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Synthesis of *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amines as antimicrobial and anticancer agents

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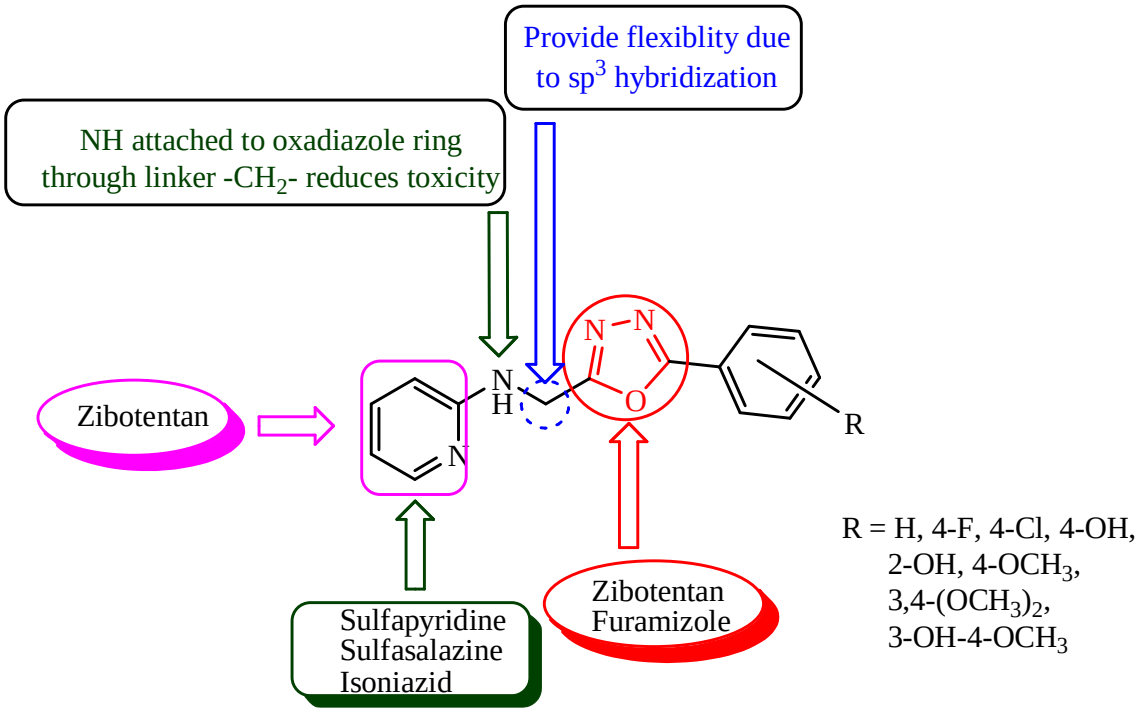
Graphical Abstract



Abstract:
A new series of oxadiazole analogues was synthesized starting from 2-aminopyridine. The compounds were characterized by infrared (IR), nuclear magnetic resonance (NMR), and mass spectral analyses followed by determination of their anticancer and antimicrobial activities. Three compounds were tested for *in vitro* anticancer activity against NCI-60 human cell lines of nine different panels including leukemia, non-small lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer according to the National Cancer Institute (NCI, USA) Protocol at 10 μM. The compounds *N*-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**5c**), *N*-{[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**5f**), and *N*-{[5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**5g**) showed anticancer with higher selectivity towards HOP-92 (Non-Small Cell Lung Cancer). *N*-{[5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**5b**) showed maximum antibacterial activity with minimum inhibitory concentration (MIC) of 4-8 μg/mL, while *N*-{[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**5f**) showed maximum antifungal activity with MIC 4 μg/mL.
Keywords: anticancer agents; antibacterial; antifungal; one-dose assay; oxadiazole analogues

Introduction

Drug resistance is a big apprehension nowadays in both anticancer and antimicrobial therapy. The indiscriminate use of antibiotics causes attribution to the emergence of drug resistance to majority of antibacterial agents .

On the other hand fungal infections like Candidiasis, Crytococcosis and Aspergillosis are more common in immuno-compromised patients.

Owing to this increased microbial resistance, new classes of antimicrobial agents with novel mechanisms of action are today need to fight against the multidrug-resistant infections.

Cancer causes nearly 13 percent total deaths globally surpassing cardiovascular disease. In India the total number of cancer cases are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020. A total of 1,658,370 new cancer cases and 589,430 cancer deaths are projected to occur in the United States in 2015 and it is expected that the new cases of cancer will jump to 19.3 million worldwide by 2025.

The biological prospects of oxadiazoles as anticancer, antitubercular, anticonvulsant, antimicrobial, anti-HIV, anti-inflammatory inspired us to go on further with the exploration of this moiety.

Introduction

In the present investigation the scaffold was designed containing oxadiazole nucleus attached to hydrophobic aryl ring (Zibotentan contains both oxadiazole and pyridine rings) through a methylene group, which imparts flexibility to the molecule due to sp^3 hybridization, so that the compound may well accommodate in their target sites, with the hope of increased biological activity.

The NH attached to the oxadiazole ring through linker (-CH_2-) is expected to play an important role in reducing toxicity (**Fig. 1**). Some of the antibacterial/ antimycobacterial drugs (sulfapyridine, sulfasalazine, isoniazid etc.) also contain pyridine. Similarly furamizole that contains oxadiazole ring exhibits a strong antibacterial activity.

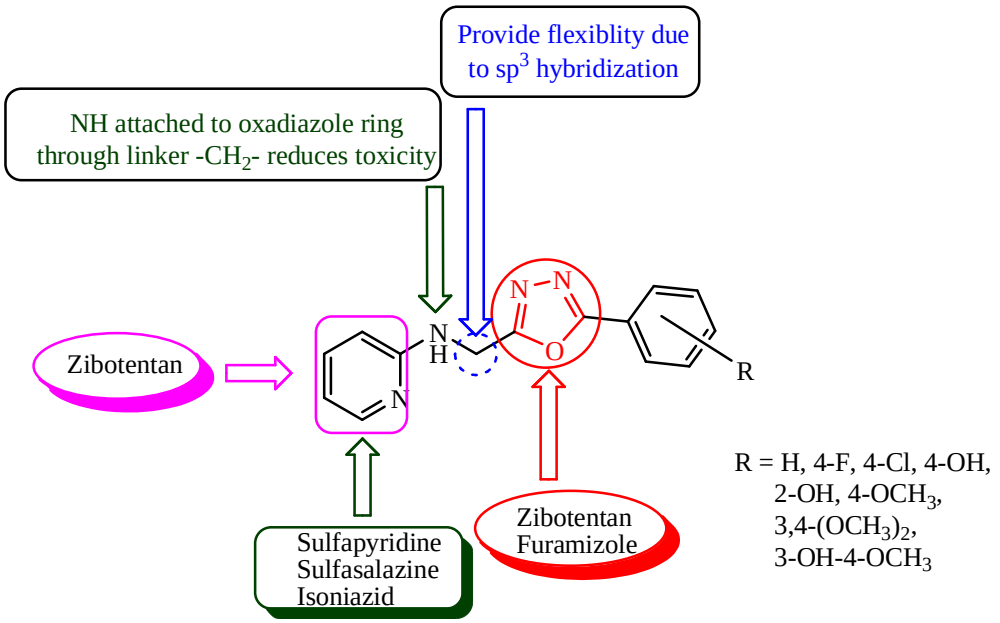


Fig. 1. Design of newer oxadiazole scaffolds as biologically active agents

Results and discussion

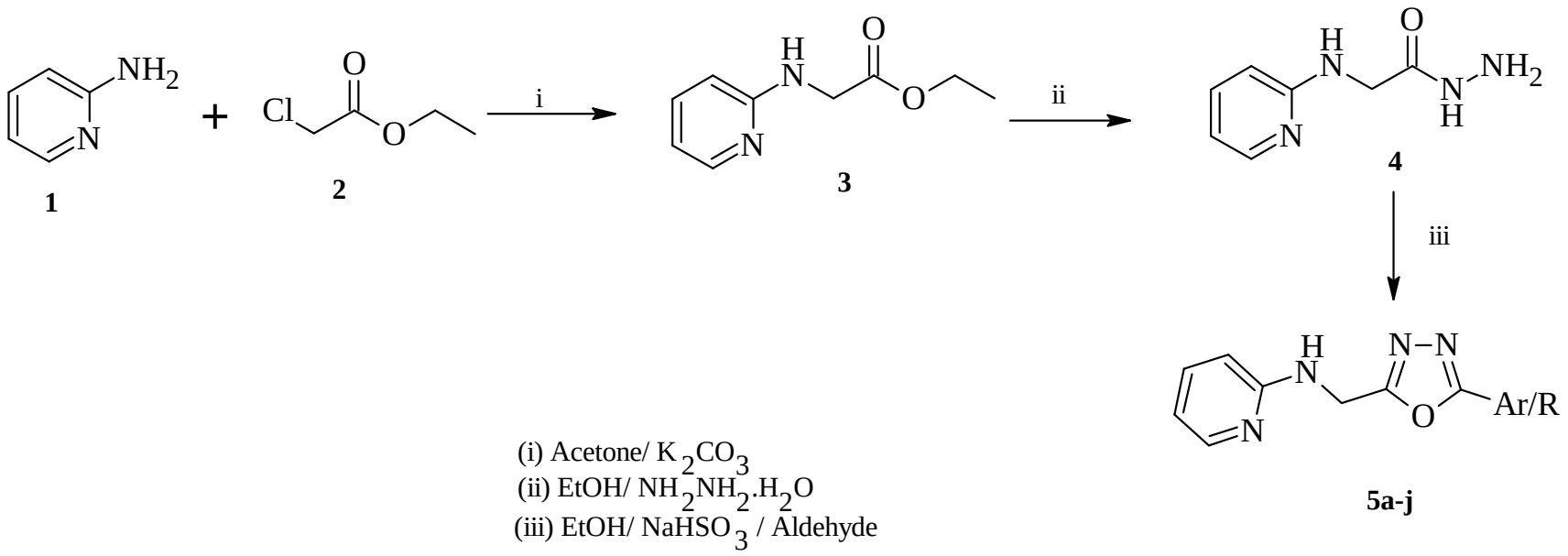
Chemistry

The 2,5-disubstituted-1,3,4-oxadiazole analogues (**5a-j**) described in this study are shown in **Table 1** and the reaction sequence for their synthesis is shown in **Scheme 1**. In the initial step 2-aminopyridine (**1**) (0.1 mol; 9.41 g) and ethyl choroacetate (**2**) (0.2 mol; ~24 ml) were taken in a round bottom flask and suspended in 80-100 ml acetone and 10 g anhydrous potassium carbonate were added to the mixture. The mixture was refluxed for 24 h on a sand bath with vigorous stirring to obtain the intermediate semi-solid ethyl (pyridine-2-ylamino)acetate (**3**). In the subsequent step compound **3** was refluxed with hydrazine hydrate in ethanol for 8-12 h to obtain 2-(pyridine-2-ylamino)acetohydrazide (**4**) as a brown semi-solid. In the final step compound **4** was refluxed with aromatic aldehydes for 12-14 h using 20 mol% $NaHSO_3$ in ethanol-water system (1:2, v/v) to obtain *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**5a-j**). The oxadiazole analogues were synthesized *as per* the reported method . The yields of the title compounds were ranging from 51% to 82% after recrystallization with absolute ethanol. The completion of reaction was monitored by thin layer chromatography (TLC) using mobile phase benzene/methanol (1:4) and cyclohexane/acetone (1:4).

Results and discussion

The purity of the synthesized compounds was checked by elemental analysis. Both the analytical and spectral data of the compounds were in full agreement with the proposed structure. The IR spectra of final compounds showed oxadiazole stretching at 1152-1169 cm^{-1}, and NH band at 3191-3209 cm^{-1}, while the C=N stretching was observed at 1531-1547 cm^{-1}. The proton NMR spectra confirmed the structures on the basis of the chemical shift, multiplicity and coupling constants in DMSO-d_6. The spectra showed a triplet at δ 1.24-1.29 ppm corresponding to CH_3; a multiplet at δ 2.54-2.56 corresponding to CH_2 group; a singlet at δ 3.32-3.35 ppm corresponding to CH_2 (methylene linker); a singlet at δ 3.80-3.81 ppm corresponding to OCH_3; a singlet at δ 8.58-8.99 ppm corresponding to the aromatic NH; a singlet at δ 10.52-11.11 ppm corresponding to the OH phenolic group, while aromatic peaks were observed as singlet, doublet and multiplet in the aromatic region according to the nature of protons. The molecular mass (M^+) and $(M+2)^+$ were observed in the mass spectra.

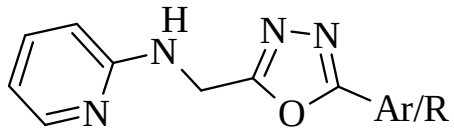
Results and discussion



Scheme 1. Protocol for the synthesis of *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**5a-j**).

Results and discussion

Table 1. Physical constants of *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**5a-j**).



5a-j

Compound	Ar	NSC Code	Yield (%)	Mp (° C)
5a	Phenyl-	-	76	86-88
5b	4-Fluorophenyl-	-	68	102-104
5c	4-Chlorophenyl-	783625	79	198-200
5d	4-Hydroxyphenyl-	-	64	72-74
5e	2-Hydroxyphenyl	-	77	138-140
5f	4-Methoxyphenyl-	783626	80	150-152
5g	3,4-dimethoxyphenyl-	782627	82	160-162
5h	3-Hydroxy-4-methoxyphenyl-		78	112-114
5i	2-Furyl-	-	72	220
5j	Ethyl-	-	51	80-82

Results and discussion

Anticancer activity

Three compounds were tested for anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, prostate and breast cancer cell lines (nearly 60 cell lines) as per the NCI US protocol and carried out at Nation Cancer Institute, USA. The compound **5f** showed maximum activity with growth percent (GP) of 94.33 followed by compound **5g** (GP = 95.12) and **5c** (GP = 96.37). The compound **5c** showed maximum selectivity towards HOP-92, MCF7, and SNB-75 with percent GI of 34.14, 21.22, 20.52 and 15.39 respectively. The compound **5f** showed maximum selectivity towards HOP-92, CCRF-CEM, HOP-62, and PC-3 with percent GI of 35.29, 24.42, 23.38, and 22.27 respectively while compound **5g** showed maximum selectivity towards HOP-92, PC-3, HOP-62, and SNB-75 with percent GI of 31.59, 25.76, 23.61, and 23.04 respectively. The anticancer activity is given in **Table 2**. Compounds **5c**, **5f**, and **5g** showed maximum selectivity towards HOP-92 (Non-Small Cell Lung Cancer). The maximum percent GI was recorded on HOP-92 by compound **5g**. No clear cut structure activity relationship (SAR) was observed with anticancer data however 4-methoxyphenyl substitution on position 5 of oxadiazole ring showed significant better results than 3,4-dimethoxyphenyl and 4-chlorophenyl substitutions.

Results and discussion

Table 2. *In vitro* anticancer activity of *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues.

Compound	60 cell lines assay in one dose 10 μM concentration				
	Mean Growth percent (GP)	**Range of GP**	**The most sensitive cell lines**	**GP of the most sensitive cell line**	**% Growth Inhibition (GI)**
5c	96.37	65.86 to 112.35	HOP-92 (Non-Small Cell Lung Cancer)	65.86	**34.14**
			T-47D (Breast Cancer)	78.78	21.22
			MCF7 (Breast Cancer)	79.48	20.52
			SNB-75 (CNS Cancer)	84.61	15.39
5f	94.33	64.71 to 119.37	HOP-92 (Non-Small Cell Lung Cancer)	64.71	**35.29**
			CCRF-CEM (Leukemia)	75.58	24.42
			HOP-62 (Non-Small Cell Lung Cancer)	76.62	23.38
			PC-3 (Prostate cancer)	77.73	22.27
5g	95.12	68.41 to 119.53	HOP-92 (Non-Small Cell Lung Cancer)	68.41	**31.59**
			PC-3 (Prostate cancer)	74.34	25.76
			HOP-62 (Non-Small Cell Lung Cancer)	76.39	23.61
			SNB-75 (CNS cancer)	76.96	23.04

Results and discussion

Antibacterial activity

Some of the compounds showed significant antibacterial activity. Compound **5b** showed maximum antibacterial activity with minimum inhibitory concentration (MIC) of 8 μg/mL against *S. aureus* and *B. subtilis* and 4 μg/mL against *E. coli*. The compounds **5c**, **5d**, **5e**, **5f**, and **5h** showed moderate activity with MIC between 32 and 64 μg/mL while compounds **5a**, **5i** and **5j** showed lower activity. The activity of compound 5b was found to be nearly equal to the standard drug ciprofloxacin. The antibacterial activity of the compounds is reported in **Table 3**. Electron withdrawing groups like 4-fluoro, 4-chloro increased the activity in a greater extent than electron releasing the hydroxy and methoxy groups. The furyl and ethyl substitutions at position 5 of oxadiazole ring were not favorable.

Results and discussion

Antifungal activity

Some of the compounds (**5a-j**) showed significant antifungal activity. The compound **5f** showed maximum antifungal activity with MIC of 4 μg/mL against *A. niger* and *C. albicans* followed by compound **5g** with MIC of 8 μg/mL against *A. niger* and 16 μg/mL against *C. albicans*. The antifungal activity of compounds is reported in **Table 3** with fluconazole as the standard drug. Compounds **5d**, **5f** and **5g** showed significant antifungal activity while rest of the compounds showed moderate to low antifungal activity. The electron releasing methoxy and hydroxy groups confers better antifungal activity than electron withdrawing fluoro and chloro groups. The activity was found to be less important with furyl or ethyl substituents at position 5 of the oxadiazole ring.

Results and discussion

Table 3. Antimicrobial activity of *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**5a-j**).

Compound	Minimum inhibitory concentration (µg/mL)				
	Antibacterial			Antifugal	
	S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
5a	128	64	128	256	128
5b	8	8	4	64	32
5c	32	32	16	64	64
5d	64	64	32	8	8
5e	64	64	32	32	64
5f	64	64	64	4	4
5g	32	64	32	8	16
5h	64	64	32	64	32
5i	256	128	256	128	64
5j	256	256	256	256	>256
Ciprofloxacin	4	4	4	-	-
Fluconazole	-	-	-	2	1

Conclusions

All the synthesized compounds were obtained in satisfactory yields and evaluated for their anticancer and antimicrobial activities. The compound **5f**, which expressed maximum anticancer activity on human cancer cell lines at 10 μM concentration could be considered as lead for further optimization and drug discovery. Similarly compounds **5b** and **5f** showed maximum antimicrobial activity. All these derivatives can be further modified to exhibit more potency. Synthesis of other series of oxadiazole analogues is in progress in our laboratory. The oxadiazole derivatives discovered in this study may provide valuable information in the field of drug design and discovery.

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