Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: informahealthcare.com/journals/ienz20

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M. Gopalakrishnan, P. Sureshkumar, J. Thanusu & V. Kanagarajan

To cite this article: M. Gopalakrishnan, P. Sureshkumar, J. Thanusu & V. Kanagarajan (2008) Synthesis, spectral analysis, antibacterial and antifungal activities of some 4,6-diaryl-4,5-dihydro-3-hydroxy-2[*H*]-indazole—a novel fused indazole derivative, Journal of Enzyme Inhibition and Medicinal Chemistry, 23:6, 974-979, DOI: 10.1080/14756360701811254

To link to this article: https://doi.org/10.1080/14756360701811254

Published online: 20 Oct 2008.

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Journal of Enzyme Inhibition and Medicinal Chemistry, December 2008; 23(6): 974–979

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Synthesis, spectral analysis, antibacterial and antifungal activities of some 4,6-diaryl-4,5-dihydro-3-hydroxy-2[*H*]-indazole—a novel fused indazole derivative

M. GOPALAKRISHNAN, P. SURESHKUMAR, J. THANUSU, & V. KANAGARAJAN

Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

(Received 8 August 2007; accepted 30 October 2007)

Abstract
A novel class of 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazoles **25-32** were synthesized and evaluated for their *in vitro* antibacterial and antifungal activities. Four Compounds, which all possessed electron withdrawing functional groups (*—Cl, —NO_2, —Br*) **27**, **28**, **30** and **32** were more potent against the tested bacterial/fungal strains than the standard bacterial and fungal drugs ciprofloxacin and fluconazole respectively.

Keywords: *6-Carbethoxy-3,5-diarylcyclohex-2-enone, 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole, hydrazine hydrate, antibacterial activity, antifungal activity*

Introduction

Various structurally diverse indazole nucleus have aroused great interest in the past and recent years due to their wide variety of biological properties such as antimicrobial activity [1], inhibitors of protein kinase B/Akt [2], antiprotozal agents [3], antichagasic activity [3], leishmanocidal activity [3], trypanocidal activity [3], inhibitors of S-adenosyl homocysteine/methylthio adenosine (SAH/MTA) nucleosides [4], potent activator of the nitric oxide receptor [5], inhibit platelet aggregation [5].

In recent years there has been a great deal of interest in exploiting more than one proximal functional groups for designing novel structures capable of performing a variety of functions. The present study describes the use of 6-carbethoxy-3,5-diarylcyclohex-2-enone [6], an intermediate with three versatile functional groups i.e., ketone, olefin and ester for the synthesis of fused indazole derivatives. In continuation of our earlier work on the synthesis of various bio active heterocyclic nucleus [7–11], we wish to report the development of indazoles on 6-carbethoxy-3,5-diarylcyclohex-2-enone derivatives thus paving the way for a novel class of 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole**.**

Experimental

Microbiology

Materials. All the bacterial strains namely *Staphylococcus aureus*, β-*Heamolytic streptococcus, Vibreo cholerae*, *Salmonella typhii*, *Shigella felxneri*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* and fungal strains namely *Aspergillus flavus*, *Mucor*, *Rhizopus* and *Microsporum gypsuem* are clinical strains and are get hold of from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

Correspondence: M. Gopalakrishnan, Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India. Tel: 91 4144 228 233. E-mail: profmgk@yahoo.co.in

ISSN 1475-6366 print/ISSN 1475-6374 online © 2008 Informa UK Ltd.
DOI: 10.1080/14756360701811254

In vitro *antibacterial and antifungal activity (Disc Diffusion method)*. The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB) (Hi-media, Mumbai) for fungi and nutrient broth (NB) (Hi-media, Mumbai) for bacteria by the Disc Diffusion method [12]. The respective hydrochlorides of the test compounds **25-32** were dissolved in water to obtain 1 mg mL^{-1} stock solution and the different concentrations [100, 200, 500 ppm (μg/mL)] are prepared from the stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7 days old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. Sterile paper disc of 5 mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated in BOD incubator at 37°C for bacteria and at 28°C for fungi. The zone of inhibition was recorded by visual observations after 24 h of inhibition for bacteria and after 72-96 h of inhibition for fungi. Moreover, the zone of inhibition was measured by excluding the diameter of the paper disc. Ciprofloxacin was used as standards for bacteria and fluconazole as standard for fungi under analogous conditions.

In vitro *antibacterial and antifungal activity (Minimum inhibitory concentration (MIC) method)*. Minimum inhibitory concentration (MIC) in μg/mL values is carried out by two-fold serial dilution method [13]. The respective hydrochlorides of the test compounds **23-27** were dissolved in water to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 hrs old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7 days old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4-10^5 cfu/mL. The final inoculums size was 10^5cfu/mL for antibacterial assay and 1.1-1.5 X 10^2 cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH 5.6 for fungi (SDB). 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 72-96 h for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72-96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacteria studies and Fluconazole was used as standards for fungal studies.

Chemistry

Performing TLC assessed the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Nicolet-Avatar−330 FT-IR spectrophotometer and note worthy absorption values (cm^{-1}) alone are listed. ^1H, D_2O exchanged ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer using DMSO-*d* as solvent. Two-dimensional HSQC spectra were recorded at 500 MHz and on Bruker DRX 500 NMR spectrometer using DMSO-*d* as solvent. The ESI + ve MS spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory micro-analysis was obtained on Carlo Erba 1106 CHN analyzer.

By adopting the literature precedent, 1,3-diaryl-prop-2-en-1-ones **9-16** [14] and 6-carbethoxy-3,5-diarylcyclohex-2-enone **17-24** [6] were prepared.

*Typical procedure for the synthesis of 4,6-diphenyl-4,5-dihydro-3-hydroxy-2[H]-indazole **25***. A solution of 6-carbethoxy-3,5-diarylcyclohex-2-enone, **17** (0.1 mol) in methanol (40 mL) was treated with hydrazine hydrate (0.15 mol) and refluxed for 5 h. The reaction mixture was cooled and then poured over crushed ice. The crude product **25** was recrystallized twice using methanol as solvent. IR (KBr) (cm^{-1}): 3425, 3060, 2922, 2863, 1607, 1515, 1446, 1369, 758, 695; ^1H NMR (δ ppm): 2.90, 3.1-3.2 (m,2H,H_5), 4.19 (dd,1H,H_4), 6.75 (d,1H,H_7), 9.7 (s,1H,H_2), 11.53 (s,1H,OH), 7.10-7.48 (m,10H,H-Arom.); ^{13}C NMR (δ ppm): 34.2 C-4, 36.2 C-5, 98.3 C-9, 113.4 C-7; 136.2 C-8; 125.0-128.5 C-Arom.; 140.2, 145.3 *ipso*-C's; 157.2 C-3.

In the D_2O exchanged ^1H NMR spectrum, a broad peak at 11.53 ppm due to —OH proton at C-3 and a broad peak at 9.74 ppm due to —NH proton at C-2 disappeared.

In the HSQC spectrum, one bond correlation (34.2/4.19) between C-4 and H_{4a} occurs. The ^{13}C resonance at 36.2 ppm has correlations with methylene protons H_{5a} (36.2/2.90; 36.2/3.20) and hence C-5 resonates at 36.2 ppm. The ^{13}C resonance at 113.4 ppm correlations with doublet at 6.75 ppm. The doublet at 6.75 ppm is conveniently assigned to H_7. The cross peak (113.4/6.75 ppm) confirms that the ^{13}C resonance at 113.4 ppm is due to C-7. The ^{13}C resonances at 98.3, 136.2, 157.2 ppm have no correlations with protons and hence it is due

to quaternary carbons. Among the quaternary carbon resonances, the ^{13}C resonance at 140.2, 143.2 ppm is assigned to *ipso* carbons. The ^{13}C resonance at 136.2 and 157.2 ppm are due to the C-8 and C-3 carbons. The signal at 98.3 ppm is due to C-9 carbon and the C-6 carbon is merged with aromatic carbons.

*The compounds **26-32** were synthesized similarly.*

*6-phenyl-4,5-dihydro-4-p-tolyl-3-hydroxy-2[H]-indazole **26***. IR (KBr) (cm^{-1}): 3419, 3060, 3062, 2919, 2858, 1593, 1516, 757, 695; ^1H NMR (δ ppm): 2.21 (s,3H,CH_3 at phenyl ring); 2.87, 3.1-3.2 (m,2H,H_5), 4.14 (dd,1H,H_4), 6.75 (d,1H,H_7), 8.30 (s,1H,H_2), 10.98 (s,1H,OH), 7.02-7.47 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 20.5 CH_3 at phenyl ring; 33.8 C-4, 36.3 C-5, 98.5 C-9, 113.3 C-7; 136.3 C-8; 125.0-128.5 C-Arom.; 134.8,140.2,141.1,142.2 *ipso*-C's; 156.4 C-3.

*4-(4-chlorophenyl)-4,5-dihydro-6-phenyl-3-hydroxy-2[H]-indazole **27***. IR (KBr) (cm^{-1}): 3404, 3054, 2928, 1600, 1535, 1491, 757, 694; ^1H NMR (δ ppm): 2.87, 3.1-3.2 (m,2H,H_5), 4.20 (dd,1H,H_4), 6.76 (d,1H,H_7), 8.35 (s,1H,H_2), 11.5 (s,1H,OH), 7.16-7.47 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 33.7 C-4, 36.1 C-5, 97.8 C-9, 113.4 C-7; 136.2 C-8; 125.1-128.5 C-Arom.; 130.5, 140.1, 141.2, 144.2 *ipso*-C's; 156.3 C-3.

*4-(4-nitrophenyl)-4,5-dihydro-6-phenyl-3-hydroxy-2[H]-indazole **28***. IR (KBr) (cm^{-1}): 3422, 3076, 2924, 2847, 1599, 1515, 1437, 1345, 753, 695; ^1H NMR (δ ppm): 2.86, 3.20-3.25 (m,2H,H_5), 4.34 (dd,1H,H_4), 6.76 (d,1H,H_7), 9.70 (s,1H,H_2), 11.70 (s,1H,OH), 7.25-8.11 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 34.6 C-4, 36.0 C-5, 97.3 C-9, 113.5 C-7; 136.2 C-8; 123.4-128.8 C-Arom.; 140.1, 146.2 *ipso*-C's; 157.2 C-3.

*4-(4-methoxyphenyl)-4,5-dihydro-6-phenyl-3-hydroxy-2[H]-indazole **29***. IR (KBr) (cm^{-1}): 3417, 3065, 3052, 2930, 2836, 1608, 1511, 1443, 1373, 760, 696; ^1H NMR (δ ppm): 2.85, 3.10-3.20 (m,2H,H_5), 3.64 (s,3H,OCH_3 at phenyl ring); 4.11 (dd,1H,H_4), 6.73 (d,1H,H_7), 8.40 (s,1H,H_2), 10.70 (s,1H,OH), 7.03-7.45 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 33.4 C-4, 36.4 C-5, 54.9 —OCH_3 at phenyl ring; 98.7 C-9, 113.4 C-7; 136.2 C-8; 125.0-128.5 C-Arom.; 137.2, 140.3, 141.0, 157.5 *ipso*-C's; 156.3 C-3.

*6-(4-bromophenyl)-4,5-dihydro-4-phenyl-3-hydroxy-2[H]-indazole **30***. IR (KBr) (cm^{-1}): 3402, 3093, 3000, 2927, 2830, 1608, 1526, 1439, 1349, 807, 736; ^1H NMR (δ ppm): 2.85, 3.10-3.20 (m,2H,H_5), 4.16 (dd,1H,H_4), 6.76 (d,1H,H_7), 9.70 (s,1H,H_2), 11.72 (s,1H,OH), 7.10-7.82 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 34.4 C-4, 36.1 C-5, 97.8 C-9, 113.3 C-7; 135.2 C-8; 126.0-129.7 C-Arom.; 131.4, 131.9, 135.2, 139.2 *ipso*-C's; 157.5 C-3.

*6-(4-methylphenyl)-4,5-dihydro-4-phenyl-3-hydroxy-2[H]-indazole **31***. IR (KBr) (cm^{-1}): 3422, 3062, 3051, 2928, 2834, 1603, 1510, 1441, 1370, 764, 691; ^1H NMR (δ ppm): 2.81, 3.13-3.18 (m,2H,H_5), 2.24 (s,3H,CH_3 at phenyl ring); 4.08 (dd,1H,H_4), 6.70 (d,1H,H_7), 8.38 (s,1H,H_2), 11.21 (s,1H,OH), 7.08-7.38 (m,9H,H-Arom.); ^{13}C NMR (δ ppm): 33.0 C-4, 36.7 C-5, 21.8 —CH_3 at phenyl ring; 99.2 C-9, 112.6 C-7; 134.2 C-8; 124.7-127.9 C-Arom.; 137.0, 140.5, 141.5, 157.2 *ipso*-C's; 157.2 C-3.

*4-(4-tolylphenyl)-4,5-dihydro-6-(3-nitrophenyl)-3-hydroxy-2[H]-indazole **32***. IR (KBr) (cm^{-1}): 3428, 3022, 2925, 2852, 1609, 1525, 1489, 1448, 818, 701; ^1H NMR (δ ppm): 2.85, 3.10-3.25 (m,2H,H_5), 4.16 (dd,1H,H_4), 6.77 (d,1H,H_7), 9.82 (s,1H,H_2), 10.90 (s,1H,OH), 6.95-8.24 (m,8H,H-Arom.); ^{13}C NMR (δ ppm): 33.4 C-4, 36.3 C-5, 99.0 C-9, 113.5 C-7; 137.0 C-8; 116.0-131.4 C-Arom.; 137.0, 141.9, 148.2, 157.6 *ipso*-C's; 156.1 C-3.

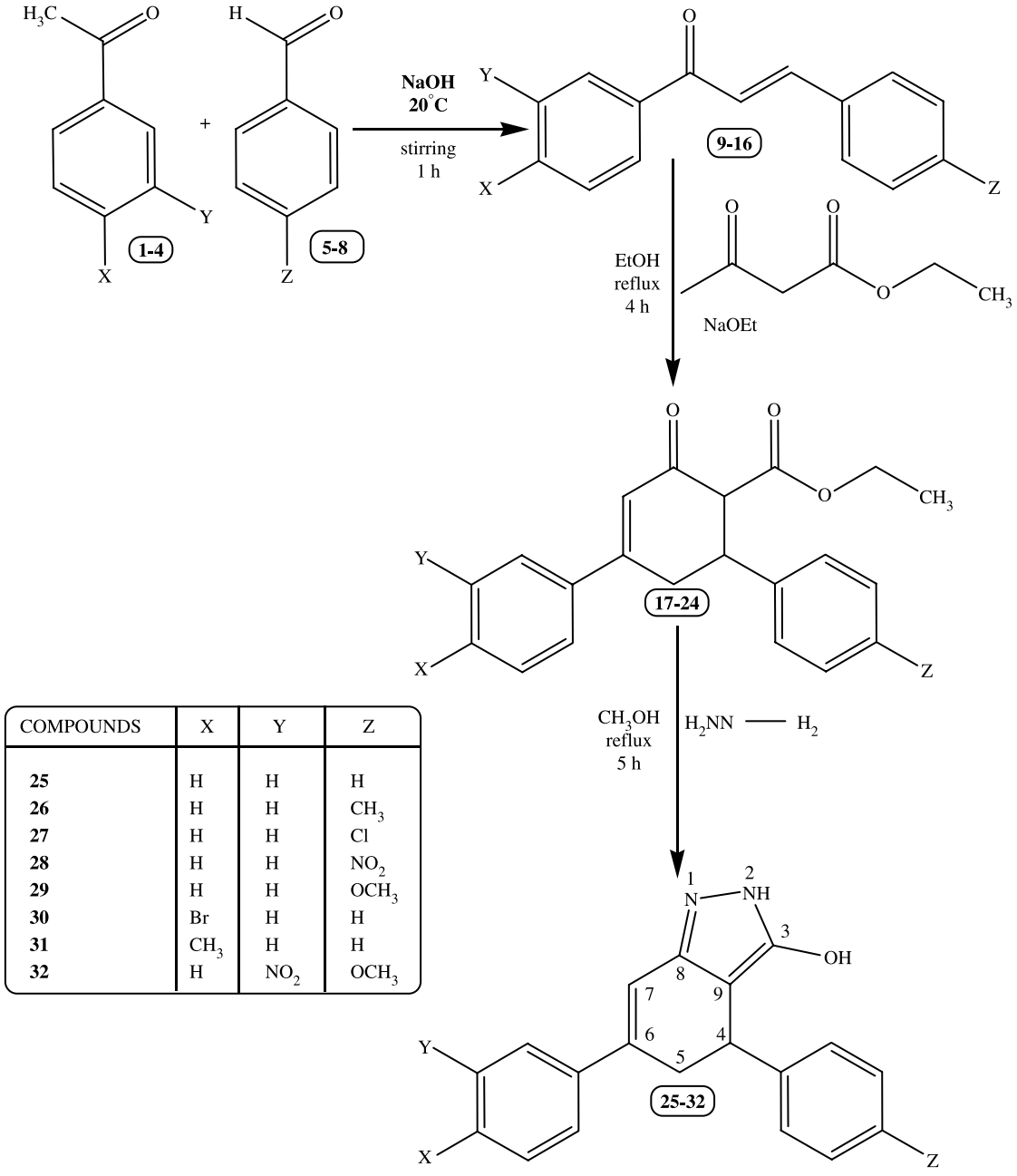
Results and discussion

Chemistry

The synthetic strategy for the formation of 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole, a fused indazole derivative involves three steps which are described as follows: Condensation of appropriate acetophenone **1-4** and appropriate benzaldehyde **5-8** in the presence of sodium hydroxide yields the respective 1,3-diaryl-prop-2-en-1-ones **9-16**. The respective α,β-unsaturated ketones **9-16** on treatment with ethyl acetoacetate in the presence of sodium ethoxide gives 6-carbethoxy-3,5-diarylcyclohex-2-enone **17-24**. The formed ketones **17-24** on treatment with hydrazine hydrate in refluxing methanol gives 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole **25-32**. The schematic representation and analytical data for the synthesized compounds **25-32** are furnished in Scheme 1 and Table I respectively. The structures of the compounds are elucidated by melting points, elemental analysis, MS, FT-IR, NMR (^1H & ^{13}C), D_2O exchanged ^1H-NMR, two-dimensional HSQC spectroscopic data.

Antibacterial activity

All the newly synthesized novel target molecule 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole **25-32** were tested for their antibacterial activity *in vitro* (Tables II and III) against *S. aureus*, *β-H. streptococcus*, *V. cholerae*, *S. typhii*, *S. felxneri*, *E. coli*, *K. pneumonia* and *Pseudomonas*. Ciprofloxacin was used as standard drug; whose zone of inhibition (mm) values for *S. aureus*, *β-H. streptococcus*, *V. cholerae*, *S. typhii*,



COMPOUNDS	X	Y	Z
25	H	H	H
26	H	H	CH_3
27	H	H	Cl
28	H	H	NO_2
29	H	H	OCH_3
30	Br	H	H
31	CH_3	H	H
32	H	NO_2	OCH_3

Scheme 1. Synthetic pathway for the formation of 4,6-diaryl-4,5-diydro-3-hydroxy-2[*H*]-indazole

S. felxneri, *E. coli*, *K. pneumonia* and *Pseudomonas was 25*, *28*, *23*, *22*, *23*, *24*, *26 and 23 mm respectively.* Minimum inhibitory concentration (MIC) in μg/mL values is reproduced in Tables IV and V. In general all the synthesized novel 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazoles **25-32** exerted a wide range of modest antibacterial activity *in vitro* against the tested organisms. All the compounds **25-32** were active against all the tested bacterial strains. Four Compounds, which all possessed electron withdrawing functional groups (*—Cl, —NO_2, —Br*) **27**, **28**, **30** and **32** were more potent against the tested bacterial strains than the standard drug Ciprofloxacin.

Antifungal activity

The *in vitro* antifungal activity (Tables II and III) of the synthesized novel heterocyclic compounds, **25-32** was studied against the fungal strains viz., *A. flavus, Mucor, Rhizopus* and *M. gypsuem.* Fluconazole was used as a standard drug whose zone of inhibition (mm) values for *A. flavus, Mucor, Rhizopus* and *M. gypsuem* was 20 ± 0.5 zone of inhibition (mm) against all the tested fungi. Minimum inhibitory concentration (MIC) in μg/mL values is reproduced in Table VI. In general, all the synthesized compounds exerted a wide range of modest *in vitro* antifungal activity against all the tested

Table I. Analytical data of compounds **25-32**.

Compound	Yield (%)	m.p°C	Elemental analysis (%) C Found (calculated)	H Found (calculated)	N Found (calculated)	m/z ($M^{+\cdot}$) Molecular formula
25	73	226	78.55 (78.59)	6.21 (6.25)	9.61 (9.65)	(291) $C_{19}H_{18}N_2O$
26	70	230	78.88 (78.92)	6.59 (6.62)	9.17 (9.20)	(305) $C_{20}H_{20}N_2O$
27	68	234	70.21 (70.26)	5.26 (5.28)	8.58 (8.62)	(326) $C_{19}H_{17}Cl\,N_2O$
28	72	212	68.01 (68.05)	5.07 (5.11)	12.50 (12.53)	(336) $C_{19}H_{17}N_3O_3$
29	70	204	74.63 (74.98)	6.27 (6.29)	8.71 (8.74)	(321) $C_{20}H_{20}N_2O_2$
30	69	232	61.76 (61.80)	4.61 (4.64)	7.56 (7.59)	(370) $C_{19}H_{17}BrN_2O$
31	60	225	78.89 (78.92)	6.59 (6.62)	9.17 (9.20)	(305) $C_{20}H_{20}N_2O$
32	68	210	65.70 (65.74)	5.20 (5.24)	11.46 (11.50)	(366) $C_{20}H_{19}N_3O_4$

Table II. *In vitro* profile of compounds **25-28** against test bacteria and fungi.

Micro organisms	Compound 25			Compound 26			Compound 27			Compound 28		
	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm
S. aureus	–	+	+++	–	+	+++	–	++	++++	–	++	+++
β-H.streptococcus	–	+	++	+	+	+++	–	+	+++	+	++	+++
V.cholerae	–	+	+++	+	++	+++	–	+	++++	–	++	+++
S. typhii	+	+	+++	+	+	+++	–	++	++++	+	++	++++
S. felxneri	–	+	+++	–	++	+++	+	+	++++	+	++	++++
E.coli	–	+	++	–	++	++	–	+	+++	–	++	+++
K.pneumonia	–	+	+++	+	++	+++	+	++	++++	+	++	++++
Pseudomonas	–	+	+++	+	+	+++	+	++	++++	+	++	++++
A.flavus	–	+	+++	–	+	+++	+	++	++++	+	++	++++
Mucor	+	+	++	–	+	+++	–	+	++++	+	++	++++
Rhizopus	–	+	+++	+	++	+++	–	++	++++	+	++	++++
M. gypsuem	–	+	+++	–	+	+++	+	++	++++	–	+++	++++

(–) = inactive, (+) = weakly active (12-16 mm), (+)(+) = moderately active (17-21 mm), (+)(+)(+) = strong active (22-29),(+)(+)(+)(+) = highly active (30-33). Ciprofloxacin (5μg/disc) was used as standard drug; whose zone of inhibition (mm) values for *S. aureus, β-H.streptococcus, V.cholerae, S.typhii, S.felxneri, E.coli, K.pneumonia* and *Pseudomonas are 25, 28, 23, 22, 23, 24, 26 and 23 mm respectively.* Fluconazole (100 units/disc) was used as a standard drug whose zone of inhibition (mm) values for *A.flavus, Mucor, Rhizopus* and *M.gypsuem* are 20 ± 0.5 zone of inhibition (mm) against all the tested fungi.

Table III. *In vitro* profile of compounds **29-32** against test bacteria and fungi.

Micro organisms	Compound 29			Compound 30			Compound 31			Compound 32		
	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm
S. aureus	+	++	+++	–	++	+++	–	++	+++	–	++	++++
β-H.streptococcus	–	+	+++	–	+	+++	–	+	+++	+	++	++++
V.cholerae	–	+	+++	–	+	++++	–	+	++++	+	++	++++
S. typhii	–	++	++++	+	++	++++	+	++	++++	+	+	+++
S. felxneri	+	++	+++	–	++	+++	–	++	+++	+	++	+++
E.coli	+	++	+++	+	++	+++	+	+	+++	+	+	+++
K.pneumonia	–	+	+++	+	++	+++	+	+	+++	+	+	+++
Pseudomonas	+	+	++	+	+	+++	+	++	++	+	++	++++
A.flavus	+	+	+++	–	+	++	–	+	++	+	++	++++
Mucor	–	++	+++	–	++	+++	–	+	++	+	++	++++
Rhizopus	–	+	+++	+	++	+++	+	++	+++	+	+	++++
M. gypsuem	+	++	++++	+	++	+++	+	++	+++	+	++	++++

(–) = inactive, (+) = weakly active (12-16 mm), (+)(+) = moderately active (17-21 mm), (+)(+)(+) = strong active (22-29),(+)(+)(+)(+) = highly active (30-33). Ciprofloxacin (5μg/disc) was used as standard drug; whose zone of inhibition (mm) values for *S. aureus, β-H.streptococcus, V.cholerae, S.typhii, S.felxneri, E.coli, K.pneumonia* and *Pseudomonas are 25, 28, 23, 22, 23, 24, 26 and 23 mm respectively.* Fluconazole (100 units/disc) was used as a standard drug whose zone of inhibition (mm) values for *A.flavus, Mucor, Rhizopus* and *M.gypsuem* are 20 ± 0.5 zone of inhibition (mm) against all the tested fungi.

Table IV. *In vitro* antibacterial activities (MIC) values for compounds **25-32**.

	Minimum Inhibitory Concentration (MIC) in μg/mL			
Compound	*S.aureus*	*β-H.streptococcus*	*V.cholerae*	*S. typhii*
25	50	200	200	100
26	25	100	200	100
27	12.5	100	100	50
28	50	50	50	50
29	25	100	50	25
30	50	50	50	25
31	25	100	25	25
32	12.5	25	12.5	200
Ciprofloxacin	25	50	50	50

Table V. *In vitro* antibacterial activities (MIC) values for compounds **25-32**.

	Minimum Inhibitory Concentration (MIC) in μg/mL			
Compound	*S. felxneri*	*E.coli*	*K.pneumonia*	*Pseudomonas*
25	50	200	50	25
26	50	200	50	25
27	12.5	100	12.5	12.5
28	12.5	100	12.5	12.5
29	50	12.5	50	100
30	50	12.5	50	200
31	50	50	100	200
32	200	50	100	12.5
Ciprofloxacin	25	25	50	25

organisms. Moreover, of all the compounds tested, compounds **27**, **28**, **30** and **32** are more effective against the tested fungal strains than the standard drug, Fluconazole.

Conclusion

A close examination of the *in vitro* antibacterial and antifungal activity profile in differently substituted novel 4,6-diaryl-4,5-dihydro-3-hydroxy-2[H]-indazole **25-32** against the tested bacterial strains *viz*. *S. aureus*, *β-H. streptococcus*, *V. cholerae*, *S. typhii*, *S. felxneri*, *E. coli*, *K. pneumonia* and *Pseudomonas* and the fungal strains viz., *A. flavus*, *Mucor*, *Rhizopus* and *M. gypsuem* respectively, provides a better structure activity relationship correlate, which may be summarized as follows:

Results of this study show that the nature of substituent on the phenyl ring viz., chloro, nitro as well as the bromo functions at the *meta* and *para* positions of the aryl moieties are determinant for the nature and extent of the activity of the synthesized compounds, which might have influences on their inhibiting

Table VI. *In vitro* antifungal activities (MIC) values for compounds **25-32**.

	Minimum Inhibitory Concentration (MIC) in μg/mL			
Compound	*A.flavus*	*Mucor*	*Rhizopus*	*M. gypsuem*
25	100	200	50	50
26	100	100	50	50
27	12.5	12.5	12.5	12.5
28	12.5	12.5	12.5	12.5
29	100	100	50	12.5
30	200	100	50	25
31	200	200	50	25
32	12.5	12.5	6.25	6.25
Fluconazole	50	50	25	25

mechanism of actions. The method of action of these compounds is unknown. These observations may promote a further development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection.

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