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Synthesis and anti-microbial activity of pyrazolylbisindoles—Promising anti-fungal compounds

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Abstract—A series of pyrazolylbisindole derivatives have been synthesized by reacting substituted pyrazole aldehydes with substituted indoles using phosphotungstic acid, a Keggin type heteropoly acid as catalyst. The synthesized pyrazolylbisindoles were evaluated for anti-microbial activities. The effect of pyrazolylbisindoles on the mycelial growth of plant pathogenic fungi is revealed. Entries **3c** and **3d** emerged as the most interesting compounds in this series exhibiting excellent anti-fungal activity.

Bisindolyl metabolites affect the central nervous system and are used as tranquilizers.[1] Various indolyl derivatives display diverse pharmacological activities and are useful in the treatment of fibromyalgia, chronic fatigue and irritable bowel syndrome.[2] Vibrindole, a bisindolylmethane, was demonstrated to exhibit anti-bacterial activity. Pyrazole nucleus has pronounced pharmacological applications as anti-anxiety, anti-pyretic, analgesic and anti-inflammatory drugs. Certain pyrazoles show significant bacteriostatic, bactericidal and fungicidal activities, antiviral/antitumour activities. In continuation of our work in the biological activities of pyrazole moiety[3] we herein report the screening results of the anti-bacterial and anti-fungal activities of pyrazolylbisindoles. The present study was carried out to investigate the anti-bacterial and anti-fungal inhibitions of pyrazolylbisindoles.

The electrophilic substitution reaction of indoles with aromatic aldehydes yields the corresponding bisindolylmethanes.[4] Protic acids,[5] Lewis acids,[6] clays,[7] LDPE,[8] iodine,[9] amberlyst[10] as well as ionic liquids[11] are known to promote these reactions. However, many Lewis acids are deactivated or sometimes decomposed by nitrogen containing reactants. Even when the desired reactions proceed, more than stoichiometric amounts of Lewis acids are required because the acids are trapped by nitrogen. Many of the procedures involve expensive reagents,[12] long reaction time,[13] and low yield of the products,[14] strongly acidic conditions and cumbersome experimental and product isolation procedures.[15]

In this paper, we report the synthesis of pyrazolyl bisindoles using readily available phosphotungstic acid, Keggin type heteropoly acid. Among a wide variety of heteropoly acids, the Keggin is the most stable and more easily available. The phosphotungstic acid has the highest acid strength and fairly high thermal stability in the series.[16] Heteropoly acids are stable, relatively nontoxic, crystalline and preferable with regard to safety and ease of handling. The heteropoly acids are efficient catalysts for the synthesis of vitamins E, K1 and C,[17] pinacol rearrangement,[18] esterification,[19] and cyclotrimerisation of aldehydes,[20] Prins reaction,[21] Friedel-Crafts reaction and Beckman rearrangement under mild conditions.[22] Silica supported phosphotungstic acid was found to be an active and recyclable catalyst for the Diels–Alder reaction.[23]

In view of the emerging importance of the use of heterogeneous solid acids as reusable catalysts in organic synthesis, we wish to disclose a simple and efficient procedure for the synthesis of pyrazolylbisindoles using phosphotungstic acid, a Keggin type heteropoly acid. Accordingly, treatment of pyrazolyl aldehyde[24] (**1a**–**h**) with substituted indoles (**2a**–**h**) in the presence of phosphotungstic acid resulted in the formation of pyrazolylbi-

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sindoles (**3a**–**h**) in 93% yield (Scheme 1). The structure of each of the compounds was identified from spectroscopic data.[25]

The scope and generality of this procedure is illustrated with respect to various substituted pyrazole aldehydes and indoles and the results are summarized in Table 1.

The catalyst offers several advantages including mild reaction conditions, cleaner reactions, shorter reaction times, high yield of the products as well as simple experimental and isolation procedures, which make it useful for the synthesis of bisindolylmethanes.

In the present study, anti-microbial activities of eight different newly synthesized pyrazlolylbisindoles were evaluated[26] against three human pathogens such as *Candida albicans* (yeast), and *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (bacteria). Anti-fungal activity of these compounds was also tested against two plant pathogenic fungi viz., *Rhizoctonia solani* and *Curvularia lunata* under in vitro condition. The biological screening results of pyrazoylbisindoles with 10% DMSO as a control for anti-microbial inhibition are tabulated below (Tables 2 and 3).

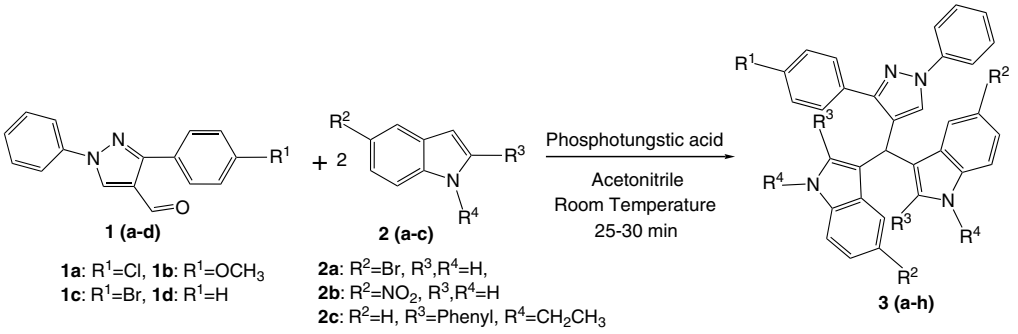
Effect of pyrazolylbisindoles on the growth of human pathogens. Out of the eight compounds, seven exhibited different levels of inhibitory effects against three human pathogens namely *C. albicans*, *S. epidermidis* and *P. aeruginosa* at a concentration of 1 mmol. The compound **3e** (0.622 mg/mL) significantly inhibited the growth of human pathogens compared to rest of the compounds and control. The inhibition ranged from 20% to 30% as compared to control. Excluding the compound **3b** other compounds inhibited the growth of one or more human pathogens ranging from 6.7% to 24.4% (Table 2).

Table 2. Effect of pyrazolylbisindoles on the growth of human pathogens

Compound	Zone of inhibition (cm)		
	C. albicans	*S. epidermidis*	*P. aeruginosa*
3a	0.6^{c}(6.7)	0.0^{d}(0.0)	0.0^{d}(0.0)
3b	0.0^{d}(0.0)	0.0^{d}(0.0)	0.0^{d}(0.0)
3c	0.0^{d}(0.0)	0.0^{d}(0.0)	1.4^{b}(15.6)
3d	2.2^{b}(24.4)	0.0^{d}(0.0)	1.5^{b}(16.7)
3e	2.7^{a}(30.0)	2.1^{a}(23.3)	1.8^{a}(20.0)
3f	0.0^{d} (0.0)	0.0^{d}(0.0)	1.3^{c}(14.4)
3g	0.5^{c}(5.6)	1.4^{b}(15.6)	1.4^{b} (15.6)
3h	0.0^{d}(0.0)	0.7^{c}(7.9)	0.0^{d}(0.0)
Control	0.0^{d}(0.0)	0.0^{d}(0.0)	0.0^{d}(0.0)
CD (0.05%)	0.11	0.08	0.12

In a column, means followed by the same letter do not differ significantly (LSD test; $P \leqslant 0.05$). Figures in brackets are % inhibition as compared to control.

Effect of pyrazolylbisindoles on the growth of plant pathogenic fungi. Among the eight compounds, seven exhibited anti-fungal activity against both the plant pathogens ranged from 13.3% to 96.7% at a concentration of 1 mmol. Among the eight compounds tested, the compound **3c** (0.652 mg/mL) significantly inhibited *C. lunata* (96.7%) and *R. solani* (93.9%) compared to rest of the compounds and control. The compound **3d** was rated as the second best with remarkable anti-fungal activity. Among the eight compounds evaluated, **3f** did not show any anti-fungal activity (Table 3).



Scheme 1. Synthesis of pyrazolylbisindoles.

Table 1. Synthesis of pyrazolylbisindoles

S.No.	Compound	From	R^1	R^2	R^3	R^4	Time (min)	Yield (%)
1	**3a**	**(1a + 2a)**	Cl	Br	H	H	25	90
2	**3b**	**(1b + 2b)**	OCH_3	NO_2	H	H	30	88
3	**3c**	**(1b + 2a)**	OCH_3	Br	H	H	25	91
4	**3d**	**(1c + 2a)**	Br	Br	H	H	25	90
5	**3e**	**(1d + 2a)**	H	Br	H	H	25	89
6	**3f**	**(1d + 2b)**	H	NO_2	H	H	30	87
7	**3g**	**(1a + 2c)**	Cl	H	Ph	CH_3CH_2	25	85
8	**3h**	**(1c + 2c)**	Br	H	Ph	CH_3CH_2	25	83

Table 3. Effect of pyrazolylbisindoles on mycelial growth of plant pathogenic fungi

Compound	Mycelial growth (cm)	
	C. lunata	*R. solani*
3a	7.6[d] (15.6)	7.4[d] (17.8)
3b	1.4[b] (84.4)	4.8[c] (53.3)
3c	0.3[a] (96.7)	0.5[a] (93.9)
3d	0.8[ab](91.1)	1.7[b] (81.1)
3e	4.7[c] (47.8)	5.3[c] (41.1)
3f	9.0[e] (0.0)	9.0[e] (0.0)
3g	5.2[c] (42.2)	7.3[d] (18.8)
3h	7.8[d] (13.3)	7.5[d](16.7)
Control	9.0[e] (0.0)	9.0[e] (0.0)
CD (0.05%)	0.71	0.73

In a column, means followed by the same letter do not differ significantly (LSD test; $P \leqslant 0.05$). Figures in brackets are % inhibition of fungal growth as compared to control.

The results obtained clearly indicate that the series of pyrazolylbisindoles discussed here are active towards growth inhibition of pathogenic plant fungi and select human pathogens under this investigation. In general, most of the compounds exhibited excellent anti-fungal activity against plant pathogens. The compounds **3c** and **3d** may be promoted as fungicides for the control of crop diseases in agriculture as they showed more than 80% inhibitory activity against the test plant pathogens. However, further studies are required to determine their potential against wide range of plant pathogens, MIC and mode of actions.

References and notes

1. (a) Foldeak, S.; Czombas, J.; Matkovis, B. *Acta Univ. Sjeged. Acta Phys. Chem.* **1965**, *11*, 115; (b) Povszasz, J.; Katalin, G. P.; Foleat, S.; Malkovics, B. *Acta Phys. Acad. Sci. Hung.* **1996**, *29*, 299.
2. (a) Kathleen, A.; Merrill, A.G. PCT Int Appl. WO 99, *Chem. Abstr.* **1999**, *130*, 276765p; (b) Bradfield, C. A.; Bjeldanes, L. F. *J. Toxicol. Environ. Health* **1987**, *21*, 311; (c) Dashwood, R. H.; Uyetake, L.; Fong, A. T.; Hendricks, J. D.; Bailey, G. S. *Food Chem. Toxicol.* **1987**, *27*, 385.
3. Sridhar, R.; Perumal, P. T.; Etti, S.; Shanmugam, G.; Ponnusamy, M. N.; Prabavathy, V. R.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6035.
4. (a) Remers, W. *Chem. Heterocycl. Com.* **1972**, *1*, 25; (b) Nair, V.; Thomas, S.; Mathew, S. C.; Abhilash, K. G. *Tetrahedron* **2006**, *62*, 6731.
5. Kamal, A.; Qureshi, A. A. *Tetrahedron* **1963**, *19*, 513.
6. Chatterjee, A.; Manna, S.; Banerji, J.; Pracard, C.; Prange, T.; Shoolery, J. *J. Chem. Soc., Perkin Trans. 1* **1980**, 553.
7. Yadav, J. S.; Reddy, B. V. S.; Satheesh, G. *Tetrahedron Lett.* **2004**, *45*, 3673.
8. Yadav, J. S.; Reddy, B. V. S.; Murthy, V. S. R.; Kumar, G. M.; Madan, C. *Synthesis* **2001**, 783.
9. Ji, S.-J.; Wang, S.-Y.; Zhang, Y.; Loh, T.-P. *Tetrahedron* **2004**, *60*, 2051.
10. Farhanullah, S. A.; Maulik, P. R.; Ram, V. J. *Tetrahedron Lett.* **2004**, *45*, 5099.
11. (a) Ji, S.-J.; Zhou, M.-F.; Wang, S.-Y.; Loh, T.-P. *Synlett* **2003**, 2077; (b) Gu, D.-G.; Ji, S.-J.; Jiang, Z.-Q.; Loh, T.-P. *Synlett* **2005**, 959.
12. (a) Areadi, A.; Bianchi, G.; Chiarini, M.; D'Anniballe, G.; Marinalli, F. *Synlett* **2004**, 944; (b) Evans, D. A.; Scheidt, K. A.; Fandrick, K. R.; Lam, H. W.; Wu, J. J. *J. Am. Chem. Soc.* **2003**, *125*, 10780; (c) Zhou, J.; Ye, M.-C.; Huang, Z. Z.; Tang, Y. *J. Org. Chem.* **2004**, *69*, 1309; (d) Banik, B. K.; Fernandez, M.; Alvarez, C. *Tetrahedron Lett.* **2005**, *46*, 2577.
13. Kamal, A.; Qureshi, A. A. *Tetrahedron* **1963**, *19*, 512.
14. Osawa, T.; Namiki, M. *Tetrahedron Lett.* **1983**, *24*, 4719.
15. (a) Szmuszkovicz, J. *J. Am. Chem. Soc.* **1975**, *79*, 2819; (b) Iqbal, Z.; Jackson, A. H.; Rao, K. R. N. *Tetrahedron Lett.* **1988**, *29*, 2577; (c) Alam, M. M.; Varala, R.; Adapa, S. R. *Tetrahedron Lett.* **2003**, *44*, 5115; (d) Shi, M.; Cui, S.-C.; Li, Q.-J. *Tetrahedron* **2004**, *60*, 6679; (e) Bartoli, G.; Bartolcci, M.; Foglia, G.; Giuliani, A.; Marcontoni, E.; Sambri, L.; Torregiani, E. *J. Org. Chem.* **2003**, *68*, 4594.
16. Kozhevnikov, I. V. *Russ. Chem. Rev.* **1987**, *56*, 811.
17. (a) Kozhevnikov, I. V.; Kulikov, S. M.; Chukaeva, N. G.; Kirsanov, A. T.; Letunova, A. B.; Blinova, V. I. *React. Kinet. Catal. Lett.* **1992**, *47*, 59; (b) Kozhevnikov, I. V.; Vasilieva, I. B.; Zarutsky, V. V. *React. Kinet. Catal. Lett.* **1992**, *47*, 83.
18. Torok, B.; Bucsi, T.; Beregszaszi, T.; Kapocsi, I.; Molnar, A. *J. Mol. Catal. A* **1996**, *107*, 305.
19. (a) Kozhevnikov, I. V. *Stud. Surf. Sci. Catal.* **1994**, *90*, 21; (b) Kozhevnikov, I. V. *Russ. Chem. Rev.* **1993**, *62*, 473.
20. (a) Sato, S.; Sakurai, C.; Furuta, H.; Sodesawa, T.; Nozaki, F. *J. Chem. Soc., Chem. Commun.* **1991**, 1327; (b) Sato, S.; Furuta, H.; Sodesawa, T.; Nozaki, F. *J. Chem. Soc., Perkin Trans. 2* **1993**, 385.
21. Izumi, Y.; Urabe, K.; Onake, M. *Zeolite, Clay and Heteropoly Acid in Organic Reactions*; Kodansha/VCH: Tokyo, 1992, p 99.
22. Izumi, Y.; Fujita, T. *J. Mol. Catal.* **1996**, *106*, 43.
23. Meuzelaar, G. J.; Maat, L.; Sheldon, R. A.; Kozhevnikov, I. V. *Catal. Lett.* **1997**, *45*, 249.
24. Kira, M. A.; Rahman, A.; Gadella, K. Z. *Tetrahedron Lett.* **1969**, *10*, 109.
25. General procedure for the synthesis of pyrazolylbisindoles: a mixture of corresponding pyrazole aldehyde (1 mmol), indole (2 mmol) and phosphotungstic acid (20 mol%) in acetonitrile was stirred at room temperature for 25–30 min. After completion of the reaction, as indicated by TLC, the solvent was removed under reduced pressure. The residue was poured on crushed ice and filtered to give the crude product which was column chromatographed with a mixture of ethyl acetate and pet. ether (20: 80) using silica gel to afford pure pyrazolyl bisindoles.
5-Bromo-3-{(5-bromo-1*H*-indol-3-yl)[3-(4-chlorophenyl)-1-phenyl -1*H*-pyrazol-4-yl]methyl}-1*H*-indole, compound **3a**: orange colour solid (20% ethyl acetate–petroleum ether); mp 134–136 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.91 (s, 1H), 6.98 (s, 2H), 6.99 (t, J = 7.45, 2H), 7.11 (dd, J = 7.45, 2.3, 2H), 7.22 (t, J = 7.45, 1H), 7.29 (d, J = 7.45, 2H), 7.30–7.40 (m, 6H), 7.65 (d, J = 7.45, 2H), 7.75 (d, J = 7.45, 2H), 7.98 (s, 1H), 11.06 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.21, 139.88, 135.89, 133.05, 132.60, 129.98, 129.73, 129.08, 128.84, 128.56, 126.76, 126.00, 125.41, 124.07, 121.50, 118.70, 117.65, 114.26, 111.53, 30.56; IR (KBr): 3428, 2923, 1598, 1500, 1453, 1093, 757 cm^{-1}; MS: *m/z* 656 (M^+). Anal. Calcd for $C_{32}H_{21}Br_2Cl\ N_4$: C, 58.52; H, 3.22; N, 8.53. Found: C, 58.51; H, 3.23; N, 8.54.
5-Nitro-3-{(5-nitro-1*H*-indol-3-yl)[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]methyl}-1*H*-indole, compound **3b**: orange colour solid (20% ethyl acetate–petroleum ether); mp 166–168 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.88 (s, 1H), 6.83 (t, J = 7.45, 2H), 6.94 (s, 2H), 6.99 (t, J = 7.45, 2H), 7.21 (t, J = 7.45, 1H), 7.23, (d, J = 7.45, 2H),

7.32 (d, J = 7.45, 2H), 7.37 (t, J = 7.45, 4H), 7.66 (d, J = 8.0, 2H), 7.74 (d, J = 7.45, 2H), 8.05 (s, 1H), 10.83 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.53, 140.74, 140.47, 129.96, 129.43, 128.37, 128.22, 126.03, 125.97, 125.00, 124.46, 124.14, 120.81, 118.57, 117.14, 116.54, 116.47, 114.52, 112.64, 55.16, 30.51; IR (KBr): 3401, 2930, 1604, 1518, 1464, 1095, 751 cm^{-1} ; MS: m/z 584 (M^+). Anal. Calcd for $C_{33}H_{24}N_6O_5$: C, 67.80; H, 4.14; N, 14.38. Found: C, 67.78; H, 4.13; N, 14.36.

5-Bromo-3-{(5-bromo-1*H*-indol-3-yl)[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]methyl}-1*H*-indole, compound **3c**: orange colour solid (20% ethyl acetate–petroleum ether); mp 146–148 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.72 (s, 3H), 5.84 (s, 1H), 6.89 (d, J = 7.45, 2H), 6.98 (s, 2H), 7.11 (dd, J = 7.45,1.75, 2H), 7.21 (t, J = 7.45, 1H), 7.30–7.32 (m, 2H), 7.39 (t, J = 7.45, 2H), 7.55 (d, J = 7.45, 2H), 7.75 (d, J = 7.45, 2H), 7.97 (s, 1H), 11.06 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.51, 150.27, 135.93, 135.40, 129.93, 129.39, 128.51, 126.14, 125.89, 124.92, 124.02, 121.44, 121.28, 118.49, 117.92, 114.50, 114.22, 111.48, 100.00, 55.10, 30.48; IR (KBr): 3423, 2925, 1603, 1501, 1451, 1096, 758 cm^{-1}; MS: m/z 652 (M^+). Anal. Calcd for $C_{33}H_{24}Br_2N_4O$: C, 60.76; H, 3.71; N, 8.59. Found: C, 60.78; H, 3.72; N, 8.58.

5-Bromo-3-{(5-bromo-1*H*-indol-3-yl)[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methyl}-1*H*-indole, compound **3d**: Pink colour solid (20% ethyl acetate–petroleum ether); mp 214–216 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.91 (s, 1H), 6.97 (d, 2H, J = 7.45), 7.11 (dd, J = 7.45, 1.75, 2H), 7.24 (t, J = 7.45, 1H), 7.24 (t, J = 7.45, 1H), 7.29 (d, J = 7.45, 2H), 7.37 (d, J = 1.10, 2H), 7.41 (t, J = 7.45, 2H), 7.50 (d, J = 7.45, 2H), 7.59 (d, J = 7.45, 2H), 7.76 (d, J = 7.45, 2H), 7.98 (s, 1H), 11.05 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.48, 139.86, 137.63, 135.89, 135.16, 131.98, 129.99, 129.81, 129.13, 128.77, 126.72, 126.05, 124.12, 121.47, 119.40, 119.03, 118.73, 118.27, 111.50, 30.45; IR (KBr): 3422, 2925, 1596, 1501, 1453, 1094, 757 cm^{-1}; MS: m/z 701 (M^+). Anal. Calcd for $C_{32}H_{21}Br_3N_4$: C, 54.81; H, 3.02; N, 7.99. Found: C, 54.79; H, 3.01; N, 7.97.

5-Bromo-3-[(5-bromo-1*H*-indol-3-yl)(1,3-diphenyl-1*H*-pyrazol-4-yl)methyl]-1*H*-indole, compound **3e**: red colour solid (20% ethyl acetate–petroleum ether); mp 248–250 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.89 (s, 1H), 7.01 (dd, J = 7.45, 2.3, 2H), 7.22 (t, J = 7.45, 1H), 7.31–7.35 (m, 7H), 7.39 (t, J = 7.45, 2H), 7.64 (d, J = 7.45, 2H), 7.76 (d, J = 7.45, 2H), 8.02 (s, 1H), 11.09 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.46, 139.98, 135.92, 133.72, 129.99, 129.04, 128.53, 128.51, 128.42, 128.13, 126.56, 125.94, 125.34, 124.06, 121.38, 118.63, 117.91, 114.26, 111.55, 29.99; IR (KBr): 3417, 2923, 1597, 1500, 1452, 1097, 753 cm^{-1}; MS: m/z 622 (M^+). Anal. Calcd for $C_{32}H_{22}Br_2N_4$: C, 61.76; H, 3.56; N, 9.00. Found: C, 61.74; H, 3.55; N, 8.99.

5-Nitro-3-[(5-nitro-1*H*-indol-3-yl)(1,3-diphenyl-1*H*-pyrazol-4-yl)methyl]-1*H*-indole, compound **3f**: yellow colour solid (20% ethyl acetate–petroleum ether); mp 176–178 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.23 (s, 1H), 7.19 (t, J = 7.45, 1H), 7.24 (d, J = 1.55, 2H), 7.28–7.34 (m, 3H), 7.39 (t, J = 7.45, 2H), 7.50 (d, J = 7.45, 2H), 7.67 (d, J = 7.45, 2H), 7.77 (d, J = 7.45, 2H), 7.93 (dd, J = 7.45, 2.3, 2H), 8.05 (s, 1H), 8.26 (d, J = 1.55, 2H), 11.66 (br s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.44, 140.76, 140.44, 139.93, 133.60, 129.95, 129.03, 128.71, 128.46, 128.32, 128.13, 126.63, 125.96, 124.84, 120.76, 118.70, 117.16, 116.50, 112.70, 29.83; IR (KBr): 3350, 2925, 1597, 1516, 1472, 1095, 739 cm^{-1}; MS: m/z 554 (M^+). Anal. Calcd for $C_{32}H_{22}N_6O_4$: C, 69.31; H, 4.00; N, 15.15. Found: C, 69.29; H, 4.01; N, 15.14.

1-Ethyl-2-phenyl -3-{(1-ethyl-2-phenyl -1*H*-indol-3-yl)[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methyl}-1*H*-indole, compound **3g**: yellow colour solid (20% ethyl acetate–petroleum ether); mp 194–196 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.11 (t, 6H), 3.76 (s, 3H), 3.86 (q, J = 7.45, 4H), 5.68 (s, 1H), 6.72–6.78 (m, 5H), 6.90 (t, J = 7.45, 2H), 7.08–7.12 (m, 4H), 7.13–7.19 (m, 5H), 7.22 (d, J = 7.45, 2H), 7.25 (s, 1H), 7.28 (t, J = 7.45, 2H), 7.33–7.37 (m, 4H), 7.53–7.56 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.05, 152.02, 140.21, 137.37, 135.94, 132.37, 130.40, 129.70, 129.29, 129.19, 128.67, 128.59, 127.94, 127.73, 126.44, 125.47, 120.96, 120.63, 119.15, 118.72, 115.74, 113.55, 109.36, 47.52, 38.36, 15.43; IR (KBr): 3429, 2928, 1599, 1500, 1456, 1088, 742 cm^{-1}; MS: m/z 707 (M^+). Anal. Calcd for $C_{48}H_{39}Cl\,N_4$: C, 81.51; H, 5.56; N, 7.92. Found: C, 81.50; H, 5.55; N, 7.91.

1-Ethyl-2-phenyl -3-{(1-ethyl-2-phenyl-1*H*-indol-3-yl)[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]methyl}-1*H*-indole, compound **3h**: brown colour solid (20% ethyl acetate–petroleum ether); mp 202–204 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.13 (t, J = 7.45, 6H), 3.87 (q, J = 7.45, 4H), 5.72 (s, 1H), 6.79–6.93 (m, 6H), 7.10 (t, J = 7.45, 4H), 7.14–7.25 (m, 10H), 7.30 (d, J = 7.45, 2H), 7.37 (t, J = 7.45, 2H), 7.41–7.43 (m, 2H), 7.58 (d, J = 3.02, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.22, 150.33, 140.16, 136.35, 135.84, 133.71, 132.36, 131.35, 130.40, 129.16, 128.75, 128.24, 127.88, 127.70, 127.35, 125.86, 120.94, 120.73, 120.64, 119.20, 118.82, 115.73, 109.39, 38.43, 31.34, 15.49; IR (KBr): 3429, 2928, 1599, 1499, 1457, 1063, 742 cm^{-1}; MS: m/z 751 (M^+). Anal. Calcd for $C_{48}H_{39}Br\,N_4$: C, 76.69; H, 5.23; N, 7.45. Found: C, 76.68; H, 5.21; N, 7.46.

26. Materials and methods for anti-microbial activity: test organisms and their maintenance: the human pathogenic bacterial cultures such as *Candida albicans*, *S. epidermidis* and *P. aeruginosa* and plant pathogenic fungi viz., *R. solani* and *C. lunata* were obtained from the Centre for Advanced Studies in Botany, University of Madras, Chennai, India, and the biological screening of pyrazolylbisindoles was performed there itself. The human pathogens viz., *Candida albicans*, *S. epidermidis* and *P. aeruginosa* were maintained on nutrient agar (NA) consisting of the following (g/L): beef extract 1.0; yeast extract 2.0; peptone 5.0; NaCl 5.0; agar 15.0; distilled H_2O 1 L (pH 7.2) and the plant pathogens namely, *R. solani* and *C. lunata*, were maintained on potato dextrose agar (PDA) that contained (g/L) potato 200.0; dextrose 20; agar 15.0; distilled H_2O 1 L (pH 6.5) in slants or Petriplates at room temperature (28 ± 2 °C). Effect of pyrazolylbisindoles on the growth of human pathogenic bacteria: The anti-bacterial activity of the compounds against human pathogens was evaluated by the agar diffusion method.[27] About 1 mL of inoculum of each test pathogen was added to the molten NA medium and poured into sterile Petriplates under aseptic conditions. After solidification, a 5 mm well was made in the centre of each plate using a sterile cork borer. Each compound was dissolved in 10% DMSO at 1 mmol concentration and filter sterilized using 0.25 μm filter paper. Each well received 50 μL solution of each compound and the plates were incubated at room temperature. Sterile DMSO (10%) was used as control. After 48 h, the appearance of inhibition zone around the well was observed. Effect of pyrazolylbisindoles on the growth of plant pathogenic fungi: The anti-fungal activity of compounds was tested by measuring the mycelial growth of test fungi using poison plate technique.[27] Each compound amended PDA was poured into 9 cm Petriplates and they were inoculated with 5 mm mycelial discs of the test fungi. PDA containing 10% DMSO served as control. The plates were incubated at room temperature for 6 days and the mycelial growth was measured.

27. Pandurangan, A.; Mathivanan, N.; Prabavathy, V. R.; Kabilan, V.; Murugesan, V.; Murugesan, K. *Indian J. Exp. Biol.* **1995**, *33*, 357.