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Short communication

Synthesis and microbiological activity of 5-methyl-2-[*p*-substituted phenyl]benzoxazoles

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Summary — The synthesis and microbiological activity of a series of 5-methyl-2-(*p*-substituted phenyl)benzoxazoles **7a–7g** are described. The *in vitro* microbiological activity of these compounds was determined against some Gram-positive and Gram-negative bacteria and *C albicans*. The observed minimum inhibitory concentration (MIC) values of the derivatives were compared to some other 2,5-disubstituted benzoxazoles and clinically used drugs. The *in vitro* microbiological data showed that the derivatives **7a–7g** exhibited a broad spectrum of antibacterial activity and **7a–c** and **7g** were significantly active against *E coli* and/or *K pneumoniae* at an MIC of 6.2 μg/ml.

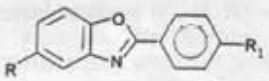
Résumé — Synthèse et activité microbiologique de 5-méthyl-2-phényl benzoxazoles substitués en para. *On a défini la synthèse et l'activité microbiologique d'une série de 5-méthyl-2-phényl benzoxazoles substitués en para 7a–7g. On a étudié l'activité microbiologique de ces composés sur les bactéries Gram-positif et négatif et* C albicans. *On a comparé la concentration minimale inhibitrice de ces dérivés avec celle de certains benzoxazoles 2,5-disubstitués déjà utilisés en clinique. Les données microbiologiques* in vitro *ont montré que ces dérivés ont une action antibactérienne de très large spectre. La concentration minimale inhibitrice des dérivés 7a–c et 7g est de 6,2* μg/ml *sur* E coli *et/ou* K pneumoniae.

2,5-disubstituted benzoxazoles / anti-microbiological activity

Introduction

Benzoxazole derivatives are the structural isosters of naturally occurring nucleotides such as adenine and guanine, which allows them to interact easily with the biopolymers of living systems and different kind of biological activity have been obtained [1–5]. Besides, due to their kind of action, it has been reported that they have shown low toxicity in warm-blooded animals [4, 6]. Considering these features and the small amount of published data on the anti-bacterial and anti-fungal activities of these compounds, we were encouraged to undertake research on the microbiological activity of 2,5-disubstituted benzoxazoles.

5-Substituted-2-(*p*-substitutedphenyl)benzoxazole derivatives **1–4**, bearing nitro, chloro and amino groups which possess electronegative properties on position R have been studied in our previous papers [7–10].



1 R = H R_1 = H, OCH_3, $C(CH_3)_3$, Cl, Br, NH_2, $NHCH_3$
2 R = Cl R_1 = CH_3, C_2H_5, $C(CH_3)_3$, $NHCOCH_3$, $NHCH_3$, Cl, NO_2
3 R = NO_2 R_1 = H, CH_3, $C(CH_3)_3$, NH_2, Cl, Br
4 R = NH_2 R_1 = H, C_2H_5, Br, F, $N(CH_3)_2$, NO_2

In this present study, in order to improve the microbiological activity, 5-methyl-2-(*p*-substituted phenyl)-benzoxazole derivatives have been selected as the target compounds because of the electropositive property of the methyl group.

Chemistry

For the synthesis of the benzoxazole derivatives using aqueous mineral acids as the condensation reagent did not provide successful results, because of the oxazole

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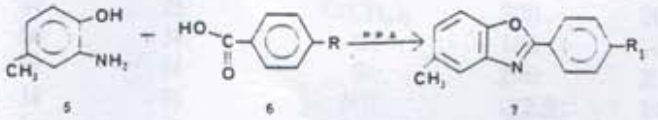
ring which was easily hydrolysed under these conditions. Reactions which occurred in pyridine or xylene also had low yields. Finally, polyphosphoric acid (PPA) was chosen as the reagent for cyclodehydration in the synthesis of the compounds as it has a good solvent power and contains anhydride groups which combine with the water formed in the reaction centre in order to prevent effective acidity. The stability of the oxazole ring increases under these conditions and high temperature could be used.

The compounds **7** were prepared by heating 2-hydroxy-5-methylanilin **5** with the appropriate carboxylic acid **6** in PPA.



The compounds **7** were synthesized as new products, except **7a** [11] and **7c** [12]. The structures of the derivatives **7a–7g** were supported by elemental analysis and spectral data. The UV, IR and ^{1}H BNMR spectra are in agreement with the proposed structures.

Results and Discussion

The compounds **7a–7g** were tested for their *in vitro* growth inhibitory activity against different bacteria and the fungus *Candida albicans*. The bacteria used were *Staphylococcus aureus* and *Streptococcus faecalis* as Gram-positive and *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as Gram-negative. The minimum inhibitory concentrations (MIC) were determined using the method of 2-fold double dilution technique [13, 14]. Ampicillin **8**, amoxicillin **9**, erythromycin **10**, chloramphenicol **11**, haloprogin **12** and clotrimazole **13** were used as standard drugs in antibacterial and antifungal tests. The data on the antimicrobial activity of the compounds **7a–7g** are given in table I with the comparison of the previously studied 2,5-disubstituted benzoxazoles.

When the activity of the compounds with the same substituents at the *para* position of the phenyl ring, but holding different substituents at position R is compared, it is found that substitution with NO_2 and Cl at position R causes a decrease whereas substitution with CH_3 group at the same position produces an increase in the activity against *S faecalis* **2a**, **2e**, **3b**, **7a**, **7e**. This situation may be explained by the inductive effect of NO_2 and Cl. In *E coli* the electronegative groups Cl, NO_2 and NH_2 at position R also reduce the activity when compared to the CH_3 substituted ones **2a**, **2b**, **2d**, **2e**, **3b**, **4b**, **4d**, **4e**, **7a**, **7b**, **7d**, **7g**. That can be considered as an effect of the electropositive property of the methyl group. The derivatives **7a**, **7b**, **7e** and **7g** are also found to be more potent than **2a**, **2b**, **2d** and **2e** against *P aeruginosa*.

The compounds **7a–7g** indicate significant antimicrobial activity (MIC 6.2–25 μg/ml). As shown in table I, the compounds **7a–7g** exhibit better MIC values against *P aeruginosa* and **7a**, **7b**, **7c** and **7e** are also found more active against *K pneumoniae* than the standard drugs **8–11** for their *in vitro* anti-bacterial tests. The antibacterial activity results show that the 5-methyl-2-(*p*-substituted phenyl)benzoxazoles, in general, possess better activity against Gram-negative than Gram-positive bacteria.

Experimental protocols

Chemistry

Kieselgel HF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent system was only chloroform. Melting points were determined on a Mettler FP-51 apparatus and were uncorrected. IR spectra were recorded with Pye Unicam SP-1025 with KBr discs. ^{1}H NMR spectra were obtained with a Perkin–Elmer R-32 spectrometer in trifluoroacetic acid and tetramethylsilane as internal standard. UV maxima were measured on a Pye Unicam SP-1700 spectrophotometer in methanol at 10^{-3} M concentration. Elemental analysis were carried out with a Perkin–Elmer model 240-C apparatus. The results of elemental analysis (C, H, N) were within ± 0.4% of the calculated amounts. The starting compounds and the solvents were commecially available products.

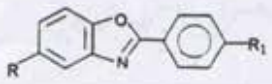
5-Methyl-2-(p-substituted phenyl)benzoxazoles 7a–7g

General procedure. A mixture of 2-hydroxy-5-methylanilin **5** (0.01 mol) and appropriate carboxylic acids **6** (0.02 mol) was heated in PPA (12 g) with stirring for 2.5 h. At the end of the reaction period, the residue was poured into ice-water and neutralized with excess of 10% NaOH solution. After extracted with benzene, the benzene solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. The filtrate was left to crystallize by addition of water. Chemical and physical data of the compounds **7a–7g** are reported in table II.

Microbiology

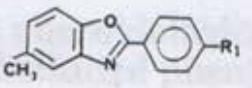
For both antibacterial and antifungal assays, the compounds were dissolved in absolute ethanol (0.8 mg/ml) [14]; further control dilutions for the compounds and standard drugs in the test medium were furnished at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 μg/ml concentrations by the 2-fold serial dilution method. The origins of bacterial and fungal strains which were used for antimicrobial tests are *S aureus* ATCC 6538, IS faecalis ATCC 10541, IE coli PATCC 10536, *K Pneumoniae* NTCC 52211, *P aeruginosa* RSKK 355 and *C albicans* RSKK 628.

Table I. The antimicrobial activity of 2,5-disubstituted benzoxazole derivatives against some Gram-positive and Gram-negative bacteria and *Candida albicans* (MIC in μg/ml).



Com	*R*	*R1*	S aureus	S faecalis	E coli	K pneumoniae	P aeruginosa	C albicans
1a	H	H	12.5	25	25	12.5	12.5	25
1b	H	OCH_3	12.5	100	50	100	12.5	100
1c	H	$C(CH_3)_3$	200	200	200	12.5	200	25
1d	H	Cl	100	100	100	100	100	200
1e	H	Br	200	200	200	100	200	200
1f	H	NH_2	12.5	100	25	12.5	12.5	25
1g	H	$NHCH_3$	50	50	50	12.5	25	25
2a	Cl	CH_3	50	50	50	25	25	50
2b	Cl	C_2H_5	25	25	25	25	25	25
2c	Cl	$C(CH_3)_3$	100	50	50	25	25	50
2d	Cl	$NHCOCH_3$	25	25	50	25	25	25
2e	Cl	$NHCH_3$	50	100	50	25	25	25
2f	Cl	Cl	25	50	25	25	25	25
2g	Cl	NO_2	25	25	12.5	25	25	25
3a	NO_2	H	12.5	100	12.5	12.5	12.5	12.5
3b	NO_2	CH_3	12.5	100	12.5	12.5	12.5	12.5
3c	NO_2	$C(CH_3)_3$	100	100	100	100	12.5	12.5
3d	NO_2	NH_2	6.25	25	12.5	12.5	12.5	12.5
3e	NO_2	Cl	12.5	12.5	25	12.5	25	12.5
3f	NO_2	Br	6.25	12.5	12.5	6.25	12.5	12.5
4a	NH_2	H	25	25	25	6.25	12.5	12.5
4b	NH_2	C_2H_5	25	25	25	6.25	12.5	25
4c	NH_2	Br	25	25	25	6.25	12.5	25
4d	NH_2	F	25	25	25	6.25	12.5	25
4e	NH_2	$N(CH_3)_2$	25	25	25	6.25	12.5	25
4f	NH_2	NO_2	25	25	25	12.5	12.5	12.5
7a	CH_3	CH_3	25	25	6.25	6.25	12.5	25
7b	CH_3	C_2H_5	25	25	12.5	6.25	12.5	25
7c	CH_3	OCH_3	25	25	12.5	6.25	12.5	25
7d	CH_3	F	25	25	12.5	12.5	12.5	25
7e	CH_3	$NHCH_3$	25	25	12.5	6.25	12.5	25
7f	CH_3	$N(CH_3)_2$	25	25	12.5	12.5	12.5	25
7g	CH_3	$NHCOCH_3$	25	25	12.5	12.5	12.5	25
8	Ampicillin		0.39	0.39	1.56	12.5	>400	—
9	Amoxicillin		0.39	0.39	1.56	12.5	>400	—
10	Erythromycin		25	1.56	50	50	25	—
11	Chloramphenicol		12.5	6.25	25	12.5	25	—
12	Haloprogin	—	—	—	—	—	—	3.12
13	Clotrimazole		—	—	—	—	—	6.25

Table II. Physical and spectral data of 5-methyl-2-(*p*-substitutedphenyl)benzoxazoles **7a–7g**.



Comp	R_1[a]	Mp (°C)	Yield (%)	Reaction temp (°C)	UV λ_{max}	UV log ε	NMR δ ppm	IR cm^{-1}
7a	CH_3	137.9[b]	61	135-140	214 307	3.13 3.26	2.73 (s, 6H) 7.62-8.44 (m, 7H)	1620 (C=N) 1265, 1060 (C-O-C)
7b	C_2H_5	79.1[b]	57	135-140	214 308	3.13 3.26	1.25-1.55 (t, 3H) 2.65 (s, 3H) 2.77-3.08 (q, 2H) 7.50-8.45 (m, 7H)	1620 (C=N) 1265, 1060 (C-O-C)
7c	OCH_3	99.6[b]	35	190-195	212 314	3.13 3.28	2.64 (s, 3H) 4.28 (s, 3H) 7.23-8.58 (m, 7H)	1615 (C=N) 1260, 1060 (C-O-C)
7d	F	128.9[b]	64	185-190	214 301	3.12 3.25	2.62 (s, 3H) 7.26-8.63 (m, 7H)	1610 (C=N) 1265, 1060 (C-O-C)
7e	$NHCH_3$	179.1[b]	52	165-170	214 326 354[c]	3.12 3.27 3.25	2.68 (s, 3H) 3.49 (s, 3H) 7.59-8.89 (m, 7H)	3320 (N-H) 1620 (C=N) 1265, 1060 (C-O-C)
7f	$N(CH_3)_2$	183.2[b]	55	245-250	214 328 363[c]	3.13 3.27 3.26	2.78 (s, 3H) 3.71 (s, 6H) 7.40-8.92 (m, 7H)	1620 (C=N) 1265, 1060 (C-O-C)
7g	$NHCOCH_3$	205.1[b]	48	255-260	214 324	3.13 3.28	2.60 (s, 3H) 2.68 (s, 3H) 7.90-8.50 (m, 7H)	3360 (N-H) 1685 (C=O) 1610 (C=N) 1265, 1060 (C-O-C)

[a]The spectral data of the compounds **7a–7g** have been obtained in this research. [b]Crystallization solvent; EtOH–H_2O. [c]Shoulder.

Antibacterial assay

The cultures were obtained in nutrient broth (Difco) for all the bacteria after 24 h of incubation at 37 ± 1°C. Testing was done in Mueller–Hinton broth (Difco) at pH 7.4 and the 2-fold serial dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 h at 37 ± 1°C, the last tube with no growth of the microorganism was recorded to represent MIC expressed in μg/ml.

Antifungal assay

The fungus *C albicans* was maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 1°C. Testing was performed in Sabouraud dextrose broth at pH 7.4 and the 2-fold serial dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1°C, the last tube with no growth of fungus was recorded to represent MIC expressed in μg/ml.

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