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Synthesis and antimicrobial activity of some 2-phenyl-benzoxazole derivatives

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ABSTRACT

In the present study, a new series of Schiff's bases derived from (4-Benzoxazol-2-yl-phenyl)-isopropylidine-amine (2a-j) have been synthesized by reacting the amino group of the 4-Benzoxazol-2-yl-phenylamine (1) with different aromatic/ hetero aromatic aldehydes in presence of glacial acetic acid. The starting material 4-Benzoxazol-2-yl-phenylamine was synthesized by condensation of o-aminophenol and p-amino benzoic acid, catalyzed by polyphosphoric acid. The structural assessment of the compounds (2a-j) was made on the basis of spectral data. The synthesized compounds were screened for their in vitro growth inhibiting activity against different strains of bacteria and fungi viz., Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Aspergillus niger, Rhizopus oryzae, Candida albicans and Penicillium chrysogenum were compared with standard agents such as Ciprofloxacin (10µg/ml) and Fluconazole (10µg/ml) using agar diffusion technique. Compounds 2b, 2c, and 2d exhibit highest antibacterial activity and compounds 2b, 2c, 2d and 2h showed good antifungal activity.

Keywords: Benzoxazole, Amines, Antibacterial Activity, Antifungal Activity

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INTRODUCTION

Benzoxazoles possess most remarkable and a wide range of biological activities [1]. The 2-substituted benzoxazoles have been shown to exhibit antimicrobial [2-6], fungicidal [7], analgesic [8-9], insecticidal, antiviral [10-11], anticonvulsant [12] and anticancer [13-15] activities and serve as topoisomerase I poisons. In the last few years, it has been reported that 2, 5-disubstituted benzoxazoles, benzimidazoles, thiocarbazides and thiocarbamides and oxazoles have potent antimicrobial activities against some Gram-positive, Gram-negative bacteria and the yeast *Candida albicans*, providing a wide variety of *in-vitro* antimicrobial effects, especially indicating significant activity against the enterobacter *Pseudomonas aeruginosa*. These examples

highlight the level of interest in new synthetic approaches to benzoxazole derivatives and have prompted researches around the globe to synthesize and explore the wide applicability of this important pharmacophoric scaffold.

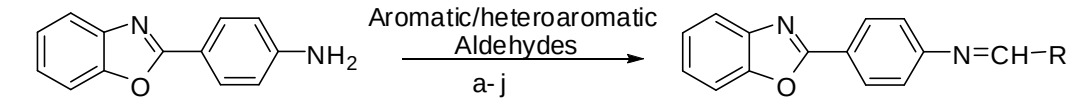
MATERIALS AND METHODS

The identification and purity of the products was checked by TLC with different combination and strength of mobile phases, i.e. hexane: ethyl acetate (2:8) or methanol: chloroform (1:9) using iodine vapours and UV light as detecting agents. Melting points were measured in open capillaries in a liquid paraffin bath and are uncorrected. IR Spectra were recorded on a SHIMADZU FTIR 8400 Spectrophotometer using potassium bromide pellets. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/ Data System using Argon/Xenon (6kV, 10 m A) as the FAB gas. The NMR spectra were recorded on Bruker DRX-300 spectrometer and Elemental analysis was performed on Elemental Vario EL III analyzer. All the chemicals used were of synthetic grade and were procured from S.D. Fine Chem. Ltd and Merck, Mumbai, India.

Synthesis of 4-Benzoxazol-2-yl-phenylamine (1):
Equimolar quantities of *o*-aminophenol (0.002mol, 218 mg) and *p*-amino benzoic acid (0.002 mol, 274 mg) were mixed with polyphosphoric acid (10 ml) in a RBF and a stirrable paste was prepared and refluxed on dimmer-stat. The reaction mixture was heated slowly to 200°C. Heating was continued for 4 hours at 200°C (± 3°C). At the end of the reaction, the resulting solution was cooled to 100°C and then poured on crushed ice with constant stirring. The product was extracted using ethyl acetate and then washed with dilute solution of 10% sodium bicarbonate, then with brine and citric acid solution. Ethyl acetate portion was concentrated and the residue was decolourized and purified by passing through a silica gel column to get the 4-Benzoxazol-2-yl-phenylamine which was recrystallized from ethyl alcohol. The characterization data, yield and melting point of the product was determined and is summarized below.

Yield: 85.42%; Melting range: 160-164°C; IR (KBr, cm^{-1}): 3193, 2921 (Aromatic C-H $_{str}$),1606, 1498, 1454 (Aromatic C=C $_{str}$), 746 (Meta substituted Benzene), 1290 (Asymmetric C-O-C $_{str}$), 1054 (5-Membered C-O $_{str}$), 1311(Tertiary aromatic amine C-N $_{str}$) 3471, 3299 (Primary amine); MS (FAB) m/z: 210 (M^+), 211 (M^++1).

General procedure for the synthesis of (4-Benzoxazol-2-yl-phenyl)-benzylidine-amine (2a-j):
Equimolar quantities of 4-Benzoxazol-2-yl-phenylamine (0.002 mol, 420 mg) and the respective aromatic aldehydes were dissolved in 10 ml of warm ethyl alcohol, containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 2-8 hrs and set aside for 24 hrs at room temperature. The resultant solid was filtered and washed with sodium bisulphite solution to remove excess of aldehyde. The progress of the reaction was monitored by TLC using ethyl acetate: acetone (9:1) as eluent. The reaction mixture was cooled and poured on crushed ice with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% w/v). The resulting solid thus obtained was collected by filtration, washed well with cold water, dried in vacuum and recrystallized from absolute ethanol (95%) and characterized. Adopting the above procedure, ten different (4-Benzoxazol-2-yl-phenyl)-substituted benzylidine-amines (2a-j) were synthesized and their characterization data, yield and melting points were determined and are summarized below.



4-(benzo[*d*]oxazol-2-yl)aniline
(1)

(2a- j)

4-(benzo[d]oxazol-2-yl)-N-benzylideneaniline (2a) :
Equimolar quantities of 4-Benzoxazol-2-yl-phenylamine (0.002 mol, 420 mg) and 2-hydroxy benzaldehyde (244mg =209µL) were dissolved in 10 ml of warm ethyl alcohol, containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 2 hrs and set aside for 24 hrs at room temperature. The resultant solid was filtered and washed with sodium bisulphite solution to remove excess of aldehyde.

Yield: 78.67%; Melting Range: 132-134°C; IR (KBr, cm^{-1}): 3058 (Aromatic C-H $_{str}$), 1575, 1496, (Aromatic C=C $_{str}$), 1610 (HC=N $_{str}$) 1247 (Asymmetric C-O-C $_{str}$), 1056 (5-Membered C-O $_{str}$), 1311(Tertiary aromatic amine C-N $_{st}$); MS (FAB) m/z **:** 298 (M^+); 1H-NMR(δ ppm): 7.7, 7.4 (4H Benzoxazole) , 7.9, 7.6 (4H aromatic), 8.4 1H CH, 7.8, 7.5(5H benzylidine).

2-((4-(benzo[d]oxazol-2-yl)phenylimino)methyl)phenol (2b):
Yield:75.28%; Melting Range: 226-230°C; IR (KBr, cm^{-1}): 3452 (OH $_{str}$), 3093 (Aromatic C-H $_{str}$), 1631 (HC=N $_{str}$), 1587, 1488,1454 Aromatic C=C $_{str}$), 1410 (Asymmetric C-O-C $_{str}$), 1051 (5-Membered C-O $_{str}$), 1310 (Tertiary aromatic amine C-N $_{st}$) ; MS (FAB) m/z **:** 315 (M^++1); 1H-NMR (δ ppm): 7.6, 7.3 (4H Benzoxazole) , 7.9, 7.6 (4H aromatic), 8.8 (1H CH), 7.1, 7.5, 7.6(4H benzylidine), 11.3 (aromatic OH).

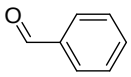
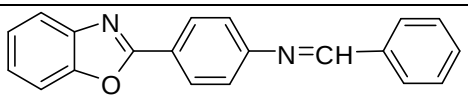
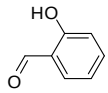
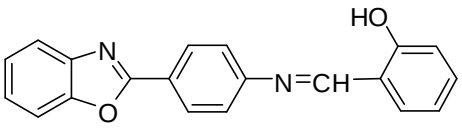
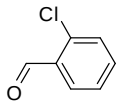
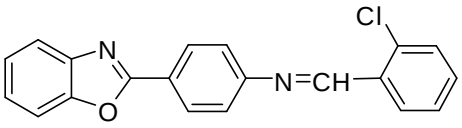
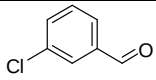
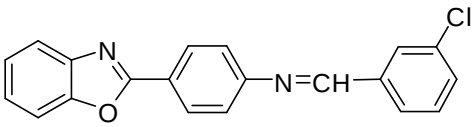
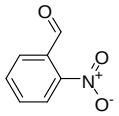
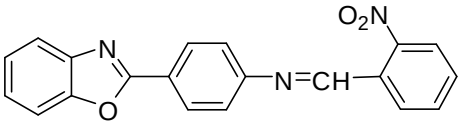
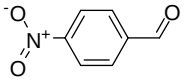
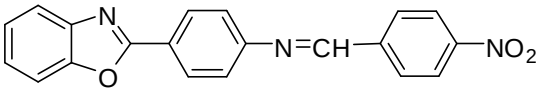
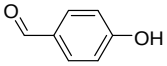
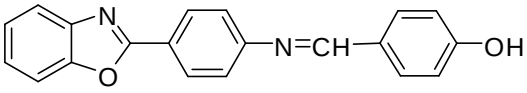
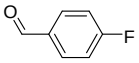
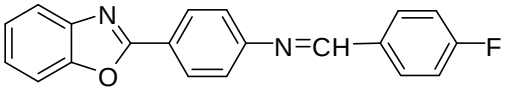
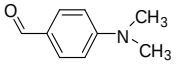
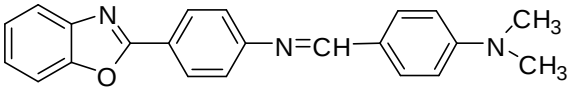
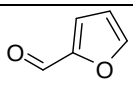
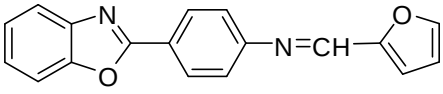
4-(benzo[d]oxazol-2-yl)-N-(2-chlorobenzylidene)aniline (2c) :
Yield: 72.25%; Melting Range: 150-152°C; IR (KBr, cm^{-1}): 2921 (Aromatic C-H $_{str}$), 1614 (HC=N $_{str}$), 1558, 1488,1452 (Aromatic C=C$_{str}$), 1051 (5-Membered C-O $_{str}$), 1325 (Tertiary aromatic amine C-N $_{st}$), 804 (C-Cl $_{str}$) ; MS (FAB) m/z **:** 333 (M^++1); 1H-NMR (δ ppm): 7.7, 7.4 (4H Benzoxazole) , 7.9, 7.6 (4H aromatic), 8.4 (1H CH), 7.4, 7.5, 7.7(4H benzylidine).

4-(benzo[d]oxazol-2-yl)-N-(3-chlorobenzylidene)aniline (2d) :
Yield: 81.05%; Melting Range: 148-150°C; IR (KBr, cm^{-1}): 2927 (Aromatic C-H $_{str}$), 1610(HC=N $_{str}$), 1580, 1490,1452 (Aromatic C=C $_{str}$), 1054 (5-Membered C-O $_{str}$), 1320 (Tertiary aromatic amine C-N $_{st}$), 840 (C-Cl $_{str}$) ; MS (FAB) m/z **:** 333 (M^++1); 1H-NMR (δ ppm): 7.7, 7.4 (4H Benzoxazole) , 7.9, 7.6 (4H aromatic), 8.4 (1H CH), 7.4, 7.5, 7.7, 7.9 (4H benzylidine).

4-(benzo[d]oxazol-2-yl)-N-(2-nitrobenzylidene)aniline (2e) :
Yield: 74.03%; Melting Range: 170-172°C; IR (KBr, cm^{-1}): 3072 (Aromatic C-H$_{str}$), 1610 (HC=N$_{str}$), 1610, 1588,1529 (Aromatic C=C$_{str}$), 1452 (Ar-NO_2) 752 (Meta substituted Benzene), 1440 (Asymmetric C-O-C$_{str}$), 1053 (5-Membered C-O$_{str}$), 1309 (Tertiary aromatic amine C-N$_{str}$); MS (FAB) m/z **:** 343 (M^+), 344(M^++1); 1H-NMR (δ ppm): 7.4, 7.7 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 7.6, 7.9, 8.0, 8.1(4H benzylidine).

4-(benzo[d]oxazol-2-yl)-N-(4-nitrobenzylidene)aniline (2f) :
Yield: 69.35%; Melting Range: 172-176°C; IR (KBr, cm^{-1})**::** 3191 (Aromatic C-H$_{str}$), 1606 (HC=N$_{str}$), 1521,1496 (Aromatic C=C$_{str}$), 1454 (Ar-NO_2) 746 (Meta substituted Benzene), 1245 (Asymmetric C-O-C$_{str}$), 1054 (5-Membered C-O $_{str}$), 1344 (Tertiary aromatic amine C-N$_{str}$); MS (FAB) m/z **:** 344 (M^{+1}); 1H-NMR (δ ppm): 7.5, 7.8 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 8.1, 8.3(4H benzylidine).

Table 1: Physical and analytical data of compounds (2a-j)

Cpd	Aldehydes	Structure
2a	 Benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-benzylideneaniline
2b	 2-Hydroxy-benzaldehyde	 2-((4-(benzo[*d*]oxazol-2-yl)phenylimino)methyl)phenol
2c	 2-Chloro-benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(2-chlorobenzylidene)aniline
2d	 3-Chloro-benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(3-chlorobenzylidene)aniline
2e	 2-Nitro-benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(2-nitrobenzylidene)aniline
2f	 4-Nitro-benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(4-nitrobenzylidene)aniline
2g	 4-Hydroxy-benzaldehyde	 4-((4-(benzo[*d*]oxazol-2-yl)phenylimino)methyl)phenol
2h	 4-Fluoro-benzaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(4-fluorobenzylidene)aniline
2i	 4-Dimethylamino-benzaldehyde	 4-((4-(benzo[*d*]oxazol-2-yl)phenylimino)methyl)-*N*,*N*-dimethylaniline
2j	 Furan-2-carbaldehyde	 4-(benzo[*d*]oxazol-2-yl)-*N*-(furan-2-ylmethylene)aniline

4-((4-(benzo[d]oxazol-2-yl)phenylimino)methyl)phenol (2g):

Yield: 70.25%; Melting Range: 210-214°C; IR (KBr, cm^{-1}): 3400 (OH $_{str}$), 3224 (Aromatic C-H $_{str}$), 1610 (HC=N $_{str}$), 1502, 1456 (Aromatic C=C $_{str}$), 1244 (Asymmetric C-O-C$_{str}$), 1047 (5-Membered C-O$_{str}$), 1244 (Tertiary aromatic amine C-N$_{str}$); MS (FAB) m/z **:** 314(M^+); 1H-NMR (δ ppm): 7.4, 7.7 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 6.8, 7.8 (4H

benzylidine), 9.4 (aromatic OH), Anal. Calcd for C_{20} $H_{14}N_2O_2$: C, 76.42; H, 4.49; N, 8.91, Found: C, 76.34; H, 4.45; N, 8.88.

4-(benzo[d]oxazol-2-yl)-N-(4-fluorobenzylidene)aniline (2h) :
Yield: 66.35%; Melting Range: 150-156°C; IR (KBr, cm^{-1}): 3056 (Aromatic C-H *str*), 2889 (Aliphatic methyl C-H *str*), 1631 (HC=N *str*), 1587, 1506, 1452 (Aromatic C=C *str*), 750 (Meta substituted Benzene), 1413 (AsymmetricC-O-C *str*), 1056 (5-Membered C-O *str*), 1325 (Tertiary aromatic amine C-N *st*); MS (FAB) m/z **:** 317 (M^++1); 1H-NMR (δ ppm): 7.4, 7.7 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 7.4, 7.8 (4H benzylidine), Anal. Calcd for C_{20} H_{13} FN_2O: C, 75.94; H, 4.14; N, 8.86, Found: C, 75.86; H, 4.12; N, 8.80.

4-((4-(benzo[d]oxazol-2-yl)phenylimino)methyl)-N,N-dimethylaniline (2i) :
Yield: 62.67 %; Melting Range: 72-75°C; IR (KBr, cm^{-1}): 2920 (Aromatic C-H *str*), 1581(HC=N *str*), 1658, 1552, 1413 (Aromatic C=C *str*), 744 (Meta substituted Benzene), 1242 (Asymmetric C-O-C *str*), 1052 (5-Membered C-O *str*), 1340 (Tertiary aromatic amine C-N *st*); MS (FAB) m/z **:** 341 (M^+); 1H-NMR (δ ppm): 7.4, 7.7 (4H Benzoxazole), 7.6, 7.9 (4H aromatic), 8.4 (1H CH), 6.8, 7.5, (4H benzylidine), 3.1 (CH_3), Anal. Calcd for C_{22} $H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31, Found: C, 77.36; H, 5.54; N, 12.28.

4-(benzo[d]oxazol-2-yl)-N-(furan-2-ylmethylene)aniline (2j):
Yield: 58.05%; Melting Range: 160-164°C; IR (KBr, cm^{-1}): 2962 (Aromatic C-H *str*), 1616 (HC=N*str*), 1683, 1558, 1506 (Aromatic C=C*str*), 746 (Meta substituted Benzene), 1261 (Asymmetric C-O-C *str*), 1097 (5-Membered C-O *str*), 1317 (Tertiary aromatic amine C-N *st*) ; MS (FAB) m/z **:** 289 (M^++1); 1H-NMR (δ ppm): 7.4, 7.7 (4H Benzoxazole) , 7.6, 7.9 (4H aromatic), 7.5 (1H CH), 6.5, 6.9, 7.7 (3H 2-furan), Anal. Calcd for C_{18} $H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72, Found: C, 74.86; H, 4.16; N, 9.68.

Screening for antimicrobial activity:
The antimicrobial activity of all the newly synthesized compounds was determined by agar well plate method [16]. Nutrient agar (Hi-Media) was used for antibacterial activity and Sabouraud dextrose agar (SDA) (Hi-Media) was used for antifungal activity. The bacterial strains used were *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* MTCC (1573), *Staphylococcus aureus* (MTCC1430), *Streptococcus pneumoniae* (MTCC 655) and *Klebsiella pneumoniae* (MTCC 618), and fungal strains used were *Aspergillus niger* (MTCC 2546), *Rhizopus oryzae* (MTCC 2775), *Candida albicans* (MTCC 183) *and Penicillium chrysogenum* (MTCC 161). All strains were procured as pure cultures from Institute of Microbial Technology, Chandigarh. The compounds were tested at a concentration of 100 µg/ml and the solutions were prepared in dimethylformamide (DMF). A solution of DMF (10%) was used as a control. The petri dishes used for antibacterial screening were incubated at 37±1°C for 24 hrs, while those used for antifungal activity were incubated at 28°C for 48-72 hrs. The diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared to Ciprofloxacin (10µg/ml) and Fluconazole (10µg/ml**)** for antibacterial and antifungal activity respectively. The antimicrobial screening results are presented in Table 2.

RESULTS AND DISCUSSION

The compounds (2a-j) were synthesized by the condensation of 4-Benzoxazol-2-yl-phenylamine and different aromatic aldehydes. The physical and analytical data of the compounds (2a-j) were collected and are presented in Table 1. The yields of 2a-j fall in the range of 66-88%. The spectral (IR, MS and NMR) data are in good agreement with their structures [17-18].

A close look at results of antimicrobial activity (Table 2) reveal that the known standard antibiotics Ciprofloxacin (10µg/ml) and Fluconazole (10µg/ml) show zone of inhibition of 20-23 mm and 18-20 mm against bacterial and fungal strains. Compounds 2b, 2c and 2d displayed activity against *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*. Compounds 2b, 2c, 2d and 2h exhibited good antifungal activity.

Table 2: Results of antimicrobial study of synthesized compounds

Comp.	**Antibacterial activity**						**Antifungal activity**			
	Zone of inhibition (mm)									
	BS	*SA*	*SP*	*EC*	*PA*	*KP*	*CA*	*AN*	*PC*	*RO*
2a	NA									
2b	16	13	13	15	12	14	16	15	14	NA
2c	15	NA		16	NA	13	12	14	12	NA
2d	13	NA		14	NA	13	15	16	14	NA
2e	NA									
2f	NA									
2g	NA									
2h	NA						14	15	12	NA
2i	NA									
2j	NA									
STD	22	21	20	23	22	20	20	20	19	18

Comp.: Compound; BS: Bacillus subtilis; SA: Staphylococcus aureus; SP: Streptococcus pneumoniae; EC: Escherichia coli; PA: Pseudomonas aeruginosa; KP: Klebsiella pneumoniae; CA: Candida albicans, AN: Aspergillus niger; PC: Penicillium chrysogenum; RO: Rhizopus oryzae. STD: Standard, ***NA: Not Active.***

CONCLUSION

It can be concluded that 2-phenyl benzoxazole derivatives exhibit potent activity against both, bacteria and fungi. The presence of halogen atom increases the activity. The most potent compound of the series was 2-((4-(benzo[d]oxazol-2-yl)phenylimino)methyl)phenol (2b). This could be due to the better penetration of the microbial cell-wall or selective uptake of the compound by the micro-organisms.

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