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DESIGN, SYNTHESIS OF BISINDOLYLOXADIAZOLES AS POTENT ANTIMICROBIAL AGENTS

Parveen Rajesab[1*],

[1*]H.K.E. Society's Smt. Veeramma Gangasiri Degree College and P. G. Centre for Women, Kalaburagi-585102
Email: parveenr.2008@rediffmail.com, prchem7@gmail.com

Corresponding author: Dr. Parveen Rajesab,
Department of Chemistry,
H.K.E.Society's,
Smt. Veeramma Gangasiri Degree College and P. G.
Centre for Women, Kalaburagi-585 102.
Karnataka State, INDIA.

ABSTRACT
A series of bisindolyl oxadiazoles were synthesized by reacting 3,5-disubstituted-indole-2-caboxyhydrazides with 3,5-disubstituted indol-2-carboxylic acids in the presence of phosphorus oxy chloride as the solvent. The products were obtained in good yields. The structures of the synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}CNMR, mass and spectral analysis. The target compounds were evaluated for antimicrobial activities.

Keywords: Bisindole, Oxadiazole, Indole, Antimicrobial Activity, 3,5-disubstituted-indole-2-caboxyhydrazide, 3,5-disubstituted indol-2-carboxylic acids.

INTRODUCTION

The indole ring system is an important biologically active compound and is present in many natural products, pharmaceuticals and agrochemicals. The indole unit forms Bisindolylmethane and Trisindolyl methane structures. BIMs exhibit antimicrobial and antifungal activities, exhibit antibiotic activity and antibacterial activity [1], anticancer activ ity against several common cancer cell lines [2]

The discovery of privileged structures in drug discovery is an emerging trend in the field of medicinal chemistry. These structures represent a class of molecules capable of binding to multiple receptors with high affinity. This type of molecules let the medicinal chemist to discover biologically active compounds [3-5].

Recently reported the synthesis and the antitumor activity of bis-indolyl thiophene [6] pyrazoles [7] furans [8] and isoxazoles [8] that showed inhibitory activity against a wide range of human tumor cell lines, generally in the micro- and sub micromolar range. Even more recently bis-indolyl pyrroles [9] have exhibited concentration-dependent antitumor activity towards a panel of 42 human tumor cell lines, with mean IC50 values of 1.54 μM and 0.67 μM, respectively. Moreover, investigating human tumor xenografts in an *ex vivo* clonogenic assay revealed selective antitumor activity.

Oxadiazoles are important classes of heterocyclic compounds which have attracted attention due to their significant biological and pharmacological properties [10–18].

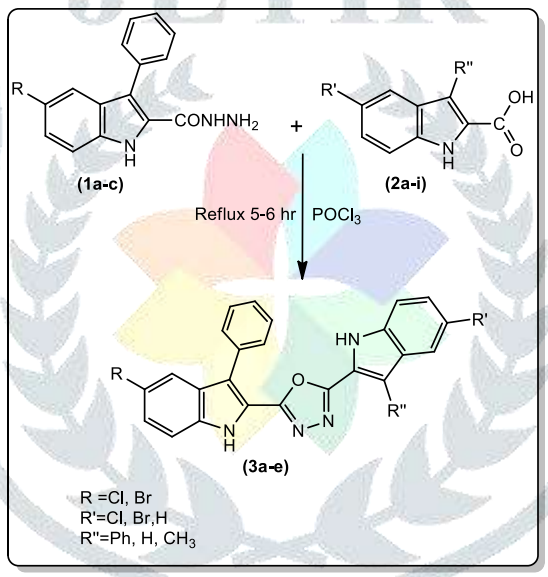
2, 5-disubstituted 1, 3, 4-oxadiazole attracted the considerable attention due to biological activity. The pharmaceutical importance of these compounds can be utilizing as antibacterial, antitubercular and insecticidal agents [19-22]. Some of these compounds have also possess analgesic, antiinflammatory, anticancer, anti-HIV agent, antiparkinsonian and antipriliferative agent [23-27]. 1,3,4-oxadiazole also have played a vital role in heterocyclic chemistry and also used in organic synthesis [28,29]. The

methods used in synthesis of 1,3,4-oxadiazole is condensation of hydrazide and its derivatives with substituted acids and bases [30,31]. 2,5-disubstituted 1,3,4-oxadiazole can be conveniently synthesized by the treatment of pyridine-4-carbohydrazide with different acids and bases and carbon disulfide in basic and acidic media [32,33]. The other method is pyridine-4-carbohydrazide intermediates which can be subsequently cyclized to 2,5-disubstituted 1,3,4-oxadiazole in the presence of a suitable cyclizing reagent like phosphoryl chloride [34-36]. In our earlier approaches, we have synthesized some new derivatives of indole with highly potent antioxidant, DNA cleavage and antimicrobial activities [37-40].

RESULT AND DISCUSSION

CHEMISTRY. Molecules were designed with the aim of exploring their antimicrobial activities. The target compounds were synthesized as outlined in **(Scheme 1)**. A novel and efficient method for the synthesis of 2,5-bis(3,5-disubstituted-1H-indol-2-yl)-1,3,4-oxadiazoles **(3a-e)** by reacting 3,5-disubstituted-indole-2-caboxyhydrazides**(1a-c)** with 3,5-disubstituted indol-2-carboxylic acids **(2a-e)**. The IR spectrum of 2-(5-chloro-3-phenyl-1H-indol-2-yl)-5-(1H-indol-2-yl)-1,3,4-oxadiazole **3a** shown a strong absorption at 3374 cm^{-1} and 3177 cm^{-1} corresponds to indole NH and absorption at 1650 cm^{-1} corresponds to C=N stretching respectively. The ^{1}HNMR spectrum of **3a** has exhibited a singlets at δ 12.60 and δ 11.90 ppm is due to indole NH which are also D_2O exchangeable. A multiplet between δ 7.34-7.70ppm corresponds to thirteen aromatic protons present in the molecule. The ^{13}CNMR spectrum of **3a** has displayed signal at δ 109, 112, 115, 115, 116, 120, 120, 122, 125, 128, 136, 158 and159.

The mass spectrum of compound **3a** has shown isotopic peaks at m/z 410 (100%), 412 (30%). The molecular ion has undergone into fragmentation to give fragment $\mathbf{m_1}$ at m/z 375 (15%) by the loss of chlorine radical. This cation $\mathbf{m_1}$ has lost C_6H_5 to give radical cation $\mathbf{m_2}$ at m/z 298 (5%). The radical cation $\mathbf{m_2}$ has lostC_8H_6N to give $\mathbf{m_3}$ at m/z 182 (20%). This fragmentation pattern and above spectral data supports the formation of compound **3a**.



Scheme-1

Table-01: Physical and analytical data of the 2-(5-chloro-3-phenyl-1H-indol-2-yl)-5-(1H-indol-2-yl)-1,3,4-oxadiazoles (3a-e).

Comp. No.	Substituents			M.P.	Yield (%)	Nature (Solvent)	Molecular Formula	Found (%) Calcd.		
	R	R'	R''					C	H	N
3a	Cl	H	H	252-54	65	Brown Crystals (Ethanol)	$C_{24}H_{15}N_4ClO$	70.13 (70.16)	03.40 (03.68)	13.55 (13.64)
3b	Cl	Br	Ph	280-82	75	Green solid (Ethanol)	$C_{30}H_{18}N_4BrClO$	63.63 (63.68)	03.19 (03.21)	09.84 (09.90)
3c	Cl	Cl	Ph	290-92	70	Green Solid (Ethanol)	$C_{30}H_{18}N_4Cl_2O$	69.03 (69.11)	03.42 (03.48)	10.67 (10.75)
3d	Br	H	H	210-12	60	Black solid (Ethanol)	$C_{24}H_{15}N_4BrO$	63.27 (63.31)	03.24 (03.32)	12.23 (12.31)
3e	Br	Br	CH_3	258-60	70	Green Crystals (Ethanol)	$C_{25}H_{16}N_4Br_2O$	55.72 (54.77)	02.91 (02.94)	10.02 (10.22)

Table-02: Spectral data of the 2-(5-chloro-3-phenyl-1H-indol-2-yl)-5-(1H- indol-2-yl)-1,3,4-oxadiazoles(3a-e).

Comp. No.	IR (KBr, cm^{-1})			^{1}HNMR and ^{13}CNMR
	C=N	Indole NH	Indole NH	
3a	1650	3177	3374	12.60 (s, 1H, Indole NH), 11.90 (s, 1H, Indole NH), 7.34-7.70 (m, 13H, Ar-H). 109, 112, 115, 115, 116, 120, 120, 122, 125, 128, 136, 158 &159.
3b	1653	3061	3086	12.60(s, 1H, Indole NH), 11.24 (s, 1H, Indole NH), 7.35-7.89 (m, 16H, Ar-H).
3c	1622	3223	3261	12.53 (s, 1H, Indole NH), 11.02 (s, 1H, Indole NH), 7.28-7.58 (m, 16H, Ar-H).
3d	1637	3227	3296	12.65 (s, 1H, Indole NH), 12.12 (s, 1H, Indole NH), 7.35-7.92 (m, 13H, Ar-H).
3e	1654	3103	3151	12.55 (s, 1H, Indole NH), 12.54 (s, 1H, Indole NH), 7.36-7.66 (m, 12H, Ar-H), 2.20 (s, 3H, CH_3).

BIOLOGICAL ACTIVITIES.
ANTIMICROBIAL ACTIVITY.
ANTIBACTERIAL ACTIVITY

Applying the agar plate diffusion technique [41], series of analogues were screened for *in vitro* antibacterial activity against **(Table 3)** gram-negative bacteria *Escherichia coli* (*E. coli*) *and Klebsiella pneumoniae* (*K. pneumoniae*) and gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and Gentamycin was used as standard. The zone of inhibitions was measured in mm for each concentration. Amongst the compounds screened against ***S. aureus***, compounds 3**b-c** and **3e** have shown highest activity. Compounds **3a**, **3d** have shown moderate activity. Amongst the compounds tested against ***E. coli*** compounds **3b-c**and **3e** have shown very good activity. Compounds **3a** and **3d** are moderately active and compounds. Amongst the compounds screened against ***K. pneumonia***, **3b-c** and **3e** have shown highest activity. Compounds, **3a**, have shown moderate inhibitory activity.

ANTIFUNGAL ACTIVITY

Antifungal activities of test compounds were compared with the activities of standard drug, fluconazole against ***C. albicans*** *and* ***C. tropicalism*** **(Table-4)**. Amongst the compounds screened against ***C. albicans***, compounds **3b-c** and **3e** have shown highest activity. Compounds **3a** and **3d** shown moderate inhibitory activity and remaining compounds have exhibited weak activities. Amongst the compounds tested against ***C. tropicalis*** compounds **3b-c** and **3e** have shown highest activity. Compounds **3a** and **3d** shown moderate inhibitory activity and remaining compounds have exhibited weak activities.

Table-03: Antibacterial activity results of synthesized compounds (3a-e).

Comp. No.	Substituent's			Antibacterial activity		
	R	**R'**	**R''**	***S. aureus***	***E. coli***	***K. Pneumoniae***
3a	Cl	H	H	11	12	13
3b	**Cl**	**Br**	**Ph**	**15**	**16**	**15**
3c	**Cl**	**Cl**	**Ph**	**16**	**15**	**16**
3d	Br	H	H	12	11	12
3e	**Br**	**Br**	**CH_3**	**16**	**14**	**15**
Standard	-	-	-	16	18	17

Std.- Gentamycin,

Table-4: Antifungal activity results of synthesized compounds (3a-e).

Comp. No.	Substituents			Antifungal activity	
	R	**R'**	**R''**	***C. albicans***	***C. tropicalis***
3a	Cl	H	H	15	16
3b	**Cl**	**Br**	**Ph**	**20**	**21**
3c	**Cl**	**Cl**	**Ph**	**21**	**20**
3d	Br	H	H	15	16
3e	**Br**	**Br**	**CH_3**	**20**	**21**
Standard	-	-	-	21	23

Std.- Fluconazole

EXPERIMENTAL

CHEMISTRY. All chemicals used in this investigation were analytical grade and were purified whenever necessary. Melting points of the synthesized compounds are measured in open capillaries and are uncorrected. Reactions are monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets (MERCK). Iodine vapour was used as detecting agent. IR spectra are recorded in KBr on Perkin Elmer and FTIR spectrophotometer (]max in cm^{-1}). ^{1}HNMR and ^{13}C NMR spectra are recorded on BRUKER AVENE II 400-MHz NMR spectrometer (chemical shift in δ ppm down field from TMS as internal reference).The mass spectra are recorded on LC-MSD-Trap-SL instruments. The elemental analysis was determined on FLASH EA 1112 SERIES instrument.

General Procedure for the Synthesis of Compound (1a–e).

The precursors 3,5-disubstituted-indole-2-caboxyhydrazides **(1a-c)** and 3,5-disubstituted indol-2-carboxylic acid (**2a–i**) were obtained from 3,5-disubstituted indol-2-carboxylates by reported method [42].

2,5-bis(3,5-disubstituted-1H-indol-2-yl)-1,3,4-oxadiazoles (3a-e).

A mixture of 3,5-disubstituted indole-2-caboxyhydrazides**(1a-c)** (0.01mol) and appropriate 3,5-disubstitutedindol-2-carboxylic acids **(2a-e)** (0.01mol) were dissolved in phosphorus oxy chloride and refluxed for 5-6hrs .The mixture was then cooled and poured into ice cold water under constant stirring. The mass obtained was filtered and washed with water and recrystallized from suitable solvent to get bisindolyloxadiazoles **(3a-e)**.

ANTIMICROBIAL ACTIVITY.

A series of novel bisindolyl analogues are tested for *in vitro* antimicrobial activity against gram-negative bacteria *Escherichia coli* and *Klebsiella Pneumoniae* and grampositive bacteria *Staphylococcus aureus* and antifungal activity against *Candida tropicalis* and *Candida albicans* by applying the agar plate diffusion technique [41]. The activity is compared with reference drugs Gentamycin for antibacterial and Fluconazole for antifungal activity. The zone of inhibition after 24 hr of incubation at 37^0C, in case of antibacterial activity and 48 hr in case of antifungal activity, was compared with that of standards.

CONCLUSION

The novel method is employed for the synthesis of bisindolyl oxadiazole derivatives obtained in good to excellent yields and is one step synthesis approach highlighted the benefits of using this procedure. The target compounds were screened for antimicrobial activity. Amongst the compounds screened for antibacterial activity, compounds with halogen substitution at five positions and a phenyl ring at third position of indole ring have exhibited very good activity when compared standard. Compounds with halogen substitution at five positions and phenyl ring at third position of indole ring have exhibited very potent antifungal activity when compared standard.

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REFERENCE

1. Shiri, M., Ali Zolfigol, M., Gerhardus ruger, H., and Tanbakouchian, Z., 2010. Bis- and Trisindolylmethanes (BIMs and TIMs) Chemical Review, 110:2250–2293.
2. Safe, S. Papineni, S. Chintharlapalli, S. 2008. Cancer chemotherapy with indole-3-carbinol, bis(3-indolyl)methane and synthetic analogs. Cancer Letters 269:326–338. doi:10.1016/j.canlet.2008.04.021.
3. Freidinger, R. M., Whitter, W. L., Lundell, G. F., Veber, D. F., Anderson, P. S., Chang, R. S. L., Lotti, V. J., Cerino, D. J., Chen, T. B., Kling, P. J., Kunkel, K. A., Springer, J. P., Hirshfield, J., 1988. Journal Medicinal Chemistry, *31:* 2235.
4. Nicolaou, K. C., Pfefferkorn, J. A., Roecker, A. J., Cao, G.-Q., Barluenga, S., Mitchell, H. J., 2000. *Journal of American Chemical Society*, *122*: 9939**.**
5. Douglas A. H., Gregory T. B., Mark L. S. 2003. The Combinatorial Synthesis of Bicyclic Privileged Structures or Privileged Substructures Chemical Review.**,** *103, (*893-930).
6. Diana, P., Carbone, A., Barraja, P., Montalbano, A., Martorana, A., Dattolo, G., Gia, O., Dalla Via, L., Cirrincione, G., 2013. Synthesis and antitumor properties of 2,5-bis(3'-indolyl)thiophenes:analogues of marine alkaloid nortopsentin. Bioorganic and Medicinal. Chemistry Letters, *17:* 2342–2346.
7. Diana, P., Carbone, A., Barraja, P., Martorana, A., Gia, O., Dalla Via, L., Cirrincione, G., **2007**. 3,5-Bis(3'-indolyl)pyrazoles, analogues of marine alkaloid nortopsentin: synthesis and antitumor properties. Bioorganic and Medicinal. Chemistry Letters, *17*: 6134–6137.
8. Diana, P., Carbone, A., Barraja, P., Kelter, H.H., Fiebig, G., Cirrincione, G., 2010. Synthesis and antitumor activity of 2,5-bis(3'-indolyl)-furans and 3,5-bis(3'-indolyl)-isoxazoles, nortopsentin analogues. Bioorganic and Medicinal. Chemistry Letters,*18*: 4524–4529.
9. Carbone, A., Parrino, B., Barraja, P., Spanò, V., Cirrincione, G., Diana, P., Maier, A., Kelter, G., Fiebig, H-H., 2013**.** Synthesis and antiproliferative activity of 2,5-bis(3'-Indolyl)pyrroles, analogues of the marine alkaloid Nortopsentin. Marine Drugs, 11, doi:10.3390/md110x000x.
10. Joshi, S. D., Vagdevi, H. M, Vaidya, V. P., Gadaginamath, G. S., 2008. European Journal of Medicinal Chemistry, 43, 1989.
11. Mullican, M. D., Wilson, M. W., Connor, D. T., Kostlan, C. R., Schrier, D. J., Dyer, R. D. 1993. Journal of Medicinal Chemistry 361: 1090.

12. Boschelli, D. H., Connor, D. T., Bornemeier, D. A., Dyer, R. D., Kennedy, J. A., Kuipers, P. J., Okonkwo, G. C., Schrier, D. J., Wright, C. D., 1993. Journal of Medicinal Chemistry, 36: 1802.
13. Raman, K., Singh, K. H., Salzman, S. K., Parmar, S. S., 1993. Journal of Pharmaceutical Science, 821: 167.
14. Sahin, G., Palaska, E., Kelicen, P., Demirdamar, R., Altinok, G., Arzneim, F., 2001, Drug Research, 51: 478.
15. Gaonkar, S. L., Rai, K. M. L., Prabhuswamy, B., 2006. European Journal of Medicinal Chemistry, 41: 841.
16. El-Emam, A. A., Al-Deeb, O. A., Al-Omar, M., Lehmann, 2004, Journal of Bioorganic Medicinal Chemistry, 12: 5107.
17. Bakht, M. A., Yar, M. S., Abdel-Hamid, S. G., Al-Qasoumi, S. I., Samad, A.,2010 European Journal of Medicinal Chemistry 45: 5862.
18. Shi, W., Qian, X., Zhang, R., Song, G., 2001 Journal Agrictural Food Chemistry, 49:124.
19. Grover,G., Kini S.G., 2003 Indian Journal of Heterocyclic Chemistry., 12: 289.
20. Hazarika, J., Kataky, J.C.S, 1997 Indian Journal of Heterocyclic Chemistry. 7: 47.
21. Kagthara, P.R., Shah, N.S., Doshi, R.K., Parekh, H.H., 1999, Indian. Journal of Chemistry. 38B: 572.
22. Shivarama Holla, B., Prasanna, C.S., Boja Poojary, Rao, K.S., Shridhara, K,. Ganesha Bhat U.,2004, Indian Journal of Chemistry. 43B: 864.
23. Zheng Li, Xicum Wang. 2003. Indian Journal of Chemistry. 42B: 941.
24. Amir, M., Shahani S., 1998. Indian Journal of Heterocyclic Chemistry. 8:107.
25. Shah, H.P., Shah, B.R., Bhatt, J.J., Desai, N.C., Trivadi, P.B., Undavia. N.K. 1996. Indian Journal of Chemistry.37B: 180.
26. Hazarika, J., Kataky, J.C.S., 1998, Indian Journal of Heterocyclic Chemistry. 7: 83.
27. Liszkiewicz, H., Kowalska, M.W., Wietrzyk J., Opolski, A., 2003, Indian Journal of Chemistry. 42B:2846.
28. K.Mogilaiah, N.Vasudeva Reddy,.Indian Journal of Chemistry.2003; 42B:2124.
29. Chande, M.S., Godbole, A.A., Coutinho, E., Desai P., 2003. Indian Journal of Chemistry. 42B: 397.
30. Subrahmanya Bhat, K., Karthikeyan, M.S., Shivarama Holla, B., Suchetha Shetty, N.,. 2004. Indian Journal of Chemistry2004; 42B: 1765.
31. Khan, M.S.Y., Akhtar, M., 2003. Indian Journal of Chemistry. 42B: 900.
32. Mishra, P., Rajak, H., Mehta, A., 2005. Journal of General Applied Microbials 15: 133.
33. Rajak, H., Kharya, M., Mishra, P., 2007. International Journal of Chemical Science. 5: 365.
34. Frank, P.V., Kalluraya, B., 2005. Indian Journal of Chemistry. 44B:1456.
35. Xio-Wen Sun, Xin-Ping Hui, Chang-Hu Chu, ,Zi-Xi Zhang. 2002. Indian Journal of Chemistry. 41B: 2176.
36. Reddy, C.H.K. Reddy, P.C.S., Reddy, P.S.N., Ratnam C.V, 1997. Indian Journal of Heterocyclic Chemistry. 7:17.
37. Sasidhar, B. S., Biradar, J. S., "Synthesis of some bisindolyl analogs for in vitro cytotoxic and DNA cleavage studies," Medicinal Chemistry Research, 22(7): 3518–3526, 2013.
38. Biradar, J. S., Sasidhar, B. S., 2011. "Solvent-free, microwave assisted Knoevenagel condensation of novel 2,5-disubstituted indole analogues and their biological evaluation," European Journal of Medicinal Chemistry, 46(12): 6112–6118, 2011.
39. Biradar, J. S., Sasidhar, B. S., Parveen, R., 2010. "Synthesis, antioxidant and DNA cleavage activities of novel indole derivatives," European Journal of Medicinal Chemistry, 45 (9): 4074–4078, 2010.
40. Biradar, J. S., Parveen, R., Sasidhar, B. S., Praveen, S. M. 2010. "Synthesis of new indolyl benzodiazepines and their DNA cleavage and antimicrobial activities," Indian Journal of Heterocyclic Chemistry, 20:181–182, 2010.
41. Praveen, C., Ayyanar, A., Perumal, P. T. 2011. "Practical synthesis, anticonvulsant, and antimicrobial activity of N-allyl and Npropargyl di(indolyl)indolin-2-ones," Bioorganic & Medicinal Chemistry Letters, 21 (13):4072–4077, 2011.
42. Biradar, J. S. , 1982. Studies in the indole field [Ph.D. thesis], Gulbarga University, Gulbarga, India.