

Antifungal and antimycobacterial activity of 1-(3,5-diaryl-4,5-dihydro-1H-pyrazol-4-yl)-1H-imidazole derivatives[☆]

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Abstract—1-(3,5-Diaryl-4,5-dihydro-1H-pyrazol-4-yl)-1H-imidazole derivatives were synthesized and tested for their in vitro antifungal and antimycobacterial activities. These imidazole derivatives showed an excellent antifungal activity against a clinical strain of *Candida albicans* and an interesting antitubercular activity against *Mycobacterium tuberculosis* H₃₇Rv reference strain.

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1. Introduction

Considering the increased incidence of severe opportunistic fungal infections in immunocompromised patients together with the development of resistance among pathogenic *Candida* spp., there is a great need for new antifungal compounds. On the other hand, the increase of tuberculosis due to emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*,^{1–4} together with the increased incidence of severe disseminated infections produced by mycobacteria other than tuberculosis (MOTT), particularly *Mycobacterium avium*⁵ in immunocompromised patients, has prompted the search for new antimycobacterial drugs.

Molecular modelling investigations confirmed that antifungal azole derivatives synthesized by us^{6,7} act interacting at the active site of the fungal cytochrome P450-dependent lanosterol 14 α -demethylase (P450_{14DM}, CYP51) but exhibit simultaneously antimycobacterial activity which may be due to a similar inhibitory interaction with the corresponding mycobacterial cytochrome. In effect, it was established, using genomic DNA from a strain of *M. tuberculosis* (MT) H₃₇Rv, that a CYP51-

like gene encodes a bacterial sterol 14 α -demethylase (MT P450_{14DM}) which acts on 14 α -methyl sterols and binds known antifungal azole derivatives.⁸ On the basis of these considerations and because many imidazole derivatives showed potent antifungal activity associated with good antimycobacterial activity,^{6,7,9,10} we synthesized a series of compounds **1a–t** (Scheme 1) in which the imidazolyl moiety, present in many azole antifungal drugs, is directly linked at 4 position of a pyrazoline ring 3,5-disubstituted **1a–e**, and 1,3,5-trisubstituted **1f–t** whose importance with respect to the antifungal^{11–14} and antimycobacterial¹⁵ activities has been previously described.

All the synthesized compounds were tested in vitro for their antifungal and antitubercular activity towards *Candida albicans* 685 and *M. tuberculosis* H₃₇Rv, respectively.

The synthesized compounds showed a high antifungal activity associated with a remarkable antimycobacterial activity.

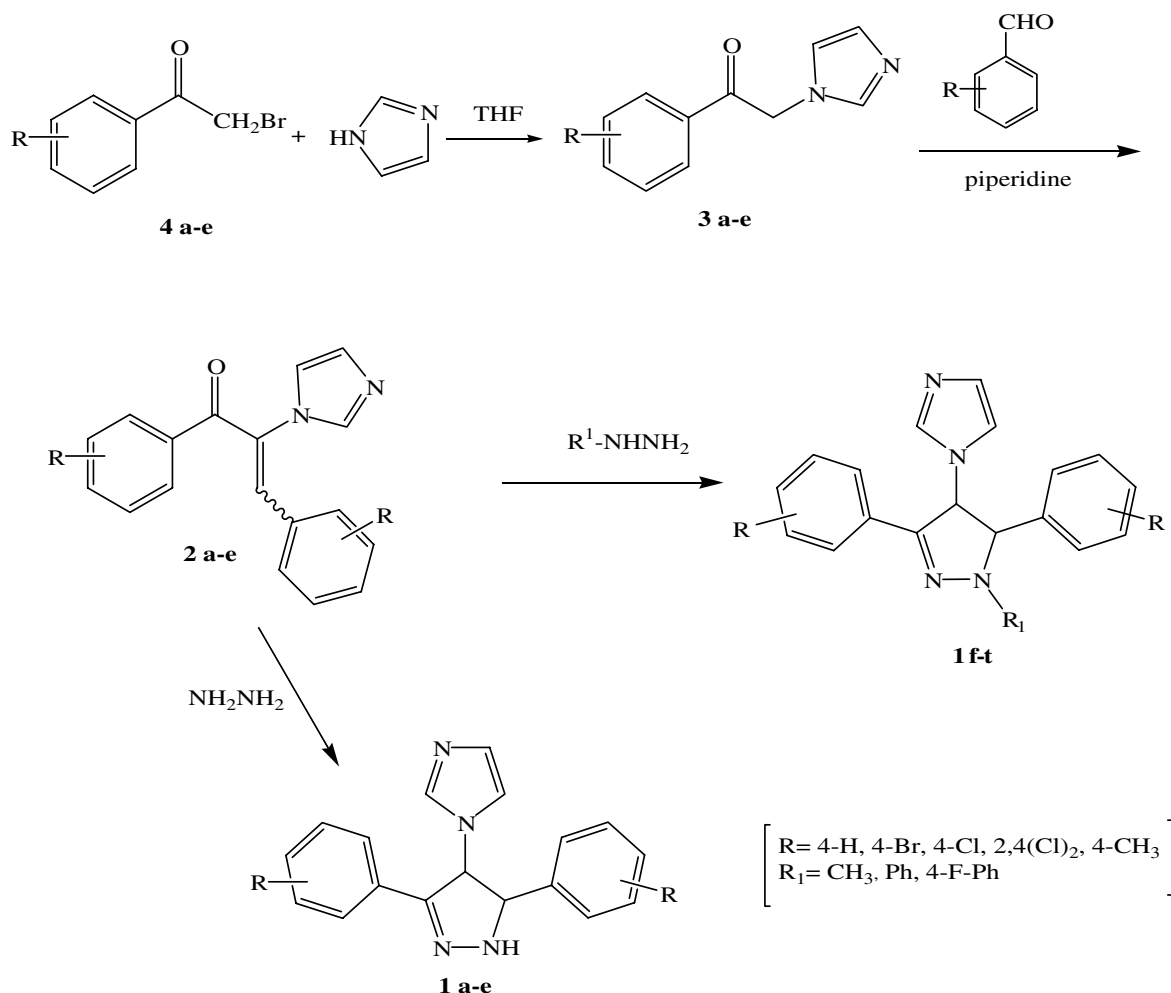
2. Chemistry

The synthesis of compounds **1a–t** was carried out via a three-step reaction which involved an N-alkylation of imidazole with the substituted-2-bromoacetophenones **4a–e** to afford the corresponding 1-aryl-2-(1H-imidazol-1-yl)-ethanones **3a–e** in accordance with the

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Scheme 1.

literature procedure⁶ followed by condensation with substituted benzaldehydes and the final cyclization of the obtained α,β -unsaturated ketones **2a–e**, with hydrazine hydrate, methylhydrazine sulfate, phenylhydrazine and 4-fluorophenylhydrazine to give the corresponding compounds **1a–e**, **1f–j**, **1k–o**, and **1p–t**, respectively.

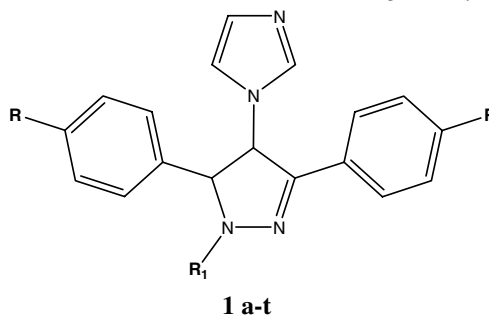
From the ¹H NMR spectra of the obtained compounds it appears that only the derivatives **1f–j** show a *cis* isomerism while all the other compounds (**1a–e**, **1k–t**) show a *trans* isomerism. The values of the coupling constants for the H₄ and H₅ pyrazoline protons for the *trans* isomers and for the *cis* isomers are in the range of 3.30–8.06 Hz and 10.37–10.99 Hz, respectively, according to the values of the coupling constants reported in the literature for other pyrazoline derivatives.^{16–18}

3. Results and discussion

A series of 1-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-4-yl)-1*H*-imidazole **1a–e** and 1-[(1-*aralkyl*)-3,5-diaryl-4,5-dihydro-1*H*-pyrazol-4-yl]-1*H*-imidazole derivatives **1f–t** were synthesized with the aim to evaluate their antifungal and antimycobacterial activities. The *in vitro*

activities (Table 1) of derivatives **1a–t** were tested against a clinical strain of *C. albicans* 685 in comparison with miconazole and amphotericin B, and against a strain of *M. tuberculosis* H₃₇Rv in comparison with isoniazid.

The antifungal activity of compounds **1g**, **1h**, **1k** and **1p** was remarkable reaching MIC values of 0.06 $\mu\text{g/mL}$ even at 48 h (**1g**, **1h**). Their activities were superior than that of the reference drugs miconazole and amphotericin B. From the obtained data it seems that the presence of the electron-accepting chloro or bromo groups in the *para* position at the phenyl residues enhance the activity (**1g**, **1h**), as same as the electron-donating methyl group at N-1 position of the pyrazoline moiety, even if the 3,5-diphenyl derivatives **1k** and **1p**, bearing a phenyl or 4-fluorophenyl substituent at the N-1 position of the pyrazoline cycle maintain a very high potency. On the other hand, the presence of the electron-donating methyl group at the *para* position of phenyl residues, reduces the activity of compounds **1e**, **1j**, **1o** and **1t**. Moreover, compounds **1a–t** were tested against a strain of *M. tuberculosis* H₃₇Rv and showed a good antimycobacterial activity reaching MIC values of 4 $\mu\text{g/mL}$ for 6 compounds of the series. In this case the substitution at

Table 1. Activity of compounds **1 a-t** against *Candida albicans* 685 clinical strain and against *Mycobacterium tuberculosis* reference strain H₃₇Rv

Compound	R	R ₁	<i>C. albicans</i> 685		H ₃₇ Rv
			MIC µg/mL after 24 h	MIC µg/mL after 48 h	
Miconazole	—	—	0.06	0.12	—
Amphotericin B	—	—	1	1	—
Isoniazid	—	—	—	—	0.5
1a	H	H	16	>64	16
1b	Br	H	4	8	8
1c	Cl	H	4	16	8
1d	2,4-(Cl) ₂	H	8	64	8
1e	CH ₃	H	8	64	16
1f	H	CH ₃	1	64	8
1g	Br	CH ₃	0.06	0.06	8
1h	Cl	CH ₃	0.06	0.06	8
1i	2,4-(Cl) ₂	CH ₃	0.12	32	16
1j	CH ₃	CH ₃	4	64	16
1k	H	Ph	0.06	1	8
1l	Br	Ph	1	64	8
1m	Cl	Ph	2	64	4
1n	2,4-(Cl) ₂	Ph	2	>64	4
1o	CH ₃	Ph	8	64	4
1p	H	4-F-Ph	0.06	1	8
1q	Br	4-F-Ph	8	>64	4
1r	Cl	4-F-Ph	2	>64	4
1s	2,4-(Cl) ₂	4-F-Ph	1	>64	16
1t	CH ₃	4-F-Ph	64	64	4

N-1 position of the pyrazoline ring with a phenyl or 4-fluorophenyl moiety, rather than a methyl or hydrogen, improves the antimycobacterial activity of compounds **1m**, **1n**, **1q**, **1r** and **1t**. The substitution at the phenyl groups at 3, 5 position of the pyrazole ring, did not appear to be influent for the activity.

According to the results obtained for other imidazole derivatives,^{6,7,9,10,19} the newly synthesized imidazole derivatives showed both in vitro antifungal and antimycobacterial property, suggesting an inhibitory interaction of these compounds with similar enzymatic sites present in fungi and mycobacteria.

4. Experimental

Mps were determined with a Buchi 510 capillary apparatus, and are uncorrected. Infrared spectra in Nujol mulls were recorded on a Jasco FT 200 spectrophotometer. Proton nuclear magnetic resonance (¹H NMR) spectra and carbon nuclear magnetic resonance (¹³C NMR) spectra were determined on a Varian Gemini 200 spectrometer, chemical shifts are reported

as δ (ppm) in CDCl₃ solution (0.05% v/v TMS). Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F₂₅₄ Merck plates. ESI-MS spectra were obtained on a PE-API I spectrometer by infusion of a solution of the sample in MeOH. Elemental analyses (C, H, N) were performed on a Carlo Erba analyzer and were within ±0.3 of the theoretical value.

4.1. Synthesis

4.1.1. 1,3-Bis(4-bromophenyl)-2-(1H-imidazol-1-yl)-prop-2-en-1-one (2b). To a solution of 1-(4-bromophenyl)-2-(1H-imidazol-1-yl)-ethanone **3b** (2.5 g, 9.4 mmol) in 80 mL of toluene, 4-bromobenzaldehyde (1.74 g, 9.4 mmol) and piperidine (0.8 g, 9.4 mmol) were added. The reaction mixture was stirred under reflux for 16 h. The solvent was removed under reduced pressure and the residue was extracted with CHCl₃ (3 × 100 mL) and the organic phase was washed with distilled water. The collected organic phases were dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was crystallized from diethyl ether. Mp 148–150 °C; yield 65%. IR (Nujol, cm⁻¹): 1655. ¹H

NMR (CDCl₃/TMS): δ 6.84 (s, 1H, =CH–), 6.87 (s, 1H, CH imid.), 6.99–7.88 (m, 10H, arom. and imidazole). MS: m/z 431 [MH⁺], 433 [MH⁺+2]. Anal. Calcd for C₁₈H₁₂N₂OBr₂ (MW 432.11): C, 50.03; H, 2.80; N, 6.48%; found: C, 49.80; H, 2.95; N, 6.60%.

In an analogous way compounds **2a** and **2c–e** were obtained.

4.1.2. 2-(1H-Imidazol-1-yl)-1,3-diphenylprop-2-en-1-one (2a).²⁰ Mp 109 °C; yield 32%. Solvent crystallization: ethyl acetate. IR (Nujol, cm^{−1}): 1665. ¹H NMR (CDCl₃/TMS): δ 6.84 (s, 1H, =CH–), 6.87 (s, 1H, CH imid.), 6.99–7.88 (m, 12H, arom. and imid.). MS: m/z 275 [MH⁺]. Anal. Calcd for C₁₈H₁₄N₂O (MW 274.32): C, 78.81; H, 5.14; N, 10.21%; found: C, 78.70; H, 5.19; N, 10.07%.

4.1.3. 1,3-Bis(4-chlorophenyl)-2-(1H-imidazol-1-yl)-prop-2-en-1-one (2c).²⁰ Mp 123 °C; yield 49%. IR (Nujol, cm^{−1}): 1656. ¹H NMR (CDCl₃/TMS): δ 6.84 (s, 1H, =CH–), 6.87 (s, 1H, CH imid.), 6.99–7.88 (m, 10H, arom. and imidazole). MS: m/z 343 [MH⁺], 345 [MH⁺+2]. Anal. Calcd for C₁₈H₁₂N₂OCl₂ (MW 343.21): C, 62.99; H, 3.52; N, 8.16%; found: C, 62.80; H, 3.35; N, 8.30%.

4.1.4. 1,3-Bis(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)-prop-2-en-1-one (2d). Mp 119 °C; yield 41%. IR (Nujol, cm^{−1}): 1678. ¹H NMR (CDCl₃/TMS): δ 6.84 (s, 1H, =CH–), 6.87 (s, 1H, CH imid.), 6.99–7.88 (m, 8H, arom. and imidazole). MS: m/z 411 [MH⁺], 413 [MH⁺+2]. Anal. Calcd for C₁₈H₁₀N₂OCl₄ (MW 412.11): C, 52.46; H, 2.45; N, 6.80%; found: C, 52.40; H, 2.35; N, 6.80%.

4.1.5. 1,3-Bis(4-methylphenyl)-2-(1H-imidazol-1-yl)-prop-2-en-1-one (2e). Mp 122 °C; yield 40%. IR (Nujol, cm^{−1}): 1648. ¹H NMR (CDCl₃/TMS): δ 2.26 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 6.60–7.75 (m, 12H, =CH–, arom. and imidazole). MS: m/z 303 [MH⁺]. Anal. Calcd for C₂₀H₁₈N₂O (MW 302.37): C, 79.44; H, 6.00; N, 9.26%; found: C, 79.50; H, 6.05; N, 9.00%.

4.1.6. (±) trans-1-(4,5-Dihydro-3,5-diphenyl-1H-pyrazol-4-yl)-1H-imidazole (1a). To an ethanolic (20 mL) solution of **2a** (0.6 g, 2.1 mmol), hydrazine hydrate (98% sol., 0.13 g, 2.6 mmol) was added under stirring. The reaction was allowed to stirring at room temperature and monitored by TLC. The solvent was evaporated under reduced pressure and the solid obtained was crystallized from diethyl ether. Mp 124–126 °C; yield 32%. IR (Nujol, cm^{−1}): 3350. ¹H NMR (CDCl₃/TMS): δ 4.75 (d, 1H, CH, H₅ pyrazole, J_{H5-H4} = 4.70 Hz); 5.58 (d, 1H, CH, H₄ pyrazole, J_{H4-H5} = 4.70 Hz); 6.50 (s, broad signal, 1H, NH disappearing on deuteration); 7.09–8.44 (m, 13H, 10H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 69.4, 72.5, 117.2, 125.6, 126.2, 129.0, 129.1, 129.2, 129.4, 130.7, 132.0, 136.8, 139.4, 145.7. MS: m/z 289 [MH⁺]. Anal. Calcd for C₁₈H₁₆N₄ (MW 288.35): C, 74.98; H, 5.59; N, 19.43%; found: C, 75.14; H, 5.37; N, 19.58%.

Compounds **1b–e** were obtained similarly.

4.1.7. (±) trans-1-[3,5-Bis-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-4-yl]-1H-imidazole (1b). Mp 173–175 °C; yield 71%. IR (Nujol, cm^{−1}): 3348. ¹H NMR (CDCl₃/TMS): δ 4.79 (dd, 1H, CH, H₅ pyrazole, J_{H5-H4} = 5.86 Hz; J_{H5-NH} = 2.20 Hz); 5.54 (d, 1H, CH, H₄ pyrazole, J_{H4-H5} = 5.86 Hz); 6.46 (d, 1H, NH disappearing on deuteration; J_{NH-H5} = 2.20 Hz); 6.94–7.64 (m, 11H, 8H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 70.1, 73.2, 116.9, 125.8, 126.4, 127.8, 128.6, 129.5, 129.7, 130.7, 131.8, 136.8, 139.4, 145.7. MS: m/z 445 [MH⁺], 447 [MH⁺+2]. Anal. Calcd for C₁₈H₁₄N₄Br₂ (MW 446.14): C, 48.36; H, 3.16; N, 12.56%; found: C, 48.15; H, 3.37; N, 12.58%.

4.1.8. (±) trans-1-[3,5-Bis-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-4-yl]-1H-imidazole (1c). Mp 175–177 °C; yield 15%. IR (Nujol, cm^{−1}): 3289. ¹H NMR (CDCl₃/TMS): δ 4.80 (d, 1H, CH, H₅ pyrazole, J_{H5-H4} = 6.10 Hz); 5.54 (d, 1H, CH, H₄ pyrazole, J_{H4-H5} = 6.10 Hz); 6.41 (s, broad signal, 1H, NH disappearing on deuteration); 6.91–7.57 (m, 11H, 8H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 70.0, 73.1, 117.4, 126.4, 127.2, 129.2, 129.5, 129.7, 130.7, 131.8, 134.4, 136.8, 139.4, 145.7. MS: m/z 357 [MH⁺], 359 [MH⁺+2]. Anal. Calcd for C₁₈H₁₄N₄Cl₂ (MW 357.24): C, 60.52; H, 3.95; N, 15.68%; found: C, 60.55; H, 3.90; N, 15.40%.

4.1.9. (±) trans-1-[3,5-Bis-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-4-yl]-1H-imidazole (1d). Mp 155–157 °C; yield 13%. IR (Nujol, cm^{−1}): 3330. ¹H NMR (CDCl₃/TMS): δ 5.34 (dd, 1H, CH, H₅ pyrazole, J_{H5-H4} = 8.06 Hz; J_{H5-NH} = 2.93 Hz); 6.32 (d, 1H, CH, H₄ pyrazole, J_{H4-H5} = 8.06 Hz); 6.41 (d, 1H, NH disappearing on deuteration, J_{NH-H5} = 2.93 Hz); 6.56–7.75 (m, 9H, 6H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 62.4, 65.5, 116.1, 127.6, 127.8, 128.0, 129.3, 129.7, 129.9, 130.3, 130.6, 130.8, 131.4, 131.7, 136.3, 136.4, 140.7, 152.7. MS: m/z 425 [MH⁺], 427 [MH⁺+2]. Anal. Calcd for C₁₈H₁₂N₄Cl₄ (MW 426.13): C, 50.73; H, 2.84; N, 13.15%; found: C, 50.55; H, 2.90; N, 13.40%.

4.1.10. (±) trans-1-[4,5-Dihydro-3,5-bis-(4-methylphenyl)-1H-pyrazol-4-yl]-1H-imidazole (1e). Mp 158–160 °C; yield 58%. IR (Nujol, cm^{−1}): 3318. ¹H NMR (CDCl₃/TMS): δ 2.31 (s, 3H, CH₃); 2.35 (s, 3H, CH₃); 4.75 (d, 1H, CH, H₅ pyrazole, J_{H5-H4} = 4.88 Hz); 5.58 (d, 1H, CH, H₄ pyrazole, J_{H4-H5} = 4.88 Hz); 6.30 (s, broad signal, 1H, NH disappearing on deuteration); 6.90–7.65 (m, 11H, 8H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 21.3, 21.6, 70.3, 72.9, 117.8, 125.6, 127.4, 128.1, 129.5, 129.6, 130.0, 134.4, 136.7, 138.7, 139.8, 145.5. MS: m/z 317 [MH⁺]. Anal. Calcd for C₂₀H₂₀N₄ (MW 316.13): C, 75.92; H, 6.87; N, 17.71%; found: C, 75.80; H, 6.20; N, 17.65%.

4.1.11. (±) cis-1-(4,5-Dihydro-3,5-diphenyl-1-methyl-1H-pyrazol-4-yl)-1H-imidazole (1f). To an ethanolic (60 mL) solution of **2a** (0.5 g, 1.8 mmol), triethylamine (0.18 g, 1.8 mmol) and methylhydrazine sulfate (0.26 g, 1.8 mmol) were added under stirring. The reaction mixture was allowed to reflux for 3 h. The solvent was evap-

orated under reduced pressure and the residue was extracted with CHCl_3 and washed with distilled water. The collected organic phases were dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was then purified by column chromatography (eluted with CH_2Cl_2) to afford an oil. Yield 43%. ^1H NMR (CDCl_3/TMS): δ 2.99 (s, 3H, CH_3); 4.20 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 10.98$ Hz); 5.63 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 10.98$ Hz); 6.90–7.52 (m, 13H, 10H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 41.3, 71.5, 80.6, 117.7, 125.5, 126.4, 128.8, 129.0, 129.2, 129.4, 130.6, 132.3, 136.8, 139.4, 146.2. MS: m/z 303 [MH^+]. Anal. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_4$ (MW 302.40): C, 74.47; H, 6.00; N, 18.53%; found: C, 74.24; H, 6.17; N, 18.48%.

Compounds **1g–j** were obtained similarly.

4.1.12. (\pm) *cis*-1-[3,5-Bis(4-bromophenyl)-(4,5-dihydro-1-methyl-1H-pyrazol-4-yl)-1H-imidazole (1g**).** Mp 148–150 °C; yield 34%. ^1H NMR (CDCl_3/TMS): δ 2.96 (s, 3H, CH_3); 4.16 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 10.98$ Hz); 5.55 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 10.98$ Hz); 6.92–7.60 (m, 11H, 8H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 41.5, 71.3, 80.7, 117.3, 125.8, 126.3, 127.7, 128.7, 129.5, 129.8, 130.9, 131.9, 136.7, 139.4, 145.9. MS: m/z 359 [MH^+], 361 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{Br}_2$ (MW 360.17): C, 49.59; H, 3.50; N, 12.18%; found: C, 49.30; H, 3.33; N, 12.05%.

4.1.13. (\pm) *cis*-1-[3,5-Bis(4-chlorophenyl)-(4,5-dihydro-1-methyl-1H-pyrazol-4-yl)-1H-imidazole (1h**).** Mp 134–136 °C; yield 89%. ^1H NMR (CDCl_3/TMS): δ 2.95 (s, 3H, CH_3); 4.17 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 10.98$ Hz); 5.54 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 10.98$ Hz); 6.88–7.50 (m, 11H, 8H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 41.5, 71.3, 80.7, 117.4, 126.4, 127.0, 129.3, 129.5, 129.7, 130.7, 131.9, 134.5, 136.8, 139.9, 145.8. MS: m/z 371 [MH^+], 373 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{Cl}_2$ (MW 371.26): C, 61.47; H, 4.34; N, 15.09%; found: C, 61.35; H, 4.27; N, 15.00%.

4.1.14. (\pm) *cis*-1-[3,5-Bis(2,4-dichlorophenyl)-4,5-dihydro-1-methyl-1H-pyrazol-4-yl]-1H-imidazole (1i**).** Mp 173–175 °C; yield 44%. ^1H NMR (CDCl_3/TMS): δ 3.01 (s, 3H, CH_3); 4.87 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 10.37$ Hz); 6.00 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 10.37$ Hz); 6.80–7.60 (m, 9H, 6H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 41.3, 70.9, 80.9, 116.8, 127.6, 127.9, 128.1, 129.4, 129.7, 130.0, 130.3, 130.6, 130.7, 131.4, 131.8, 136.0, 136.4, 140.8, 152.5. MS: m/z 439 [MH^+], 441 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{Cl}_4$ (MW 440.15): C, 58.85; H, 3.21; N, 12.73%; found: C, 58.75; H, 3.20; N, 12.50%.

4.1.15. (\pm) *cis*-1-[4,5-Dihydro-1-methyl-3,5-bis(4-methylphenyl)-1H-pyrazol-4-yl]-1H-imidazole (1j**).** Oil; yield 35%. ^1H NMR (CDCl_3/TMS): δ 2.31 (s, 3H, CH_3); 2.39 (s, 3H, CH_3); 2.96 (s, 3H, $\text{N}-\text{CH}_3$); 4.14 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 10.98$ Hz); 5.58 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 10.98$ Hz); 6.90–7.54 (m,

11H, 8H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 21.4, 21.6, 41.4, 71.3, 80.7, 117.7, 125.4, 127.1, 128.1, 129.5, 129.6, 130.0, 130.8, 134.4, 136.7, 138.7, 144.5. MS: m/z 331 [MH^+]. Anal. Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_4$ (MW 330.41): C, 76.33; H, 6.71; N, 19.96%; found: C, 76.45; H, 6.80; N, 20.07%.

4.1.16. (\pm) *trans*-(4,5-Dihydro-1,3,5-triphenyl-1H-pyrazol-4-yl)-1H-imidazole (1k**).** To an ethanolic (60 mL) solution of **2a** (0.3 g, 1.1 mmol), phenylhydrazine (0.14 g, 1.3 mmol) was added under stirring. The reaction mixture was refluxed for 3 h and monitored by TLC. The mixture was allowed to crystallized from ethanol to afford a white solid. Mp 183–185 °C; yield 38%. ^1H NMR (CDCl_3/TMS): δ 5.16 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 3.63$ Hz); 5.66 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 3.63$ Hz); 6.82–7.70 (m, 18H, 15H phen. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 70.0, 73.3, 113.7, 117.1, 120.8, 125.7, 125.8, 128.9, 129.1, 129.3, 129.4, 129.9, 130.7, 131.3, 135.9, 138.3, 142.5, 143.1. MS: m/z 365 [MH^+]. Anal. Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_4$ (MW 364.44): C, 79.10; H, 5.53; N, 15.37%; found: C, 78.94; H, 5.37; N, 15.48%.

Compounds **1l–o** were obtained similarly.

4.1.17. (\pm) *trans*-1-[3,5-Bis(4-bromophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-4-yl]-1H-imidazole (1l**).** Mp 182–184 °C; yield 34%. ^1H NMR (CDCl_3/TMS): δ 5.13 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 3.30$ Hz); 5.61 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 3.30$ Hz); 6.85–7.70 (m, 16H, 13H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 70.2, 73.4, 114.0, 117.1, 120.6, 125.8, 126.4, 128.2, 128.6, 129.2, 129.5, 129.7, 130.7, 131.8, 136.8, 139.4, 142.3, 145.7. MS: m/z 521 [MH^+], 523 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{24}\text{H}_{18}\text{N}_4\text{Br}_2$ (MW 522.23): C, 55.20; H, 3.47; N, 10.73%; found: C, 55.15; H, 3.37; N, 10.68%.

4.1.18. (\pm) *trans*-1-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-4-yl]-1H-imidazole (1m**).** Mp 183–185 °C; yield 28%. ^1H NMR (CDCl_3/TMS): δ 5.18 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 3.40$ Hz); 5.64 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 3.40$ Hz); 6.85–7.72 (m, 16H, 13H arom. and 3H imidazole); δ 71.0, 73.3, 114.3, 117.7, 120.6, 125.8, 128.2, 128.6, 129.2, 129.5, 129.7, 130.7, 131.8, 132.5, 136.8, 139.5, 142.3, 145.7. MS: m/z 433 [MH^+], 435 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{24}\text{H}_{18}\text{N}_4\text{Cl}_2$ (MW 433.33): C, 66.52; H, 4.19; N, 12.93%; found: C, 66.75; H, 4.42; N, 12.88%.

4.1.19. (\pm) *trans*-1-[3,5-Bis(2,4-dichlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-4-yl]-1H-imidazole (1n**).** Mp 163–165 °C; yield 16%. ^1H NMR (CDCl_3/TMS): δ 5.70 (d, 1H, CH, H_5 pyrazole, $J_{\text{H}_5-\text{H}_4} = 3.50$ Hz); 5.97 (d, 1H, CH, H_4 pyrazole, $J_{\text{H}_4-\text{H}_5} = 3.50$ Hz); 6.83–7.77 (m, 14H, 11H arom. and 3H imidazole). ^{13}C NMR (CDCl_3/TMS): δ 66.4, 69.5, 114.9, 117.4, 120.0, 125.5, 127.5, 127.9, 128.4, 130.6, 130.9, 131.1, 131.3, 131.5, 132.0, 133.1, 133.5, 135.8, 135.9, 142.0, 155.6, 160.4. MS: m/z 501 [MH^+], 503 [$\text{MH}^+ + 2$]. Anal. Calcd for $\text{C}_{24}\text{H}_{16}\text{N}_4\text{Cl}_4$ (MW 502.22): C, 57.40; H, 3.21; N, 11.16%; found: C, 57.35; H, 3.42; N, 11.18%.

4.1.20. (±) *trans*-1-[4,5-Dihydro-3,5-bis-(4-methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1*H*-imidazole (1o). Mp 184–186 °C; yield 25%. ¹H NMR (CDCl₃/TMS): δ 2.35 (s, 3H, CH₃); 2.39 (s, 3H, CH₃); 5.13 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 3.45 Hz); 5.65 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 3.45 Hz); 6.80–7.70 (m, 16H, 13H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 21.4, 21.6, 70.1, 73.0, 113.7, 117.1, 120.4, 125.5, 125.7, 127.9, 129.3, 129.7, 130.5, 131.2, 135.4, 135.9, 138.7, 139.4, 142.6, 143.3. MS: *m/z* 393 [MH⁺]. Anal. Calcd for C₂₆H₂₄N₄ (MW 392.50): C, 79.56; H, 6.16; N, 14.27%; found: C, 79.70; H, 6.12; N, 14.33%.

4.1.21. (±) *trans*-1-(4,5-Dihydro-3,5-diphenyl-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1*H*-imidazole (1p). To an ethanolic (60 mL) solution of **2a** (0.4 g, 1.46 mmol), triethylamine (0.16 g, 1.46 mmol) and 4-fluorophenylhydrazine hydrochloride (0.24 g, 1.46 mmol) were added under stirring. The reaction mixture was allowed to reflux for 5 h. The solvent was evaporated under reduced pressure and the residue was extracted with CHCl₃ and washed with distilled water. The collected organic phases were dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was then purified by column chromatography (eluted with CH₂Cl₂) and crystallized from diethyl ether to afford a white solid. Mp 173–175 °C; yield 36%. ¹H NMR (CDCl₃/TMS): δ 5.11 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 4.29 Hz); 5.67 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 4.29 Hz); 6.87–7.60 (m, 17H, 14H phen. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 70.3, 73.7, 114.9, 115.1, 115.6, 116.3, 117.2, 125.7, 126.0, 129.1, 129.2, 129.4, 130.0, 130.7, 131.5, 136.0, 138.1, 139.7, 142.7, 155.4, 160.2. MS: *m/z* 383 [MH⁺]. Anal. Calcd for C₂₄H₁₉N₄F (MW 382.43): C, 75.37; H, 5.01; N, 14.65%; found: C, 75.54; H, 5.16; N, 14.48%.

Compounds **1q–t** were obtained similarly.

4.1.22. (±) *trans*-1-(3,5-Bis(4-bromophenyl)-4,5-dihydro-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1*H*-imidazole (1q). Mp 183–185 °C; yield 30%. ¹H NMR (CDCl₃/TMS): δ 5.07 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 4.27 Hz); 5.61 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 4.27 Hz); 6.85–7.72 (m, 15H, 12H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 70.2, 73.1, 115.2, 115.3, 115.6, 116.3, 117.4, 125.8, 126.4, 129.1, 129.5, 129.7, 130.7, 131.8, 136.9, 142.2, 155.4, 160.0. MS: *m/z* 539 [MH⁺], 541 [MH⁺+2]. Anal. Calcd for C₂₄H₁₇N₄Br₂F (MW 540.22): C, 53.36; H, 3.17; N, 10.37%; found: C, 53.34; H, 3.16; N, 10.37%.

4.1.23. (±) *trans*-1-(3,5-Bis(4-chlorophenyl)-4,5-dihydro-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1*H*-imidazole (1r). Mp 180–183 °C; yield 15%. ¹H NMR (CDCl₃/TMS): δ 5.10 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 4.30 Hz); 5.63 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 4.30 Hz); 6.85–7.65 (m, 15H, 12H arom. and 3H imidazole). δ 70.0, 72.8, 114.8, 115.4, 115.5, 116.6, 117.6, 129.2, 129.5, 129.7, 130.7, 131.5, 131.8, 134.4, 137.2, 142.4, 155.0, 158.9. MS: *m/z* 451 [MH⁺], 453 [MH⁺+2]. Anal. Calcd for C₂₄H₁₇N₄Cl₂F (MW 451.32): C, 63.87; H, 3.80; N, 12.41%; found: C, 63.70; H, 3.60; N, 12.35%.

4.1.24. (±) *trans*-1-(3,5-Bis(2,4-dichlorophenyl)-4,5-dihydro-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1*H*-imidazole (1s). Mp 123–125 °C; yield 27%. ¹H NMR (CDCl₃/TMS): δ 5.66 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 4.34 Hz); 5.95 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 4.34 Hz); 6.84–7.65 (m, 13H, 10H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 67.2, 69.6, 114.7, 115.0, 116.2, 116.7, 117.3, 117.5, 127.5, 127.9, 128.5, 130.6, 130.9, 131.1, 131.4, 131.5, 132.0, 133.0, 133.6, 135.7, 136.0, 142.1, 155.7, 160.5. MS: *m/z* 519 [MH⁺], 521 [MH⁺+2]. Anal. Calcd for C₂₄H₁₅N₄Cl₄F (MW 520.21): C, 55.41; H, 2.91; N, 10.77%; found: C, 55.40; H, 2.88; N, 10.65%.

4.1.25. (±) *trans*-1-[4,5-Dihydro-1-(4-fluorophenyl)-3,5-bis-(4-methylphenyl)-1*H*-pyrazol-4-yl]-1*H*-imidazole (1t). Mp 175–178 °C; yield 29%. ¹H NMR (CDCl₃/TMS): δ 2.34 (s, 3H, CH₃); 2.36 (s, 3H, CH₃); 5.07 (d, 1H, CH, H₅ pyrazole, *J*_{H5-H4} = 4.39 Hz); 5.65 (d, 1H, CH, H₄ pyrazole, *J*_{H4-H5} = 4.39 Hz); 6.85–7.65 (m, 15H, 12H arom. and 3H imidazole). ¹³C NMR (CDCl₃/TMS): δ 21.3, 21.6, 70.1, 72.7, 114.8, 115.1, 116.3, 116.7, 117.3, 128.8, 129.6, 129.7, 130.7, 131.5, 134.4, 137.2, 140.0, 142.4, 155.3, 159.8. MS: *m/z* 411 [MH⁺]. Anal. Calcd for C₂₆H₂₃N₄F (MW 410.49): C, 76.08; H, 5.65; N, 13.65%; found: C, 76.00; H, 5.72; N, 13.90%.

4.2. Microbiology

All the new imidazole derivatives were evaluated for antifungal activity against *C. albicans* 685, clinical isolate; for antitubercular activity against the reference strain *M. tuberculosis* H₃₇Rv. Stock solutions of chemicals were prepared in DMSO at a concentration of 2 mg/mL. Antifungal activity was always evaluated by reference methods (NCCLS, 1997); miconazole and amphotericin B were chosen as a standard in antifungal activity measurements, each MIC was determined twice in duplicate experiments after 24 and 48 h incubation time. Antitubercular activity was evaluated by MRA, a recently developed, 1-week duration, micro-dilution Resazurin assay.²¹ The minimum inhibitory concentration, MIC, was defined as the lowest drug concentration that prevented Resazurin colour change from blue to pink and was determined by visual inspection twice in duplicate experiments; viable counting from control wells and from test wells performed onto agar plates confirmed bactericidal and bacteriostatic activity of compounds. Isoniazid was always included as a standard in antitubercular activity measurements, having a MIC of 0.5 µg/mL; DMSO was also evaluated and was always devoid of inhibiting activity up to the concentration of 2% (v/v). The antimicrobial activity of the compounds **1a–t** is reported in Table 1.

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