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Synthesis and studies on antimicrobial, antiinflammatory and antiproliferative activities of heterocycles derived from 4-/5-/6-/7-nitro/5-fluoro/chloro/bromoindole-2-carbohydrazides

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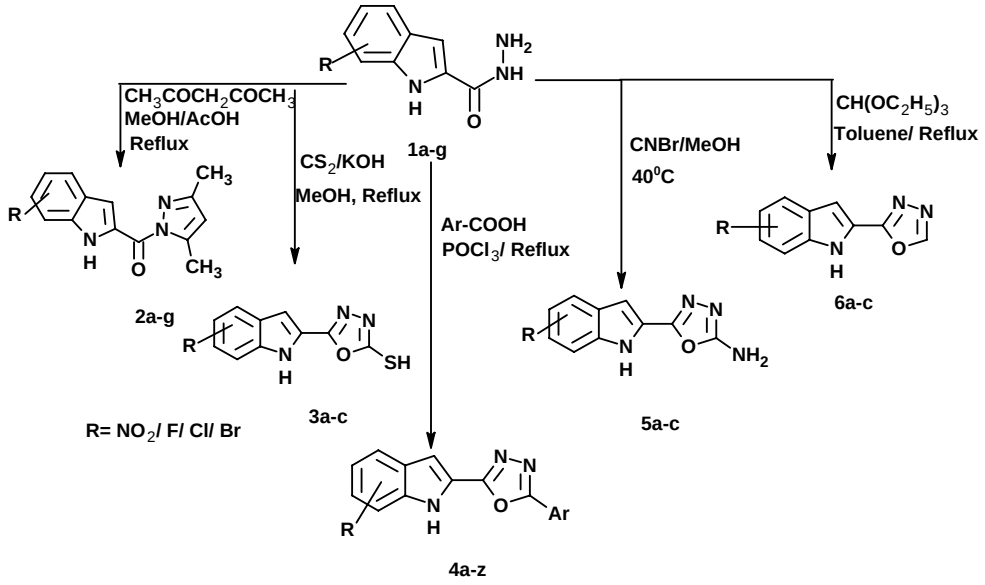
Synthesis and studies on antimicrobial, antiinflammatory and antiproliferative activities of heterocycles derived from 4-/5-/6-/7-nitro/5-fluoro/chloro/bromoindole-2-carbohydrazides are discussed. 4-/5-/6-/7-nitroindole-2-carbohydrazides and 5-fluoro/chloro/bromoindole-2-carbohydrazides on treatment with acetyl acetone in methanol have resulted in 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-4-/5-/6-/7-nitro-1*H*-indoles and 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl) carbonyl]-5-fluoro/chloro/bromo-1*H*-indoles respectively. 4-/6-/7-nitroindole-2-carbohydrazides on treatment with CS_2/KOH in methanol have yielded 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol and with cyanogen bromide yielded 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amine. Nitroindole-2-carbohydrazides are also treated with various aromatic carboxylic acids and triethyl orthoformate yielded 2-[5-(aryl)-1,3,4-oxadiazol-2-yl]-4-/5-/6-/7-nitro-1*H*-indoles and 4-/6-/7-nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indoles, respectively. Structures of the newly synthesized compounds are characterized by analytical and spectral data.

Keywords: Synthesis, heterocycles, indoles, antimicrobial, anti-inflammatory, antiproliferative activity

It is well known fact that the indole nucleus is present as a structural unit in many natural products[1]. Synthesis and biological evaluation of indole derivatives have been a topic of special interest to organic and medicinal chemists. A number of indole derivatives are reported to exhibit antibacterial, antifungal, antituberculosis, antithrombotic, anticancer and antiinflammatory activities[2-12]. Indole thiazoles were reported for their CNS depressant, antiinflammatory and anticancer activities[13-15]. Many researchers[16-19] have extensively studied the antifungal and antibacterial activities of thiazole derivatives. We have reported the antiinflammatory activity of 2-[5-(aryl)-1,3,4-oxadiazol-2-yl]-4-/5-/6-/7-nitro-1*H*-indoles recently[20]. In continuation of earlier work[21,22] on indole derivatives and to explore their bioactivities, herein antimicrobial, antiinflammatory and antiproliferative activities of a few heterocycles derived from 4-/5-/6-/7-nitro/5-fluoro/chloro/bromoindole-2-carbohydrazides are reported. Out of fifteen compounds selected for primary 3-cell line anticancer screening twelve compounds were selected for 60-cell line *in vitro* antitumour assay. Almost all the compounds exhibited moderate to good antiproliferative activity.

Results and Discussion

Ethyl 4-/5-/6-/7-nitro and methyl 5-fluoro/chloro/bromoindole-2-carboxylates on reaction with hydrazine hydrate yielded corresponding 4-/5-/6-/7-nitroindole-2-carbohydrazides and 5-fluoro/chloro/bromoindole-2-carbohydrazides **1a-g**. 4-/5-/6-/7-Nitroindole-2-carbohydrazides and 5-fluoro/chloro/bromoindole-2-carbohydrazides **1a-g** on treatment with acetyl acetone in methanol yielded 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-4-/5-/6-/7-nitro and 5-fluoro/chloro/bromo-1*H*-indoles **2a-g**. 4-/6-/7-Nitroindole-2-carbohydrazides **1a-d** on treatment with CS_2/KOH in methanol resulted in the formation of 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol **3a-3c** and with cyanogen bromide in methanol yielded 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amines **5a-c**. The 4-/5-/6-/7-nitroindole-2-carbohydrazides **1a-d** were also refluxed with



Scheme I

substituted aromatic acids in presence of phosphorous oxychloride yielded 2-[5-(aryl)-1,3,4-oxadiazol-2-yl]-4-/5-/6-/7-nitro-1*H*-indoles **4a-z** in 60-86% yields[20]. 4-/6-/7-Nitroindole-2-carbohydrazides on treatment with triethyl orthoformate resulted in the formation of 4-/6-/7-nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indole **6a-c** (**Scheme I**). The newly synthesized compounds were characterized by elemental, IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. Characterization data of the compounds are given in **Table I**. Spectral data of the newly synthesized compounds are given in the experimental section.

The ^{1}H NMR spectrum of 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-bromo-1*H*-indole **2g** displayed two singlets at δ 2.37 and 2.66 were due to three protons of two CH_3 groups and a singlet appeared at δ 6.05 was due to CH proton of pyrazole ring. Aromatic proton resonated as δ 7.37 (s, 1H), 7.39 (dd, 2H *J* = 2.0, 8.0 Hz), 7.62 (d, 1H *J* = 2.0 Hz) and 7.85 (s, 1H) respectively. The NH proton of the indole ring appeared as a downfield singlet at δ 11.12. The ^{13}C NMR spectrum displayed a pattern δ 14.01 (CH_3), 14.97 (CH_3), 96.19 (CH), 110.89, 113.22, 113.92, 125.06, 128.41, 128.86, 130.61, 135.78, 146.20, 152.73 and 159.0 (C=O) which exactly accounts for 14 carbon atoms present in the molecule. The FABMS showed molecular ion peak at *m/z* 318 (60%) and an isotopic peak at *m/z* 320 (60%, M+2). Other prominent peaks were at 239 (10%, M-Br), 224 (100%, M-CH_3Br), 222 (90%) and 154 (65%) respectively.

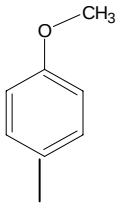
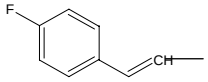
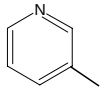
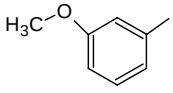
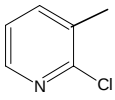
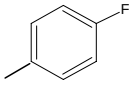
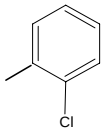
The FABMS of 5-(4-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol **3a** showed molecular ion peak at *m/z* 262 (40%, M^+) and *m/z* 263 (60%, M+1) in accordance with its molecular formula, $C_{10}H_6N_4O_3S$. The ^{1}H NMR spectrum 5-(6-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amines **5b** exhibited a singlet at δ 7.46 due to two protons of the NH_2 group. The aromatic protons resonated as a singlet at 7.05, two doublets at 7.81 (*J* = 8.7Hz) and 7.93 (*J* = 8.7Hz) and a singlet at 8.29 respectively. The NH proton appeared as a singlet at δ 12.72. The ^{13}C NMR displayed a pattern δ 102.44, 108.24, 115.00, 121.45, 125.69,127.98, 132.33, 135.68 , 143.26, 169 (C-NH_2) account for carbon atoms in the molecule and DEPT 135º(75 MHz, DMSO-d_6): δ 102.45 (CH), 108.36 (CH), 115.02 (CH), 121.47 (CH) confirmed the presence of four C(CH)-atoms of indole nucleus (C-3, C-4, C-5, C-7) as signals due to CH carbons only will appear in the DEPT spectrum. The IR spectrum of 4-nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indole **6a** displayed bands at 3100 cm^{-1} accounts for NH stretching and 1650 cm^{-1} accounts for C=N stretch. The FABMS displayed a protonated molecular ion peak at *m/z* 231 (10%, M+1), which is in accordance with its molecular formula, $C_{10}H_6N_4O_3$.

Biological studies

Antiinflammatory activity

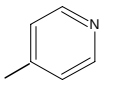
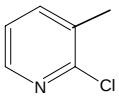
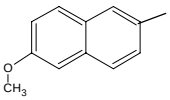
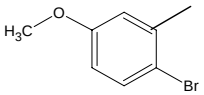
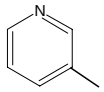
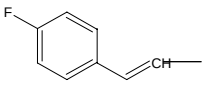
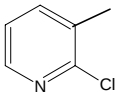
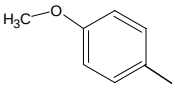
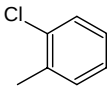
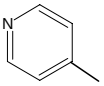
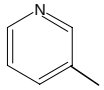
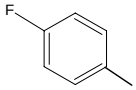
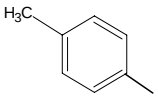
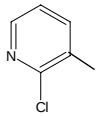
Seven of the newly synthesized compounds **2c, 2d**, **3a**, **3b**, **3c, 5a** and **5b** were evaluated for their anti-inflammatory activity against carrageenin-induced

Table I — Characterization data of the compounds **2a-g**, **3a-c**, **4a-z***, **5a -c** and **6a-c**

Compd	R	Ar	Yield (%)	m.p (ºC)	Colour of the crystals(crude)	N Found(Calcd)%
2a	4-NO_2	-	75	200-202	Yellow powder	19.54 (19.71)
2b	5-NO_2	-	75	168-70	Brown microcrystals	19.63 (19.71)
2c	6-NO_2	-	74	223-25	Light yellow needles	19.58 (19.71)
2d	7-NO_2	-	75	186-88	Light yellow needles	19.72 (19.71)
2e	5-Fluoro	-	72	116-17	Brown microcrystals	16.17 (16.33)
2f	5-Chloro	-	81	134-36	Cream powder	15.24 (15.35)
2g	5-Bromo	-	79	148-49	White microcrystals	13.09 (13.21)
3a	4-NO_2	-	70	218-20	Orange crystals	21.12 (21.36)
3b	6-NO_2	-	68	230-32	Orange yellow crystals	21.28 (21.36)
3c	7-NO_2	-	70	210-15	Orange yellow crystals	21.25 (21.36)
4a	4-NO_2		80	>250	Yellow crystals	16.58 (16.66)
4b	4-NO_2		75	150	Yellow crystal	15.89 (15.99)
4c	4-NO_2		74	>250	Orange yellow crystals	22.54 (22.79)
4d	4-NO_2		80	180-82	Yellow crystals	16.50 (16.66)
4e	4-NO_2		85	>250	Orange crystals	20.49 (20.50)
4f	4-NO_2		75	>260	Yellow crystals	17.25 (17.28)
4g	5-NO_2		86	>250	Light brown crystals	16.38 (16.47)

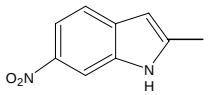
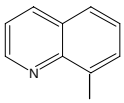
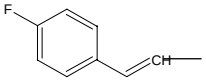
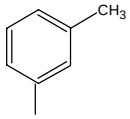
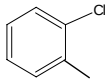
—Contd

Table I — Characterization data of the compounds **2a-g**, **3a-c**, **4a-z***, **5a -c** and **6a-c**(*—Contd*)

Compd	R	Ar	Yield (%)	m.p (ºC)	Colour of the crystals(crude)	N Found(Calcd)%
4h	5-NO_2		78	>250	Brown crystals	22.83 (22.79)
4i	5-NO_2		76	194-96	Brown crystals	20.50 (20.49)
4j	5-NO_2		75	>250	Dark brown crystals	14.46 (14.50)
4k	5-NO_2		78	200-202	Brown crystals	13.35 (13.49)
4l	6-NO_2		68	>250	Light brown crystals	22.74 (22.79)
4m	6-NO_2		75	>250	Yellow crystals	16.04 (15.99)
4n	6-NO_2		70	>250	Orange yellow crystals	20.46 (20.50)
4o	6-NO_2		80	>250	Dark yellow crystals	16.62 (16.66)
4p	6-NO_2		87	>250	Yellow crystals	16.40 (16.47)
4q	7-NO_2		83	238-30	Yellow crystals	22.78 (22.79)
4r	7-NO_2		70	248-50	Yellow crystals	22.74 (22.79)
4s	7-NO_2		68	230-32	Yellow crystals	17.19 (17.28)
4t	7-NO_2		65	182(Dec)	Yellow crystals	17.45 (17.49)
4u	7-NO_2		65	198-200	Beige crystals	20.45 (20.50)

—Contd

Table I — Characterization data of the compounds **2a-g**, **3a-c**, **4a-z***, **5a -c** and **6a-c**(**—***Contd*)

Compd	R	Ar	Yield (%)	m.p (ºC)	Colour of the crystals(crude)	N Found(Calcd)%
4v	7-NO_2		80	>250	Yellow crystals	21.50 (21.53)
4w	7-NO_2		68	248-50	brown microcrystals	19.56 (19.59)
4x	7-NO_2		60	240-42	Beige crystals	16.05 (15.99)
4y	7-NO_2		75	222-25	Yellow crystals	17.43 (17.48)
4z	7-NO_2		75	210-12	Yellow crystals	16.40 (16.44)
5a	4-NO_2	-	70	>250	Yellowish crystals	28.49 (28.56)
5b	6-NO_2	-	78	>250	Yellow powder	28.34 (28.56)
5c	7-NO_2	-	68	215-18	Brown microcrystals	28.49 (28.56)
6a	4-NO_2	-	74	195	Yellow powder	24.27 (24.34)
6b	6-NO_2	-	69	>250	Yellow crystals	24.26 (24.34)
6c	7-NO_2	-	75	200-202	Yellow powder	24.38 (24.34)

*Taken from previously reported paper.

acute paw edema in rats weighing 150- 200g (ref.23). Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) were used for the experiment. They were housed in standard polypropylene cages and kept under RT (24 ± 2ºC), relative humidity (60-70%) in a 12 hr light-dark cycle. The animals were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 hr before and during experimental hr. Institutional ethics committee approved all the experiments.

As shown in **Table II,** the rats were divided in to nine groups of one each as. Group-1 received 10 mL/kg of 2% gum acacia, group-2 received Indomethacin at a dose of 1.5 mg/kg. 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} , 8^{th} and 9^{th} group administered the test compounds **2c**, **2d**, **3a**, **3b**, **3c, 5a** and **5b** respectively at a dose of 50 mg/kg suspended in 10 mL/kg of 2% gum acacia orally by gavage feeding. Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan with gum *acacia* in normal saline in the left hind paw of the rats, one hr after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0 hr and 3 hr after carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage antiinflammatory activity was calculated using the formula,

$$\% \text{ of edema inhibition} = 100 - (V_{test} / V_{control}) \times 100$$

Where, $V_{control}$ = Volume of paw edema in control group

V_{test} = Volume of paw edema in drug treated group

The results were expressed as% inhibition of edema over the untreated control group. **Table II** shows the effect of drug and extract treatment on

Table II — Antiinflammatory activity compounds **2c, 2d, 3a, 3b, 3c, 5a** and **5b**

Drug/Compd 2% gum *acacia*	Dose (mg/kg, p.o)	Increase in paw% edema volume in mL	Inhibition of paw edema
(Control)	10 mL/kg	0.53	
Indomethacin (Standard drug)	1.5	0.25	53
2c	50	0.51	3.8
2d	50	0.53	0
3a	50	0.41	22.6
3b	50	0.53	0
3c	50	0.53	0
5a	50	0.47	11.3
5b	50	0.53	0

carrageenan induced edema. The percentage of inhibition was compared with that of standard drug indomethacin (1.5 mg/kg). The compound 5-(4-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol **3a** exhibited maximum activity in comparison with other test compounds. The compound 5-(4-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amine **5a** exhibited a moderate activity. It can be seen from the activities that presence of thiol group has enhanced activity in comparison to amino group. The exact structure activity relationship cannot be drawn based on these preliminary data.

Antibacterial studies

The newly synthesized compounds **2a-g, 3a-c, 4a-z, 5a-c** and **6a-c** were screened for their antibacterial activity against *Escherichia coli, Staphyllococcus aureus* (Smith), *Psuedomonus aeruginosa* (Gessard), *and Klebsiella pneumoniae* (Friedlander) bacterial strains by disc diffusion method[24-26]. The discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140ºC for an hr. The test compounds were prepared with different concentrations such as < 10μg/mL and >10 μg/mL in dimethylformamide. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37ºC for 24 hr. Ampicillin was used as standard drug. Solvent and growth controls were kept. The zones of inhibition and minimum inhibitory concentrations [MIC] were noted. The results of such studies are given in **Table III**.

The compounds 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-chloro-1*H*-indole 2f, 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-bromo-1*H*-indole 2g, 5-(7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol 3c, 2-[5-(4-pyridinyl)-1,3,4-oxadiazol-2-yl]-5-nitro-1H-indole 4h and 2-[5-(4-pyridinyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1H-indole 4q, 5-(7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amines 5c were found to exhibit highest antibacterial activity compared to all other compounds. Presence of halo group on the indole ring and pyridine substituent on the oxdiazole rind has enhanced the antibacterial activity.

Antifungal studies

The newly synthesized compounds 2a-g, 3a-c, 4a-z, 5a-c and 6a-c were screened for their antifungal activity against *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigatus* (NCIM No.902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[24-26]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the *p*H to 5.7. Normal saline was used to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL Saline to get a suspension of corresponding species. 20 mL of agar media was poured in to each petridishes. Excess suspension was decanted and the plates were dried by placing in an incubator at 37ºC for 1hr. Using an agar punch wells were made on these seeded agar plates and <10 μg/mL and >10 μg/mL of the test

Table III — Antibacterial and antifungal screening data of the compounds **2a-g**, **3a-c**, **4a-z** and **5a-c** at 6.25 – 100 μg/mL

Compd	Antibacterial activity (Zone of inhibition in mm)					Antifungal activity (Zone of inhibition in mm)		
	E.coli,	*S. aureus*	*P.aeruginosa*	*K. pneumoniae*	*A.flavus*	*A.fumigatus*	*P. arneffe*	*T.mentagrophytes*
2b	6	-	8	-	-	-	6	-
2c	6	-	8	-	6	-	6	-
2d	18	6	-	8	-	-	-	6
2f	18	20	19	20	18	20	18	18
2g	18	18	18	18	18	8	8	10
3a	-	-	-	-	-	8	-	9
3b	-	-	-	-	-	-	8	-
3c	-	18	18	18	18	18	18	18
4a	12	6	-	6	5	18	19	18
4b	18	-	5	10	-	6	5	-
4c	10	7	-	-	-	18	-	-
4d	10	-	-	-	18	-	-	-
4e	18	-	-	10	-	-	18	-
4f	18	-	-	12	-	-	18	-
4g	-	19	-	-	-	19	-	19
4h	19	18	18	18	-	-	-	-
4i	6	5	-	17	16	-	16	-
4j	6	6	-	-	-	-	-	-
4k	6	5	6	-	16	10	6	10
4l	4	4	17	-	6	-	6	-
4m	6	4	-	-	-	-	-	-
4n	5	10	18	-	18	19	-	-
4o	6	19	-	-	6	-	5	-
4p	-	-	-	-	6	-	5	-
4q	18	18	-	18	-	18	-	-
4r	-	-	-	-	18	18	18	18
4s	-	5	6	-	22	19	18	19
4t	10	-	-	-	18	19	18	18
4u	-	5	6	-	18	18	18	-
4v	-	-	5	-	-	19	-	19
4w	10	-	6	10	18	18	-	-
4x	16	-	6	18	18	-	18	-
4y	12	-	-	-		18	18	18
4z	-	10	10	-	18	18	-	-
5a	-	-	-	-	-	-	-	-
5b	-	-	10	-	-	-	-	-
5c	18	22	18	8	18	19	18	20
6c	-	18	-	-	-	-	-	-
Ampicillin	20	28						
Amphotericin B	-	-	-	>20	>20	>20	>20	

compounds in DMSO were added in to each well labeled. A control was also prepared for the plates in the same way using solvent DMSO. The petridishes were prepared in triplicate and maintained at 37ºC for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with amphotericin B as standard.

The results are given in Table III. The compounds 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-chloro-1*H*-indole 2f, 5-(7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol 3c, 2-[5-(4-methoxyphenyl)-1,3,4-oxadia-

Table IV— Three cell line *in vitro* antiproliferative screening **4a-x**

Sl.no.	Compd	NCI code	Activity
1	**4a**	NSC: 735450	Active
2	**4b**	NSC: 735463	Active
3	**4c**	NSC: 735454	Active
4	**4d**	NSC: 735453	Active
5	**4e**	NSC: 734879	Active
6	**4f**	NSC: 735452	Active
7	**4i**	NSC: 736260	Inactive
8	**4j**	NSC: 736953	Inactive
9	**4l**	NSC: 736257	Inactive
10	**4n**	NSC: 736259	Active
11	**4q**	NSC: 734881	Active
12	**4r**	NSC: 734882	Active
13	**4s**	NSC: 734885	Active
14	**4u**	NSC: 734883	Active
15	**4x**	NSC: 734884	Active

Fixed concentration (100 μM; standard NCI protocol)

zol-2-yl]-4-nitro-1*H*-indole **4a**, 2-[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole **4r**, 2-[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole **4s**, 2-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole **4t**, 2-[5-(2-chloronicotinyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole 4**u** and 2-[5-(3-methylphenyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole **4y**, 5-(7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amines 5c were found to exhibit excellent antifungal activity as compared to all other compounds. It is also interesting to note that oxadiazole bearing 7-nitro-indoles possessed excellent antifungal activity compared to other nitroindole derivatives. Halo indole derivatives did not exhibit promising activity unlike it was found in the case of antibacterial activity.

Antiproliferative activity

Among the newly synthesized 2-[5-(aryl)-1,3,4-oxadiazol-2-yl]-4-/5-/6-/7-nitro-1*H*-indoles **4a-z**, about fifteen compounds **4a-f**, **4q-s**, **4u** and **4x** were selected for their *in vitro* antiproliferative activity by National Institute of Health, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI as per the procedure suggested by Boyd and Paul[27] in a primary three cell line one dose antitumor assay against NCI-H (Lung), MCF-7 (Breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on a pre-incubated microtiter plate. Test agents are added at a single concentration and culture is incubated for 48 hr. End point of determinations was made with sulpharhodamine B, a protein binding dye. Compounds which reduce the growth of any one of the cell line to 32% or less (negative numbers indicate cell kill) are passed for evaluation in a panel of 60 cell lines over a 5-long dose range.

Among the fifteen tested compounds, twelve compounds were selected for 60-cell line screening and considered as active which are given in **Table IV**. The 60 cell screening results showed that, except 2-[5-(2-chloro-3-pyridinyl)-1,3,4-oxadiazol-2-yl]-5-nitro-1*H*-indole **4i,** 2-[5-(6-methoxy-2-naphthyl)-1,3,4-oxadiazol-2-yl]-5-nitro-1*H*-indole **4j** and 2-[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]-6-nitro-1*H*-indole **4l** all other compounds showed promising anti-proliferative activity on the whole panel of 60 cells derived from nine cancer cells namely leukemia, lung, colon, melanoma, renal, ovarian, CNS, prostrate and breast cells. Their GI_{50} values were determined. The 60 cell results are presented in **Table V**.

It can be seen from the **Table V** that the compound 2-[5-(6-methoxy-2-naphthyl)-1,3,4-oxadiazol-2-yl]-5-nitro-1*H*-indole **4j** and 2-[5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl]-6-nitro-1*H*-indole **4p** exhibited least antiproliferative activity compared to other compounds. The compounds 2-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-4-nitro-1*H*-indole **4a**, 2-[5-(4-fluoro-cinnamyl)-1,3,4-oxadiazol-2-yl]-4-nitro-1*H*-indole **4b**, 2-[5-(3-pyridinyl)-1,3,4-oxadiazol-2-yl]-4-nitro-1*H*-indole **4c**, 2-[5-(4-fluorocinnamyl)-1,3,4-oxadiazol-2-yl]-6-nitro-1*H*-indole **4m** and 2-[5-(2-chloronicotinyl)-1,3,4-oxadiazol-2-yl]-6-nitro-1*H*-indole **4n** exhibited moderate antiproliferative activity.

Experimental Section

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by TLC using Merck silica gel G60 F_{254} coated aluminium plates. IR spectra were recorded on a Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Varian spectrometer using TMS as internal standard and ^{13}C NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian spectrometer at IISc., Bangalore. FABMS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas at CDRI, Lucknow and elemental analysis was done on VARIO EL-III (Elementar Analysensysteme GmbH) at Department of Chemistry, Mangalore University.

Table V — 60-Cell line *in vitro* antiproliferative screening data of **4a-f, 4q-s, 4u** and **4x** (GI_{50} in μM)

Panel/cell line	GI_{50}, μM										
Leukemia	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4q**	**4r**	**4s**	**4u**	**4x**
CCRF-CEM	-	-	-	-	-	-	27.1	81.2	>100	>100	22.4
HL-60 (TB)	26.8	11.0	11.2	17.7	13.4	-	-	-	0.861	-	-
K-562	16.1	0. 90	24.3	6.91	7.68	10.3	23.1	>100	>100	26.9	53.6
MOLT-4	33.2	25.6	39.8	20.0	10.8	21.4	44.2	61.3	>100	>100	>100
RPMI-8226	18.1	26.3	33.1	17.2	7.48	3.10	28.6	>100	>100	34.5	54.2
SR	-	-	-	-	-	-	38.7	>100	-	53.9	41.1
Non-small cell lung Cancer											
A549/ATCC	18.6	50.8	>100	35.5	16.4	12.3	>100	>100	>100	12.9	>100
EKVX	>100	>100	>100	>100	>100	40.2	87.0	51.6	>100	27.9	>100
HOP-62	20.4	>100	>100	23.9	31.8	17.0	67.30	15.8	>100	20.2	.0518
HOP-92	16.1	42.1	36.9	13.8	12.9	15.0	0.306	34.5	>100	17.7	6.21
NCI-H226	28.2	>100	>100	46.3	64.9	26.2	>100	60.2	>100	29.0	>100
NCI-H23	36.8	>100	>100	33.4	54.5	14.9	>100	48.0	>100	24.6	45.6
NCIH322M	40.9	81.4	>100	22.5	35.2	17.5	>100	29.1	>100	23.1	>100
NCI-H460	41.3	52.7	77.3	45.3	33.2	19.4	>100	>100	>100	>100	>100
NCI-H	522	67.1	>100	63.1	30.5	99.7	16.9	>100	51.2	>100	16.2
A549/ATCC	-	-	-	-	-	-	-	-	-	-	-
EKVX	-	-	-	-	-	-	-	-	-	-	-
Colon cancer											
COLO 205	>100	>100	>100	>100	85.3	>100	55.3	>100	>100	19.8	>100
HCC-2998	>100	>100	>100	>100	42.3	18.6	>100	>100	>100	24.6	>100
HCT-116	23.8	47.8	>100	17.0	16.8	10.3	44.9	71.5	>100	42.8	.0211
HCT-15	77.0	97.0	>100	35.8	11.7	13.0	35.4	92.1	>100	30.9	>100
HT29	>100	47.1	>100	49.8	10.5	14.6	28.7	>100	>100	25.8	>100
KM12	34.2	52.1	99.3	19.2	17.2	12.8	>100	>100	>100	14.4	>100
SW-620	65.9	>100	>100	47.5	26.5	18.9	42.5	>100	>100	43.1	>100
CNS cancer											
SF-268	44.1	47.9	>100	91.6	53.4	28.2	>100	26.4	>100	81.2	<0.01
SF-295	37.4	40.6	89.5	27.5	32.5	18.4	97.4	46.0	>100	10.7	61.3
SF-539	23.3	>100	>100	35.8	>100	17.7	37.4	42.8	>100	23.6	45.5
SNB-19	61.2	>100	>100	-	2.03	>100	44.0	20.5	>100	19.1	20.5
SNB-75	-	24.7	>100	-	-	5.07	15.0	13.9	>100	15.2	96.0
U251	16.8	71.4	53.6	16.9	14.1	12.8	44.4	25.0	>100	20.0	28.0
Melanoma											
LOX IMVI	39.7	14.2	>100	19.3	55.0	14.2	24.8	46.4	95.1	39.6	>100
MALME-3M	>100	7.2	>100	12.0	16.3	7.20	88.6	20.2	>100	15.8	>100
M14	26.8	40.2	>100	>100	37.6	17.2	>100	87.3	>100	>100	0.233
SK MEL-2	>100	>100	>100	>100	65.4	40.4	>100	18.9	>100	19.3	31.1
SK MEL-28	>100	51.2	>100	>100	0.01	13.1	>100	82.3	>100	23.8	>100
SK MEL-5	95.3	>100	>100	60.8	25.5	21.0	49.4	>100	>100	25.7	>100
UACC-257	28.6	60.8	99.8	47.1	17.1	12.5	>100	>100	>100	42.2	>100
UACC-62	76.1	46.6	>100	>100	41.1	19.0	>100	21.0	>100	21.3	>100

—*Contd*

Table V — 60-Cell line *in vitro* antiproliferative screening data of **4a-f, 4q-s, 4u** and **4x** (GI_{50} in μM) (**—***Contd*)

Ovarian cancer											
IGROV1	27.7	23.0	98.4	12.8	16.5	15.8	7.15	4.12	>100	1.84	33.7
OVCAR-3	25.5	50.3	98.7	22.1	30.1	20.1	64.7	>100	>100	16.9	>100
OVCAR-4	37.9	34.1	>100	15.7	<0.01	2.90	>100	>100	>100	17.9	>100
OVCAR-5	27.4	>100	>100	20.2	13.6	14.1	>100	37.0	>100	22.5	51.5
OVCAR-8	14.6	>100	26.2	17.2	6.81	4.58	40.1	24.6	>100	5.46	53.4
SK-OV-3	76.7	>100	>100	<0.01	28.5	>100	>100	17.0	>100	10.8	18.4
Renal cancer											
786-O	18.8	>100	>100	19.5	20.4	13.0	33.3	24.0	>100	19.5	0.049
A498	>100	>100	>100	>100	>100	14.1	>100	35.4	>100	22.8	45.6
ACHN	40.2	>100	>100	>100	20.2	19.1	38.6	31.2	>100	15.0	44.6
CAKI-1	41.8	>100	>100	76.6	28.8	20.8	>100	>100	>100	16.9	>100
RXF-393	17.3	52.4	67.6	18.5	13.0	11.1	16.2	12.7	>100	5.0	16.6
SN12C	20.8	72.4	>100	53.1	16.2	17.9	>100	39.0	>100	25.5	>100
TK-10	22.7	>100	>100	21.8	23.8	19.4	28.7	42.9	>100	20.3	19.3
UO-31	21.2	71.1	>100	62.1	21.0	16.4	16.8	5.89	42.3	1.94	18.8
Prostate cancer											
PC-3	35.1	38.7	73.9	18.3	7.66	15.8	>100	41.8	>100	22.3	>100
DU-145	44.2	>100	>100	44.4	21.6	15.6	>100	53.9	>100	19.2	50.7
Breast cancer											
MCF7	63.2	32.4	>100	36.8	36.8	21.5	74.9	>100	>100	>100	-
NCI/ADR-RES	28.6	>100	>100	35.3	19.9	10.5	>100	50.8	>100	12.9	>100
MDA-MB-231	16.2	36.1	46.1	22.5	14.4	13.9	23.2	11.4	>100	23.2	23.2
/ATCC											
HS-578T	24.7	>100	49.9	39.6	44.9	14.8	>100	34.9	>100	29.1	58.7
MDA-MB435	73.4	29.2	>100	41.9	28.3	16.8	>100	>100	>100	11.0	>100
BT-549	33.7	>100	>100	59.3	>100	21.0	-	-	>100	-	-
T-47D	>100	>100	>100	<0.01	4.75	>100	60.8	22.1	>100	16.4	34.5

General procedure for the synthesis of 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-4-/5-/6-/7-nitro and 5-fluoro/chloro/bromo-1*H*-indoles 2a-g

4-/5-/6-/7-Nitroindole-2-carbohydrazides or 5-fluoro/chloro/bromoindole-2-carbohydrazides (0.001 mole) were refluxed with acetyl acetone (0.0015 mole) in methanol (10 mL) and catalytic amount of acetic acid for 3-4 hr. Solids were separated on cooling, washed with *n*-hexane and recrystallised from DMF-methanol mixture.

General procedure for the synthesis of 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol derivatives 3a-c

4-/6-/7-Nitroindole-2-carbohydrazide (0.001 mole) was refluxed with alcoholic KOH and carbon disulphide (0.0015 mole) for 5 hr the reaction-mixture was then cooled and acidified with conc. HCl. The solid separated was filtered, washed with cold water and recrystallised using DMF-methanol mixture.

General method for the synthesis of 2-[5-(aryl)-1,3,4-oxadiazol-2-yl]-4-/5-/6-/7-nitro-1*H*-indoles 4a-z

A mixture of 4-/5-/6-/7-nitroindole-2-carbohydrazide (0.001mole), aromatic carboxylic acid (0.0015 mole) in 4 mL phosphorus oxychloride was refluxed on an oil- bath for 6 hr. The excess phosphorus oxychloride was distilled under reduced pressure. The cooled reaction mass was then poured into ice-cold water with stirring. The separated solid was filtered and washed with sodium bicarbonate solution and then with water. The product was then recrystallised from ethanol-dimethylformamide mixture.

General procedure for the synthesis of 5-(4-/6-/7-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amines 5a-c

4-/6-/7-Nitroindole-2-carbohydrazide (0.001mole) was stirred with cyanogen bromide (0.0015 mole) in methanol for 1-2 hr and then added saturated sodium bicarbonate solution (10 mL). Filtered the solid and washed with water and recrystallised from DMF-methanol mixture.

General procedure for the synthesis of 4-/6-/7-nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indole 6a-c

A mixture of 4-/6-/7-nitroindole-2-carbohydrazide (0.001mole), triethyl orthoformate (0.0015 mole) in toluene was refluxed on an oil-bath for 6 hr. The excess solvent was distilled under reduced pressure. The reaction mass was cooled and the separated solid was filtered and washed with acetone. The product was then recrystallised from ethanol – dimethylformamide mixture.

Spectral data

2-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-nitro-1*H*-indole 2b. FABMS: *m/z* 284 (55%, M^+), 286 (75%, M+1), 286 (25%, M+2).

2-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-7-nitro-1*H*-indole 2d. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 6.36 (s, 1H, =CH), 7.36 (t, 1H, ArH *J* = 6.9Hz), 7.82 (s, 1H, ArH), 8.30 (t 1H, ArH *J* = 8.1Hz), 12.39 (s, -NH); FABMS: *m/z* 284 (85%, M^+), 286 (75%, M+1), 286 (20%, M+2).

2-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-fluoro-1*H*-indole 2e. FABMS: *m/z* 256 (10%, M-1), *m/z* 257 (100%, M^+), 258 (95%, M+1), 259 (20%, M+2H), 161 ($C_9H_{11}N_3$, 25%), 162 (($C_9H_{11}N_3$)+H, 75%).

2-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-chloro-1*H*-indole 2f. IR(KBr): 3332 (-NH), 1651 cm^{-1} (-C=O).

2-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-bromo-1*H*-indole 2g. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 2.66(s, 3H, CH_3), 6.05(s, 1H, =CH), 7.37 (s, 1H, ArH), 7.39 (dd, 2H, ArH *J* = 2.0, 8.0Hz), 7.62 (d, 1H, ArH, *J* = 2.0Hz), 7.85 (s, 1H, ArH), 11.12 (s, -NH); ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 14.01 (CH_3), 14.97 (CH_3), 96.19 (CH), 110.89, 113.22, 113.92, 125.06, 128.41, 128.86, 130.61, 135.78, 146.20, 152.73, 159.0 (C=O); FABMS: *m/z*, 317 (65%, M-1), 318 (60%, M^+), 319 (64%, M+1) 320 (60%, M+2), 239 (10%, M-Br), 224 (100%, M-CH_3Br), 222 (90%), 154 (65%).

5-(4-Nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol 3a. FABMS: *m/z* 262 (40%, M^+), 263 (60%, M+1), 154 (100%), 136 (80%).

5-(7-Nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol 3d. FABMS: *m/z* 262 (65%, M^+), 189 (100%, $C_9H_7N_3O_2$, protonated 7-nitro-1*H*-indole-2-carbonitrile cation), 220 (4%, 7-nitro-1*H*-indole-2-carbohydrazonic acid cation).

5-(4-Nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amine 5a. FABMS: *m/z* 245 (20%, M^+), 246 (55%, M+1).

5-(6-Nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amine 5b. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05 (s, 1H, ArH) 7.46 (s, 2H, NH_2), 7.81 (d, 1H, ArH, *J* = 8.7Hz), 7.93 (d, 1H, ArH, *J* = 8.7Hz), 8.29 (s, 1H, ArH), 12.72 (s, -NH); ^{13}C NMR 135°C (75MHz, DMSO-d_6): δ 102.44, 108.24, 115.00, 121.45, 125.69, 127.98, 132.33, 135.68, 143.26,169; DEPT(135 MHz, DMSO-d_6): δ 102.45 (CH), 108.36 (CH), 115.02 (CH), 121.47 (CH).

5-(7-Nitro-1*H*-indol-2-yl)-1,3,4-oxadiazol-2-amine 5c. IR (KBr): 3458 (NH_2), 3136 (CH), 1675 cm^{-1} (C=N); FABMS: *m/z* 245 (20%, M^+); 246 (45%, M+1), 176 (25%), 154 (100%), 136 (80%).

4-Nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indole 6a. IR (KBr) 3100 (NH), 1650 cm^{-1} (C=N); FABMS: *m/z* 231 (10%, M+1), 221 (10%), 189 (20%), 154 (100%), 136 (90%).

7-Nitro-2-(1,3,4-oxadiazol-2-yl)-1*H*-indole 6c. IR (KBr) 3200, 1650 cm^{-1} (C=N); FABMS: *m/z* 232 (10%, M+2), 221(10%), 189(25%), 154 (100%), 136(70%).

Conclusion

Synthesis and studies on antimicrobial, antiinflammatory and antiproliferative activities of heterocycles derived from 4-/5-/6-/7-nitro/5-fluoro/chloro/bromo-indole-2-carbohydrazides are discussed. The new compounds were screened for their antibacterial, antiinflammatory and antiproliferative activities. The compounds 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-chloro-1*H*-indole **2f** and 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl) carbonyl]-5-bromo-1*H*-indole **2g** were found to exhibit highest antibacterial activity and 2-[(3,5-dimethyl-1*H*-pyrazol-1-yl)carbonyl]-5-chloro-1*H*-indole **2f** and 2-[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1*H*-indole **4s** were found to exhibit highest antifungal activity. The compound 5-(4-nitro-1*H*-indol-2-yl)-1,3,4-oxadiazole-2-thiol derivatives **3a**

exhibited promising antiinflammatory activity. The compound 2-[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-4-nitro-1H-indole **4f** exhibited highest anti-proliferative activity and the compounds 2-[5-(2-chloronicotinyl)-1,3,4-oxadiazol-2-yl]-4-nitro-1H-indole **4e**, 2-[5-(2-chloronicotinyl)-1,3,4-oxadiazol-2-yl]-7-nitro-1H-indole **4u** exhibited moderate anti-proliferative activity.

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