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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2-(2-(BENZO [D] OXAZOL-2-YL) PHENYLAMINO)-N-(SUBSTITUTED PHENYL) ACETAMIDES

Priyanka*, N.K. Sharma, K. K. Jha and M. Vijaya Kumar

Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Bagarpur, Moradabad, Uttar Pradesh, India

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Correspondence to Author:

Priyanka

Assistant Professor, College of Pharmacy, Teerthanker Mahaveer University, Bagarpur, Moradabad, Uttar Pradesh, India

E-mail: priya_its@rediffmail.com

ABSTRACT: In the present work six new benzoxazole derivatives were synthesized adopting the suitably selected scheme. The synthetic methodology included the synthesis of 2-(2-aminophenyl) benzoxazole (D1) by the reaction of anthranilic acid with 2-aminophenol in poly phosphoric acid. Substituted chloroacetanilides (A1-A6) were synthesized by the reaction of substituted anilines with chloroacetyl chloride. Finally compound 2-(2-(benzo[d]oxazol-2-yl) phenylamino)-N-(substituted phenyl) acetamide (2A-2F) were synthesized by the fusion of 2-(2-aminophenyl) benzoxazole (D1) and substituted chloroacetanilides (A1-A6). All the aynthesized compounds were subjected to antimicrobial screening against the bacterial strains *i.e.*, *Bacillus subtilis* (MTCC-619), *Streptococcus pneumonia* (recultured), *Escherichia coli* (NCTC 6571) and *Staphylococcus aureus* (NCTC 7447). The compounds were also tested for antifungal potential against two fungal strains i.e. *Aspergilus. Niger* (NCIM NO. 618*)* and *Candida albicans* (NCYC 597). In all the testing a significant correlation existed between the antimicrobial potential and the concentration of test compound.

INTRODUCTION: Various Heterocyclic compounds have received considerable attention during last few decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. The earlier sources of drugs i.e. plant, animal and mineral sources, resulted somehow lesser therapeutic efficacy and even more toxicities comparatively. So the process of new drug discovery has been accelerated by the necessity of synthesizing compounds possessing better therapeutic and least possible toxicological parameters.

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During recent years there have been some interesting developments in the pharmacology of benzoxazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities [1].

It has been investigated in past decade that benzoxazoles possess a wide range of promising biological activities [2]. The substituted benzoxazoles have been shown to exhibit antitumor[3], antioxidant, antihelmintic [4], cyclooxygenase inhibitory [5], antifungal [6], Antitubercular [7], $5HT_3$ receptor antagonists [8], anti-inflammatory, analgesic & cyclin dependent kinase inhibitory [9], 5-lipoxygenase inhibitory [10], melatonin receptor agonist [11], anticancer [12], antibacterial [13] and anti-HIV-1[14] activities.

Recent observations suggested that substituted benzoxazoles and related heterocycles possessed potential activities with lower toxicities chemo-therapeutically [15] e.g. benzoxazole derivative, Calcimycin, is a carboxylic polyether antibiotic isolated from the culture of *Streptomyces chartreusis* (NRRL 3882). It was found to be very active against Gram-positive bacteria including some *Bacillus* and *Micrococcus* microbes. Two calcimycin analogues, Routiennocin [16] and Cezomycin [17] were found to be highly active against *Bacillus cereus, Bacillus negaterium, Micrococcus luteus* and *Streptomyces rimosus*. Additionally Frankamide [18] that is 11-demethyl cezomycin showed some activity against *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis* and against several plant pathogenic fungal strains [19].

Keeping in view of the immense biological activities exhibited by the molecule, present investigation explores the possibility of antimicrobial potential of a series of suitably substituted benzoxazole moiety.

EXPERIMENTAL: All melting points were determined in open capillaries and are uncorrected. The progress of the reaction and the purity of compounds were checked by TLC on percolated silica gel plates using n-Hexane, ethyl acetate, chloroform & benzene in different ratio as mobile phase. Detection of compounds was made by treatment with iodine vapours. IR spectra of compounds were recorded on FTIR 4100 type A spectrophotometer and HNMR spectra (DMSO) on Bruker FTAC spectrometer with TMS as internal standard.

Synthesis of substituted chloroacetanilides (A1-A6): Substituted aniline (0.1mole) was dissolved in 50ml of glacial acetic acid containing 50ml of saturated solution of sodium acetate. In case the substance did not dissolve completely, the mixture was warmed. The solution was cooled in ice bath with stirring. To this stirred solution, about 1 ml of 2-chloroacetyl chloride was added drop wise so that the vigorous reaction could not take place. After half an hour, the white product formed was separated by filtration through Whatman filter paper.

The solid precipitate was washed with distilled water, dried and recrystalized from aqueous alcohol[20].

Synthesis of 2-(2-aminophenyl) benzoxazole (D1): Equimolar quantities (0.1 mole) of 2-aminophenol and anthranilic acid were refluxed in poly phosphoric acid at 190°-195°C for 4 hrs. The completion of reaction was checked by thin layer chromatography (TLC) using benzene: chloroform (2:3) solvent system as mobile phase and iodine vapour as developing agent. The reaction mixture was poured into crushed ice, with vigorous stirring. Filtered, washed with cold water, dried and recrystalized from ethanol[21].

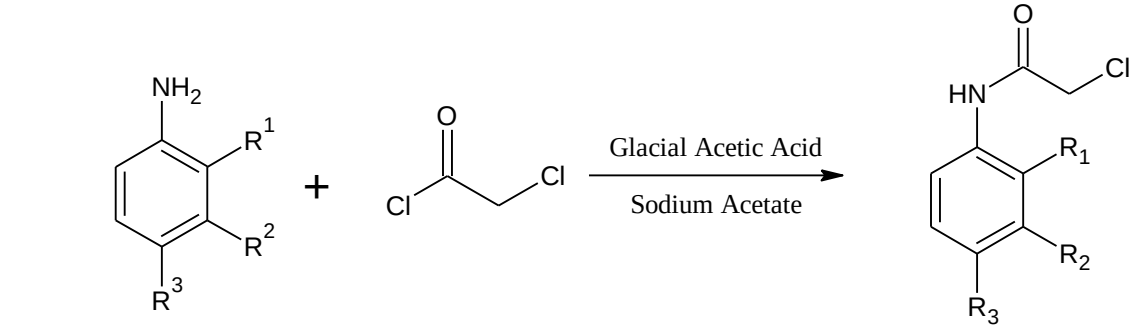
Synthesis of 2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(substituted phenyl)acetamides (2A-2F): Equimolar quantities (0.1mole) of compound 2-(2-aminophenyl) benzoxazole (D1) and substituted chloroacetanilide (A_1-A_6) were mixed in 25 ml of 1, 4-dioxane. To this 0.001 ml of triethylamine (TEA) was added and the reaction mixture was refluxed for 2 hours. The completion of reaction was checked by TLC using ethyl acetate: n-hexane (1:3) solvent system in iodine vapour. It was then cooled and poured into crushed ice. The solid product thus obtained was filtered, washed with 1% potassium bicarbonate followed by distilled water, dried & recrystallized with ethanol [20].

Reaction Scheme: (Given on the following page)

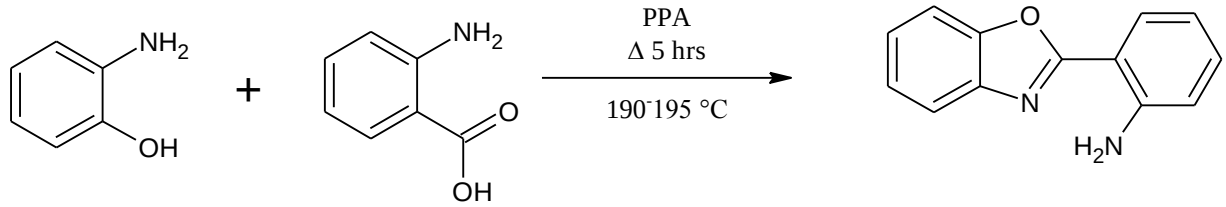
Yield = 69%, mp 115-120˚C. IR (KBr, cm^{-1}) υ; 1420 (Ar.C=C Str.); 3060(Ar.C-H Str.); 753 (Ar.C-H bend $_{out\ plane}$); 1152 (Ar.C-H bend $_{in\ plane}$); 1302 (Ar. C-N Str.); 1589 (Ar. C=N Str.); 1753 (Ar. C-O Str.); 3374 (Ali. 1° N-H Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.2057- 7.7392 (5H, m, Ar.H), 6.5177, 6.6812, 6.7392 (3H, s, Ar.H), 4.4257-4.4976 (2H, d, Ali NH_2). Anal. for $C_{13}H_{10}N_2O$, Calcd (%) C C, 74.27; H, 4.79; N, 13.33; O, 7.61%, Found C, 74.25; H, 4.77; N, 13.31; O, 7.59%.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(4-bromophenyl) acetamide (2A): Compound 2A was found as light brown solid. Yield = 63%, mp 150-160˚C. IR (KBr, cm^{-1}) υ; 1485 (Ar.C=C Str.); 3089 (Ar.C-H Str.); 741 (Ar.C-H bend $_{out\ plane}$); 1152 (Ar.C-H bend $_{in\ plane}$); 1320 (Ar. C-N Str.); 1614 (Ar. C=N Str.); 1740 (Ar. C-O Str.); 1550

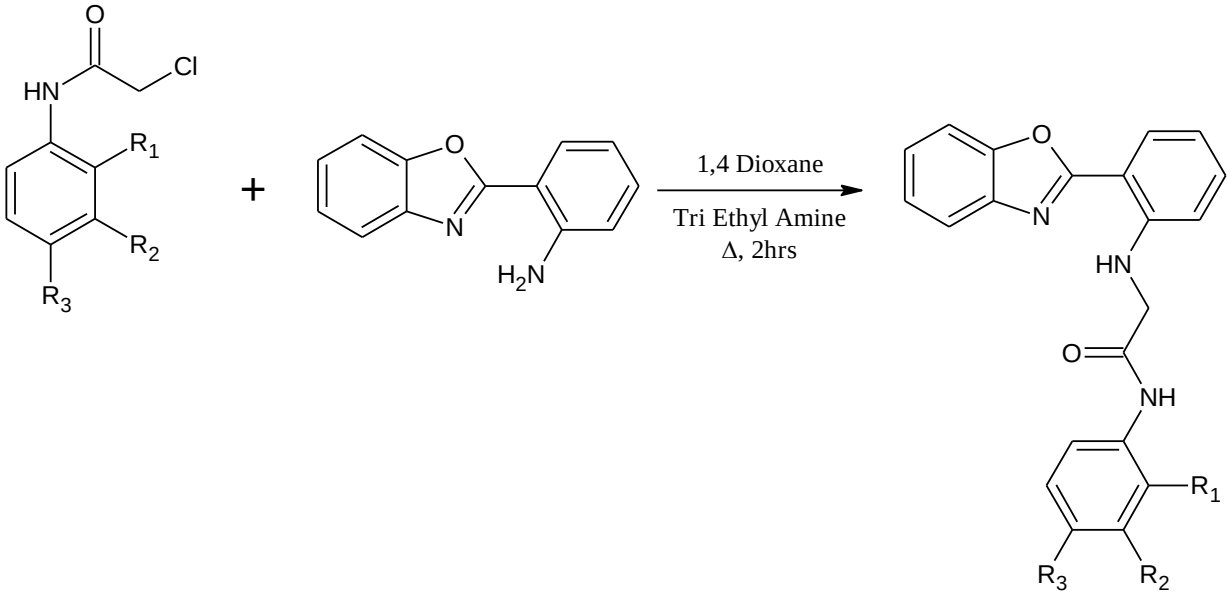
(Ali. C=O Str.); 3370 (Ar. 2° N-H Str.); 2825 (Ali.C-H Str.); 531(Ar. C-Br. Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.29 (s, 5H, ArH), 7.53 (d, 2H, CH), 7.41 (m, 2H, Ar-H), 6.64-6.69 (m, 2H, Ar-H), 4.13 (d, 3H CH_2NH), 8.0 (s, 1H, NH). Anal. for $C_{21}H_{16}BrN_3O$, Calcd (%) C, 59.73; H, 3.82; Br, 18.92; N, 9.95; O, 7.58%, Found C, 59.70; H, 3.79; Br, 18.89; N, 9.92; O, 7.55%



A1-A6



D1



2A-2F

2-(2-aminophenyl) benzoxazole (D1): Compound D1 was found as Reddish brown solid.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(4-chlorophenyl) acetamide (2B): Compound 2B was found as reddish brown solid. Yield = 62%, mp 135-140˚C. IR (KBr, cm^{-1}) υ; 1499 (Ar. C=C Str.); 3078 (Ar. C-H Str.); 821 (Ar.C-H bend $_{out\ plane}$); 1243 (Ar.C-H bend $_{in\ plane}$); 1282 (Ar. C-N Str.); 1590 (Ar. C=N Str.); 1755 (Ar. C-O Str.); 1544 (Ali. C=O Str.); 3299 (Ar. 2° N-H Str.); 2952 (Ali.C-H Str.); 1042 (Ar. C-Cl. Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.26 (s, 5H, C_6H_5), 7.25 (m, 2H, p-Cl Ar-H), 6.64-6.49 (d, 2H, ArH) 4.23

(d, 3H, CH_2NH) 8.23 (s, 1H, NH). Anal. for $C_{21}H_{16}ClN_3O_2$, Calcd (%)C, 66.76; H, 4.27; Cl, 9.38; N, 11.12; O, 8.47%, Found C, 66.72; H, 4.23; Cl, 9.34; N, 11.10; O, 8.44%.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(2,4-dichloro phenyl) acetamide (2C): Compound 2C was found as reddish brown solid. Yield = 65%, mp 82-85˚C. IR (KBr, cm^{-1}) υ; 1508 (Ar.C=C Str.); 3046 (Ar.C-H Str.); 753 (Ar.C-H bend $_{out\ plane}$); 1254 (Ar.C-H bend $_{in\ plane}$); 1272 (Ar. C-N Str.); 1601 (Ar. C=N Str.); 1725 (Ar. C-O Str.); 1664 (Ali. C=O Str.); 3266 (Ar. 2° N-H Str.) 2952 (Ali.C-H Str.); 1089 (Ar. C-Cl. Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.32-7.37 (m, 5H, C_6H_5), 6.69-6.72 (d, 2H, ArH), 7.13 (s, 1H, p-Cl), 7.26 (s, 1H, O-Cl), 4.12 (s, 3H, CH_2NH), 8.32 (s, 1H, NH). Anal. for $C_{21}H_{15}Cl_2N_3O_2$, Calcd (%)C, 61.18; H, 3.67; Cl, 17.20; N, 10.19; O, 7.76%, Found C, 61.14; H, 3.64; Cl, 17.18; N, 10.18; O, 7.75%.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(3-chlorophenyl) acetamide (2D): Compound 2D was found as reddish brown solid. Yield = 64%, mp 80-85˚C. IR (KBr, cm^{-1}) υ; 1507 (Ar.C=C Str.); 3045 (Ar C-H Str.); 753 (Ar.C-H bend $_{out\ plane}$); 1230 (Ar.C-H bend $_{in\ plane}$); 1299 (Ar. C-N Str.); 1582 (Ar. C=N Str.); 1702 (Ar. C-O Str.); 1633 (Ali. C=O Str.); 3350 (Ar. 2° N-H Str.) 2870 (Ali.C-H Str.); 1096 (Ar. C-Cl. Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 8.1104, 7.6054, 7.3046, 6.7778, 6.5540 (5H, s, Ar.H); 7.0475-7.0854 (2H, d, Ar.H); 4.0089-4.2089 (2H, d, Ar.NH); 7.4556-7.2431(6H, m, Ar.H). Anal. for $C_{21}H_{16}ClN_3O_2$, Calcd (%)C, 66.76; H, 4.27; Cl, 9.38; N, 11.12; O, 8.47%, Found C, 66.73; H, 4.25; Cl, 9.35; N, 11.11; O, 8.44%.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(3-nitrophenyl) acetamide (2E): Compound 2E was found as reddish brown solid. Yield = 63%, mp 150-155˚C. IR (KBr, cm^{-1}) υ; 1507 (Ar.C=C Str.); 3045 (Ar C-H Str.); 753 (Ar.C-H bend $_{out\ plane}$); 1230 (Ar.C-H bend $_{in\ plane}$); 1299 (Ar. C-N Str.); 1582 (Ar. C=N Str.); 1702 (Ar. C-O Str.); 1633 (Ali. C=O Str.); 3350 (Ar. 2° N-H Str.) 2870 (Ali.C-H Str.); 1096 (Ar. C-Cl. Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 8.6692, 7.227, 6.6586, 6.5272 (4H, s, Ar.H); 7.824-7.2272 (8H, m, Ar.H); 4.9089-4.9557 (2H, d, Ar.NH). Anal. for $C_{21}H_{16}ClN_3O_2$, Calcd (%)C, 64.94; H, 4.15; N, 14.43; O, 16.48%, Found C, 64.92; H, 4.14; N, 14.40; O, 16.46%.

2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-phenylacetamide (2F): Compound 2F was found as reddish brown solid. Yield = 65%, mp 146-150˚C. IR (KBr, cm^{-1}) υ; 1520 (Ar.C=C Str.); 3051 (Ar. C-H Str.); 805 (Ar.C-H bend $_{out\ plane}$); 1176 (Ar.C-H bend $_{in\ plane}$); 1297 (Ar.C-N Str.); 1593, 1606 (Ar.C=N Str.); 1737 (Ar.C-O Str.); 1770 (Ali.C=O Str.); 3360 (Ar.2° N-H Str.); 2886 (Ali.C-H Str.). ^{1}H NMR (400.13 MHz, DMSO) δ; 7.21-7.64 (9H, ArH), 6.64-6.69 (d, 2H, Ar NH), 4.29-4.32 (d, 3H, CH_2NH). Anal. for $C_{21}H_{16}ClN_3O_2$, Calcd (%) C, 73.45; H, 4.99; N, 12.24; O, 9.32%, Found C, 73.42; H, 4.97; N, 12.23; O, 9.30%.

Biological Activity: Antibacterial activity of the compounds, **2A-2F** was studied against *Staphylococcus aureus* (NCTC 7447), *Bacillus subtilis* (MTCC-619), *Escherchia coli* (NCTC 6571) and *Streptococcus pneumonia* (recultured) by disc-diffusion method and Ampicillin (100µg/ml) was used as the reference antibiotics[22, 23]. Agar media was taken in the pre-sterilized petri-dishes and the microorganisms were grown. Each test compounds were dissolved in dimethyl formamide (DMF) to get a concentration of 10 mg/ml. The disc (6 mm in diameter) was impregnated with 200 µg/ml 100 µg/ml and 50 µg/ml of each test solution, placed on the seeded agar medium and the petri-dishes were incubated at 37˚C for 24 hr. DMF alone was used as control at the equal aforementioned concentration. Zone of inhibition of each compound in mm was recorded and the results were furnished in **Table 1**.

TABLE 1: SUBSTITUTION OF COMPOUNDS

Compound code	R_1	R_2	R_3	Derivatives name
2A	H	H	Br	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(4-bromophenyl) acetamide
2B	H	H	Cl	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(4-chlorophenyl) acetamide
2C	Cl	H	Cl	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(2,4-dichloro phenyl) acetamide
2D	H	Cl	H	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(3-chlorophenyl) acetamide
2E	H	NO_2	H	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-(3-nitrophenyl) acetamide
2F	H	H	H	2-(2-(benzo[d]oxazol-2-yl)phenylamino)-N-phenylacetamide

In all the cases a significant correlation existed between the concentration of the tested compound and the inhibition of microbial intensification. As the concentration of the tested compound amplified, the growth of bacteria diminished. The antifungal activity of the compounds, 2A-2F was also determined against *Aspergillus niger* (NCIM NO. 618*)* and *C.albicans* (NCYC 597) by filter paper disc technique. The antifungal activity was studied by incubating for 48 hr at 22˚C and Fluconazole (100 µg/ml) was used as standard drug [24].

RESULTS AND DISCUSSION: Substituted chloroacetanilides (A_1-A_6) were prepared by suitably substituted aromatic amine and chloroacetyl chloride in glacial acetic acid, as catalyst. 2-(2-aminophenyl) benzoxazole (D1) was synthesized according to illustrated procedure [25] and was used as preparatory material**.** It was reacted with substituted α-chloro acetanilides (A_1-A_6) in the existence of dioxane and Tri ethyl amine (TEA) as catalyst to attain the title compounds *i.e.* 2-(2-(benzo[d]oxazol-2-yl)phenylamino)-n-(substituted phenyl) acetamides (2A-2F). All the synthesized compounds were obtained in adequate yield. The structures of the synthesized compounds were assigned on the bases of their spectral data and elemental analysis.

In the IR spectra of 2-(2-aminophenyl) benzoxazole (D1), occurrence of absorption band at 1302 cm^{-1} and at 3374 cm^{-1} strappingly recommended the existence of C-N and NH group respectively in the molecule, while the bands at 1420-1589 cm^{-1} recommended the company of C=C and C=N ring stretching respectively. Other significant peaks were observed at 1753 cm^{-1} for C-O and 3060 cm^{-1} for C-H stretching bands**.** The emergence of absorption band in the IR spectrum of the compound 2A for 2° N-H and C-Br were observed at 3370 cm^{-1} and 531 cm^{-1} respectively. The appearance of absorption band in IR spectrum of compound 2A appears for (C=O) and (C=N) at 1550 cm^{-1} & 1614 cm^{-1} respectively. Spectra of all the other compounds were found to be in full consignment with the assigned structure.

Antimicrobial activity: All compounds were screened at the concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml. The results of antimicrobial screening are presented in **Tables 2 and 3**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF BENZOXAZOLE COMPOUNDS

Sample	Zone of inhibition (in mm)											
	E. coli			*S. aureus*			*B. subtilis*			*S. pneumoniae*		
	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
2A	12	15	18	12	16	19	15	17	20	10	13	17
2B	10	14	19	14	16	19	15	18	21	12	15	17
2C	12	16	19	11	14	16	10	11	13	11	14	18
2D	07	09	12	10	14	17	11	14	16	09	12	16
2E	13	17	20	15	18	19	12	14	17	13	15	19
2F	12	15	19	10	13	15	15	19	22	10	14	17
Ampicillin (100µg/ml)	22			21			25			23		

TABLE 3: ANTIFUNGAL ACTIVITY OF BENZOXAZOLE COMPOUNDS

Sample	Zone of inhibition (in mm)					
	A. niger			*C.albicans*		
	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
2A	10	12	17	11	15	17
2B	09	15	20	10	14	18
2C	12	15	21	09	13	17
2D	10	14	17	12	15	19
2E	07	10	11	11	14	17
2F	09	12	16	13	17	22
Fluconazole (100 µg/ml)	25			23		

From the data presented, it is clear that 2E is the highly active amongst the synthesized compounds, as it displayed better inhibition against *E. coli, S. aureus and S. pneumonia* compared to others. In the case of *B. subtilis* compound 2B was proved to be very effective while 2C displayed least activity. The compound 2F and 2C exhibited significant activity against *Candida albicans* and *Aspergillus niger* respectively.

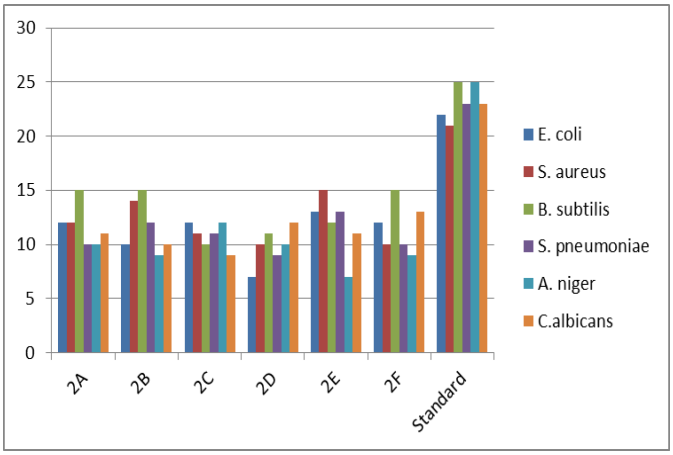


FIGURE 1: ANTIMICROBIAL ACTIVITY OF BENZOXAZOLES AT 50µg/ml

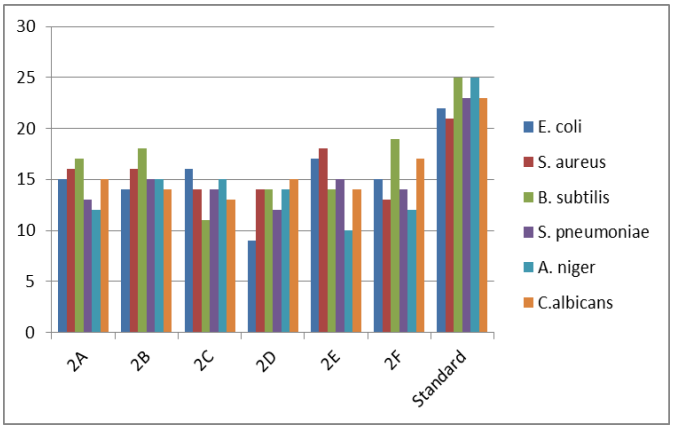


FIGURE 2: ANTIMICROBIAL ACTIVITY OF BENZOXAZOLES AT 100µg/ml

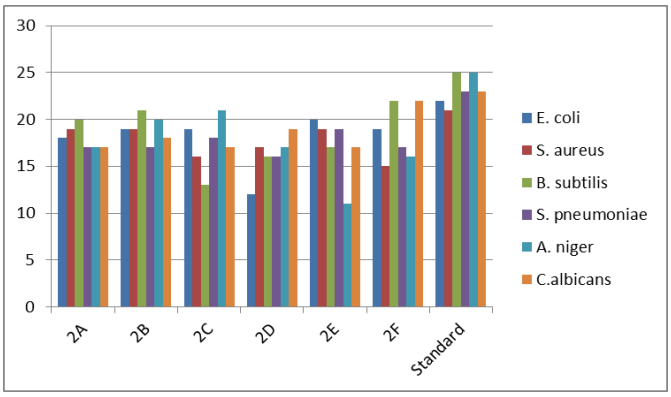


FIGURE 3: ANTIMICROBIAL ACTIVITY OF BENZOXAZOLES AT 200µg/ml

CONCLUSION

The benzoxazole moiety independently has been reported to possess potent antimicrobial activity. In the present work authors have provided a convenient synthetic method for the synthesis of newer benzoxazole compounds by utilizing various substituted anilines imparting condensation of these with 2-(2-aminophenyl) benzoxazole.

The results of antimicrobial screening were encouraging. Further investigations with appropriate structural modifications of title compounds may result in therapeutically useful outcomes for future researchers.

The above results established the fact that benzoxazole derivatives could be a rich source of potential entities in search of new generation of biologically active compounds and be worthwhile to explore the possibility in this area by fusing differently substituted moieties which may result in better pharmacological activities.

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