacetic acids, and their pyrazoline derivatives. Molecular docking studies to the target proteins are performed in an effort to understand the results obtained. The results obtained from the docking studies reveal that for optimum binding, spacing between aromatic groups is an important determinant of the activity; in the case of chalcone acids, interactions are dominated by combinations of van der Waal interactions and hydrogen bonding, whereas in the case of pyrazolines, there was only hydrogen bonding interaction which is confined to only a single side of the molecule due to steric constraints caused by adjacent aromatic rings.

2. Results and discussion

2.1. Chemistry

The synthetic strategy employed to prepare the title compounds $11a-i$ and $12a-i$ is illustrated in Scheme 1. The hydroxyaldehydes and hydroxyacetophenones were reacted to obtain chalcones using the well-known Claisen–Schmidt condensation. Then, the phenolic OH group was reacted with chloroethylacetate to give corresponding aryloxy esters. The esters were hydrolyzed to acids under basic conditions. Esters were also treated with hydrazine hydrate and glacial acetic acid in a solvent-efficient reaction to obtain 1,2-pyrazoline derivatives in good yields (Levai & Jeko, 2007; Lévai & Jekó, 2006; Lévai & Jékó, 2006; Shubhalaxmi, Geidner, Kumar, Fun, & Bhat, 2015). All the reaction steps were carried out using minimal ethanol, and formation of pyrazoline was achieved in a solvent-free system, in pure form in yields above 70–80%. The current procedure with no use of solvents or minimum portions in some reactions can be considered as green synthetic method. Excellent purity of the product was achieved in the reaction; thus, loss of material and solvent during recrystallization is also avoided, thus making it a greener and efficient strategy for synthesis of similar molecules.

Scheme 1. Schematic representation of the synthetic protocol.

R (1, 3, 7, 9, 11) = a-H, b-2,4-Cl$_2$, c-4-OCH$_3$, d-2-OCH$_3$, e-4-CH$_3$, f-4-Br, g-2-Br, h-2-Br-4-OCH$_3$, i-2-Br-4-CH$_3$.
R (4, 6, 8, 10, 12) = a-H, b-4-Cl, c-2,4-Cl$_2$, d-4-OCH$_3$, e-3,4-(OCH$_3$)$_2$, f-2,5-(OCH$_3$)$_2$, g-4-F, h-4-NO$_2$, i-4-CH$_3$. 
The structures were assigned to the title compounds with the aid of collective information drawn from TLC, measurement of melting point, and spectral techniques like FTIR, 1H NMR, and MS. The intermediates were characterized by FTIR, 1H NMR, Mass Spectrometry, and Elemental Analysis as and when needed to confirm the structure. Mass spectra of compounds showed M+, M+1, M−1, and M−2 peaks as the case may be which is in agreement with their molecular formula. IR spectra of 3a–i and 6a–i showed characteristic conjugated carbonyl stretch at 1,670–1,640 cm−1. The compounds 7a–i and 8a–i showed characteristic ester carbonyl stretch at 1,800–1,700 cm−1, in addition to the absence of 3,385–3,100 cm−1 corresponding to aryl O–H stretch. Compounds 9a–i and 10a–i absorbed in the IR band of 1,750–1,700 cm−1 characteristic to acid carbonyl stretch; the presence of broad band at 3,500–2,500 cm−1 region also supports the formation of aryloxy acid, as it is indicative of the H-bonding interactions, in addition to the fact that the carbonyl in acid appears at lower region against the ester carbonyl group.

The FT-IR spectra of pyrazolines show absorption in the range of 3,400–3,300 cm−1 due to N–H stretching, 3,350–3,100 cm−1 due to O–H stretching, peaks at 3,050–2,900 cm−1 region corresponding to C–H str of the aliphatic CH2, and Ar–H at the region 2,950–2,850 cm−1. Peaks corresponding to the carbonyl stretch of the C=O are observed between the range 1,690–1,650 cm−1, and respective peaks are observed for C=N, C=C, and C–O–C in the regions 1,620–1,590 cm−1, 1,520–1,500 cm−1, and 1,250–1,200 cm−1. The presence of NH peak and the absence of conjugated carbonyl peak corresponding to chalcone at 1,650 cm−1 region confirm the formation of pyrazolines. In 1H NMR spectrum of the representative compounds from 11a–i to 12a–i, the O–CH2 protons appear as singlet at 4.5 ppm, NH protons as doublet at 4.7–4.8 ppm, and all aromatic protons at 7.0–7.8 ppm. The absence of characteristic doublet of doublet peak due conjugated CH=CH around 7.4–7.6 ppm range is indicative of the formation of pyrazolines, as it signifies the reaction of chalcone to give the heterocycle.

In the 13C NMR spectra of the compounds, 11a representative of the targeted compounds shows peaks at δ-167.11, 157.47, 149.02, 136.03, 128.95, 128.49, 128.16, 125.84, 115.04, 66.79, 63.64, and 42.06, corresponding to the respective carbons present in the compounds. In the MS-API spectra of the compounds, M+ or M+1, peaks are shown, and are base peaks, in cases otherwise the fragment is assigned corresponding to the base peak. The conformation in chalcones and chalcone aryloxy acids, 9a–i and 10a–i, is known to be E, as established in our previous work (Shubhalaxmi et al., 2015).

2.2. Molecular docking
After application of all the docking algorithms in MOE, triangle matcher placement methodology and London dG scoring function could reproduce the bioactive conformations of the ligand molecules. Hence, docking on synthesized compounds was performed using this methodology. RMSD between co-crystallized compounds and re-docked compounds is represented in Table 1. Analysis of the docking scores suggested that chalcone acids have better affinity to antimicrobial proteins as compared to pyrazoline acids. This observation was also supported by MIC results. The reason behind low energy scores of pyrazoline carboxylic acids and aryloxy carboxylic acids could be attributed to few important structural differences. In the case of aryaminotransferase (1W6F), interactions were dominated by aromatic–aromatic van der Waal interactions as well as hydrogen bonding interactions for aryloxy acids, whereas in the case of pyrazoline acids, two factors have contributed in lesser energy: (a) chain length between two aromatic groups has been reduced; (b) interactions are dominated by hydrogen bonding interactions alone as shown in Table 2. This shows that for optimum binding, spacing between aromatic groups is an important determinant of the activity. Similar observations were found in other target proteins, where in the case of chalcone acids, interactions are dominated by combinations of van der Waal interactions and hydrogen bonding interactions and interactions with target proteins were uniformly distributed along the length of ligands, whereas in the case of pyrazoline acids, reduced spacing between two aromatic groups led to constrains in the flexibility of molecules to optimally interact with amino acid residues of target proteins and hence interactions are unequally distributed only on one side of the molecules. The presence of
pyrazolines also increased steric clashes with the amino acid residues of target proteins as shown in arylaminetransferase proteins.

2.3. Antimicrobial activity
Antimicrobial property was tested for aryloxy acetic acids and pyrazoline derivatives. Most of the tested compounds showed significant zones of inhibition against the microbes considered for the study. The zone diameters in mm are listed in Tables 3 and 4. The MIC in μg/mL is shown in Tables 5 and 6, respectively, for chalcone acids and pyrazoline acids. The MIC of the compounds of both the series is plotted and shown in Figures 2 and 3. From the data obtained from biological evaluation, it can be concluded that all tested compounds, 9a–i and 10a–i, 11a–i, and 12a–i, have good to moderate biological activity, suggesting the significance of free acidic OH in inducing enhanced antifungal and antibacterial efficiencies against the tested fungi, C. albicans, and tested Gram-positive bacteria, viz., S. aureus and M. smegmatis. Compounds 11a, 12b, and 12c among pyrazolines showed MIC of 625 μg/mL against all the tested microbes. 9f and 9g showed very good MIC values against Mycobacterium, whereas, 9g and 9h were active against S. aureus in concentrations as low as 32.5 μg/mL. All three compounds, 9f, 9g, and 9h, were active against C. albicans at 32.5 μg/mL concentrations. In comparison to the pyrazolines, all the chalcone acids showed much lower MICs. Pyrazolines showed much higher zones of inhibition against the tested microbes, but showed considerably higher MICs than the corresponding chalcone acids, which is an interesting observation. The positional isomers with electron-withdrawing group farther away from the acid function are relatively higher active among the chalcone acids.

| Compound | S | S | S | S |
|----------|---|---|---|---|
| PDB ID   | 4L6H | 4HOE | 3SRW | 1W6F |
| 9a/11a   | -18.93 | -11.52 | -17.74 | -13.27 | -23.39 | -12.29 | -20.21 | -12.14 |
| 9b/11b   | -15.73 | -13.79 | -18.64 | -13.67 | -23.07 | -13.56 | -21.58 | -12.91 |
| 9c/11c   | -19.54 | -12.34 | -18.72 | -13.77 | -19.85 | -13.36 | -20.40 | -12.20 |
| 9d/11d   | -19.40 | -11.72 | -23.66 | -13.12 | -25.92 | -13.24 | -21.68 | -12.43 |
| 9e/11e   | -17.31 | -12.02 | -20.15 | -14.03 | -25.46 | -13.13 | -20.61 | -12.97 |
| 9f/11f   | -16.44 | -12.54 | -21.46 | -15.60 | -22.45 | -12.68 | -19.07 | -12.11 |
| 9g/11g   | -16.94 | -11.55 | -23.01 | -13.18 | -20.35 | -12.72 | -20.12 | -11.89 |
| 9h/11h   | -18.87 | -11.86 | -20.32 | -13.35 | -22.01 | -12.75 | -19.73 | -13.16 |
| 9i/11i   | -16.10 | -11.48 | -19.55 | -12.87 | -22.09 | -13.51 | -20.03 | -12.19 |
| 10a/12a  | -15.73 | -10.92 | -19.12 | -15.87 | -21.94 | -13.99 | -18.49 | -11.21 |
| 10b/12b  | -16.10 | -11.88 | -19.43 | -12.32 | -22.21 | -12.82 | -19.41 | -13.34 |
| 10c/12c  | -18.51 | -11.37 | -18.20 | -13.12 | -27.70 | -12.57 | -21.68 | -12.10 |
| 10d/12d  | -16.98 | -11.24 | -19.91 | -13.40 | -23.64 | -13.66 | -19.10 | -12.78 |
| 10e/12e  | -16.38 | -12.33 | -18.79 | -14.08 | -21.78 | -13.83 | -22.80 | -13.03 |
| 10f/12f  | -17.66 | -11.35 | -20.93 | -13.42 | -24.84 | -13.78 | -20.05 | -13.29 |
| 10g/12g  | -17.34 | -11.66 | -16.42 | -13.87 | -24.02 | -12.14 | -17.87 | -12.44 |
| 10h/12h  | -19.36 | -12.90 | -19.92 | -14.01 | -22.01 | -12.24 | -19.22 | -12.73 |
| 10i/12i  | -13.62 | -12.20 | -20.63 | -12.00 | -26.38 | -12.65 | -19.21 | -11.89 |

Note: S represents binding energy in kcal/mol of ligand conformation to active site.
Table 2. The representation of binding mode of representative pyrazolines with various microbial targets in docking studies

| PDB ID | Aryloxy acid/ Pyrazoline carboxylic acid | Ligand interaction |
|--------|----------------------------------------|-------------------|
| 1W6F   | ![Image](image1.png)                   | ![Image](image2.png) |
| 3SRW   | ![Image](image3.png)                   | ![Image](image4.png) |
| 4HOE   | ![Image](image5.png)                   | ![Image](image6.png) |
| 4L6H   | ![Image](image7.png)                   | ![Image](image8.png) |

Legend:
- polar
- acidic
- basic
- greasy
- proximity contour
- sidechain acceptor
- sidechain donor
- backbone donor
- backbone acceptor
- arene-arene
- solvent residue
- solvent contact
- metal complex
- metal contact
- arene-cation
- receptor
- exposure

Note: The images are placeholders and the actual images are not provided.
3. Experimental

All reagents were used as purchased from commercial suppliers without further purification. All the melting points were determined in open capillaries, using Thomas Hoover melting point apparatus, expressed in °C, and are uncorrected. The reactions were monitored by TLC for completion and compounds were checked for purity by TLC on silica gel-G (Merck grade). Infrared spectra (IR) were recorded on Schimadzu 8400S Infrared Spectrophotometer. Samples were screened in Potassium bromide (KBr) pellets and the values are expressed in cm⁻¹; ¹H NMR and ¹³C NMR spectra of the compounds were recorded on Bruker Ascend 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm; LC-MS of the samples was recorded using Mass spectrometer, ABSciex-API4000; and Elemental analyses were performed on a Flash EA1112 CHNS analyzer (Thermo Electron Corporation).

The general procedure for synthesis of starting materials and aryloxy esters is given in the supplementary material. Their physicochemical data as well as spectral characterization are included in the same file.

3.1. General procedure for the synthesis of compounds 4-[3-oxo-3-phenylprop-1-en-1-yl]phenoxy)acetic acids and 4-[3-phenylprop-2-enoyl]phenoxy)acetic acids 9a–i and 10a–i

A solution of the appropriate esters (0.02 mol) in ethanol is refluxed with 5 ml of 30% aqueous NaOH for 2 h. The mixture was cooled to room temperature and neutralized to obtain the respective aryloxy acid. The product obtained was dried and recrystallized using acetone water system. Their physicochemical characterization data are given in Table 7. The spectral data for other compounds in the series are included in the supplementary information.

Table 3. Antimicrobial activity of chalcone acids by well diffusion assay

| Compound | Zone diameters in mm |
|----------|----------------------|
|          | M. smegmatis | S. aureus | E. coli | C. albicans |
|          | 50 µL | 25 µL | 50 µL | 25 µL | 50 µL | 25 µL |
| 9a       | -     | -     | 5.66 ± 0.57 | - | - | 14.00 ± 1.73 | 8.66 ± 0.57 |
| 9b       | -     | -     | 15.33 ± 1.15 | 11.66 ± 2.51 | - | 14.33 ± 1.52 | 9.33 ± 0.57 |
| 9c       | -     | -     | 12.00 ± 1.00 | 8.66 ± 0.57 | - | 8.66 ± 2.08 | 17.33 ± 1.15 | 11.00 ± 1.00 |
| 9d       | -     | -     | -     | 9.33 ± 1.52 | - | 16.33 ± 0.57 | 9.66 ± 0.57 |
| 9e       | -     | -     | 14.66 ± 0.57 | 13.00 ± 1.00 | - | - | 13.00 ± 1.00 | 7.66 ± 0.57 |
| 9f       | 19.66 ± 4.72 | 14.33 ± 0.57 | 13.33 ± 0.57 | 9.66 ± 0.57 | - | 17.33 ± 1.15 | 15.66 ± 1.52 |
| 9g       | 12.83 ± 0.28 | 9.66 ± 0.57 | 20.00 ± 2.00 | 15.66 ± 0.57 | - | - | 22.66 ± 2.51 | 15.00 ± 0 |
| 9h       | -     | -     | -     | - | - | 10.33 ± 0.57 | 10 ± 0 |
| 9i       | 18.00 ± 2.00 | 16.00 ± 3.46 | 16.33 ± 0.57 | 15.33 ± 0.57 | - | - | 17.33 ± 0.57 | 17 ± 0 |
| 10a      | 11.33 ± 3.05 | - | - | - | - | - | 12.00 ± 1.00 | - |
| 10b      | -     | -     | -     | - | - | - | 8.66 ± 0.57 | - |
| 10c      | 15.66 ± 1.15 | 13.00 ± 1.00 | 12.33 ± 1.52 | - | - | - | 15.33 ± 1.15 | 14.00 ± 1.73 |
| 10d      | -     | -     | -     | - | - | - | 12.00 ± 1.00 | 10.66 ± 0.57 |
| 10e      | 17.00 ± 1.00 | 13.66 ± 0.57 | - | - | - | - | 20.00 ± 1.00 | 17.33 ± 0.57 |
| 10f      | 14.66 ± 0.57 | - | - | 12.66 ± 0.57 | 13.33 ± 0.57 | - | - |
| 10g      | -     | -     | 9.00 ± 1.00 | - | - | - | - |
| ABS/AFS  | 46.67 ± 0.48 | 33.33 ± 0.58 | 28.22 ± 0.58 | 30.27 ± 1.55 |
| Control  | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

Notes: ABS: antibacterial standard, Ciprofloxacin; AFS: antifungal standard, Fluconazole; both standards used are 10 μg discs; –: no detected inhibition; control: dimethylsulfoxide; note: compounds 10f and 10h showed no inhibition zones against any tested micro-organism, E. coli was resistant to all the tested compounds at 25 µL concentration.
3.1. (4-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy)acetic acid (9a)

IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3500–2500 (O–H str), 2916 (Ar–H str), 1704 (acid C=O str), 1658 (C=O str), 1596 (C=C str); (400 MHz, DMSO-$d_6$, $\delta$ ppm): 4.76 (2H, s, O–CH$_2$), 7.70–7.82 (2H, dd, CH=CH), 6.98–8.12 (9H, m, aromatic protons) 13.05 (1H, s, acidic H); MS (API) [M+1]$^+$: m/z 283; ANAL. Calcd. for C$_{17}$H$_{14}$O$_4$: calcd: C, 72.33; H, 5.00. found: C, 72.47, H, 5.02.

### Table 4. Antimicrobial activity of pyrazolines by well diffusion assay

| Compound | Zone diameters in mm |  |
|----------|----------------------|---|
|          | M. smegmatis (50 µL) | S. aureus (50 µL) | E. coli (25 µL) | C. albicans (25 µL) |
| 11a      | 19.66 ± 1.52         | 24.00 ± 0.00       | 21.66 ± 0.57    | 18.66 ± 0.57       |
| 11b      | 18.00 ± 1.00         | 25.66 ± 0.57       | 24.33 ± 2.08    | 17.33 ± 1.15       |
| 11c      | 14.66 ± 1.15         | 21.66 ± 0.57       | 17.33 ± 1.52    | 13.33 ± 0.57       |
| 11d      | 21.33 ± 0.57         | 26.66 ± 1.15       | 24.33 ± 2.08    | 10.33 ± 0.57       |
| 11e      | 19.00 ± 2.64         | 12.00 ± 0.00       | 16.00 ± 0.00    | 13.00 ± 1.00       |
| 11f      | 15.66 ± 0.57         | 24.00 ± 0.57       | 16.00 ± 0.00    | 13.66 ± 0.57       |
| 11g      | 20.00 ± 1.00         | 22.33 ± 1.15       | 21.33 ± 1.15    | 12.00 ± 1.00       |
| 11h      | 17.66 ± 0.57         | 18.00 ± 1.00       | 12.66 ± 0.57    | 13.66 ± 0.57       |
| 11i      | 19.00 ± 2.64         | 11.66 ± 1.52       | 22.00 ± 0.00    | 13.00 ± 1.00       |
| 11j      | 15.66 ± 0.57         | 14.66 ± 1.25       | 20.66 ± 0.57    | 13.66 ± 0.57       |
| 11k      | 20.00 ± 1.00         | 17.00 ± 0.00       | 22.00 ± 0.57    | 13.00 ± 1.00       |
| 11l      | 17.66 ± 0.57         | 12.66 ± 1.52       | 21.66 ± 0.00    | 13.66 ± 0.57       |
| 12a      | 19.00 ± 2.64         | 12.00 ± 0.00       | 16.00 ± 0.00    | 13.00 ± 1.00       |
| 12b      | 18.00 ± 1.00         | 25.66 ± 0.57       | 24.33 ± 2.08    | 13.66 ± 0.57       |
| 12c      | 17.66 ± 0.57         | 15.66 ± 0.57       | 22.00 ± 0.00    | 13.00 ± 1.00       |
| 12d      | 14.66 ± 1.15         | 10.66 ± 0.57       | 17.66 ± 0.57    | 13.66 ± 0.57       |
| 12e      | 21.33 ± 0.57         | 15.66 ± 0.57       | 16.00 ± 0.00    | 13.66 ± 0.57       |
| 12f      | 20.00 ± 1.00         | 23.33 ± 1.15       | 21.66 ± 0.00    | 13.66 ± 0.57       |
| 12g      | 19.00 ± 2.64         | 12.66 ± 1.52       | 21.66 ± 0.00    | 13.66 ± 0.57       |
| 12h      | 17.66 ± 0.57         | 12.66 ± 1.52       | 18.00 ± 1.00    | 13.66 ± 0.57       |
| 12i      | 20.00 ± 1.00         | 15.00 ± 1.00       | 19.33 ± 2.08    | 13.66 ± 0.57       |
| ABS/AFS  | 46.67 ± 0.48         | 33.33 ± 0.58       | 28.22 ± 0.58    | 30.27 ± 1.55       |
| Control  | 00                   | 00                  | 00              | 00                  |

Notes: ABS: antibacterial standard, Ciprofloxacin; AFS: antifungal standard, Fluconazole; both standards used are 10 µg discs; -: no detected inhibition; control: dimethylsulfoxide.

3.2. Antimicrobial activity of chalcone acids.
3.1.2. (4-[(1E)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl]phenoxy)acetic acid (9c)

IR (KBr $\nu$ cm$^{-1}$): 3400–2576 (O–H str), 2916–2840 (Ar–H str), 1720 (acid C=O str), 1589 (C=C str), 1172 (Ar–O–CH$_2$ str); $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 3.88 (3H, s, OCH$_3$), 4.77 (2H, s, O–CH$_2$), 7.70–7.80 (2H, dd, CH=CH), 6.99–8.17 (8H, m, aromatic protons), 13.12 (1H, s, acidic H); ANAL. Calcd. for C$_{18}$H$_{16}$O$_5$: calcd: C, 69.22; H, 5.16. found: C, 69.42; H, 5.17.

Table 5. MIC of chalcone acids

| Compound | MIC in μg/mL |
|----------|--------------|
|          | M. spigmatis | S. aureus | C. albicans |
| 9a       | 125          | 62.5      | 125         |
| 9b       | 125          | 125       | 125         |
| 9c       | 125          | 125       | 125         |
| 9d       | 250          | 250       | 250         |
| 9e       | 125          | 125       | 125         |
| 9f       | 31.25        | 62.5      | 31.25       |
| 9g       | 31.25        | 31.25     | 31.25       |
| 9h       | 125          | 31.25     | 31.25       |
| 9i       | 125          | 31.25     | 125         |
| 10a      | 125          | 125       | 125         |
| 10b      | 125          | 250       | 125         |
| 10c      | 62.5         | 62.5      | 62.5        |
| 10d      | 500          | 250       | 500         |
| 10e      | 125          | 250       | 62.5        |
| 10f      | 125          | 250       | 125         |
| 10g      | 250          | 250       | 125         |
| 10h      | 125          | 125       | 125         |
| 10i      | 125          | 125       | 125         |
| ABS      | <10          | <10       | –           |
| AFS      | –            | –         | <10         |

Notes: ABS: antibacterial standard, Ciprofloxacin; AFS: antifungal standard, Fluconazole; –: no detected inhibition; control: dimethylsulfoxide. MICs for E.coli were not performed as compounds did not show any significant inhibition zone.
3.1.3. {4-[(1E)-3-(4-Methylphenyl)-3-oxoprop-1-en-1-yl]phenoxy}acetic acid (9e)

IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3,500–2,600 (O–H str), 3,000–2,900 (Ar–H str), 1719 (acid C=O str), 1,650 (C=O str), 1,600 (C=C str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 2.41 (3H, s, CH$_3$), 4.68 (2H, s, O–CH$_2$), 7.71–7.77 (2H, d, CH=CH), 6.99–8.17 (8H, m, aromatic protons); ANAL. Calcd. for C$_{18}$H$_{16}$O$_4$: calcd: C, 72.96; H, 5.44. found: C, 73.10; H, 5.45.

3.1.4. {4-[(2E)-3-phenylprop-2-enoyl]phenoxy}acetic acid (10a)

IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3500–2500 (O–H str), 1735–1704 (acid C=O str), 1662 (C=O str), 1604 (C=C str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 4.84 (2H, s, O–CH$_2$), 7.70–7.97 (2H, dd, CH=CH), 6.99–8.17 (9H, m, aromatic protons) 13.15 (1H, s, acidic H); MS (API) [M+1]$^+$: m/z 283; ANAL. Calcd. for C$_{17}$H$_{14}$O$_4$: calcd: C, 72.33; H, 5.00. found: C, 72.52, H, 5.45.

3.1.5. {4-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]phenoxy}acetic acid (10b)

IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3500–2500 (O–H str), 1731–1704 (acid C=O str), 1662 (C=O str), 1604 (C=C str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 4.73 (2H, s, O–CH$_2$), 7.67–7.98 (2H, dd, CH=CH), 6.99–8.16 (8H, m, aromatic protons) 13.21 (1H, s, acidic H); MS (API) [M+1]$^+$: m/z 283; ANAL. Calcd. for C$_{17}$H$_{13}$ClO$_4$: calcd: C, 64.46; H, 4.14. found: C, 64.45; H, 4.14.

3.2. General procedure for the synthesis of compounds 4-(3-phenyl-1H-pyrazol-5-yl)phenoxyacetic acid and 4-(5-phenyl-1H-pyrazol-3-yl)phenoxyacetic acid 11a–i and 12a–i

Respective ester (7a–i or 8a–i) of 1 mmol is reacted with gram equivalents of NH$_2$NH$_2$ (99%) in the presence of 2–3 drops of glacial acetic acid under reflux condition for 3 h. Upon cooling of the
reaction mixture to room temperature, crystals of the product are obtained in good yields. They are filtered and dried. The physicochemical characterization data of the compounds are given in Table 8.

3.2.1. [4-(3-phenyl-1H-pyrazol-5-yl)phenoxy]acetic acid (11a)
IR (KBr \( \nu \max \) cm\(^{-1}\)): 3332 (N–H str), 3250 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1681 (C=O str), 1596 (C=N str), 1512 (C=C str), 1242 (Ar–O–CH\(_2\) str); 1H NMR (400 MHz, DMSO-d6, \( \delta \)-ppm): 2.80 (1H, t, pyrazoline CH), 3.38 (1H, t, pyrazoline CH\(_2\)-H\(_a\)), 4.32 (1H, s, –NH), 4.46 (2H, s, –OCH\(_2\)), 4.78 (1H, t, pyrazoline CH\(_2\)-H\(_b\)), 6.93–7.61 (9H, m, aromatic CH), 9.32 (1H, s, acid OH); 13C NMR (100 MHz, DMSO-d6, \( \delta \)-ppm): 167.11, 157.47, 149.02, 136.03, 133.79, 128.95, 128.49, 128.16, 125.84, 115.04, 66.79, 63.64, 41.12; MS (API) [M]+: m/z 296; ANAL. Calcd. for C\(_{17}\)H\(_{16}\)N\(_2\)O\(_3\); calcd: C, 69.38; H, 4.79; N, 9.52. found: C, 69.65; H, 4.80; N, 9.55.

3.2.2. {4-[3-(2,4-dichlorophenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11b)
IR (KBr \( \nu \max \) cm\(^{-1}\)): 3332 (N–H str), 3250 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1681 (C=O str), 1596 (C=N str), 1512 (C=C str), 1242 (Ar–O–CH\(_2\) str); 1H NMR (400 MHz, DMSO-d6, \( \delta \)-ppm): 2.72 (1H, t, pyrazoline CH), 3.38 (1H, t, pyrazoline CH\(_2\)-H\(_a\)), 4.33 (1H, s, –NH), 4.50 (2H, s, –OCH\(_2\)), 5.02 (1H, s, –NH), 6.92–7.6 (7H, m, aromatic CH), 9.363 (1H, s, acid OH); ANAL. Calcd. for C\(_{17}\)H\(_{14}\)Cl\(_2\)N\(_2\)O\(_3\); calcd: C, 56.22; H, 3.33; N, 7.74. found: C, 56.44; H, 3.34; N, 7.74.

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**Table 7. Physicochemical data of aryloxy acids 9a–i and 10a–i**

| Compound | Mol. formulae | R       | Mol. weight | Yield (in %) | \( R_f \) value* | MP (in °C) |
|----------|---------------|---------|-------------|--------------|------------------|------------|
| 9a       | C\(_{17}\)H\(_{14}\)O\(_4\) | H       | 282         | 64           | 0.2              | 180–182    |
| 9b       | C\(_{17}\)H\(_{13}\)ClO\(_4\)  | 2,4-Cl\(_2\) | 351         | 71           | 0.2              | 168–170    |
| 9c       | C\(_{18}\)H\(_{16}\)O\(_5\)  | 4-OCH\(_3\) | 312         | 78           | 0.2              | 179–181    |
| 9d       | C\(_{18}\)H\(_{16}\)O\(_5\)  | 2-OCH\(_3\) | 312         | 73           | 0.2              | 201–203    |
| 9e       | C\(_{16}\)H\(_{13}\)O\(_4\)  | 4-CH\(_3\) | 296         | 91           | 0.2              | 181–183    |
| 9f       | C\(_{17}\)H\(_{14}\)BrO\(_4\)  | 4-Br     | 361         | 70           | 0.2              | 103–105    |
| 9g       | C\(_{17}\)H\(_{13}\)BrO\(_4\)  | 2-Br     | 361         | 41           | 0.2              | 142 (dec)  |
| 9h       | C\(_{18}\)H\(_{14}\)BrO\(_4\)  | 2-Br-4-OCH\(_3\) | 391 | 75 | 0.2 | 92–94 |
| 9i       | C\(_{18}\)H\(_{14}\)BrO\(_4\)  | H        | 391         | 87           | 0.2              | 110 (dec)  |
| 10a      | C\(_{17}\)H\(_{14}\)ClO\(_4\)  | H        | 282         | 40           | 0.2              | 142 (dec)  |
| 10b      | C\(_{17}\)H\(_{13}\)ClO\(_4\)  | 4-Cl     | 317         | 81           | 0.1              | 160–162(dec) |
| 10c      | C\(_{17}\)H\(_{14}\)ClO\(_4\)  | 2,4-Cl\(_2\) | 351         | 89           | 0.2              | 90–92      |
| 10d      | C\(_{17}\)H\(_{13}\)O\(_4\)  | 4-OCH\(_3\) | 312         | 73           | 0.2              | 68–70      |
| 10e      | C\(_{17}\)H\(_{13}\)O\(_4\)  | 3,4-(OCH\(_3\)\(_2\)) | 342 | 98 | 0.2 | 144–146 |
| 10f      | C\(_{18}\)H\(_{15}\)BrO\(_5\)  | 2-Br-4-OCH\(_3\) | 342 | 61 | 0.2 | 189–191 |
| 10g      | C\(_{17}\)H\(_{14}\)FO\(_4\)  | 4-F      | 300         | 80           | 0.1              | 246–248(dec) |
| 10h      | C\(_{17}\)H\(_{14}\)NO\(_4\)  | 4-NO\(_2\) | 327         | 60           | 0.1              | 41.12      |
| 10i      | C\(_{17}\)H\(_{13}\)O\(_4\)  | 4-CH\(_3\) | 296         | 84           | 0.2              | 192 (dec)  |

*Mobile phase, Ethyl acetate and Hexane in the ratio 2:1, respectively, and are rounded off to the nearest decimal.

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3.2.3. {4-[3-(4-methoxyphenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11c)
IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3348 (N–H str), 3317 (O–H str), 3047 (C–H str), 2912 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1512 (C=C str), 1242 (Ar–O–CH$_2$ str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 2.77 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$-Ha), 4.32 (1H, t, pyrazoline CH$_2$-Hb), 3.76 (3H, s, OCH$_3$), 4.46 (2H, s, –OCH$_2$), 4.74 (1H, s, –NH), 6.93–7.61 (9H, m, aromatic CH), 9.33 (1H, s, acid OH); ANAL. Calcd. for C$_{18}$H$_{18}$N$_2$O$_4$: calcd: C, 66.25; H, 5.56; N, 8.58. found: 66.44; H, 5.57; N, 8.60.

3.2.4. {4-[3-(2-methoxyphenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11d)
IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3332 (N–H str), 3256 (O–H str), 2993 (C–H str), 2908 (Ar–H str), 1674 (C=O str), 1598 (C=N str), 1504 (C=N str), 1234 (Ar–O–CH$_2$ str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 2.77 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$-H), 4.32 (1H, t, pyrazoline CH$_2$-H), 3.76 (3H, s, OCH$_3$), 4.46 (2H, s, –OCH$_2$), 4.74 (1H, t, pyrazoline CH$_2$-Hb), 6.92–7.61 (9H, m, aromatic CH), 9.32 (1H, s, acid OH); ANAL. Calcd. for C$_{18}$H$_{18}$N$_2$O$_4$: calcd: C, 66.25; H, 5.56; N, 8.58. found: 66.38; H, 5.57; N, 8.59.

3.2.5. {4-[3-(4-methylphenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11e)
IR (KBr $\nu_{\text{max}}$ cm$^{-1}$): 3332 (N–H str), 3256 (O–H str), 3039 (C–H str), 2909 (Ar–H str), 1674 (C=O str), 1596 (C=N str), 1512 (C=N str), 1242 (Ar–O–CH$_2$ str); $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$-ppm): 2.30 (3H, s, CH$_3$), 2.77 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$-H), 4.32 (1H, s, NH), 4.46 (2H, s, –OCH$_2$), 4.76 (1H, t, pyrazoline CH$_2$-H), 6.92–7.61 (9H, m, aromatic CH), 9.32 (1H, s, acid OH); $^{13}$C NMR (100 MHz,
DMSO-d6, δ-ppm): 167.11, 157.45, 149.21, 137.94, 136.08, 131.06, 129.53, 128.16, 125.84, 115.02, 114.70, 66.78, 63.57, 41.08, 21.36; MS (API) [M+1] +: m/z 311; Anal. Calcd. for C_{18}H_{18}N_{2}O_{3}; calcd: C, 69.66; H, 5.85; N, 9.03. found: C, 69.86; H, 5.86; N, 9.05.

3.2.6. {4-[3-(4-bromophenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11f)

IR (KBr υ max cm⁻¹): 3340 (N–H str), 3275 (O–H str), 3039 (C–H str), 2900 (Ar–H str), 1674 (C=O str), 1596 (C=N str), 1504 (C=C str), 1234 (Ar–O–CH, str), 825 (Ar–Br str); 1H NMR (400 MHz, DMSO-d6, δ-ppm): 2.81 (1H, t, pyrazoline CH), 3.32 (1H, t, pyrazoline CH₂-Ha), 4.43 (1H, s, NH), 4.46 (2H, s, –OCH₂), 4.79 (1H, t, pyrazoline CH₂-Hb), 6.93–7.61 (8H, m, aromatic CH), 9.34 (1H, s, acid OH); Anal. Calcd. for C_{17}H_{15}BrN_{2}O_{3}; calcd: C, 54.42; H, 4.03; N, 7.47. found: C, 54.47; H, 4.04, N, 7.49.

3.2.7. {4-[3-(2-bromophenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11g)

IR (KBr υ max cm⁻¹): 3340 (N–H str), 3275 (O–H str), 3039 (C–H str), 2912 (Ar–H str), 1674 (C=O str), 1596 (C=N str), 1504 (C=C str), 1234 (Ar–O–CH, str), 825 (Ar–Br str); 1H NMR (400 MHz, DMSO-d6, δ-ppm): 2.81 (1H, t, pyrazoline CH), 3.32 (1H, t, pyrazoline CH₂-Ha), 4.43 (1H, s, NH), 4.46 (2H, s, –OCH₂), 4.79 (1H, t, pyrazoline CH₂-Hb), 6.93–7.61 (8H, m, aromatic CH), 9.34 (1H, s, acid OH); Anal. Calcd. for C_{17}H_{15}BrN_{2}O_{3}; calcd: C, 54.42; H, 4.03; N, 7.47. found: C, 54.63; H, 4.04, N, 7.49.

3.2.8. {4-[3-(2-bromo-4-methoxyphenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11h)

IR (KBr υ max cm⁻¹): 3394 (N–H str), 3325 (O–H str), 2939 (C–H str), 2900 (Ar–H str), 1674 (C=O str), 1604 (C=N str), 1512 (C=C str), 1249 (Ar–O–CH, str), 833 (Ar–Br str); 1H NMR (400 MHz, DMSO-d6, δ-ppm): 2.81 (1H, t, pyrazoline CH), 3.32 (1H, t, pyrazoline CH₂-Ha), 3.74 (3H, s, OCH₃), 4.43 (1H, s, NH), 4.46 (2H, s, –OCH₂), 4.79 (1H, t, pyrazoline CH₂-Hb), 6.87–8.05 (7H, m, aromatic CH), 9.32 (1H, s, acid OH); Anal. Calcd. for C_{18}H_{17}BrN_{2}O_{4}; calcd: C, 53.35; H, 4.23; N, 6.91. found:53.45; H, 4.23, N, 6.92.

3.2.9. {4-[3-(2-bromo-4-methyphenyl)-1H-pyrazol-5-yl]phenoxy}acetic acid (11i)

IR (KBr υ max cm⁻¹): 3394 (N–H str), 3317 (O–H str), 3024 (C–H str), 2916 (Ar–H str), 1666 (C=O str), 1612 (C=N str), 1512 (C=C str), 1234 (Ar–O–CH, str), 817 (Ar–Br str); 1H NMR (400 MHz, DMSO-d6, δ-ppm): 2.36 (3H, s, CH₃), 2.81 (1H, t, pyrazoline CH), 3.34 (1H, t, pyrazoline CH₂-Ha), 3.74 (3H, s, OCH₃), 4.43 (1H, s, NH), 4.46 (2H, s, –OCH₂), 4.79 (1H, t, pyrazoline CH₂-Hb), 6.87–8.05 (7H, m, aromatic CH), 9.32

Table 9. The data representing details of three-dimensional X-ray crystallographic structures of microbial proteins co-crystallized with their ligands and RMSD values for validation of docking protocol

| PDB ID | Target | Ligand | Organism      | RMSD | Interaction |
|--------|--------|--------|---------------|------|-------------|
| 1W6F   | Arylamine n-acetyltransferase | Isoniazid | M. smegmatis | 1.87 | CYS70       |
| 3SRW   | Dihydrofolate 7-aryl diaminquinazolines | S. aureus | 0.398 | ASP28, LEU6, PHE93, ARG45, GLY95, ARG45, THR64, SER65, ALA8, ILE15, THR47, GLU101 |
| 4HOE   | Dihydrofolate reductase | 5-[3-(2,5-Dimethoxy-4-Phenylphenyl)-but-1-Yn-1-Yl]-6-Methyl-pyrimidine-2,4-Diamine | Candida albicans | 0.807 | GLU32, ILE9, TYR118, ILE112, |
| 4L6H   | Cobalamin-independent methionine synthase | Methotrexate | Candida albicans | 1.81 | ASP504, LYS19, TRP576, TYR531 |
(1H, s, acid OH); Anal. Calcd. for C_{18}H_{17}BrN_{2}O_{3}; calcd: C, 55.54; H, 4.40; N, 7.20. Found: C, 55.70; H, 4.41; N, 7.22.

3.2.10. [4-(5-phenyl-1H-pyrazol-3-yl)phenoxy]acetic acid (12a)
IR (KBr $\nu$ cm$^{-1}$): 3310 (N–H str), 3276 (O–H str), 2923 (C–H str), 2912 (Ar–H str), 1731 (C=O str), 1604 (C=N str), 1222 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.80 (1H, t, pyrazoline CH), 4.32 (1H, s, NH), 4.50 (2H, s, –OCH$_2$), 4.7–4.8 (1H, t, pyrazoline CH$_2$–H$_b$), 7.2–7.6 (9H, m, aromatic CH), 9.32 (1H, s, acid OH); Anal. Calcd. for C$_{18}$H$_{17}$BrN$_2$O$_3$; calcd: C, 55.54; H, 4.40; N, 7.20.

3.2.11. {4-[5-(4-chlorophenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12b)
IR (KBr $\nu$ max cm$^{-1}$): 3348 (N–H str), 3317 (O–H str), 3031 (C–H str), 2912 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1249 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.78 (1H, t, pyrazoline CH), 3.42 (1H, t, pyrazoline CH$_2$–H$_a$), 4.34 (1H, s, NH), 4.50 (2H, s, –OCH$_2$), 4.78–4.80 (1H, t, pyrazoline CH$_2$–H$_b$), 6.93–7.61 (8H, m, aromatic CH), 9.36 (1H, s, acid OH); Anal. Calcd. for C$_{17}$H$_{16}$N$_2$O$_3$; calcd: C, 61.73; H, 4.57; N, 8.47.

3.2.12. {4-[5-(2,4-dichlorophenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12c)
IR (KBr $\nu$ max cm$^{-1}$): 3310 (N–H str), 3263 (O–H str), 2967 (C–H str), 2912 (Ar–H str), 1674 (C=O str), 1612 (C=N str), 1242 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.70 (1H, t, pyrazoline CH), 3.55 (1H, t, pyrazoline CH$_2$–H$_a$), 4.33 (1H, s, NH), 4.50 (2H, s, –OCH$_2$), 5.04 (1H, t, pyrazoline CH$_2$–H$_b$), 7.0–7.6 (7H, m, aromatic CH), 9.36 (1H, s, acid OH); Anal. Calcd. for C$_{17}$H$_{14}$Cl$_2$N$_2$O$_3$; calcd: C, 55.91; H, 3.86; N, 7.67.

3.2.13. {4-[5-(4-methoxyphenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12d)
IR (KBr $\nu$ max cm$^{-1}$): 3317 (N–H str), 3310 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1249 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.77 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$–H$_a$), 3.73 (3H, s, OCH$_3$), 4.33 (1H, s, NH), 4.50 (2H, s, –OCH$_2$), 4.74 (1H, t, pyrazoline CH$_2$–H$_b$), 6.93–7.61 (9H, m, aromatic CH), 9.32 (1H, s, acid OH); $^{13}$C NMR (100 MHz, DMSO-d$_{6}$, $\delta$-ppm): 166.99, 158.88, 158.28, 149.10, 135.42, 128.20, 127.24, 127.07, 115.17, 114.89, 114.22, 66.73, 63.58, 55.54, 41.20; MS (API) [M-2] +: m/z 324; Anal. Calcd. for C$_{18}$H$_{18}$N$_2$O$_4$; calcd: C, 66.25; H, 5.56; N, 8.58.

3.2.14. {4-[5-(3,4-dimethoxyphenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12e)
IR (KBr $\nu$ max cm$^{-1}$): 3340 (N–H str), 3315 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1242 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.82 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$–H$_a$), 3.73 (6H, s, –OCH$_3$), 4.33 (1H, s, NH), 4.50 (2H, s, –OCH$_2$), 4.74–4.76 (1H, t, pyrazoline CH$_2$–H$_b$), 7.0–7.6 (7H, m, aromatic CH), 9.36 (1H, s, acid OH); Anal. Calcd. for C$_{19}$H$_{20}$N$_2$O$_5$; calcd: C, 64.04; H, 5.66; N, 7.86.

3.2.15. {4-[5-(2,5-dimethoxyphenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12f)
IR (KBr $\nu$ max cm$^{-1}$): 3335 (N–H str), 3271 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1666 (C=O str), 1604 (C=N str), 1242 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.82 (1H, t, pyrazoline CH), 3.36 (1H, t, pyrazoline CH$_2$–H$_a$), 3.66 (3H, s, –OCH$_3$), 3.77 (3H, s, –OCH$_3$), 4.35 (1H, s, NH), 4.5 (2H, s, –OCH$_2$), 4.93–4.95 (1H, td, pyrazoline CH$_2$–H$_b$), 7.0–7.6 (7H, m, Ar–H), 9.36 (1H, s, acid H); Anal. Calcd. for C$_{19}$H$_{20}$N$_2$O$_5$; calcd: C, 64.04; H, 5.66; N, 7.86. Found: C, 64.23; H, 5.67; N, 7.88.

3.2.16. {4-[5-(4-fluorophenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12g)
IR (KBr $\nu$ max cm$^{-1}$): 3356 (N–H str), 3309 (O–H str), 3039 (C–H str), 2908 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1249 (Ar–O–CH$_2$); $^1$H NMR (400 MHz, DMSO-d$_{6}$, $\delta$-ppm): 2.80 (1H, sy, pyrazoline CH), 3.42 (1H, s, pyrazoline CH$_2$–H$_a$), 4.32 (1H, broad with solvent, NH), 4.46 (2H, s, –OCH$_2$), 4.78 (1H, t, pyrazoline CH$_2$–H$_b$), 6.93–7.61 (8H, m, aromatic CH), 9.32 (1H, s, acid OH); $^{13}$C NMR (100 MHz, DMSO-d$_{6}$, $\delta$-ppm): 166.97, 162.98, 160.58, 158.35, 149.14, 139.74, 139.71, 129.07, 128.99, 127.28, 126.92,
115.64, 115.43, 115.18, 114.89, 66.73, 63.34, 41.12; MS (API) [M-2]^+: m/z 312; ANAL. Calcd. for C\textsubscript{17}H\textsubscript{15}FN\textsubscript{2}O\textsubscript{3}; calcd: C, 64.96; H, 4.81; N, 8.91. found: C, 65.21; H, 4.82; N, 8.94.

3.2.17. (4-{[5-(4-nitrophenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12h)
IR (KBr \textit{\nu} max cm\textsuperscript{-1}): 3356 (N–H str), 3255 (O–H str), 3047 (C–H str), 2912 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1519 (C=C str), 1242 (Ar–O–CH\textsubscript{2}); \textit{\delta}ppm: 2.83 (1H, t, pyrazoline CH), 3.52 (1H, t, pyrazoline CH 2-Ha), 4.33 (1H, s, NH), 4.50 (2H, s, –OCH 2), 4.96 (1H, d, pyrazoline CH 2-Hb), 6.97–8.22 (8H, m, aromatic CH), 9.36 (1H, s, acid OH); ANAL. Calcd. for C\textsubscript{17}H\textsubscript{15}N\textsubscript{3}O\textsubscript{5}; calcd: C, 59.82; H, 4.43; N, 12.31. found: C, 59.99; H, 4.44; N, 12.34.

3.2.18. (4-{[5-(4-methylphenyl)-1H-pyrazol-3-yl]phenoxy}acetic acid (12i)
IR (KBr \textit{\nu} max cm\textsuperscript{-1}): 3348 (N–H str), 3309 (O–H str), 3031 (C–H str), 2904 (Ar–H str), 1681 (C=O str), 1604 (C=N str), 1512 (C=C str), 1242 (Ar–O–CH\textsubscript{2}); \textit{\delta}ppm: 2.28 (3H, s, CH\textsubscript{3}), 2.80 (1H, t, pyrazoline CH), 3.33 (1H, s, pyrazoline CH 2-Ha), 4.32 (1H, s, CH\textsubscript{2}), 2.80 (1H, t, pyrazoline CH), 3.33 (1H, s, pyrazoline CH 2-Ha), 4.32 (1H, s, CH\textsubscript{2}), 4.50 (2H, s, –OCH\textsubscript{2}), 4.80 (1H, t, pyrazoline CH 2-Hb), 7.0–7.6 (8H, m, aromatic CH), 9.32 (1H, s, acid OH); ANAL. Calcd. for C\textsubscript{18}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}; calcd: C, 69.66; H, 5.85; N, 9.03. found: C, 69.79; H, 5.86; N, 9.04.

3.3. Docking
Molecular docking studies were performed in DOCK module of Molecular operating Environment (MOE 2008.10) package (Salam et al., 2008). Molecular docking was performed between synthesized ligands and three-dimensional X-ray crystallographic structures of proteins which act as target proteins for antimicrobial activity of the ligands. Protein structures were obtained from a protein data bank (Berman et al., 2000; Hevener et al., 2009) which are bound to a co-crystallized ligand. Protein structures derived from micro-organisms tested for antimicrobial activities were only used in the docking. Prior to docking, co-crystallized ligand structures were extracted, energy minimized, and re-docked in their binding pockets. The reproducibility of the co-crystallized ligand’s pose after docking was set as criteria for validation. Reproducibility was defined in the root mean square deviation in the Å between heavy atoms of co-crystallized and re-docked poses to be less than 2 Å is shown in Table 9. Hydrogens were added to protein structures and tautomers of amino acid residues at 7.4 pH were correctly assigned using 3D protonate tool in MOE. Proteins were energy minimized to relax constraints in crystallization process. Ligands were built in ACD/chemsketch freeware (www.acdlabs.com) and were geometry optimized using AM1 semi-empirical methods in MOPAC engine in MOE. Binding sites were defined in the vicinity of 10 Å around co-crystallized ligands.

3.4. Antimicrobial screening
The antibacterial activity of the newly synthesized compounds 11a–i and 12a–i was evaluated employing well diffusion method in nutrient agar media (Sathish, Pavithra, & Ananda, 2012). Antibacterial activity of compounds against 12-h-old bacterial culture of a tuberculosis variant bacterium Mycobacterium smegmatis (MTCC 944), Gram-positive bacteria Staphylococcus aureus (MTCC 3160), and Gram-negative bacteria Escherichia coli (MTCC 1687) was performed in vitro by measuring the zone of inhibition (Palomino et al., 2002). Antifungal activity of these compounds was also carried out against pathogenic fungi Candida albicans(MTCC 7253). All the bacterial and fungal cultures were purchased from the microbial type culture collection, IMTECH, Chandigarh, India, and maintained as per the standard protocol. Nutrient agar media (about 15–20 mL) was poured into each petri plate and allowed to solidify by placing inside the laminar air flow for 15 min. Hundred μL of 0.5 McFarland standard of bacterial/fungal suspension was inoculated on the agar media and spread on the whole surface with a sterile cotton bud. Using a sterile cork borer, 5-mm wells were made on the seeded agar plates. Working solutions of the test compounds in DMSO are made at 10 mg/mL and were transferred at different concentrations (25 and 50 μg mL\textsuperscript{-1}) into the wells in triplicates. The plates were incubated at 37 °C for 12 h and observed for the zone of inhibition in millimeter. DMSO was used as a negative control. Ciprofloxacin was used as an antibacterial standard and fluconazole as an antifungal standard (10 mcg discs).
3.5. Minimum inhibitory concentration (MIC)

Antibacterial activity of 11a–i and 12a–i compounds against 12-h-old culture of Gram-positive Staphylococcus aureus (MTCC 3160), Gram-negative Escherichia coli (MTCC 1687), and Tuberculosis variant bacteria Mycobacterium smegmatis (MTCC 994) was determined by the MIC method. Antifungal activity of these compounds was also carried out against pathogenic fungi Candida albicans (MTCC 7253). All the bacterial and fungal cultures were purchased from the microbial type culture collection, IMTECH, Chandigarh, India, and maintained as per the standard protocol. Fresh bacterial and fungal cultures grown in nutrient agar after reaching 0.5 McFarland standard were diluted 25 times with sterile distilled water. Hundred microliters of diluted culture were spread on the agar media uniformly. All the synthesized compounds were dissolved in 100% DMSO at 10-mg/mL concentration. All the synthesized compounds were also tested for their MIC against bacteria and fungi. Most of the compounds have the tendency of precipitating immediately with small amounts of water addition. To avoid this precipitation, MIC was determined with a modified method using 96-well microplates. Fifty microlitres of 10-mg/mL synthesized compound solution was diluted (4 times dilution) with 150 μL of DMSO in the first well (2.5 mg/mL) and 50 μL of this diluted solution further diluted 4 times in the next well containing by DMSO (0.625 mg/mL). After this, 50 μL of diluted compound from the second well was transferred to third well containing 50 μL of DMSO; in this manner, compounds were serially diluted up to 10 μg/mL of compound concentration.

Each of these serially diluted compounds was taken in a micropipette and 0.5 μL was dispensed on the bacterial/fungal lawn prepared, as explained earlier. The plates were incubated for 10–12 h and observed for inhibition (Harikrishna, Isloor, Ananda, Obaid, & Fun, 2015). The lowest concentration of compound showing the clear zone on the microbial plate is considered as the MIC of the compound.

4. Conclusions

A series of positional isomers of substituted [4-(3-phenyl-1H-pyrazol-5-yl)phenoxy]acetic acids were synthesized using a solvent-free reaction and were characterized using elemental analysis and spectral techniques. Docking studies were performed to predict their efficacy for antimicrobial action against a tuberculosis variant M. smegmatis, Gram-positive S. aureus, Gram-negative E. coli, and a fungus C. albicans. The carboxylic acids showed better binding energy with target proteins in comparison to the two pyrazolines. When the antimicrobial investigation was done, the prediction made by docking supported the experimental results, wherein the MICs of the chalcone acids were higher than those of corresponding pyrazoline acids. This was in contradiction to the higher zones of inhibition obtained for these class of compounds, thus establishing, though the molecules are promisingly potent, that they are active only at higher concentrations. This study will help other researchers design novel structures and find better drug candidates.

Supplementary material

Supplementary material for this article can be accessed here http://dx.doi.org/10.1080/23312009.2016.1141388.

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