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Green Synthesis, Biological Evaluation of Novel Benzoxazole Derivatives

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Abstract: Benzoxazole is one of the most important heterocyclic compound, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery. In the present study some novel benzoxazole derivatives were synthesized under green synthesis by microwave irradiation method by using Phenyliodoniumbis-trifluoroacetate (PIFA) in ethanol according to the scheme. All the synthesized benzoxazole derivatives have been characterized by using elemental analysis, FT-IR, ^{1}HNMR, ^{13}C NMR spectroscopy and further supported by Mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were evaluated for antimicrobial activity by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique and analgesic activity was examined by using the hot-plate method. All the compounds exhibited moderate to significant antimicrobial and analgesic activities.

INTRODUCTION:

Heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of hetero atoms in the ring. Heterocyclic compounds are very widely distributed in nature and are essential to life in various ways. Benzoxazole is a heterocyclic compound, is made from oxazole ring fused with benzene ring, having various biological activities and still of great scientific interest now a days. Benzoxazole compounds and their derivatives were found to numerous pharmacological activities like antitumor (Rida SM *et al*, 2005 & Klimesová V et al; 2009), anticonvulsant (Nadeem Siddiqui *et al*, 2008), antimicrobial (Gurvinder Singh*et al*, 2013), antileishmanial (Carole Di Giorgio *et al*, 2002), anti-tubercular (Mustafa ARISOY *et al*, 2009), antifungal (Samia M. Rida *et al*, 2005), anti-inflammatory (A. Srinivas*et al*, 2010 & Seetaramswamy *et al;* 2014), antipsychotic (Maria A. Siracusa *et al*, 2008), antioxidant (N. D. Jayanna*et al*, 2013) and anti-diabetic activities (K Arakawa *et al, 1997*). The present research work focuses on the synthesis of newer benzoxazole derivatives under green synthesis by microwave irradiation method by using Phenyliodoniumbis-trifluoroacetate (PIFA) in ethanol with potential activities that are now in development.

EXPERIMENTAL:

Material and Methods:

All the chemicals used were of laboratory grade and procured from E.Merck and S.D. Fine Chemicals (NSP, Guntur).Melting points were determined in digital melting point apparatus and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. All the microwave experiments were performed using RAGA's microwave synthesizer. IR spectra were recorded on BROOKER-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a BRUKER Ac 400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Purity of the synthesized compounds was checked by HPLC AGILENT. The results are in agreements with the structures assigned. All chemicals were reagent grade and used without further purification, and all solvents were freshly distilled before use.

General procedure for the synthesis of benzoxazole derivatives (BX-1 to BX-10): To a pyrex reaction vessel were added 2-aminophenol(1.1 mmol), aromatic aldehyde (1.0 mmol) and Phenyliodoniumbis-trifluoroacetate (PIFA) (1.05 mmol) in ethanol (3 ml).The reaction

vessel was then placed in the Emrys Optimizer and exposed to microwave irradiation (80 ^{0}C) for 15 minutes. The reaction mixture was then allowed to cool at room temperature and quenched with 15 ml of water. The crude reaction mixture was extracted with ethyl acetate (3 × 15 ml). The combined general experimental procedure organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated and purified by column chromatography on silica gel using petroleum ether/ ethyl acetate to afford the pure product (Naveen et al., 2016).

5-Methyl-2-(2-nitrophenyl)benzo[d]oxazole (BX-1): Pink solid; M.P 134-136 ^{0}C; R_f = 0.49 (AcOEt/petroleum ether 30%). IR (KBr): 3431, 2915, 1542, 1480, 1374, 1196, 1044, 800, 772 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 2.48 (s, 3H, -C*H*3); 7.19 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.42 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.58 (s, 1H, Ar-H); 7.65 (t, 1H, *J* = 7.6 Hz, Ar-H); 7.71 (t, 1H, *J* = 7.6 Hz, Ar-H); 7.86 (d, 1H, *J* = 7.6 Hz, Ar-H); 8.11 (d, 1H, *J* = 7.6 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 21.6, 110.4, 120.5, 121.6, 124.2, 127.3, 131.8, 132.4, 134.9, 141.7, 149.2, 149.3, 158.9. MS (EI): *m/z*=254 [M+]. Anal.Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02%. Found: C, 66.00; H, 4.02; N, 10.89%.

2-(2-Chlorophenyl)-5-methylbenzo[d]oxazole (BX-2): Colourless solid; M.P 74-76 ^{0}C; R_f = 0.59 (AcOEt/petroleum ether 25%). IR (KBr): 2921, 1734, 1590, 1548, 1468, 1423, 1325, 1263, 1194, 1019, 774, 730 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 2.49 (s, 3H, -C*H*3); 7.18 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.38 (m, 2H, Ar-H); 7.47 (d, 1H, *J* = 8.4 Hz, Ar- H); 7.54 (d, 1H, *J* = 9.2 Hz, Ar-H); 7.62 (s, 1H, Ar-H); 8.11 (d, 1H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 21.6, 110.2, 120.4, 123.5, 126.5, 126.8, 126.9, 131.4, 131.9, 133.5, 134.6, 141.9, 148.9, 161.1. MS (EI): *m/z*=245 [M$^+$], 247 [M^{+2}]. Anal.Calcd for $C_{14}H_{10}ClNO$: C, 69.00; H, 4.14; N, 5.75%. Found: C, 69.22; H, 4.25; N, 5.88%.

5-Methyl-2-(4-nitrophenyl)benzo[d]oxazole (BX-3): Pale yellow solid; M.P 218-250 ^{0}C; R_f = 0.44 (AcOEt/petroleum ether 30%). IR (KBr): 3402, 1556, 1521, 1342, 854, 706 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 2.50 (s, 3H, -CH_3); 7.22 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.48 (d, 1H, *J* = 8.4 Hz Ar-H); 7.59 (s, 1H, Ar-H); 8.35-8.41 (m, 4H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 21.6, 110.4, 120.5, 124.3, 127.6, 128.4, 133.0, 135.3, 142.2, 149.4, 160.8, 162.7. MS (EI): *m/z*=254 [M+]. Anal.Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02%. Found: C, 66.32; H, 4.10; N, 10.92%.

5-Chloro-2-(3-nitrophenyl)benzo[d]oxazole (BX-4): Colourless solid; M.P 184-186 ^{0}C; R_f = 0.52 (AcOEt/petroleum ether30%). IR (KBr): 3424, 2361, 1526, 1449, 1351, 1100, 821 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 7.38 (q, 1H, *J* = 8.4 Hz, Ar-H); 7.54 (d, 1H, *J* = 9.1 Hz,Ar-H); 7.72 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.78 (s, 1H, Ar-H); 8.39 (d, 1H, *J* = 8.8Hz, Ar-H); 8.55 (d, 1H, *J* = 7.6 Hz, Ar-H); 9.07 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) 111.8, 120.5, 122.7, 126.3, 126.5, 130.4, 130.7, 133.3,142.9, 149.5, 157.5, 161.9. MS (EI): *m/z*=274 [M$^+$], 276 [M^{+2}]. Anal.Calcdfor $C_{13}H_7ClN_2O_3$: C, 56.85; H, 2.57; N, 10.20%. Found: C, 56.75; H, 2.49; N,10.15%.

2-(2-Chlorophenyl)benzo[d]oxazole (BX-5): Colourless solid; M.P 61-64 ^{0}C; R_f = 0.53 AcOEt/petroleum ether30%). IR (KBr): 2953, 1537, 1430, 1253, 1194, 1022, 806, 738 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 7.36-7.46 (m, 4H, Ar-H); 7.56-7.57 (m, 1H, Ar- H); 7.61-7.62 (m, 1H, Ar-H); 7.84-7.86 (m, 1H, Ar-H); 8.13 (dd, 1H, *J* = 7.6,2.3 Hz, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 110.9, 120.6, 124.8, 125.7,126.2, 127.1, 131.5, 131.9, 132.0, 133.6, 141.8, 150.6, 161.1. MS (EI):*m/z*=229 [M$^+$], 231 [M^{+2}]. Anal.Calcd for $C_{13}H_6ClNO$: C, 67.99; H, 3.51; N,6.10%. Found: C, 68.11; H, 3.62; N, 5.99%.

2-(1H-Pyrrol-2-yl)benzo[d]oxazole (BX-6): Pink solid; M.P 144-146 ^{0}C; R_f = 0.51 AcOEt/petroleum ether 30%).IR (KBr): 3401, 1629, 1585, 1455, 1403, 1243, 1117, 741 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 6.36-6.38 (m, 1H, Ar-H); 7.04-7.05 (m, 1H, Ar-H); 7.28-7.33 (m, 2H, Ar-H); 7.52 (d, 1H, *J* = 7.6 Hz, Ar-H); 7.64 (d, 1H, *J* = 7.6 Hz,Ar-H); 10.25 (s, 1H, -N*H*). ^{13}C NMR (125 MHz, $CDCl_3$) C 110.5, 110.9,113.3, 118.9, 119.9, 123.1, 124.4, 124.7, 150.2, 158.2, 163.7. MS (EI):*m/z*=184 [M$^+$]. Anal.Calcd for $C_{11}H_8N_2O$: C, 71.73; H, 4.38; N, 15.21%.Found: C, 71.81; H, 4.25; N, 15.25%.

2-(1-Methyl-1H-indol-2-yl)benzo[d]oxazole (BX-7): Colourless solid; M.P 161-163 ^{0}C; R_f = 0.55 (AcOEt/petroleum ether25%). IR (KBr): 2332, 1579, 1450, 1340, 1240, 1141, 753 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 4.31 (s, 3H, -NCH_3); 7.18 (t, 1H, J = 7.6 Hz, Ar-H);7.35-7.38 (m, 3H, Ar-H); 7.42 (d, 2H, J = 10.7 Hz, Ar-H); 7.57 (d, 1H, J = 7.6Hz, Ar-H); 7.72 (d, 1H, J = 7.6 Hz, Ar-H); 7.80 - 780 (m, 1H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 32.2, 107.6, 110.2, 110.5, 119.9, 120.7, 122.1,124.5, 124.6, 125.2, 126.3, 126.9, 139.9, 142.2, 149.9, 157.8. MS (EI):m/z=248 [M$^+$]. Anal.Calcd for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28%.Found: C, 77.55; H, 4.75; N, 11.39%.

2-[3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]benzo[d]oxazole (BX-8): Colourless solid; M.P 205-207 ^{0}C; R_f = 0.44 (AcOEt/petroleum ether25%). IR (KBr): 3411, 1627, 1590, 1502, 1454, 1391, 1244, 1093, 989 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 7.32-7.33 (m, 2H, Ar-H); 7.37-7.39 (m, 1H,Ar-H); 7.45-7.53 (m, 5H, Ar-H); 7.71

(d, 1H, J = 9.1 Hz, Ar-H); 7.81 (d, 2H,J = 8.4 Hz, Ar-H); 7.99 (d, 2H, J = 8.4 Hz, Ar-H); 8.71 (s, 1H, pyrazolyl-H).^{13}C NMR (125 MHz, $CDCl_3$) C 110.3, 110.4, 119.5, 124.6, 124.9, 127.7,128.5, 129.8, 130.3, 130.6, 134.9, 139.3, 141.9, 150.2, 151.1, 158.1. MS (EI):m/z=372 [M^+], 374 [M^{+2}]. Anal.Calcd for $C_{22}H_{14}ClN_3O$: C, 71.07; H, 3.80;N, 11.30%. Found: C, 71.25; H, 3.75; N, 11.25%.

2-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-5-methylbenzo[d]oxazole (BX-9): Colorless solid; M.P 210-212^{0}C; R_f=0.52 (AcOEt/petroleum ether30%). IR (KBr): 2920, 1589, 1500, 1262, 1223, 1057, 944, 830, 799 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 2.46 (s, 3H, -C*H*$_3$); 7.11 (d, 1H, *J* = 8.4 Hz, Ar-H); 7.34-7.39 (m, 2H, Ar-H); 7.49-7.52 (m, 3H, Ar-H); 7.60 (d, 2H, *J* = 8.4Hz, Ar-H); 7.80 (d, 2H, *J* = 7.6 Hz, Ar-H); 7.91 (d, 2H, *J* = 8.4 Hz, Ar-H);8.68 (s, 1H, pyrazolyl-H). ^{13}C NMR (125 MHz, $CDCl_3$)C 21.6, 109.7, 110.4,119.8, 123.3, 126.1, 127.7, 129.7, 130.9, 131.1, 131.4. MS (EI): *m/z*=430[M^+], 432 [M^{+2}]. Anal.Calcd for $C_{23}H_{15}BrN_3O$: C, 64.20; H, 3.75; N, 9.77%.Found: C, 64.25; H, 3.69; N, 10.02%.

2-[3-(4-Ethoxyphenyl)-1-phenyl-1H-pyrazole-4-yl]benzo[d]oxazole (BX-10): Orange solid; M.P 189-191 ^{0}C; R_f=0.44 (AcOEt/petroleumether50%). IR (KBr): 3430, 2930, 1631, 1583, 1450, 1240, 1045, 750 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) H 1.43 (t, 3H, J = 6.9 Hz, -OCH_2CH_3); 4.09 (q, 2H,J = 6.9 Hz, -OCH_2CH_3); 6.98 (d, 2H, J = 9.1 Hz, Ar-H); 7.26-7.29 (m, 2H,Ar-H); 7.34 (t, 1H, J = 7.6 Hz, Ar-H); 7.46-7.54 (m, 3H, Ar-H); 7.69 (d, 1H, J= 6.9 Hz, Ar-H); 7.80 (d, 2H, J = 7.6 Hz, Ar-H); 7.94 (d, 2H, J = 8.4 Hz, Ar H); 8.68 (s, 1H, pyrazolyl-H). ^{13}C NMR (125 MHz, $CDCl_3$) C 14.9, 63.6,109.9, 110.4, 114.3, 119.4, 119.8, 124.5, 124.7, 127.4, 129.7, 130.2, 130.6,139.4, 142.0, 147.0, 150.2, 152.1, 158.6, 159.7. MS (EI): m/z=381 [M^+].Anal. Calcd for $C_{24}H_{19}N_3O_2$: C, 75.57; H, 5.02; N, 11.02%. Found: C, 75.44;H, 5.11; N, 11.09%.

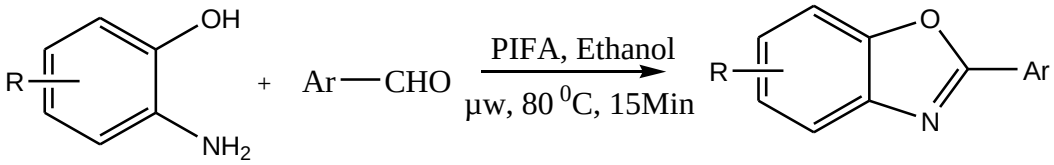
BIOLOGICAL EVALUATION:

Preparation of Culture Media: Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Sabouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture: Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^{0}C.

Standardization of Stock Culture: Stock cultures were placed in the incubator (37^{0}C for bacteria and 24^{0}C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15 × 10^8cfu/ml.

Preparation of Working Stock Culture: Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5cfu/ml. This was then used for further *in vitro* screening.



Scheme 1: Synthesis of benzothiazole derivatives (BT-1 to BT-10)

Table 1: Physical data of benzoxazole derivatives (BX-1 to BX-10)

Comp	Ar	M.F	M.W	M.P (^{0}C)	R_f
BX-1	5-Methyl-2-nitrophenyl	$C_{14}H_{10}N_2O_3$	254.24	134-136	0.49
BX-2	5-Methyl-2-chlorophenyl	$C_{14}H_{10}ClNO$	243.69	74-76	0.59
BX-3	5-Methyl-4-nitrophenyl	$C_{14}H_{10}N_2O_3$	254.24	218-220	0.44
BX-4	5-Chloro-3-nitrophenyl	$C_{13}H_7ClN_2O_3$	274.66	184-186	0.52
BX-5	2-Chlorophenyl	$C_{13}H_6ClNO$	227.65	61-63	0.53
BX-6	1H-Pyrrol-2-yl	$C_{11}H_8N_2O$	184.19	144-146	0.51
BX-7	1-Methyl-1H-indol-2-yl	$C_{16}H_{12}N_2O$	248.28	161-163	0.55
BX-8	3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl	$C_{22}H_{14}ClN_3O$	371.82	205-207	0.44
BX-9	3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-methyl-4-yl	$C_{23}H_{15}BrN_3O$	429.29	210-212	0.52
BX-10	3-(4-Ethoxyphenyl)-1-phenyl-1H-pyrazole-4-yl	$C_{24}H_{19}N_3O_2$	381.43	189-191	0.44

Table-2: Antimicrobial activity of synthesized benzoxazole derivatives (BT-1 to BT-10)

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	***S.pyogenus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
BX-1	100	100	250	250	200	1000
BX-2	100	200	100	500	250	500
BX-3	250	250	250	100	500	1000
BX-4	200	250	125	500	1000	250
BX-5	250	200	500	500	500	1000
BX-6	100	62.5	100	200	500	200
BX-7	200	500	62.5	250	250	500
BX-8	500	250	500	200	200	200
BX-9	200	500	200	100	500	200
BX-10	500	200	250	250	500	250
Streptomycin	250	100	100	100	NT	NT
Greseofulvin	NT	NT	NT	NT	500	100

Preparation of Drug Dilutions: Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening: Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

Determination of MIC (Naga Raju et al., 2015): The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 25^0C respectively for 24 hours for bacteriae and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC) (Qaralleh et al., 2010; Majali et al., 2016). The MIC values of the test compounds are recorded in Table-2.

ANALGESIC ACTIVITY (Gollapalli Naga Raju *et.al,* 2016):

All the synthesized newer benzoxazole derivatives BX-1 to BX-10were evaluate the analgesic activity. To begin with the oral toxicity of the synthesized compounds was performed by acute toxic class method. The selected adult albino rats were used to determine the dose. The animals were fasted overnight prior to the acute experimental procedure. Following the period of fasting, the animals were weighed and the synthesized compounds were orally administered at a dose of 50 mg/kg bodyweight. Immediately after dosing, the animals were observed continuously for the first 30 min for behavioural changes and for mortality at the end of 24 h, 48 h, 72 h and 96 h respectively. As no mortality was observed with the above dose even after 96 h, the LD_{50} value of the compounds expected to exceed 50mg/kg body weight. Toxicity assays showed that all the compounds proved tobe nontoxic at tested dose levels and well tolerated by the experimental animals as their LD_{50} cut – off values > 50 mg/kg body weight. Analgesic activity of the synthesized compounds was determined during tail immersion method. Healthy Swiss mice (n=6) of either sex was elected by random sampling technique and placed into individual restraining cages leaving the tail hanging out freely. The animals were then allowed to adapt in the cages for 30 minutes before testing. The lower 5 cm portion of the tail was marked and immersed in a beaker of freshly filled warm water of at 55 ± 5º C. Within a few seconds the rat reacts by withdrawing the tail. There action time was recorded by a stop watch. After each determination the tail was carefully dried. This reaction was determined before oral feeding of thedrug and synthesized compounds which were recorded as zero minutes reading. The test compounds, control (2% gum acacia) and standard (Pentazocine) at a dose level of 50 mg/kg body weight were administered orally by intragastric tube. The time (in seconds) to withdraw the tail clearly out of water was taken as the reaction time. The first reading (0 min) was taken immediately after the administration of the test compound and subsequent reaction time was recorded at 15, 30, 60 and 90 min respectively. The cut-off time of the immersion is 15 seconds. The mean reaction time was recorded for each group and compared with the value of the standard drug pentazocine. The percentage analgesic activity was calculated using the formula:

% potency = $[(T_2 – T_1) / T_2] \times 100$

Where, T_1 is the reaction time (in sec) before treatment and T_2 is there action time (in sec) after treatment.

Animals: The selection of animals, caring and handling was done as per the guidelines set by the IAEC of Chalapathi Institute of Pharmaceutical Sciences, Guntur. Inbred albino mice (Swiss strain) of adult gender weighing 120-150 g were used for the study. The mice were housed individually in clean polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. All animals were fed with sterile commercial pelleted rat chow supplied by Hindustan Lever Ltd (Mumbai, India) with free access towater (ad libitum) under standardized housing conditions (natural light-dark cycle, temperature 23 ± 1 ^{0}C, relative humidity 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to 12experimental groups of 5 mice each. Each mouse was used only once. All tests were performed between 08:00 and 16:00 h. All efforts were made to minimize animal suffering and to use only the minimum number of animals necessary to produce reliable scientific data. The experimental protocols and procedures listed below conformed to the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee (IAEC/CIPS/08/2015).Mice equivalent doses in mg/kg body weight of clinical doses were calculated as mg/kg body weight with the help of standard tables.

Dose and administration of compounds: The synthesized compounds (50 mg/kg), Pentazocine as a reference opiod analgesic drug (50 mg/kg) and 2% gum acacia as control were administered orally by intragastric tube. The relative potencies to Pentazocine were determined in Table 3.

RESULTS AND DISCUSSION:

To synthesize newer benzoxazole derivatives, proceeded to explore the PIFA(1.05 mmol) promoted oxidative cyclization reaction of 2-aminothiophenol (1.1 mmol) with aromatic aldehyde (1.0 mmol) in ethanol at 80 ^{0}C under microwave irradiation. To our delight the reaction was complete after 5 min and showed a good conversion towards benzoxazole, which was isolated in 60% yield after aqueous work-up followed by column chromatography. This positive initial result prompted us to further investigate the conditions suitable for this reaction under microwave irradiation. Extension of the irradiation time from to 15 min resulted in the complete conversion and it was isolated in 80% yield. Further irradiation up to 30 min did not lead to further increase in product yield. Attempts to decrease the reaction temperature were unsuccessful. From these observations, selected a microwave irradiation of the substrates with PIFA (1.05 mmol) in ethanol at 80 ^{0}C for 15 min as the standard reaction conditions for the synthesis of a wide range of benzoxazoles.

The versatility of this methodology was demonstrated with respect to variation in the aldehyde and amine by synthesis of a small family of newer benzoxazole derivatives (BX-1 to BX-10). As shown in scheme-1, microwave assisted oxidative cyclization worked well for a variety of aldehydes and 2-aminothiophenols, giving good to excellent yields of the corresponding newer benzoxazole derivatives (BX-1 to BX-10). However, compounds containing heterocyclic cores like pyridine, thiophene and furan, respectively were obtained only in moderate yields. These results can be attributed to the cleavage of these heterocycles under microwave condition. However, other microwave assisted protocols resulted in excellent yield of similar products. The structure of all the synthesized compounds was confirmed by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy.

The results of analgesic activity are presented in Table-3, which demonstrate that 2-aryl benzoxazole analogues were generally found to be less potent than their corresponding heteroaryl analogues. Among the heteroaryl analogues, pyrazolyl groups exhibited good analgesic activity and their values are comparable to the standard Pentazocine..

CONCLUSION:

In summary, we have explored a useful and practical approach to benzoxazoles by PIFA promoted cyclo condensation of 2-aminothiophenol with different aromatic aldehydes. The current protocol is noteworthy, since it has advantages like wide substrate scope, short reaction time, microwave condition and satisfactory yields.

Table 3: Analgesic activities of newer benzoxazolederivatives

Compd code	Comparative analgesic potency to Pentazocine after time in minutes				
	10 min.	15 min.	30 min.	60 min.	90 min.
BX-1	1.30±0.01	2.45±0.02	2.56±0.03	2.60±0.02	2.63±0.01*
BX-2	1.30±0.01	2.46±0.01	2.50±0.02	2.59±0.01	2.65±0.01*
BX-3	1.30±0.01	2.47±0.01	2.60±0.01	2.64±0.01	2.67±0.01*
BX-4	1.31±0.01	2.49±0.03	2.59±0.2	2.64±0.01	2.69±0.02*
BX-5	1.30±0.01	2.38±0.02	2.44±0.01	2.51±0.01	2.60±0.01*
BX-6	1.30±0.01	2.37±0.02	2.43±0.03	2.70±0.09	2.86±0.08*
BX-7	1.30±0.01	2.69±0.01	3.01±0.01	3.13±0.01	3.22±0.08*
BX-8	1.36±0.01	3.36±0.02	4.37±0.03	4.72±0.01	5.99±0.01*
BX-9	1.38±0.01	3.47±0.01	4.57±0.03	4.81±0.01	5.82±0.01*
BX-10	1.40±0.01	3.57±0.02	4.59±0.01	4.85±0.03	6.00±0.02*
Gum acacia	1.30±0.01	1.24±0.01	1.12±0.01	1.15±0.01	1.30±0.01*
Pentazocine	1.30±0.01	3.31±0.03	4.39±0.04	4.74±0.03	5.72±0.02*

All results were significantly different from the standard and normal control. Value at P = 0.05.
2% (w/v) of gum acacia was used as control

Evaluation of analgesic activity of twenty compounds was performed by tail immersion test. All the tested compounds displayed varying degrees of analgesic activity. Benzoxazole derivatives bearing pyrazolyl system exhibited comparable to or slightly more potent activity than the standard Pentazocine.

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