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Synthesis and Microbiological Activity of 5(or 6)-Methyl-2-substituted Benzoxazole and Benzimidazole Derivatives

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Summary

The synthesis and microbiological activity of a new series of 5(or 6)-methyl-2-substituted benzoxazoles (**IVa–n**) and benzimidazoles (**Va–h**) were described. The in vitro microbiological activity of the compounds was determined against gram-positive, gram-negative bacteria and the yeast Candida albicans in comparison to reference drugs. Microbiological results indicated that the derivatives possessed a broad spectrum of activity against the tested microorganisms and the compounds **IVa–g**, **IVi–k**, **IVn**, **Vb–c** and **Vh** showed significant activity against Pseudomonas aeruginosa having MIC values of 25 μg/ml, providing higher potencies than the reference drugs tetracycline and streptomycin. Moreover, the derivative 5-methyl-2-(p-chlorobenzyl)benzoxazole (**IVb**) possessed the same potency against Candida albicans as the reference drugs oxiconazole and haloprogin having a MIC value of 6.25 mg/ml. However, none of the derivatives showed a better spectrum of activity than the reference drugs.

Zusammenfassung

Synthese und antimikrobielle Wirkung 5(oder 6)-Methyl-2-substituierter Benzoxazol- und Benzimidazol-Derivate

Die Synthese und antimikrobielle Wirkung einer neuen Serie 5(oder 6)-Methyl-2-substituierter Benzoxazole (**IVa–n**) und Benzimidazole (**Va–h**) werden dargestellt. Die antimikrobielle Wirkung dieser Verbindungen in vitro wurde gegen gram-positive und gram-negative Bakterien sowie Candida albicans im Vergleich zu einigen Standardarzneimitteln untersucht. Die antimikrobiellen Resultate zeigten, daß die Derivate ein breites Wirkungsspektrum gegen die untersuchten Mikroorganismen hatten. Die Derivate **IVa–g**, **IVi–k**, **IVn**, **Vb–c** und **Vh** zeigten gute Wirkungen gegen Pseudomonas aeruginosa mit einem MHK-Wert von 25 μg/ml und damit bessere Wirkung als die Standardarzneimittel Tetracyclin und Streptomycin. 5-Methyl-2-(p-chlorobenzyl)benzoxazol (**IVb**) zeigt eine mit Oxiconazol und Haloprogin vergleichbare Wirkung gegen Candida albicans mit einem MHK-Wert von 6,25 μg/ml. Keines der Derivate zeigte jedoch ein besseres Wirkungsspektrum als die Standardarzneimittel.

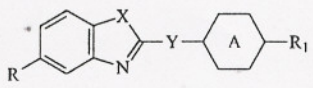
Key words Benzimidazole, microbiological activity, synthesis · Benzoxazoles, microbiological activity, synthesis

1. Introduction

Since thiabendazole (2-[4-thiazolyl]benzimidazole) has been found effective in the treatment of helmintic diseases with blood clinical efficacy [1], several substituted benzimidazole derivatives have been reported for their antimicrobial, antitumor and antiviral activities [2–7]. The inhibitory property of 2-(α-hydroxybenz)benzimidazole (HBB) on the replication of poliovirus has been fully demonstrated [8, 9]. In addition, 1,2-bis(2-benzimidazolyl)-1,2-ethanediol derivatives were also exhibited selective inhibitory actions on the multiplication of poliovirus possessing no toxicity [10]. Moreover, a series of substituted 2-phenylbenzimidazole-4-carboxamides were synthesized and evaluated for in vitro and in vivo antitumor activities. These potent antitumor agents had less DNA binding affinities and showed much lower levels of cytotoxicity than usual compounds which act as DNA-binding drugs. Despite very low cytotoxicities several of the compounds had moderate levels of antileukemic effects. However, the most interesting aspect of their biological activity was the lack of cross-resistance to the amsacrine resistance P388 cell line, suggesting the mechanism of cytotoxicity of these compounds either may not involve inhibition of topoisomerase II or may be via the altered enzyme [11]. Currently, substituted pyrimido[1,6-a]benzimidazoles were found to be a new class of potent DNA gyrase inhibitors. Their antibacterial activity is, however, inferior to the quinolone DNA gyrase inhibitors and antibacterial agents like norfloxacin or fleroxacin [12]. On the other side, recent observations suggest that besides the benzimidazole derivatives, the isoster of this ring system, benzoxazoles also indicate potential in vitro and/or in vivo antimicrobial activity with lower toxicity [13–16]. An antibiotic calcimycin (A23187), isolated from a strain of Streptomyces chartreusis, encludes benzoxazole ring in its molecular structure, was found very active especially against some gram-positive bacteria by acting as a good ionophore that forms dimeric complexes with divalent to transport them across the bioligical membranes [17–199. Furthermore, the compound, 3-([benzoxazol-2-yl]ethyl)-5-ethyl-6-methylpyridin-2-(1-H)-on (L-696,299), which was a highly selective antagonist of the reverse transcriptase enzyme and inhibited the spread of HIV-1 IIIb infection by > 95 % in MT4 human T-lymphoid cell culture, was selected for clinical evaluation as an antiviral agent [20–22].

Although these ring systems are the structural isoesters of naturally occurring nucleotides such as adenine and guanine, it allows them to interact easily with the biopolymers of the living systems and to possess specific selectivity at their mechanisms of activity concerning reduction of cytotoxicity. Consequently, the view of these observations, including the fact of the demand for new chemotherapeutic drugs, encouraged us to build some expectations upon these heterocyclic compounds anticipating that they would be the candidates of a new class of antimicrobial agents.

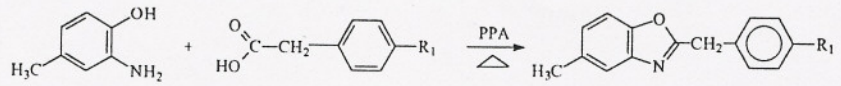
Recently, we reported the synthesis and the antimicrobial activity of various 5-substituted-2-(p.substitutedphenyl)benzoxazoles (**I**), 5-substituted-2-(p.substitutedbenzyl)benzoxazoles (**II**), 5-substituted-2-(2-phenylethyl)benzoxazoles and benzimidazoles (**III**), given in Scheme 1, against some gram-positive, gram-negative bacteria and the yeast Candida albicans providing a wide variety of in vitro antibacterial activity especially against the gram-negative rods Klebsiella pneumoniae and Pseudomonas aeruginosa [23–28].



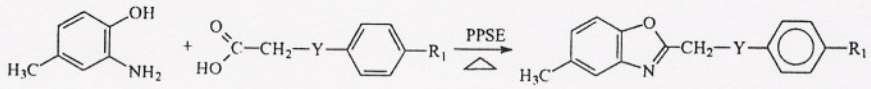
I X = O Y = - A = Phenyl R = H, Cl, NO_2, NH_2, CH_3
R_1 = H, CH_3 , C_2H_5 , F, Br, Cl, $NHCH_3$, NO_2, NH_2, $C(CH_3)_3$, $NHCOCH_3$, $NH(CH_3)_2$, OCH_3
II X = O Y = CH_2 A = Phenyl R = H, Cl, NO_2
R_1 = H, OCH_3 , Cl, Br, NO_2
III X = O, NH, S, Y = C_2H_4 A = Phenyl or Cyclohexyl
R = H, Cl, NO_2, NH_2

Scheme 1

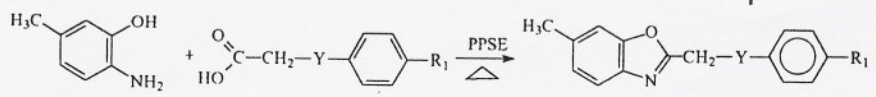
In the present study, 5(or 6)-methyl-2-substituted benzoxazoles (**IVa–n**) and benzimidazoles (**Va–h**) (Scheme 2) were synthesized as the target compounds in order to examine their antimicrobial activity together with the structure-activity relationships.



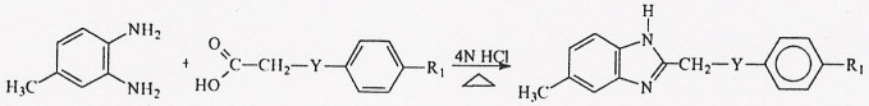
IVa R_1= H
IVb R_1= Cl
IVc R_1= Br
IVd R_1= NO_2
IVe R_1= NH_2



IVf Y= O R_1= H
IVg Y = S R_1= H
IVh Y = O R_1= Cl



IVi Y = - R_1= H
IVj Y = - R_1= Cl
IVk Y = - R_1= Br
IVl Y = O R_1= H
IVm Y = S R_1= H
IVn Y = O R_1= Cl



Va Y = - R_1= H
Vb Y = - R_1= Cl
Vc Y = - R_1= Br
Vd Y = - R_1= NH_2
Ve Y = CH_2 R_1= H
Vf Y = O R_1= H
Vg Y = S R_1= H
Vh Y = O R_1= Cl

Scheme 2

2. Material and methods

2.1. Chemistry

The synthesis of 5(or 6)-methyl-2-substituted benzoxazoles (**IV**) and 5(or 6)-methyl-2-substituted benzimidazoles (**V**) was performed through heating carboxylic acids with appropriate o-substituted anilines by means of several dehydrating agents in a one-step procedure.

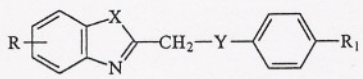
Polyphosphoric acid (PPA) or trimethylpolyphosphate esters (PPSE), which contain anhydride groups, prevent the solvolysis of the oxazolo ring under hot aqueous acidic conditions, and were used as dehyerating agents in the synthesis of compounds **IV** [26, 29]. For the synthesis of the derivatives **IVf–n**, PPSE were found to be more suitable and easier to handle as a cyclodehydration reagent than PPA.

During the synthesis of compounds **V**, aqueous hydrochloric acid was used as the condensation reagent [30], however, this route was not successful for the preparation of compounds **IV**.

Compounds **IV–V** were prepared as new products except **Va, Vg, Vh** [31]. The structures of all the derivatives **IVa-n** and **Va–h** were supported by elemental analysis and spectral data. The IR and ^{1}H-NMR spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Table 1.

Silicagel HF_{254} (E. Merck, Darmstadt, Germany) chromatoplates (0.3 mm) were used for TLC and the solvent systems were dichloromethan : hexane (5 : 1) for compounds **IV**, chloroform : methanol : petrolum ether (4 : 0.3 : 1) for **V**. All melting points were taken on a Büchi SMP 20 capillary apparatus (Büchi Laboratoriums-technik AG, Flawil, Switzerland). IR

Table 1: Physical properties, preparation and spectral data of the compounds **IV–V**.



Comp. No.	R	R_1	X	Y	Method	Reac. temp. (°C)	Reac. time (h)	Yield (%)	M.P. (°C)	IR (cm^{-1})	^{1}H NMR δ ppm
IVa	5-CH_3	H	O	–	A	120–125	2	36.43	44	3000–3100, 2900–3000, 1620–1570, 1480, 1290, 1090, 770–800	2.50 (3H, s), 4.30 (sH, s), 7.00–7.60 (8H, m)
IVb	5-CH_3	Cl	O	–	A	130–135	1.5	41.51	48	3000–3100, 2920–3000, 1620–1470, 1480, 1250, 1080, 770–810	2.45 (3H, s), 4.20 (2H, s), 7.00–7.60 (7H, m)
IVc	5-CH_3	Br	O	–	A	120–130	2.5	43.68	51–52	3000–3100, 2920–3000, 1630–1570, 1485, 1260, 1080, 770–820	2.50 (3H, s), 4.20 (2H, s), 7.00–7.60 (7H, m)
IVd	5-CH_3	NO_2	O	–	A	140–145	1	49.67	91–92	3000–3100, 6860–2980, 1620–1520, 1480, 1260, 1160, 870–790	2.50 (3H, s), 4.40 (2H, s), 7.00–7.70 (5H, m), 8.10–8.40 (2H, d)
IVe	5-CH_3	NH_2	O	–	A	190–195	1.5	21.36	64	3250–3420, 3010–3100, 2980–2840, 1625–1520, 1480, 1260, 1160, 810–790	2.50 (3H, s), 4.20 (2H, s), 3.60 (2H, s), 6.55–9.85 (2H, d), 7.00–7.50 (5H, m)
IVf	5-CH_3	H	O	O	B	110–120	2.5	41	52.5–53.5	3100, 2900–2960, 1605–1500, 1465, 1245–1059, 950–695	2.40 (3H, s), 5.20 (2H, s), 6.80–7.50 (8H, m)
IVg	5-CH_3	H	O	S	B	90–100	3	43.13	65–66	3100, 2950, 1575–1482, 1405, 1260–1030, 960–690	2.40 (3H, s), 4.20 (2H, s), 7.00–7.50 (8H, m)
IVg	5-CH_3	Cl	O	O	B	110–120	3	62.86	93–94	3105, 2900–2950, 1605–1500, 1380, 1245–1042, 945–652	2.47 (3H, s), 5.26 (2H, s), 6.90–7.03 (2H, dd, $J_{2,3}$, = $J_{5,6}$,= 9.26 Hz)
IVi	6-CH_3	H	O	–	B	70–80	2.5	48	35	3095, 2975, 1630, 1590, 1470, 1260	2.43 (3H, s), 4.22 (2H, s), 7.03–7.58 (8H, m)
IVj	6-CH_3	Cl	O	–	B	140–150	2	6.0	42–43	3100, 2980, 1625, 1580, 1500, 1260	2.44 (3H, s), 4.19 (2H, s), 7.64–7.05 (7H, m)
IVk	6-CH_3	Br	O	–	B	140–150	2	10	69–70	3100, 2965, 1620, 1560, 1490, 1240	2.43 (3H, s), 4.19 (2H, s), 7.68–7,02 (7H, m)
IVl	6-CH_3	H	O	O	B	130	2.5	53.55	63–64	3090, 2958, 1605–1500, 1455–1385, 1245–1045, 940–690	2.40 (3H, s), 5.27 (2H, s), 6.90–7.65 (8H, m)
IVm	6-CH_3	H	O	S	B	130	5	50.20	48–49	3100, 2940, 1615–1570, 1440, 1245–1030, 950–700	2.43 (3H, s), 4.25 (2H, s), 6.95–7.62 (8H, m)
IVn	6-CH_3	Cl	O	O	B	100–110	2.5	38.23	84–85	3100, 2950, 2890, 1625–1580, 1495, 1250–1040, 965–606	2.48 (3H, s), 5.26 (2H, s), 6.90–7.00 (2H, dd, $J_{2,3}$, = $J_{5,6}$, = 9.27 Hz), 7.10–7.30 (4H, m), 7.60 (1H, d, $J_{4,5}$ = 8.03 Hz)
Va	5-CH_3	H	NH	–	C	90–100	15	44	130–131	3100, 2970, 1640, 1600, 1500, 1440, 1260	2.40 (3H, s), 4.17 (2H, s), 6.96–7.42 (8H, m), 7.78 (1H, m)
Vb	5-CH_3	Cl	NH	–	C	90–100	10	18.5	147–148	3100, 300, 1635, 1500, 1450, 1270	2.40 (3H, s), 4.40 (2H, s), 6.97–7.42 (7H, m)
Vc	5-CH_3	Br	NH	–	C	90–100	12	63.5	129–131	3100, 2980, 1640, 1600, 1500, 1440, 1260	2.41 (3H, s), 4.16 (2H, s), 7.02–7.39 (7H, m)
Vd	5-CH_3	NH_2	NH	–	C	90–100	12	24.5	149	3420, 3080, 2960, 1635, 1525, 1460, 1280	2.42 (3H, s), 4.05 (2H, s), 5.21 (2H, s), 7.32–7.01 (7H, m)
Ve	5-CH_3	H	NH	CH_2	C	100	5	36.01	127–128	3100–2700, 1640–1545, 1460, 1340–1020, 942–700	2.44 (3H, s), 3.15 (4H, s), 6.30–7.46 (8H, m)
Vf	5-CH_3	H	NH	O	C	100	5	18.98	167–168	3080–2680, 1600–1500, 1460, 1310–1030, 945–695	2.44 (3H, s), 5.30 (2H, s), 6.90–760 (8H, m)
Vg	5-CH_3	H	NH	S	C	100	6.5	26.37	105–106	3100–2700, 1635–1550, 1440, 1300–1020, 940–695	2.42 (3H, s), 4.34 (2H, s), 6.90–7.50 (8H, m)
Vh	5-CH_3	Cl	NH	O	C	100	14	14	150–151	3100–2700, 1630–1500, 1460, 1300–1020, 970–650	2.45 (3H, s), 5.31 (2H, s), 6.54–7.42 (7H, m)

spectra were recorded on Pye Unicam SP-1025 (Pye Unicam Ltd., Chambridge, England) with KBr discs. ^{1}H-NMR spectra were obtained with a Bruker 80 MHz spectrometer (Bruker Instruments Inc., Billerica, USA) in d_6-chloroform or d_6-dimethylsulfoxid and tetramethylsilan (TMS) was used as an internal standard. UV maxima were measured on a Pye Unicam SP-1700 spectrometer (Pye Unicam Ltd.) in methanol at a concentration of 10^{-4}–10^{-5} mol/l. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C, H, N) were within ± 0.4 % of the calculated amounts.

The compounds were prepared by three general methods according to the dehydrating agent used. The cyclodehydration reagent PPSE was prepared in our laboratory by the method described in [29]. Data on the preparation of the compounds are summarized in Table 1. The reaction mixtures were protected from moist air by means of a calcium chloride drying tube and stirred magnetically. The starting compounds and the solvents were commercially available products.

2.2. Synthetic methods

2.2.1. Preparation of PPSE

A mixture of phosphorus pentoxide (10 g, 35 mmol), hexamethyldisiloxane (25 ml, 132.5 mmol), and 1,2-dichlorobenzene (50 ml) was refluxed for 5 min under nitrogen atmosphere until the solution became clear. The obtained solution was used.

2.2.2. General procedure for 5-methyl-2-substituted benzoxazoles IVa–IVe (Method A)

A mixture of 2-hydroxy-5-methyl aniline (0.01 mol) and p-substituted phenylacetic acid (0.015 mol) was heated in PPA (12 g). At the end of the reaction period, the mixture was poured into ice-water and neutralized with an excess of 10 % NaOH solution. The precipitate was collected, washed, dried and extracted with benzene to separate from impurities. After the evaporation of the solvent in vacuo. The crude product was obtained and crystallized.

2.2.3. General procedure for 5(or 6-methyl-2-substituted benzoxazoles IVf–IVn (Method B)

p-Supstituted phenylacetic acid or p-substituted phenoxyacetic acid or thiophenoxyacetic or 3-phenylpropionic acid (0.005 mol) and 2-hydroxy-4-methyl aniline or 2-hydroxy-5-methyl aniline (0.0069 mol) were added to a solution of PPSE in 1,2-dichlorobenzene (15 ml) prepared as above, and the mixture was heated under reflux with vigorous stirring for 1–2.5 h, before being taken up in dichloromethane (30 ml). 1N NaOH (50 ml) was then added, the organic layer was separated, and the aqueous solution extracted with dichloromethane (3 × 25 ml). The combined extracts were dried on Na_2SO_4 and the solvent evaporated to give crude product. The crude products were crystallized from n-hexane.

2.2.4. General procedure for 5-methyl-2-substituted benzimidazoles Va–Vh (Method C)

A mixture of 4-methyl-o-phenylenediamine (0.01 mol), p-substituted phenylacetic acid or p-substituted phenoxyacetic acid or thiophenoxyacetic acid or 3-phenylpropionic acid (0.005 mol) and 4N HCl (10 ml) were boiled under reflux. At the end of the reaction period, the reaction mixture was poured into ice-water and neutralized with excess of $NaHCO_3$. The precipitate was collected, washed, dried and extracted with benzene to separate from impurities. After the evaporation of solvent in vacuo, the crude product was obtained and crystallized.

2.3. Microbiology

For both, the antibacterial and the antimycotic assays, the compounds were dissolved in absolute ethanol (0.8 mg/ml) [32]. Further dilutions of the compounds and control drugs in the test medium were performed to obtain the required concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/ml. The minimum inhibitory concentration (MIC) was determined by using the method of two-fold serial dilution technique [32, 33]. In order to ensure that the solvent per se had no effect on bacterial growth, a control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions used in our experimental and found inactive in culture medium. All the compounds were tested for their in vitro growth inhibitory against different bacteria and Candida albicans RSKK 628. The following bacterial strains were tested: Staphylococcus aureus RSKK 250 and Streptococcus faecalis RSKK 500 as gram-positive and Escherichia coli RSKK 313, Klebsiella pneumoniae RSKK 256, and Pseudomonas aeruginosa RSKK 356 as gram-negative bacteria. RSKK strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of Faculty of Pharmacy of Ankara University. Ampicillin, amoxycillin, tetracycline, gentamycin, streptomycin, oxiconazole, and haloprogin (Oxoid L., Hampshire, England) were used as reference drugs.

2.3.1. Antibacterial assay

The cultures were obtained from Mueller-Hinton broth (Difco Lab., Detroit, MI; USA) of all the bacterial strains after 24 h of incubation at 37 ± 1 °C. Testing was crried out in Mueller–Hinton broth at pH 7.4 and the two-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 microorganism was recorded to represent the MIC expressed in µg/ml.

2.3.2. Antimycotic assay

Candida albicans was obtained from Sabouraud dextrose broth (Difco Lab.) after incubation for 24 h at 25 ± 1 °C. Testing was performed in Sabouraud dextrose broth at pH 7.4 and the two-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 yeast was recorded to represent the MIC expressed in µg/ml.

3. Results

The chemical, physical and spectral data of the synthesized compounds **IV** and **V** are reported in Table 1. The antimicrobial activity of the compounds also in comparison to some control drugs is shown in Table 2 and indicates that the compounds **IV–V** are able to inhibit in vitro growth of a number of microorganisms exhibiting MIC values between > 200 and 6.25 µg/ml.

Table 2 shows that the synthesized compounds provided a wide range of antibacterial activity against the tested

Table 2: In vitro antimicrobial activity (MIC, µg/ml) of the compounds **IV–V** and the standard drugs.

Compounds	Microorganismus[a]						
	Gram-positive			Gram-negative			Fungus
	Sa	Sf	Bs	Ec	Kp	Pa	Ca
IVa	12.5	50	12.5	50	12.5	25	12.5
IVb	6.25	50	12.5	25	12.5	25	6.25
IVc	12.5	50	12.5	50	12.5	25	12.5
IVd	12.5	50	6.25	50	12.5	25	25
IVe	50	50	6.25	50	12.5	25	12.5
IVf	25	25	25	25	25	25	25
IVg	25	25	25	25	25	25	25
IVh	50	50	25	50	12.5	50	50
IVi	50	50	>200	50	50	25	12.5
IVj	50	50	12.5	50	50	25	50
IVk	50	50	12.5	50	50	25	50
IVl	50	50	25	50	25	50	12,5
IVm	50	50	12.5	50	25	50	12,5
IVn	50	50	25	50	25	25	50
Va	50	50	25	50	50	50	12.5
Vb	25	50	25	50	25	25	12.5
Vc	12.5	50	>200	25	12.5	25	12.5
Vd	50	50	25	50	25	50	12.5
Ve	25	50	12.5	50	25	50	12.5
Vf	50	50	25	50	25	50	12.5
Vg	50	50	25	50	25	50	25
Vh	100	100	100	50	50	25	25
Ampicillin	0.78	0.78	0.78	3.12	12.5	>200	–
Amoxycillin	0.78	0.78	0.78	3.12	12.5	>200	–
Tetracycline	0.78	0.78	0.78	3.12	3.12	50	–
Gentamycin	0.78	12.0	0.78	3.12	1.56	12.5	–
Streptomycin	3.12	100	50	1.56	1.56	100	–
Oxiconazole	–	–		–	–	–	6.25
Haloprogin	–	–		–	–	–	6.25

[a] Abbreviations: Sa, Staphylococcus aureus; Sf, Streptococcus faecalis; Bs, Bacilus subtilis; Ec, Escherichia coli; Kp, Klebsiella pneumoniae; Pa, Pseudomonas aeruginosa; Ca, Candida albicans.

microorganisms. Besides the compounds **IVi,** and **Vh**, which showed lack in activity against B. subtilis, the derivatives 5-methyl-2-(p-nitrobenzyl)benzoxazoles (**IVd**) and 5-methyl-2-(p-aminobenzyl)benoxazole (**IVe**) showed good MIC values of 6.25 µg/ml. However, all of the syntesized compounds exhibited lower potency than the compared control drugs such as ampicillin, amoxycillin and tetracycline for the tested gram-positive bacteria.

4. Discussion

The activity of the synthesized compounds were also tested against E. coli, K. pneumoniae and P. aeruginosa as gram-negative bacteria. They exhibited MIC values between 25–50 µg/ml against E. coli indicating less activity than the compared control drugs. **IVa-e**, **IVh** and **Vc** were found to be more active than the other synthesized compounds having MIC values of 12.5 µg/ml against K. pneumoniae, but they showed lower potency than the reference drugs tetracycline, gentamycin and streptomycin.

On the other hand, for the determination of the antibacterial activity against P. aeruginosa, a microorganism causing nosocomial infections and often resistant to antibiotic therapy, the derivatives **IVa–g**, **IVi–k**, **IVn**, **Vb–c**, and **Vh** showed good activities providing higher potency than the compared drugs tetracycline and streptomycin however, they possessed one dulution step lower MIC values than gentamycin having MIC values of 25 µg/ml.

Moreover, synthesized compounds were tested against C. albicans to observe the antimycotic activity and the derivative 5-methyl-2-(p-chlorobenz)benzoxazole (**IVb**) was found to be more active than the other tested compounds showing the same potency as the reference drugs oxiconazole and haloprogin having a MIC value of 6.12 µg/ml.

In conclusion, Table 2 reveals that the two different heterocyclic nuclei at the synthesized compounds indicate bioisosteric effects for the antimicrobial activity in the tested microorganisms. Holding a methyl group at position 5 instead of 6 on the benzoxazole ring increases the efficacy especially against S. aureus, B. subtilis and C. albicans. Substitution of the para position of the 2-benzyl moiety on the heterocyclic ring with a chlorine atom causes an increase in the intensity of the activity against S. aureus and C. albicans. In contrast, having a nitro or amino group at the same position is found to be more important to improve the antibacterial activity in B. subtilis.

5. References

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