SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF 5-AMINO-2-(p-SUBSTITUTED-PHENYL) BENZOXAZOLE DERIVATIVES

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SUMMARY: *5 Amino 2 (p substituted phenyl)benzoxazole derivatives were synthesized by heating 2,4- diaminophenol with the appropriate carboxylic acids in the presence of polyphosphoric acid. Their structures were elucidated using UV, IR, NMR and Elemental Analysis methods.*

The activities of these compounds against some gram (+) bacteria such as K. pneumoniae, Ps. aeruginosa and some gram (−) bacteria such as Staph. aureus, S. faecalis, E. coli and the fungus C. albicans were determined (MIC: 6.2—25 μg/ml).

5-AMİNO-2-(p-SUBSTITUTE-FENİL)BENZOKSAZOL TÜREVLERİNİN SENTEZ VE ANTİMİKROBİYAL ETKİLERİ

ÖZET: *5 Amino 2 (p sübstitüe fenil)benzoksazol türevleri 2,4 diaminofenol ile uygun karboksilik asitlerin polifosforik asit varlığında ısıtılması ile sentezlenmişlerdir. Bileşiklerin yapıları UV, IR, NMR ve Elementel Analiz yöntemleri kullanılarak açıklanmıştır.*

Sentezlenen bileşiklerin K. pneumoniae, Ps. aeruginosa gibi bazı gram (+) ve Staph. aureus, S. faecalis, E. coli gibi gram (−) bakteriler ve fungus olarak da C. albicansa karşı etkileri saptanmıştır (MIK: 6,2—25 μg/ml).

INTRODUCTION

Since benzoxazole derivatives are the isosters of naturally occuring nucleotids (1), they easily intereact with the biopolymers of the living system. As a consequence of this feature, benzoxazole derivatives which have multiple biological activity have been known for a long time.

2-Substituted benzoxazoles were prominently studied (2-10) trusting that this position is decisive for the biological activity whereas position 5 (4, 6, 10, 11) prevailing the intensity of the activity. It was reported that para substituted 2-aryl-5-benzoxazolealkanoic acid derivatives had the highest activity compared to analogs (4, 6). Benoxa-

profen (3, 6) and Zoxazolamin (6) are also the kind of benzoxazole derivatives which are substituted at both 2 and 5 positions.

As a result, 5-amino-2-(p-substituted-phenyl)benzoxazoles have been selected as the target compounds in this research. We decided to synthesize these compounds by the same method which we prepared some 5-substituted-2-(p-substituted-phenyl) benzoxazole derivatives earlier using polyphosphoric acid (12-14).

It was stated by Davis et all. that five-membered heterocyles carrying two benzene rings were chemotherapeutically active (15). From this phenomenon, we decided to study antimicrobial activity of 2-phenylbenzoxazoles having 2 benzene rings. We previously determined the microbiological activities of some 2-(p-substituted-phenyl) benzoxazole derivatives (12-14). In this research, we decided to study the activity of 5-amino-2(p-substituted-phenyl) benzoxazoles against some gram (+), gram (-) bacteria and Candida albicans.

EXPERIMENTAL

Material

Kieselgel HF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent system was chloroform only. Melting points were determined on a Mettler FP-5, FP-51 and Buchi SMP-20 capillary melting point apparates with dried samples. IR spectra were recorded with Pye Unicam SP-1025 with KBr discs. NMR spectra were obtained with a Perkin Elmer R-32 Spectrometer in trifluoroacetic acid. Elemental analysis were carried out with a Perkin-Elmer model 240-C. UV maxima were measured on a Pye Unicam SP-1700 in methanol at 10^{-3} M concentration. The starting compounds and the solvents were commercially available.

Method

Synthezis of the Compounds

The compounds were synthesized by heating 0.01 mol 2,4-diaminophenol.2 HCl with 0.01 mol appropriate carboxylic acids in 24 g polyphosphoric acid with stirring for 2.5 hours. The compounds and the data for the preparation were listed in Table 1. At the end of the reaction period, the residue was poured into ice-water and neutralized with excess of 10 % NaOH solution, extracted with benzene, the benzene solution was dried over anhydrous sodium sulphate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and after filtered left to crystallize. The spectral and elemental analysis of the compounds were given in Tables 2 and 3.

Microbial Activity

The microorganisms used for antimicrobial activity are as follows:

1. Staphylococcus aureus ATCC 6538
2. Streptococcus faecalis ATCC 10541
3. Escherichia coli ATCC 10536
4. Klebsiella pneumoniae NTCC 52211
5. Pseudomonas aeruginosa RSKK 628
6. Candida albicans RSKK 628

The microbiological activity were tested as described earlier (12-14).

Antimicrobial Activity

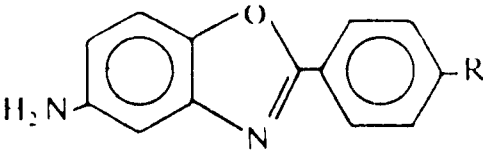
All the bacteria were prepared in Nutrient broth (Beef extract: 3 g Peptone: 5 g Sodium Chloride, 5 g Agar, 5 g Distilled water: 1000 ml). Testing was done in Mueller Hinton broth (Meet infusion: 6 g Casein hydrolysate, 17.5 g Starch, 1.5 g Distilled water, 1000 ml). The gradual double dilution technique was applied. After inoculation with 0.2 ml of culture from the nutrient broth, the seeded broths were incubated at 37 °C for 24 hours. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 hours,

the last tube with no growth of the microorganism was taken to represent the minimum inhibitory concentration (MIC, expressed in μg/ml). A compound inhibiting the growth of the microbe at 25 μg/ml concentration was considered to be active.

Antifungal Activity

The activity of the compounds against C. albicans were tested in Sabouraud's broth (Neopeptone: 10 g, Dextrose: 40 g, Distilled water: 1000 ml). 0.2 ml of fungal culture were inoculated into broth and the respective inoculated broths were used for testing after incubation for 5 days at 25°C. The compounds were tested by the gradual double dilution technique as described in the case of antibacterial testing.

Table 1. 5 Amino 2 Phenylbenzoxazoles and the Data on Their Preparations



Comp. No.	R	Acid Used as Starting Compound	Reaction Temperature, C	Mp, °C	Yield %
1	H	Benzoic acid	130	153.1	62.58
2	C_2H_5	p—Ethylbenzoic acid	140—145	126.1	57.82
3	Br	p- Bromobenzoic acid	210	201.2	61.50
4	F	p Fluorobenzoic acid	150	156.8	57.26
5	$N(CH_3)_2$	p Dimethylaminobenzoic acid	200	205	64.47
6	NO_2	p Nitrobenzoic acid	180	217	15.45

Table 2. UV, NMR and Elemental Analysis of 5—Amino 2 Phenylbenzoxazoles

Comp. No.	UV λ max (nm)	Log ε	NMR (δ ppm)	Elemental Analysis Calculated (%)	Observed (%)
1	214*	3.1953	7.75 — 8.85 (8H, m)	C = 74.27	74.30
	233	3.2240		H = 4.79	4.68
	280	3.2175		N = 13.32	13.51
	330*	3.060			
2	216	3.2118	1.2 — 1.8 (3H, t)	C = 76.77	77.01
	235*	3.2011	2.8 — 3.2 (2H, q)	H = 5.64	5.78
	277	3.2557	7.5 — 8.7 (7H, m)	N = 11.19	10.92
	329	3.0835			
3	216	3.2266	7.48 — 8.43 (7H, m)	C = 59.10	58.89
	239*	3.2076		H = 2.79	2.77
	284	3.2685		N = 8.62	8.55
	336*	3.0658			
4	219*	3.2282	7.38 — 8.80 (7H, m)	C = 72.72	72.77
	234	3.2679		H = 3.43	3.49
	276	3.2725		N = 10.60	10.82
	329*	3.1079			
5	223	3.2271	3.58 (6H, s)	C = 72.43	72.19
	330	3.2853	7.70 — 8.90 (7H, m)	H = 5.70	5.68
	368*	3.2776		N = 15.83	15.91
6	228	3.2574	7.80 — 8.30 (7H, m)	C = 65.98	66.05
	329	3.2874		H = 3.12	3.12
	345*	3.2643		N = 14.43	14.52

* Shoulder

The antimicrobial activity of Amoxicillin, Ampicillin, Erythromycin and Chloramphenicol agaist same microorganisms were tested using the same methods and MIC values were given. The activity of the compounds was tested by dissolving the compounds in absolute ethanol (12–16). For that reason, the activity of ethanol against same microorganisms were tested in the same dilutions and found inactive. The MIC values of the compounds were given in Table 4.

RESULTS AND DISCUSSION

The well-known Phillips method (17) which was used for the synthesis of benzoxazoles, benzothiazoles and especially benzimidazoles has not been successful for the preparation of 2-substituted benzoxazole derivatives (12, 18), because these compounds were unstable to hot aqueous acids and the oxazole ring was easily hydrolysed under this condition (19). It was found that high temperature and excessive time also caused ring opening (20). The ring closure reactions carried out in pyridine or xylene had low yields (12, 18). Therefore polyphosphoric acid was prefered as the reagent (12–14, 21, 22). Totally 6 compounds, 5 of them being for the first time were synthesized. The structures of the compounds were elucidated using UV, IR, NMR and Elemental Analysis Methods. IR spectra were similar for all the compounds. NMR spectra were taken using trifluoroacetic acid as the solvent, because of that we couldn't sweep above 10 ppm and we couldn't obtain the resonance of the N–H protons of the compounds.

Table 3. IR Spectral Data of 5—Amino—2—(p—Substituted—Phenyl)Benzoxazole Derivatives

Absorption Region (cm^{-1})	Bond
3490 – 3250	N—H
3120 – 3080	=C—H
1630 – 1565	C=N and C=C
1270 – 1060	C—O—C
860 – 700	C—H
1510	NO_2 (Comp. no: 6)
1350	NO_2 (Comp. no: 6)

The antimicrobial activity of the compounds were tested by dissolving the compounds in absolute ethanol. The activity of ethanol against the microorganisms were tested in the same dilutions. It was found that ethyl alcohol did not exhibit any activity in that dilutions.

All the compounds were found active against the gram (-), the gram (+) bacteria and the fungus which were tested. The compounds exhibit the same activity against Stap. aureus with erythromycin. Their activity against E. coli are the same as chloramphenicol and more than erythro-

Table 4. Antimicrobial Activity of 5—Amino—2—(p—Substituted—Phenyl)benzoxazole Derivatives (MIC in μg/ml)

Comp. No.	Staph. aureus	S. faecalis	E. coli	K. pneumoniae	Ps. aeruginosa	C. albicans
1	25	25	25	6.2	12.5	12.5
2	25	25	25	6.2	12.5	25
3	25	25	25	6.2	12.5	25
4	25	25	25	6.2	12.5	25
5	25	25	25	6.2	12.5	25
6	25	25	25	12.5	12.5	12.5
7*	0.3	0.3	1.5	12.5	500	—
8**	0.3	0.3	1.5	12.5	1000	—
9***	25	1.5	50	50	25	—
10****	12.5	6.2	25	12.5	25	—

* Ampicillin ** Amoxicillin *** Erythromycin **** Chloramphenicol

mycin. The compounds are more potent than all of the antibiotics tested against K. pneumoniae. For Ps. aeruginosa these derivatives also show higher antimicrobial activity than all of the antibiotics used.

The antimicrobial activity and structure relationships of these benzoxazole derivatives in K. pneumoniae indicate us that if the compound carries NO_2 group at the para position on the phenyl ring, the activity decreases as compared to other substituents. On the contrary, in C. albicans the substition of the para position by the NO_2 group increases the activity. These differences can be explained by the physicochemical properties of the NO_2 group. The inductive or/and the resonance effects of the substituents may be important for the activity.

The quantitative structure – activity relationships covering the physicochemical properties will be studied later.

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