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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PYRAZOLYL BIS-INDOLYLMETHANE

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ABSTRACT

Pyrazolyl bis-indolylmethane has emerged as structurally novel antibacterial and anti-inflammatory. Therefore various substituted pyrazolyl bis-indolylmethane were synthesized by addition of pyrazolyl aldehyde with different substituted indoles. The structures of the synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR and Mass Spectrum. All the synthesised compounds were showed maximum zone of inhibition againt both Gram positive and Gram negative organisms. In the anti-inflammatory activity, compounds **1, 3** and **5** produced significant activity in a dose dependant manner.

Keywords: Pyrazolyl bis-indolylmethane, Antibacterial, Anti-inflammatory.

INTRODUCTION

The potent physiological properties of various traditional medicines containing indole based alkaloids have been utilized in added stimulus to research, and many important indole syntheses were developed. During this period, the essential amino acid tryptophan[1] as well as the plant growth hormone indole-3-acetic acid [2, 3] were discovered. The present work deals with the synthesis of nitrogen heterocyclic indoles, which are known to be present in many naturally occurring alkaloids. The synthesis and reaction of indoles have been an interesting research topic for over a century since a number of their derivatives occur in nature and possess a variety of important biological activities. Some of the natural (from terrestrial and marine sources) or synthetic indole derivatives have promising activity in various types of pharmacological assays and may prove to be potential candidates for drug development [4]. A number of di-indolyl alkanes have been reported to be isolated from terrestrial and marine natural sources, *viz*. parasitic bacteria, tunicates and sponges [5] and some of these possess significant biological activities. The first report of the occurrence of di-indolyl alkane in nature was published when three indolic metabolites were isolated from the fungus, *Balarisia epichloe*, a parasite to pasture grasses which were known to elicit ergot-type syndrome in cattle grazing on these infected grasses. The 3, 3'-di-indolylmethane has been gaining increasing importance in recent years because of its potent anti-carcinogenic properties.

The search for anti-bacterial and anti-inflammatory compounds with a more selective activity and lower toxicity continues to be an area of invistigation in medicinal chemistry. The chemical diversity and mechanisms of action of anti-bacterial ant anti-inflammatory [6] make it difficult to find a common way of identifying new drugs. Compound containing bis-indole have shown as potent inhibitors of [7] PKCβ and are currently under development for the treatment of diabetic complications [8]. The hapal-indole alkaloids represent a series of 20 compounds isolated from the blue green algae. They exhibit significant antibacterial and antimycotic activity and have attracted the interest of both synthetic and pharmaceutical chemistry. The present work deals with the synthesis of nitrogen heterocyclic indoles, which are known to be present in many naturally occurring alkaloids. The synthesis and reaction of indoles have been an interesting research topic for over a century since a number of their derivatives occur in nature and possess a variety of important biological activities. Some simple, but very important essential amino acids tryptophan, serotonin, and the dyestuff indigo and the plant growth hormone indole-3-acetic acid and several groups of important alkaloids are indole derivatives [9].

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. In addition, primary and opportunistic microbial infections continue to increase the number of immunes compromised patients, those suffering from such as AIDS or cancer or who have undergone organ transplantation [10]. Inflammatory diseases take a heavy toll of millions of people all over the world. These are of autoimmune origin, and the body's own defense mechanisms aggravate the consequence of the initial noxious stimulus in these chronic diseases [11]. Inflammatory diseases are classified into four categories, namely rheumatoid arthritis, respiratory asthma, cutaneous psoriasis & inflammatory bowel disease (ulcerative colitis). Hence, in the present study we were interested to prepare a series of bis-indolyl methane derivatives and investigate their antimicrobial and anti-inflammatory activities [12].

MATERIALS AND METHODS

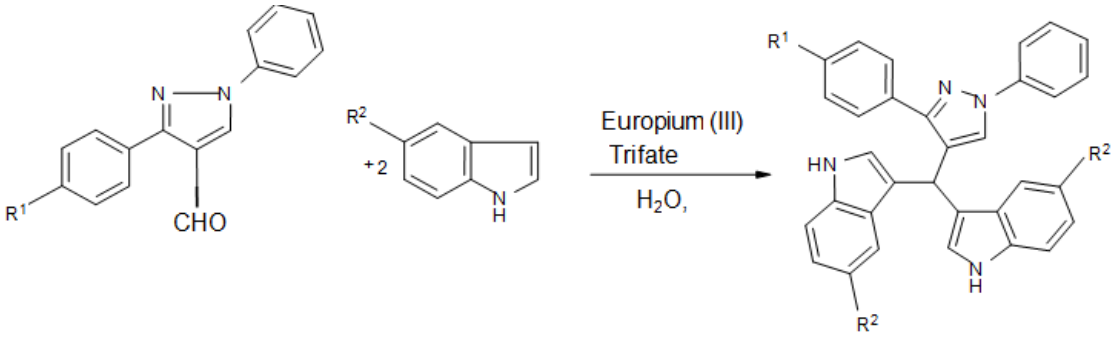
Experimental chemical part

IR spectrum was recorded using FT-IR, Perkin Elmer 8400 series instrument. NMR spectrum was obtained on a JEOL 500 MHz Bruker Daltonics, Germany. Mass spectrum was recorded by using Shimadzu MS-2010 A, Koyoto, Japan. Melting points (uncorrected) were obtained on a melting point apparatus, Lab India, Mumbai. Carrageenan was obtained from Sigma Aldrich Co, St Louis, USA. All other chemicals used were of analytical grade.

Synthesis of pyrazolyl bis-indolyl methane

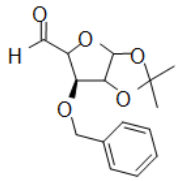
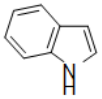
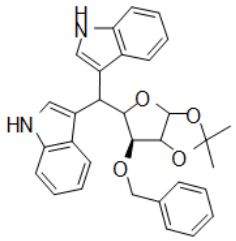
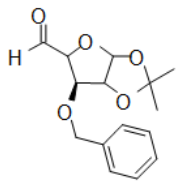
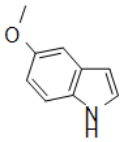
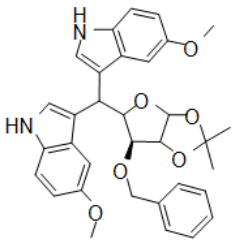
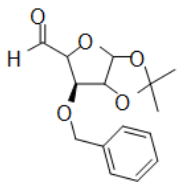
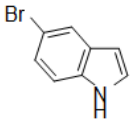
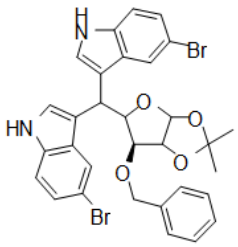
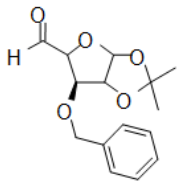
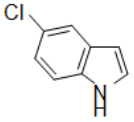
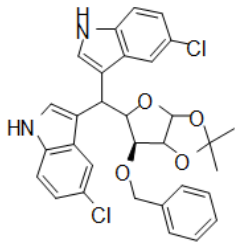
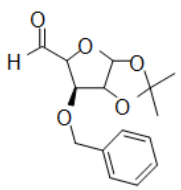
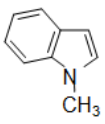
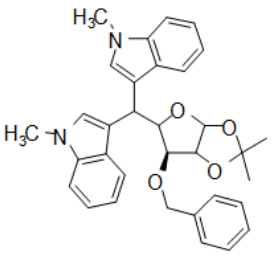
In the presence of 0.2 gm Europium trifate, pyrazolyl aldehyde was treated with indole in water for 2-3 h. After completion of the reaction as indicated by TLC, the reaction mixture was extracted with ethyl acetate (2×30 mL) and the organic layers were separated carefully from the aqueous layer. The combined organic layers were dried over anhydrous Na_2SO_4 by which the water present after the extraction can be removed and further, the organic layer is concentrated in vacuum [13]. The crude was purified by column chromatography on silica gel (Merck, 100-200 mesh, ethyl acetate-petroleum ether (10:90).to obtained pure pyrazolyl bis-indolylmethane.

Mass spectra were recorded on Varian VG 70-70H mass spectrometer. Analytical TLC was performed on precoated sheets of silica gel G of 0.25 mm thickness containing PF 254 indicator (Merck, Darmstadt). IR spectra were recorded as solids in KBr pellets on a Perkin-Elmer FTIR spectrometer. ^{1}H NMR spectra were recorded on a Jeol 500 MHz spectrometer using TMS as internal standard. ^{13}C NMR spectra were recorded on 125z spectrometer in $CDCl_3$ and chemical shifts are given in δ relative to the solvent peak. The enol ether used was brought from Lancaster. The procedure does not require dry solvent and inert atmosphere [14, 15]. All the products obtained were purified by column chromatography using silica gel (Merck, 100–200 mesh).The elution with ethyl acetate: petroleum ether (9.5:0.5) yielded the products 1-5, shown in Table-1.



Scheme-1

Table 1: Structures of different synthesized bis-indole derivatives

Entry	Aldehyde	Indole	Product	Time (hour)	Yield%
1				3.0	78
2				3.0	75
3				4.5	60
4				3.5	66
5				2.5	60

3-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) (1H-indol-3-yl) methyl)-1H-indole

Obtained as Pink color solid, mp 166–168 ºC; yield 0.46 g, 91 %; ^{1}H NMR (500 MHz, DMSO-d6):δ 5.87 (s, 1H), 6.83 (t, J = 7.65, 2H), 6.93 (s, 2H), 6.99 (t, J = 7.65, 2H), 7.23 (m, 3H), 7.31 (d, J = 8.40, 2H), 7.39 (t, J = 7.65, 2H), 7.51 (d, J = 8.40, 2H), 7.60 (d, J = 8.40, 2H), 7.76 (d, J = 7.65, 2H), 8.06 (s, 1H), 10.83 (br s, 2H). ^{13}C NMR (125 MHz, CDCl3): δ 149.28, 139.91, 137.18, 133.06, 131.98, 130.07, 130.00, 128.81, 126.70, 126.05, 124.24, 121.68, 121.47, 119.23, 118.87, 118.63, 118.19, 112.11, 55.16; IR (KBr): 3412, 2915, 1597, 1539, 1448, 1089, 746 cm^{-1}; MS: m/z 499 (M+). Anal. Calcd for $C_{32}H_{23}ClN_4$: C, 77.02; H, 4.65; N, 11.23. Found: C, 77.28; H, 4.58; N, 11.36.

3-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole

Obtained as Orange color solid, mp 166–168 ºC; yield 0.42 g, 85 %; ^{1}H NMR (500 MHz, DMSO-d6): δ 5.84 (s, 1H), 6.83 (t, J = 7.65, 2H), 6.89-6.92 (m, 4H), 7.00 (t, J = 7.65, 2H), 7.20 (m, 3H), 7.32 (d, J = 8.45, 2H), 7.38 (t, J = 8.40, 2H), 7.58 (d, J = 8.4, 2H), 7.75 (d, J = 7.65, 2H), 8.03 (s, 1H), 10.83 (br s, 2H). ^{13}C NMR (125 MHz, CDCl3): δ 159.47, 150.32, 140.07, 137.21, 129.96, 129.39, 128.74, 126.75, 126.24, 125.59, 124.18, 121.44, 119.21, 118.85, 118.55, 118.40, 114.46, 112.11, 55.16; IR (KBr): 3411, 2924, 1599, 1539, 1452, 1089, 748 cm-1 ; MS: m/z 543 (M+). Anal. Calcd for $C_{32}H_{23}BrN_4$: C, 70.02; H, 4.27; N, 10.31. Found: C, 70.09; H, 4.38; N, 10.23.

5-Nitro-3-{(5-nitro-1H-indol-3-yl)[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]methyl}-1H-indole

Obtained as Orange color solid, mp 166–168 ºC; yield 0.44 g, 88 %; ^{1}H NMR (500 MHz, DMSO-d6): δ 3.84 (s, 3H), 5.88 (s, 1H), 6.83 (t, J = 7.45, 2H), 6.94 (s, 2H), 6.99 (t, J = 7.45, 2H), 7.21 (t, J = 7.45, 1H), 7.23, (d, J = 7.45, 2H), 7.32 (d, J = 7.45, 2H), 7.37 (t, J = 7.45, 4H), 7.66 (d, J = 8.0, 2H), 7.74 (d, J = 7.45, 2H), 8.05 (s, 1H), 10.83 (br s, 2H). ^{13}C NMR(125 MHz, CDCl3): δ 159.53, 140.74, 140.47, 129.96, 129.43, 128.37, 128.22, 126.03, 125.97, 125.00, 124.46, 124.14, 120.81, 118.57, 117.14, 116.54, 116.47, 114.52, 112.64, 55.16, 30.51; IR (KBr): 3410, 2931, 1602, 1524, 1465, 1099, 751 cm^{-1} ; MS: m/z 584 (M+). Anal. Calcd for $C_{33}H_{24}N_6O_5$: C, 67.80; H, 4.14; N, 14.38. Found: C, 67.78; H, 4.13; N, 14.36.

5-Bromo-3-[(5-bromo-1H-indol-3-yl)(1,3-diphenyl-1Hpyrazol-4-yl)methyl]-1H-indole

Obtained as Red color solid, mp 248–250 ºC; yield 0.47 g, 93 %; ^{1}H NMR (500 MHz, DMSO-d6): δ 5.89 (s, 1H), 7.01 (dd, J = 7.45, 2.3, 2H), 7.22 (t, J = 7.45, 1H), 7.31–7.35 (m, 7H), 7.39 (t, J = 7.45, 2H), 7.64 (d, J = 7.45, 2H), 7.76 (d, J = 7.45, 2H), 8.02 (s, 1H), 11.09 (br s, 2H). ^{13}C NMR (125 MHz, CDCl3): δ 150.46, 139.98, 135.92, 133.72, 129.99, 129.04, 128.53, 128.51, 128.42, 128.13, 126.56, 125.94, 125.34, 124.06, 121.38, 118.63, 117.91, 114.26, 111.55, 29.99; IR (KBr): 3417, 2923, 1597, 1500, 1452, 1097, 753 cm^{-1}; MS: m/z 622 (M+). Anal. Calcd for $C_{32}H_{22}Br_2N_4$: C, 61.76; H, 3.56; N, 9.00. Found: C, 61.74; H, 3.55; N, 8.99.

5-Bromo-3-{(5-bromo-1H-indol-3-yl)[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methyl}-1H-indole

Obtained as Orange color solid, mp 134–136 ºC; yield 0.45 g, 90 %; ^{1}H NMR (500 MHz, DMSO-d6): δ 5.91 (s, 1H), 6.98 (s, 2H), 6.99 (t, J = 7.45, 2H), 7.11 (dd, J = 7.45, 2.3, 2H), 7.22 (t, J = 7.45, 1H), 7.29 (d, J = 7.45, 2H), 7.30–7.40 (m, 6H), 7.65 (d, J = 7.45, 2H), 7.75 (d, J = 7.45, 2H), 7.98 (s, 1H), 11.06 (br s, 2H). ^{13}C NMR (125 MHz, CDCl3): δ 149.21, 139.88, 135.89, 133.05, 132.60, 129.98, 129.73, 129.08, 128.84, 128.56, 126.76, 126.00, 125.41, 124.07, 121.50, 118.70, 117.65, 114.26, 111.53, 30.56; IR (KBr): 3428, 2923, 1598, 1500, 1453, 1093, 757 cm^{-1}; MS: m/z 656 (M+). Anal. Calcd for $C_{32}H_{21}Br_2Cl\,N_4$: C, 58.52; H, 3.22; N, 8.53. Found: C, 58.51; H, 3.23; N, 8.54.

Experimental Biological Part

Anti-bacterial activity by using Cup Plate Method

Cup plate method is based on the diffusion of compound from a vertical cylinder or a cavity through the solidified agar layer of a Petri dish or plate to an extent such that growth of the added bacteria is prevented entirely in a circular area or "zone" around the cylinder or cavity containing a solution of the compound. The compounds **1**, **2**, **3**, **4** and **5** were tested at 100 µg/ml against one Gram-positive and three Gram-negative bacterial strains. Sterile nutrient agar plates were prepared, by pouring the sterile agar into Petri dishes in aseptic conditions. 1 ml of each standardized test organism culture was spread on to agar plates. Cavity was done by using a sterile borer of diameter 6 mm. The test compounds **1**, **2**, **3**, **4** and **5** as well as the standard drug solutions and DMSO solvent control was placed in the cavity separately. Then the plates were kept for 1h to allow the diffusion of solution into the medium. All the bacterial plates were incubated at 37 °C for 24 h and fungal plates were incubated at 28 °C for 48 hrs. The zone of inhibition was measured in mm. The result was shown in Table-2.

Table 2: Anti- bacterial activity of compounds 1-5 in cup plate method

Compounds	Concentrations (µg/ml)	Zone of inhibition in mm			
		Tested organisms			
		S. aureus (G+ve)	*E. coli* (G-ve)	*P. vulgaris* (G-ve)	*P. aeruginosa* (G-ve)
1	500	10.0	9.3	12.4	13.0
	250	7.4	7.2	8.3	9.2
	125	4.2	6.0	4.6	5.1
2	500	12.0	7.6	12.6	12.8
	250	8.4	6.3	8.4	8.6
	125	5.6	5.8	4.6	4.8
3	500	13.8	6.5	12.0	11.0
	250	6.3	5.4	8.0	7.8
	125	4.0	5.0	4.4	4.0
4	500	9.2	5.1	9.0	11.4
	250	7.5	4.5	6.8	8.0
	125	4.1	3.9	3.5	4.4
5	500	9.7	5.0	11.9	9.6
	250	8.0	4.0	8.2	7.0
	125	4.2	3.9	4.5	3.9
Gentamycin	100	21.0	17.6	19.0	19.8

Anti-inflammatory activity by Carrageenan induced paw edema in rats

The animals were obtained from the animal house of IRTT Perundurai Medical College, Perundurai, Tamil Nadu, India, maintained under standard conditions (12 h light / dark cycle; 25 ± 3 ºC, 45-65% humidity) and had free access to standard rat feed and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of the experiment. The experiments were performed during the light portion between

07:00-18:00 h to avoid circadian influences. Animal studies were performed according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

Wistor albino rats (150-200 g) were divided into five groups with six animals in each group. Group I was served as control and received 0.5 % w/v dispersion of CMC in distilled water. Groups II-IV animals were treated with compounds **1**, **3** and **5** at 75 and 150 mg/kg, respectively and Group V animals was treated with standard indomethacin at 10 mg/kg. All the treatments were administered orally. The initial hind paw volume of rats was determined volumetrically by using a plethysmometer. A solution of Carrageenan in CMC (1%, 0.05 ml/rat) was injected subcutaneously into the left hind paw 30 min after the treatments. The animals in the control group received the vehicle only. Paw volumes were measured up to 6 h at intervals of 30, 60, 120, 180, 240, 300 and 360 min and percent increase in edema between the control and treated groups were compared shown in Table 3. The percentage protection was calculated as

$$\% \text{ Paw edema inhibition} = 1 - \frac{\text{Edema volume in drug treated group}}{\text{Edema volume in control group}} \times 100$$

Table 3: Anti-inflammatory effect of compounds 1, 3 and 5 in carrageenan induced paw edema in rats

Treatments	Dose (mg/kg)	Paw volume, ml after min						
		30	60	120	180	240	300	360
Control	-	0.57 ± 0.02	0.62 ± 0.03	0.69 ± 0.02	0.78 ± 0.02	0.76 ± 0.03	0.72 ± 0.06	0.68 ± 0.04
Compound **1**	75	0.50 ± 0.03	0.52 ± 0.04	0.64 ± 0.02	0.68 ± 0.02	0.66 ± 0.03	0.64 ± 0.01	0.62 ± 0.06
	150	0.49 ± 0.04	0.54 ± 0.05	0.60 ± 0.03	0.64 ± 0.01	0.62 ± 0.05	0.61 ± 0.02	0.59 ± 0.09
Compound **3**	75	0.47 ± 0.01	0.50 ± 0.01	0.54 ± 0.01	0.57 ± 0.02	0.59 ± 0.01	0.57 ± 0.02[c]	0.55 ± 0.01[c]
	150	0.40 ± 0.02	0.44 ± 0.02[c]	0.48 ± 0.02[c]	0.52 ± 0.01[c]	0.54 ± 0.02[c]	0.52 ± 0.01[c]	0.46 ± 0.02[b]
Compound **5**	75	0.39 ± 0.03[b]	0.41 ± 0.02[b]	0.40 ± 0.01[b]	0.40 ± 0.08 [b]	0.43 ± 0.06[b]	0.45 ± 0.05[b]	0.47 ± 0.06[b]
	150	0.36 ± 0.02[b]	0.38 ± 0.04[b]	0.37 ± 0.02[b]	0.34 ± 0.06 [b]	0.37 ± 0.05[b]	0.40 ± 0.06[b]	0.42 ± 0.05[b]
Indomethacin	10	0.28 ± 0.03[a]	0.26 ± 0.05[a]	0.30 ± 0.03[a]	0.22 ± 0.04[a]	0.28 ± 0.03[a]	0.20 ± 0.04[a]	0.22 ± 0.02[a]

Values are given as mean ± S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at [a]P<0.001, [b]P<0.01, [c]P<0.05 between control and treated groups.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by multiple comparison using the Dunnet's test. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

All the synthesis of pyrazolyl bis-indolyl methane's makes use of the achiral aldehyde and hence results in the formation of achiral bis-indolyl methane's [16]. Only few reports of chiral bis-indolyl methane is present in the literature. Carbohydrates play prominent role in the human biological system. A synthesis that results in the clubbing of indole with a suitably modified pyrazolyl aldehyde moiety will be important in terms of the biological activity the resultant molecule is expected to display. All the compounds (**1**-**5**) were evaluated for anti bacterial activity with Gentamycin as standard. All the synthetic derivatives of pyrazolyl bis-indolyl methane (1-**5**) were showed inhibitory potential at different concentration levels in both Gram positive and Gram negative organisms. Compounds **1**, **3** and **5** showed maximum zone of inhibition at 500 µg/ml against *S. aureus, E. coli, P. vulgaris* and *P. aeruginosa.* All the other concentrations showed moderate to weak activity against all the organisms. However, the standard Gentamycin showed potent activity against all the organisms when compared to all the compounds. Results revealed that synthesized pyrazolyl bis-indolyl methane derivatives exerted inhibitory effects against certain pathogenic bacteria (both Gram positive and Gram negative) associated with severe infections justify the reasoning behind the use of these pyrazolyl bis-indolyl methane derivatives against bacterial diseases [17,18].

Against Carrageenan induced paw edema in rats, compounds **1**, **3** and **5** at 150 mg/kg significantly reduced the paw edema after 30-360 min when compared to control. Compound **5** exhibited significant activity at 75 mg/kg after the 30-360 min. However, compounds **1, 3** and **5** produced significant activity in a dose dependant manner. The standard indomethacin at 10 mg/kg produced better results than the tested samples. Carrageenan induced inflammation is a non-specific inflammation resulting from a complex of diverse mediators. This model is conventional, sensitive, accepted for screening of newer anti-inflammatory agents and reliably predicts the anti-inflammatory efficacy based on inhibition of prostaglandin amplification [19].

CONCLUSION

In the present study, compounds **1, 3** and **5** exhibited potent effect indicating it to be a good candidate for anti- bacterial and anti-inflammatory activity. Further research would be of interest to explain the exact mechanism of these compounds and toxicity studies can also be explored.

REFERENCES

1. Kershaw MJ, Leigh DA. Journal of Antimicrobial Chemotherapy 1975; 311-315.
2. Uchiyama K, Ono A, Hayashi Y. Chem. Soc. Jpn1998; 71: 2945.
3. Gribble GWJ, Trans Handy, Okello ST. Chemistry of indoles 2000; 221-237.
4. Sundberg RJ. The Chemistry of Indoles, Academic press New York 1970.
5. Reddy YJS, Murthy BVS, Mahesh Kumar VSR. Ch. Synthesis 2001; 783.
6. Nitinkumar SS, Rajesh SK, Ravi SL. Journal of Sulfur Chemistry 2008; 29: 539 – 547.
7. Sreekanth T, Subhas SK, Babu RB. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1.
8. Hansch C. Comprehensive Medicinal Chemistry, Pergamon press. I st Edn 1990.
9. Akila S, Selvi S, Balasubramanian K. Tetrahedron Lett 2001; 57: 3465.
10. Andreani A, Burnelli S, Granaiola MAU. J. Org. Chem 2002; 67: 3700.
11. Danheiser RL, Brisbois RG, Kowalczyk JJ. J. Am. Chem Soc 1990; 112: 3093.
12. Kershaw MJ, Leigh DA. Journal of Antimicrobial Chemo 1975; 1: 311 – 315.
13. Imperato G, Eibler E, Neidermarer J, Manabe K, Kobayashi S. Eur.J. 2008; 8: 4094-4101.
14. Li CJ, Cahn TH, John Wiley & Sons. Organic reactions in aqueous Media. New York 1997.
15. Thomas AE, Kenneth OL, Wenying CY. J. Org. Chem. 2002; 67: 7884.
16. Yadav JS, Reddy BVS, Murthy VSR. Ch. Synthesis. 2001; 783.
17. Fehnel E. Heterocycl, A. J. Chem. 1967; 4: 5654.
18. Chakrabarty M, Ghosh N, Basak R. Tetrahedron Lett 2002; 43: 4075.
19. Prabhakar KR, Veerapur VP, Bansal P. Bioorg Med Chem 2006; 14: 7113 – 7120.