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Synthesis and biological evaluation of some substituted benzoxazole derivatives as antibacterial agents

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ABSTRACT

Synthesis of benzoxazoles by treating 4-ethylsulphonyl-2-aminophenols or 4-Methyl-2-aminophenols with substituted benzoic acid derivatives is reported in this piece of research to establish new candidates with improved antimicrobial property. These reactions are operationally simple and proceed in average yield. Compounds are biologically evaluated against gram positive and gram negative bacteria S. aureus and E. coli. The synthesized compounds possessed a broad spectrum of activity with zone of inhibition in concentration 10-80 µg/ml. The range for percentage relative inhibition is observed to be 32.1% to 45.5 % in S. aureus, the range for percentage relative inhibition is observed to be 40 % to 51.8 % in E. coli.

Keywords: Benzoxazole, synthesis, antimicrobial agents

INTRODUCTION

The antimicrobial agents are amongst the most important and frequently used group of drugs to treat disorders of microbes. Literature reveals that substituted benzoxazoles possess diverse chemotherapeutic activities including antibiotics, antimicrobials, anti viral, topoisomerase I and II and anti-tumour activities. Benzoxazole is an aromatic organic with a molecular formula C_7H_5NO, a benzene-fused oxazole ring structure, and an odour similar to pyridine. Benzoxazole is used primarily in industry and research, and has no household use. Being a heterocyclic compound, benzoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. It is found within the chemical structures of pharmaceutical drugs such as flunoxaprofen. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization. Benzoxazole ring is one of the most common heterocycles in medicinal chemistry. Previous reports revealed that substituted benzoxazoles possess diverse chemotherapeutic activities including antibiotics (M. Prudhomme et al., 1986) antimicrobial (I. Oren et al., 1997; 1998; O. Temiz-Arpaci et al., 2005,; Vinsová J et al., 2005, , Katla et.al., 2007, Patil et. al, 2010), antiviral (Akbay A et al., 2003) topoisomerase I and II inhibitors (Asli Pinar et al., 2004) and antitumor activities (Ukei M. et al., 1997; Varga A. et al., 2005). In previous studies, synthesis of some compounds bearing hydrogen, chlorine, methyl, nitro, amine and amide substitution at the 5^{th} position the benzoxazole ring was done. The in vitro antimicrobial activity against some Gram-positive, Gram-negative bacteria and *Candida albicans* was examined (E. Sener et al., 1986; I. Yalcin et al., 1986; ÖZDEN, S et al., 1987; E. Sener et al., 1987; I. Yalçın et al., 1990; Yalcin Iet al., 1992). As per reported literature substituents at ortho position of phenyl ring by electron withdrawing group resulted in increased potency. Similarly a substitution at para position is significant for activity. Thus on the basis of aforementioned considerations, few more compounds were designed and synthesized through

Scheme 1; the series of antimicrobial agents (I-XX) is reported in this pice of research , choosing an ethylsulphonyl and methyl fragment at the 5th position of benzoxazole with together a substituted-phenyl/benzyl/2-phenylethyl at the 2nd position. The strategy employed was to examine the effect of the second position against some gram-positive, gram-negative bacteria and its isolate in comparison to control drugs. The structure activity relationship analysis revealed that the compounds possessing a methyl group at the position 5 and 6 of benzoxazole moiety play a pivotal role in increasing the activity against *S. aureus* and *E. coli.*

MATERIALS AND METHODS

CHEMISTRY

The reactions were monitored and the purity of the products was checked by thin layer chromatography (TLC). Kieselgel HF 254 chromatoplates (0.03mm) were used for TLC and the solvent system was chloroform, methanol (9, 1). All the melting point was taken on an open capillary melting point apparatus and is uncorrected. IR spectra were recorded on FTIR, SHIMADZU-8400S spectrometer as KBr discs. ^{1}HNMR was recorded in solvent dimethylsulphoxide on JEOL AL300 FTNMR operating at 300.40 MHz in the presence of internal standard tetra methyl silane. FAB Mass Spectra of all the compounds were recorded on JEOL SX-102 Mass Spectrometer.

General procedure for the synthesis of 5-Ethylsulphonyl/methyl-2-(Substituted-phenyl/benzyl and/or phenyl ethyl) benzoxazoles

5-Ethylsulphonyl-2-(Substituted-phenyl/benzyl and/or phenyl ethyl) benzoxazole derivatives (I-XIII) were synthesized by heating 4-ethylsulphonyl-2-aminophenol (0.01 mol) with of substituted benzoic/phenyl acetic acid (0.01 mol) in 24 g polyphosphoric acid (PPA). The above solution was stirred for 2-3 hours. The residual was poured into an ice- cold water mixture and made alkaline with an excess of 10M sodium hydroxide. The resultant solution is extracted with ethyl acetate and benzene (compounds X-XIV). Then this solution was dried over anhydrous sodium sulphate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of the solvent in vacuum, the crud product was obtained and recrystallized from ethanol.

BIOLOGICAL EVALUATION, MICROBIOLOGICAL METHOD

Whatmann's filter paper (size no.1) was cut into small discs (one-quarter inch diameter) and was autoclaved for 1 hr. Accurately weighed compounds (10mg) were dissolved in 5 ml of dimethylsulphoxide and volume was made upto10 ml (Stock A, 1000 µg/ml), 5 ml of stock A was diluted upto10ml (Stock B, 500 µg/ml). Aliquots of stock B were further diluted to get conc. of 10, 20, 40, 80, 100µg/ml. All the dilutions were applied to autoclaved filter paper disc using micropipette with sterile pipette tip. Discs dried by evaporating solvent and kept in sterilized bottle in refrigerator. Agar media 3.8 g (Muller Hinton agar media, HI media) was boiled with 100ml of distilled water, the solution was then autoclaved at 121^{0}C for half an hour. Media was then cooled to 40-50^{0}C and was poured into sterilized petri plate, which was solidified at room temperature. The identified & collected bacterial strains contain two gram negative & two gram positive strains which are *E. coli*, *S. aureus*. The identified organism was applied to Muller Hinton media, for sensitivity test, by streaking on the medium with the help of swab, the plate was first streaked to left and then rotated to 90^0 and streaking was repeated right. The sensitivity disc was applied. All the reservoir discs of samples, standards and control with different dilutions were placed onto the inoculated plate (Muller-Hinton) with sterilized forceps. Sufficient space between discs was maintained so that clear zone of inhibition can be observed. Disc was pressed into medium with slight pressure so that disc gets stick to agar media. After application of disc lid of the petri plates were closed. Petri plates were inverted and incubated in incubator at 37^{0}C for 24 hours.

RESULTS AND DISCUSSION

CHEMISTRY

General procedure for the synthesis of 5-Ethylsulphonyl/methyl-2-(Substituted-phenyl/benzyl and/or phenyl ethyl) benzoxazoles

5-Ethylsulphonyl-2-(Substituted-phenyl/benzyl and/or phenyl ethyl) benzoxazole derivatives (I-XIII) were synthesized by heating 0.01 mole 4-ethylsulphonyl-2-aminophenol with 0.01 mol. of suitable (benzoic/phenyl acetic) acid in 24 g polyphosphoric acid (PPA). The above solution was stirred for 2-3 hours. The residual was poured into an ice-water mixture and neutralized with an excess of 10M NaOH. Solution was extracted with ethyl acetate while X-XIV is extracted with benzene. Then this solution was dried over anhydrous sodium sulphate and

evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of the solvent in vacuo, the crude product was obtained and recrystallized from ethanol.

Infrared Spectroscopy

The IR spectroscopy data confirmed the presence of required as shown in Table I. As per the data obtained, presence of C-Cl, C-Br and C-N functional groups is confirmed in all compounds which interpreted the presence of substituted benzoxazole. Presence of two heteroatom, nitrogen (C=N), and oxygen(C-O) in all the compounds confirmed the presence of benzoxazole ring. Presence of aromatic structure are confirmed in all compounds due to presence of C=C-C peaks.

NMR spectroscopy

The data obtained from NMR analysis, as shown in Table I, has been interpreted with standard δ-scale of various functional groups confirm the presence of groups containing specific number of hydrogen atom. In all data presence of aromatic C-SH, [CH-Cl], group was confirmed.

Elemental Analysis

Elemental analysis reveals the presence of carbon, hydrogen, and nitrogen in the compound. It helps in identification of molecular formula of compound as the number of C, H, N, and O can be determined. Results are shown in Table I.

BIOLOGICAL EVALUATION

The synthesized compounds were subjected to antibacterial activity by disc diffusion method. Tetracycline and Ampicillin were taken as standard against *S. aureus* and *E. coli* as shown in Table II.

Staphylococcus aureus (Gram Positive)

Compounds X, XX, XVI, and IV, I, has shown a significant inhibitory activity towards S. *aureus* at minimum concentration ranging from 10-80 µg/ml. Among all these compounds, X (45.5%), XX (41.2%), XVI (39.6%) and IV (39.3%) have shown maximum percentage of relative zone of inhibition while XIX (32.1%) has shown least percentage of relative zones of inhibition as compared with the standard drug. Results showed that the antibacterial efficacy of synthesized compounds against S. aureus is in the following order

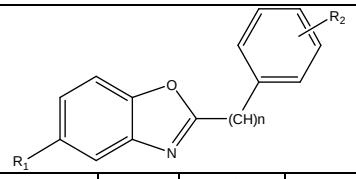
X>XX>XVI>IV>I>II>VIII>VI>XI>IX>III>XVII>VII>XIV>XIX

Substituted phenyl group has shown better activity against *S. aureus* than substituted benzyl groupand 5-ethylsulphonyl substituted benzoxazole was found slightly more active than methyl substituted benzoxazole against *S. aureus.* All the compounds have exhibited a significant activity towards *Staphylococcus aureus* in minimum concentration ranges from 10-80 µg/ml. Amongst all the compounds, XI has shown maximum zone of inhibition while and XIV has shown least inhibition at the minimum concentration. The range for percentage relative inhibition is observed to be 32.1% to 45.5 %. Results showed that the antibacterial efficacy of synthesized compounds against *S.aureus* is in the following decreasing order, 3, 4-dimethoxy> 4-NO_2> 2, 4- dichoro > 2-Cl > 4- Cl

Escherichia coli (Gram negative)

Compounds I, XI, XIV, XVII, XIX have shown a significant inhibitory activity towards E. coli at minimum concentration ranging from 10-80 µg/ml. Among all these compounds, XI (51.8%), XIV (51.8%), XVII (51.7%) and XIX (48.6%) have shown maximum percentage of relative zone of inhibition while IX (40%) has exhibited least percentage of relative zones of inhibition as compared with the standard drug. The antibacterial efficacy of synthesized compounds against E. coli is in the following order **XI>XIV>XVII> XIX > I> VIII > IV> III> X> VII> XX> II> XVI> VI> IX.** Substituted phenyl group shows better activity against E. coli than substituted benzyl group.5-Methylsubstituted benzoxazole is slightly more active than 5-ethylsulphonyl substituted benzoxazole against E. coli. Amongst all the compounds, compound no. XI and XIV shows maximum zone of inhibition while IX shows least inhibition at the minimum concentration. The range for percentage relative inhibition is observed to be 40 % to 51.8 %. Results showed that the antibacterial efficacy of synthesized compounds against E. coli is in the following decreasing order- **2, 4- dichoro > 4- C l> 2-Cl > 4- Br.**

Table I: Substituted benzoxazole derivatives



Comp. No.	R_1	R_2	N	Mol. Formula	m.p.	% Yield	I.R cm^{-1}	m/z value	^1H NMR (δ)
I	C_2H_5-SO_2	4-Cl	0	$C_{15}H_{12}ClNO_3S$	125-135^{0}C	84.9%	2924.85, 1592.13, 1454.23, 1180.35. 741.58, 696.22	-	8.337, 8.24, 7.968, 7.774, 7.54, 3.227, 1.254
II	C_2H_5-SO_2	4-Cl	1	$C_{16}H_{14}ClNO_3S$	105-110^{0}C	88.6%	3047.32, 2929.67, 1609.49, 1454.23, 1254.61, 740.61	-	8.26, 7.917-7.889, 7.110, 7.0 34 , 3.874, 1.253
III	C_2H_5-SO_2	2,4-Cl	1	$C_{16}H_{13}Cl_2NO_3S$	120-125^{0}C	97.7%	3090.32, 2927.74, 1609.49, 1255.57, 741.58	-	8.261, 7.93, 7.899, 7.737, 7.346, 7.302, 7.268, 4.351, 3.162, 1.279
IV	C_2H_5-SO_2	2-Cl	0	$C_{15}H_{12}ClNO_3S$	100-105 ^{0}C	84.9%	2975.96, 2933.53, 1454.23, 768.58, 610.43	-	8.007, 7.979, 7.896, 7.723, 7.551, 7.341, 3.137, 1.224
V	C_2H_5-SO_2	2-Cl	1	$C_{16}H_{14}ClNO_3S$	110-120^{0}C	88.6%	3092.39, 2921.96, 1617.2, 1447.48, 1238.21, 747.36	-	7.915, 7.889, 7.469, 7.465, 4.478, 3.937, 1.227
VI	C_2H_5-SO_2	4-Br	0	$C_{15}H_{12}BrNO_3S$	150-155^{0}C	96.6%	3081.07, 2922.92, 1615.27, 1456, 16, 1181.32, 690.47, 688.54	M+2h	8.336, 7.939, 7. 698, 7.264, 3.222, 1.285
VII	C_2H_5-SO_2	4-NO_2	0	$C_{15}H_{12}N_2O_5S$	80-90^{0}C	84%	2920.03, 1613.34, 1454.23, 1224.71, 668.29	M+1h	8.261,7.941, 7.768, 7.731, 3.562, 1.282
VIII	C_2H_5-SO_2	3-NO_2	0	$C_{15}H_{12}N_2O_5S$	90-100^{0}C	84%	3118.65, 1486.6, 1190, 668.29	M+1h	8.403, 8.032, 7.945, 7.839, 7.607, 3.17, 1.278
IX	C_2H_5-SO_2	3,4-OCH_3	0	$C_{17}H_{17}NO_5S$	180-185^{0}C	91%	2976.92, 2925.78, 1615.27, 1455.19, 1181.32, 647.07	M+1h	7.918, 7.654, 6.992, 6.866, 3.743, 3.389, 1.278
X	C_2H_5-SO_2	4-OCH_3	0	$C_{16}H_{15}NO_4S$	140-145^{0}C	83.7%	2925.81, 1558.38, 1457.12	M+1h, M+2h, M+3	7.915, 7.687, 7.263, 6.987, 3.729, 3.219, 1.28
XI	C_2H_5-SO_2	2,4-Cl	0	$C_{15}H_{11}Cl_2NO_3S$	130-135^{0}C	94%C	1616.24, 1457.1, 1259.43,	M+2h	8.009, 7.981, 7.633, 7.469, 7.261, 3.198, 1.288
XII	C_2H_5-SO_2	4-OCH_3	1	$C_{17}H_{17}NO_4S$	-	87.4%	-	-	
XIII	C_2H_5-SO_2	4-SO_2NH_2	0	$C_{15}H_{14}N_2O_5S_2$	150-160^{0}C	84.9%	3017.42, 2920.99, 1616.24, 1457.12, 1175.53, 754.12	-	
XIV	CH_3	4-Cl	0	$C_{14}H_{10}ClNO$	145-150^{0}C	95.6%	3034.78, 2921.96, 1616.24, 1457.12, 1175.53, 754.12	M+1h	7.433, 7.26, 7.158, 2.346
XV	CH_3	4-Cl	1	$C_{15}H_{12}ClNO$	-	98%		-	-
XVI	CH_3	2,4-Cl	1	$C_{15}H_{11}Cl_2NO$	70-80^{0}C	96%	3026.1, 2922.92, 1588.27, 1506.3, 1179.39, 750.26	M+2h	7.096, 7.123, 7.289, 4.352, 2.445,
XVII	CH_3	2-Cl	0	$C_{14}H_{10}ClNO$	100-110^{0}C	94.2%	3055.03, 2919.06, 1592.13, 1471.59, 1250.75, 735.79	M+1h	7.192, 7.219, 7.259, 7.475, 2.502
XVIII	CH_3	2-Cl	1	$C_{15}H_{12}ClNO$	70-80^{0}C	127%	3026.1, 2922.92, 1596.95, 1475.44, 1176.5, 737.37	M+1h	7.087, 7.115, 7.258, 4.4, 2.445
XIX	CH_3	4-Br	0	$C_{14}H_{10}BrNO$	140-145^{0}C	142%	3078.18, 2861.2, 1592.13, 1457.12, 1174.57, 688.54	M+2h	7.163, 7.19, 7.262, 7.46,7 .433, 7.548, 2.488
XX	CH_3	4-NO_2	0	$C_{14}H_{10}N_2O_3$	155-160^{0}C	125%	2919.06, 1605.63, 1441.69, 1178.43, 738.69	M+h	8.023, 8.051, 7.074, 7.101, 7.261, 2.466

Table II: Zone of inhibition for samples, standard & control.

Compound Name	Zone of inhibition in mm									
	Escherichia coli					*Staphylococcus aureus*				
	Conc. in (mg/ml)					Conc. in (mg/ml)				
	0.01	**0.02**	**0.04**	**0.06**	**0.08**	**0.01**	**0.02**	**0.03**	**0.04**	**0.05**
I	9.36	9.7	10.46	10.69	11.31	9.02	10.12	11.47	11.78	12.43
II	-	8.54	9.32	10.66	11.12	-	9.87	10.99	11.87	12.23
III	-	9.02	10.77	12.87	12.43	-	9.01	10.66	1162	12.83
IV	9.15	9.14	10.54	11.84	12.94	-	10.23	11.87	12.09	12.76
V	-	-	-	-	-	-	-	-	-	-
VI	-	8.22	9.75	10.66	11.77	-	9.65	10.45	10.45	11.64
VII	-	8.54	9.54	10.63	10.36	-	8.73	9.37	10.37	10.29
VIII	-	9.17	10.81	11.82	10.92	-	9.82	10.64	11.89	12.01
IX	7.18	8.02	9.77	9.83	9.02	8.20	9.23	9.82	10.18	10.83
X	8.19	8.98	9.872	10.92	10.66	8.10	9.62	10.72	11.92	12.07
XI	9.83	10.37	11.93	12.06	13.76	10.54	11.84	11.93	12.76	12.68
XII	-	-	-	-	-	-	-	-	-	-
XIII	-	-	-	-	-	-	-	-	-	-
XIV	9.04	10.37	11.47	11.83	12.38	8.30	9.38	9.98	10.74	11.48
XV	-	-	-	-	-	-	-	-	-	-
XVI	-	8.34	9.48	9.47	10.48	7.04	8.57	9.45	10.56	11.65
XVII	10.21	10.35	10.96	11.24	11.34	9.86	10.31	11.16	12.96	13.25
XVIII	-	-	-	-	-	-	-	-	-	-
XIX	8.04	9.74	10.47	10.95	11.74	7.57	8.9	9.437	9.85	10.65
XX	-	8.45	9.46	10.98	11.89	-	8.37	9.76	10.74	11.06
Tetracycline	-	20.01	-	-	-		26.01	-	-	-
Ampicillin	-	-	12.96	-	-	-	-	24.62	-	-
Control	-	3.10	-	-	-	-	7.31	-	-	-

CONCLUSION

All the above results confirms the correctness of scheme being followed for the synthesis of designed compounds, correctness of the anticipated structures drawn for the synthesized compounds and biological evaluation confirms that the designed compounds exhibited the significant antibacterial activities when compared with reference drug. Substituted phenyl group shows better activity against *S. aureus* than substituted benzyl group. 5-ethylsulphonyl substituted benzoxazole is slightly more active than methyl substituted benzoxazole against *S. aureus.* All the compounds have shown a significant activity towards *Staphylococcus aureus* in minimum concentration ranges from 10-80 µg/ml. Compound XI has shown maximum zone of inhibition while and XIV shows least inhibition at the minimum concentration. Amongst all the compounds, XI, XIV, XVII and XIX have shown maximum percentage of relative zone of inhibition while IX has shown least percentage of relative zones of inhibition as compared with the standard drug in case of in *E. coli*. The field is further open for study of these compounds with respect to toxicity studies, chronic toxicity studies, pharmacokinetic studies and clinical studies to establish molecules as novel drug moieties.

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