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Preliminary communication

Synthesis and structure–activity relationships of new antimicrobial active multisubstituted benzazole derivatives

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Abstract

A series of multisubstituted benzoxazoles, benzimidazoles, and benzothiazoles (**5–7**) as non-nucleoside fused isosteric heterocyclic compounds was synthesized and tested for their antibacterial activities against various Gram-positive and Gram-negative bacteria and antifungal activity against the fungus *Candida albicans*. Microbiological results indicated that the synthesized compounds possessed a broad spectrum of activity against the tested microorganisms at MIC values between 100 and 3.12 μg/ml. Structure–activity relationships (SAR) studies revealed that benzothiazole ring system enhanced the antimicrobial activity against *Staphylococcus aureus*. In these sets of non-nucleoside fused heterocyclic compounds electron withdrawing groups at position 5 of the benzazoles increased the activity against *C. albicans*.

Keywords: Synthesis; Antimicrobial activity; Benzoxazole; Benzimidazole; Benzothiazole

1. Introduction

The number of cases of multidrug resistant bacterial infections is increasing at an alarming rate. As well as the clinicians have become reliant on vancomycin as the antibiotic for serious infections resistant to traditional agents [1] there is still need for the new classes of antibacterial agents.

Substituted benzimidazoles, benzoxazoles, and benzothiazoles are found to be associated with various chemotherapeutical activities such as antibacterial, antifungal, antitumor, antiviral activities [2–9]. A series of 5-formyl-, 5-(aminocarbonyl)-, or 5- and 6-nitro derivatives of 2-(4-methoxyphenyl)benzimidazoles, benzoxazoles and benzothiazoles were synthesized and determined as topoisomerase I inhibitors [4]. In evaluating their cytotoxicity, these new topoisomerase I poisons exhibited no significant cross-resistance against cell lines and indicated minimum or no DNA binding affinity. Substituted pyrimido[1,6-a]benzimidazoles were also synthesized as a new class of potent DNA gyrase inhibitors; however, their antibacterial activity was inferior to the quinolone type antibacterial agents such as norfloxacin or fleroxacin [2]. Moreover, a benzoxazole derivative, 3-(4,7-dichlorobenzoxazol-2-ylmethylamino)-5-ethyl-6-methylpyridin-2(1H)-one (L-697,661), was observed as a specific non-nucleoside reverse transcriptase inhibitor for the human immunodeficiency virus HIV-1 type and combined therapy with zidovudine and L-697,661 achieved a marked decrease of viraemia in some primary HIV infected patients [3,10,11].

Additionally, a derivative of campotothecin having a benzoxazole ring system within its structure was found to be significantly more potent than campotothecin as inhibitors of topoisomerase I [12].

Currently, a new series of benzothiazoles have been synthesized as antitumor agents and showed potent inhibitory activity against human breast cancer cell lines in vitro and in vivo [6]. Among them, lysyl-amide of 2-(4-amino-3-methylphenyl)-5- fluorobenzothiazole had been selected for phase 1 clinical evaluation [13].

UK-1 is a unique natural anticancer active product having bis(benzoxazole) structure, which is isolated from a strain of *Streptomyces* sp. 517-02 [14]. Its semisynthetic derivatives such as methyl UK-1 (MUK-1) and dimethyl UK-1 (DMUK-

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1) both have activity against Gram-positive and Gram-negative bacteria [15,16].

In the last few years, we reported the synthesis of several 2,5-disubstitutedbenzoxazole and benzimidazole derivatives as the antimicrobial agents [17–25]. The structure–activity relationships (SAR) of previously synthesized compounds indicated that the fused heterocyclic nucleus was important for the antimicrobial activity.

The goal at the outset of this research was to develop more effective antimicrobial analogues of 2,5,6-trisubstituted benzoxazole, benzimidazole, and benzothiazole. The strategy employed was to examine the effect of the isosteric heterocyclic nucleus and the positions of 2, 5 and 6 against some Gram-positive and Gram-negative bacteria and the fungus *C. albicans*. Therefore, in this study, antimicrobial activity and the SAR of a series of newly (**5–7**) and previously (**10–12**) [26,27] synthesized multisubstituted benzoxazoles, benzimidazoles, benzothiazoles, and oxazolo(4,5-b)pyridines were described in order to reveal the lead optimization against the tested various bacteria and the fungus *C. albicans*.

2. Chemistry

The general synthetic procedure was employed to prepare compounds **5a–o** (except **5e,5j**), **6a-c**, and **7a-p** (except **7e, 7i, 7m**) (Table 1) involving the reaction of the appropriate carboxylic acids **1a/b/c/d** or **e** with suitable 4,5,6-trisubstituted anilines **2, 3, 4** by refluxing in the presence of dehydrating agents in one step procedure as shown in Scheme 1. For the preparation of the compounds **5** and **6**, trimethylsilyl polyphosphate ester (PPSE) was used as the cyclodehydration reagent in the ring closure reactions [28]. During the synthesis of benzimidazole derivatives **7a-p** (except **7e, 7i, 7m**) aqueous hydrochloric acid was used as the condensation reagent according to well-known Philips' method [29].

Preparation of the compounds **5e, 5j, 5p, 7e, 7i**, and **7m**, which are holding 5- carboxylmethyl group on benzoxazole and benzimidazole ring systems, was carried out as outlined in Scheme 2. The primarily synthetic steps involved the methyl esterification of 3-amino-4-hydroxybenzoic acid **8a** and 3,4-diaminobenzoic acid **8b** to obtain the compounds **9a, b**. After the treatment of **9a** or **b** with **1a/b** or **c** in PPSE, 5-carboxylmethyl-2-substitutedbenzoxazoles **5e, 5j, 5p** and benzimidazoles **7e,7i, 7m** were obtained.

The chemical, physical and spectral data of the newly synthesized compounds **5, 6, 7** are reported in Table 1.

3. Results and discussion

The minimum inhibitory concentrations (MIC) of the newly and previously synthesized compounds determined against *Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis* as Gram-positive and *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* as Gram-negative bacteria and the yeast *Candida albicans* by using twofold serial dilution technique and compared to ampicillin, amoxycillin, tetracycline, streptomycin, clotrimazole and haloprogin as standard drugs. All the biological results of the compounds were given in Table 2. The combined data reported that the compounds were able to inhibit the in vitro growth of screened microorganisms showing MIC values between 100 and 3.12 μg/ml.

Among the synthesized compounds, 2-(phenoxymethyl)benzothiazole **6a** was found the most active derivative at an MIC value of 3.12 μg/ml against *S. aureus*. The derivatives **5c, 5o, 6a-c, 7b, 7g, 7h**, and **7p** exhibited significant antibacterial activity with MIC values of 25 μg/ml for the Gram-negative enterobacter *P. aeruginosa*, which is effective in nosocomial infections and often resistant to antibiotic therapy, providing higher potencies than the compared standard drugs.

Table 2 indicated that thiazolo ring instead of oxazolo and imidazolo moieties in the fused heterocyclic system improved the potency as threefold against *S. aureus* (compounds **6a–c**). Substitution of Z with -NH-group reduced the antibacterial activity against *S. feacalis* (compounds 7**n** and **7o**).

When a nitro group was attached at position 5 of the fused heterocyclic system caused twofold better potency against *E. coli*. However, holding a carboxylmethyl group on position R_1 or possessing a pyridine ring as a six membered moiety at the fused ring system slightly reduced the activity against *K. pneumoniae*. It should be pointed out that besides benzothiazole derivatives and the compounds having a chlorine atom on position R_1 of benzoxazole and benzimidazole rings were also performed better activity against the enteric Gram-negative rod *P. aeruginosa*.

On the other hand, the SAR results against *C. albicans* revealed that the electron withdrawing groups such as chlorine or nitro attached at position 5 of the heterocyclic nucleus increased the potency. Besides, the bridge groups such as thiomethylene or ethyl between benzazole and phenyl enhanced the activity against the same fungus.

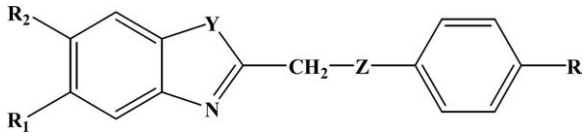
As a result, we could point out while benzothiazole as a nucleus in these series increased the activity against *S. aureus*, amino methyl group as a bridge element between fused heterocyclic ring and phenyl decreased the activity against *S. faecalis*. In these sets of non-nucleoside fused heterocyclic compounds electron withdrawing groups at position 5 of the fused ring system increased the activity against *C. albicans*. These observations provide some predictions in order to design further antimicrobial active compounds prior to their synthesis following with molecular modeling studies.

4. Experimental protocols

4.1. Chemistry

Unless otherwise noted, materials were obtained from commercially suppliers and used without further purification.

Table 1
Physical, preparation, and spectral data of the synthesized compounds **5–7**



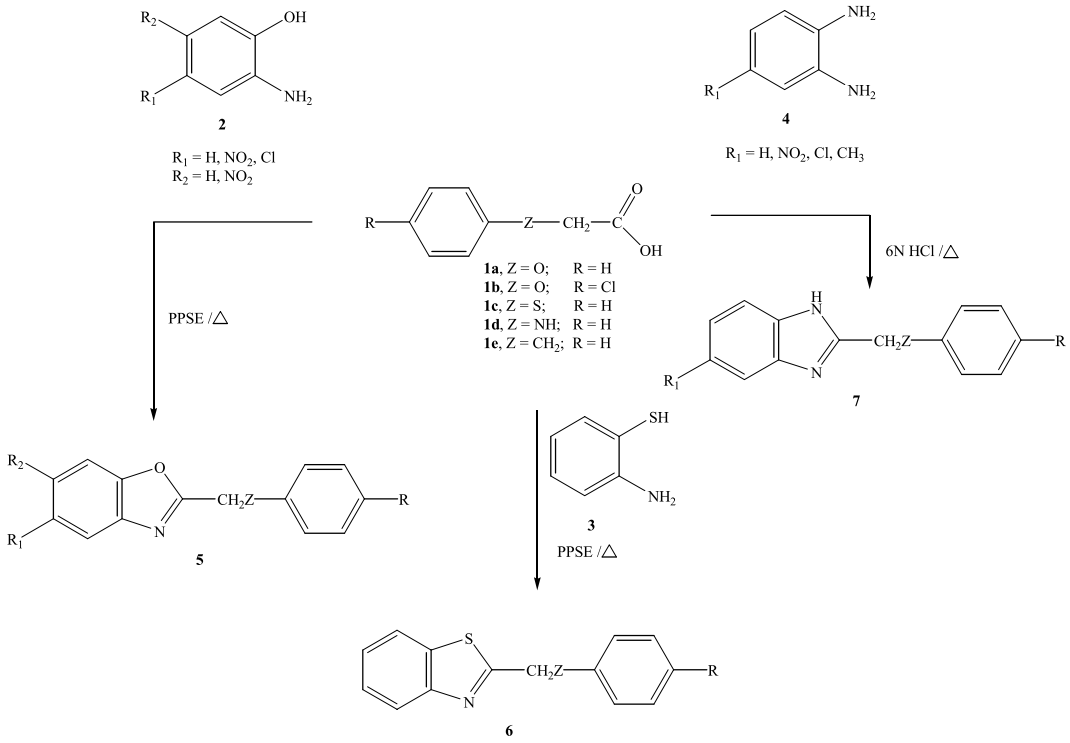
Compound number	Y	Z	R	R_1	R_2	Mp (°C) (recrystallization solvent) [a]	Yield (%)	Reaction time (h)	Reaction temperature (°C)	IR (cm^{-1})	^{1}H-NMR δ ppm(J = Hz)
5a	O	O	H	H	H	146–147 (154–156) [30] (A)	49	1.5	140	3100, 2940, 1605–1500, 1455–1390, 1300–1045, 950–700	7.70–7.20 (m, 9H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons), 5.20 (s, 2H, CH_2)
5b	O	O	H	NO_2	H	134 (B)	22	3	120	3120, 2950, 1630–1595, 1533, 1458, 1345, 1230–1060, 950–695	8.58 (d, IH, C-4 H, $J_{4,6}$ = 3.5 Hz), 8.20 and 8.45 (dd, 1H, C-6 H, $J_{6,7}$ = 8.8 Hz, $J_{6,4}$ = 3.5 Hz), 7.60 (d, IH, C-7 H, $J_{7,6}$ = 8.8 Hz), 7.40–6.80 (m, 5H, phenyl protons
5c	O	O	H	Cl	H	86 (85–88) [31] (C)	17	3	140	3110, 2975, 1610–1500, 1460–1390, 1295–1050, 945–695	δ 7.50–7.00 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 5.20 (s, 2H, CH_2)
5d	O	O	H	H	NO_2	138–139 (D)	42	2	160	3110, 2890, 1630–1585, 1525, 1458, 1348, 1270–1060, 955–700	8.50–7.50 (m, 3H, C-4 H, C-5 H, and C-7 H), 7.40–6.80 (m, 5H, phenyl protons), 5.37 (s, 2H, CH_2)
5e	O	O	H	$COOCH_3$	H	121–122 (B)	57	3	110	3100, 2995–2900, 1735, 1630–1500, 1440, 1250, 1230–1050, 985–690	8.46–8.43 (d, 1H, C-4 H, $J_{4,6}$ = 1.60 Hz), 8.19–8.06 (dd, 1H, C 6 H, $J_{6,7}$ = 8.80 Hz, $J_{6,4}$ = 1.60 Hz), 7.64–7.52 (dd, 1H, C-7 H, $J_{7,6}$ = 8.80 Hz), 7.40–6.90 (m, 5H, phenyl protons), 5.34 (s, 2H, CH_2), 3.95 (s, 3H, CH_3)
5f	O	O	Cl	H	H	81–82 (85–86) [31] (B)	56	2.5	110	3120, 2960–2900, 1630–1585, 1460–1500, 1245–1042, 949–660	δ 7.80–7.21 (m, 6H, C-4 H, C-5 H, C-6 H, C-7 H, C-3′ H and C-5′ H), 7.04–6.95 (dd, 2H, C-2′ H and C-6′ H and C-6′ H, $J_{2',3'}$ and $J_{6',5'}$ = 9.2 Hz), 5.29 (s, 2H, CH_2)
5g	O	O	Cl	NO_2	H	140–141 (D)	74	2.5	110	3110, 2960–2890, 1630–1590, 1538, 1440, 1350, 1240–1050, 940–660	8.60 (d, 1H, C-4 H, $J_{4,6}$ = 2.40 Hz), 8.40–8.20 (dd, 1H, C-6 H, $J_{6,7}$ = 8.96 Hz, $J_{6,4}$ = 2.40 Hz), 7.65 (d, 1H, C-7 H, $J_{7,6}$ = 8.96 Hz), 7.35–7.23 (dd, 2H, C-3′ H and C-5′ $J_{3',2'}$ and $J_{5',6'}$ = 9.12 Hz), 7.00–6.90 (dd, 2H, C-2′ H and C-6′ H, $J_{2',3'}$ and $J_{5',6'}$ = 9.12 Hz), 5.34 (2H, s)
5h	O	O	Cl	H	NO_2	147–148 (A)	23	5	130	3148, 2950–2880, 1635–1585, 1525, 1435, 1340, 1235–1065, 960–665	8.49–8.46 (d, 1H, C-7 H, $J_{7,5}$ = 2.24 Hz), 8.40–8.20 (dd, 1H, C-5 H, $J_{5,4}$ = 8.76 Hz, $J_{5,7}$ = 2.24 Hz), 7.90–7.80 (d, 1H, C-4 H, $J_{4,5}$ = 8.76 Hz), 7.35–7.23 (dd, 2H, C-3′ H and C-5′, $J_{3',2'}$ and $J_{5',6'}$ = 9.26 Hz), 7.03–6.90 (dd, 2H, c-2′ H and C-6′ H, $J_{2',6'}$ and $J_{6',5'}$ = 9.26 Hz), 5.35 (2H, s)
5i	O	O	Cl	Cl	NO_2	88–89 (B)	39	3	120	3130, 2940–2900, 1600–1580, 1540, 1448, 1350, 1285–1080, 965–660	8.10 (s, 1H, C-7 H), 7.90 (s, 1H, C-4 H), 7.34–7.23 (dd, 2H, C-3′ H and C-5′ H, $J_{3',2'}$ and $J_{5',6'}$ = 9.26 Hz), 7.00–6.90 (dd, 2H, C-2′ H and C-6′ H, $J_{2',3'}$ and $J_{5',6'}$ 9.26 Hz), 5.34 (s, 2H, CH_2)
5j	O	O	Cl	$COOCH_3$	H	145–146 (B)	48	4	150	3130, 2990–2880, 1743, 1630–1500, 1440, 1250, 1210–1045, 940–660	8.46–8.43 (d, 1H, C-4 H, $J_{4,6}$ = 1.60 Hz), 8.19–8.07 (dd, 1H, C-6 H, $J_{6,7}$ = 8.60 Hz, $J_{6,4}$ = 1.60 Hz), 7.63–7.52 (dd, 1H, C-7 H, $J_{7,6}$ = 8.60 Hz) 7.33–7.21 (dd, 2H, C-3′ H and C-5′ H, $J_{3',2'}$ and $J_{5',6'}$ = 9.26 Hz), 7.03–6.90 (dd, 2H, C-2′ H and C-6′ H, $J_{2',3'}$, and $J_{6',5'}$ = 9.26 Hz), 5.29 (s, 2H, CH_2) 3.95 (s, 3H, CH_3)
5k	O	S	H	H	H	37–38 (37–38) [32] (D)	33	3	140	3100, 2995–2880, 1620–1575, 1460, 1250–1020, 950–690	7.60–7.20 (m, 9H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons) 4.27 (s, 2H, CH_2)
5m	O	S	H	NO_2	H	53–54 (B)	30	3	110	3120, 2925, 1630–1575, 1531, 1490–1445, 1345, 1260–1070, 970–700	8.50 (d, 1H, C-4 H, $J_{4,6}$ = 2.08 Hz), 8.30–8.20 (dd, 1H, C-6 H, $J_{6,7}$ = 8.90 Hz, $J_{6,4}$ = 2.08 Hz), 7.60–7.50 (d, 1H, C-7 H, $J_{7,6}$ = 8.90 Hz), 7.46–7.22 (m, 5H, phenyl protons), 4.33 (s, 2H, CH_2)
5n	O	S	H	H	NO_2	57–58 (D)	32	2.5	115	3140, 2940, 1630–1579, 1535, 1425, 1345, 1275–1060, 960–695	8.30–7.80 (m, 2H, C-5 H and C-7 H), 7.60 (d, 1H, C-4 H, $J_{4,5}$ = 9.10 Hz), 7.39–7.20 (m, 5H, phenyl protons), 4.33 (s, 2H, CH_2)

(continued on next page)

Table 1
(continued)

Compound number	Y	Z	R	R_1	R_2	Mp (°C) (recrystallization solvent) [a]	Yield (%)	Reaction time (h)	Reaction temperature (°C)	IR (cm^{-1})	^{1}H-NMR δ ppm(J = Hz)
5o	O	S	H	Cl	NO_2	78–79 (B)	36	2.5	130	3125, 2970, 1630–1570, 1540, 1445, 1345, 1260–1000, 960–695	8.05 (s, 1H, C-7 H), 7.80 (s, 1H, C-4 H), 7.40–7.20 (s, 5H, phenyl protons), 4.30 (s, 2H, CH_2)
5p	O	S	H	$COOCH_3$	H	59–60 (B)	50	3	110	3120, 2995–2880, 1735, 1625–1585, 1430, 1260, 1220–1090, 975–690	8.37–8.34 (dd, 1H, C-4 H, $J_{4,6}$ = 1.60 Hz), 8.14–8.01 (dd, 1H, C-6 H, $J_{6,7}$ = 8.48 Hz, $J_{6,4}$ = 1.60 Hz), 7.50–7.10 (m, 6H, C-7 H and phenyl protons), 4.31 (s, 2H, CH_2), 3.94 (s, 3H, CH_3)
6a	S	O	H	H	H	77–78 (82–83) [33] (B)	72	3	140	3100, 2950, 1605–1500, 1445, 1260–1058, 940–670	8.00–7.80 (m, 2H, C-4 and (C-7 H), 7.40–6.90 (m, 7H, C-5 H, C-6 H and phenyl protons), 5.47 (s, 2H, CH_2)
6b	S	O	Cl	H	H	95–96 (83–86) [34] (118) [35] (B)	47	4	140	3100, 2950, 1605–1500, 1445, 1260–1050, 820–658	8.09–7.39 (m, 4H, C-4 H, C-5 H, C-6 H and C-7 H), 7.30–7.20 (dd, 2H, C-3′ H and C-5′ H, $J_{3',2'}$ and $J_{5',6'}$ = 9.11 Hz) 6.90 (dd, 2H, C-2′ H and C-6′ H, $J_{2',3'}$ and $J_{6',5'}$ = 9.11 Hz) 5.45 (s, 2H, CH_2)
6c	S	S	H	H	H	43–44 (41–42) [33] (B)	43	3.5	140	3100, 2940, 1583–1480, 1435, 1250–1030, 935–695	7.90–7.10 (m, 9H, C-4 H, C-5 H, C-5 H, C-6 H, C-7 H and phenyl protons), 4.46 (s, 2H, CH_2)
7a	NH	O	H	H	H	159–161 (160–161) [35] (A)	94	2.5	100	3095–2700, 1605–1500, 1449, 1340–1020, 922–695	7.67–6.99 (m, 9H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons), 5.35 (s, 2H, CH_2)
7b	NH	O	H	Cl	H	128–129 (129) [36] (A)	50	5	100	3100–2700, 1600–1500, 1450, 1350–1035, 925–695	7.60–6.90 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 5.29 (s, 2H, CH_2)
7c	NH	O	H	NO_2	H	185–186 (186–187) [36] (A)	37	2	100	3340, 3100, 2950, 1630–1555, 1510, 1470, 1345, 1250–1050, 1000–655	8.20–7.60 (m, 2H, C-4 H and C-6 H) 7.30–6.80 (m, 6H, C-7 H and phenyl protons), 5.20 (s, 2H, CH_2)
7d	NH	O	H	CH_3	H	167–168 (170) [36] (A)	19	5	100	3080–2680, 1600–1500, 1460, 1310–1030, 945–695	7.60–6.90 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 5.30 (s, 2H, CH_2), 2.44 (s, 3H, CH_3).
7e	NH	O	H	$COOCH_3$	H	82–83	32	3	120	3600, 3080, 2980–2880, 1729, 1630–1500, 1440, 1240, 1180–1040, 1000–695	8.20–7.00 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 4.60 (s, 2H, CH_2), 3.90 (s, 3H, CH_3)
7f	NH	O	Cl	H	H	178 (178–180) [37] (D)	29	20	100	3100–2700, 1600–1495, 1440, 1355–1055, 825–645	7.60–6.80 (m, 8H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons), 5.36 (s, 2H, CH_2)
7g	NH	O	Cl	Cl	H	182–183 (186) [36] (A)	35	19	100	3120–2650, 1620–1495, 1440, 1350–1010, 925–660	7.70 6.90 (m, 7H, C-4 H, C-7 H and phenyl protons), 5.29 (s, 2H, CH_2)
7h	NH	O	Cl	CH_3	H	150–151(148) [36] (E)	14	14	100	3100–2700, 1630–1500, 1460, 1300–1020, 970–650	7.42–6.54 (m, 7H, C-4 H, C-6 H, C-7 H and phenyl protons), 5.31 (s, 2H, CH_2), 2.45 (s, 3H, CH_3)
7i	NH	O	Cl	$COOCH_3$	H	97–98	56	3.5	130	3370, 3100, 2980–2870, 1712, 1630–1500, 1440, 1365, 1320–1040, 975–670	7.88–7.00 (m, 7H, C-4 H, C-6 H, C-6 H, C-7 H and phenyl protons), 5.30 (s, 2H, CH_2), 3.90 (s, 3H, CH_3)
7j	NH	S	H	H	H	132–133 (135–136) [32] (A)	40	4	115	3100, 3000–2500, 1600–1520, 1440, 1300–1020, 1020–670	7.50–7.07 (m, 9H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons), 4.36 (s, 2H, CH_2)
7k	NH	S	H	NO_2	H	58–60 (A)	28	5	100	3400–2800, 1630–1590, 1475, 1345, 1200–1020, 950–695	8.40 (d, 1H, C-4 H), 7.60 (dd, 1H, C-6 H, $J_{6,7}$ = 7.2 Hz, $J_{6,4}$ = 2.32 Hz), 7.40–7.10 (m, 6H, C-7 H and phenyl protons), 4.38 (s, 2H, CH_2)
7m	NH	S	H	$COOCH_3$	H	142–143	24	2	120	3100–2600, 1730, 1630–1510, 1440, 1320, 1250–1025, 970–700	8.19–7.30 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 4.70 (s, 2H, CH_2), 3.95 (s, 3H, CH_3)
7n	NH	NH	H	H	H	159–160 (163) [38] (D)	47	40	100	3490, 3100–2650, 1600–10 500, 1420, 1340–1060, 1000–695	δ 7.50–6.30 (m, 9H, C-4 H, C-5 H, C-6 H, C-7 H and phenyl protons), 4.54 (s, 2H, CH_2)
7o	NH	NH	H	CH_3	H	76–77 (F)	41	60	100	3340, 3100–2800, 1600–1510, 1450, 1315–1015, 940–695	7.60–6.50 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 4.51 (s, 2H, CH_2), 2.40 (s, 3H, CH_3)
7p	NH	CH_2	H	Cl	H	123–124 (D)	17	4	100	3150–2700, 1635–1500, 1440, 1335–1020, 930–700	7.50–6.90 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 3.19 (s, 4H, CH_2)

[a] (A), EtOH/H_2O; (B), CH_2Cl_2/hexane; (C), diethyl ether/petroleum ether; (D), benzene/petroleum ether; (E), MeOH/H_2O; (F), $CHCl_3$/hexane.

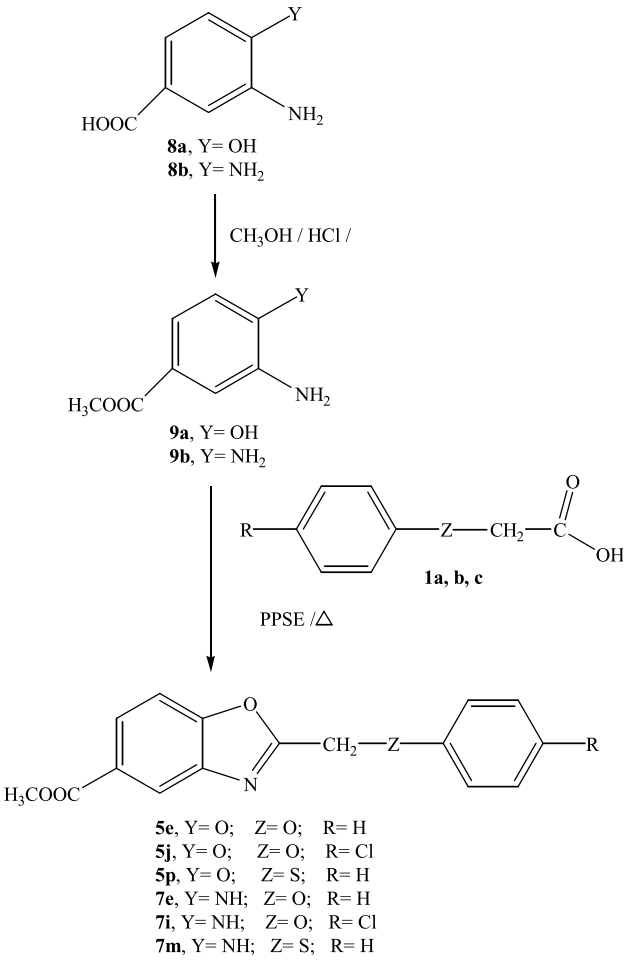


Scheme 1

The reaction mixtures were protected from moist air by means of a calcium chloride drying tube and stirred magnetically. The compounds **5, 6**, and **7** were synthesized as new products except **5a, 5c, 5f, 5k, 6a–c, 7a–d, 7f–7h**, and **7j** [30–38]. The structures of known compounds were supported only by ^{1}H-NMR spectral and elemental analyses data that are in agreement with the proposed structures. All TLC was run on Kieselgel HF_{254} chromatoplates (0.3 mm) with a fluorescent indicator, employing variety of solvents for routine monitoring of reaction mixtures and confirming the homogeneity of analytical samples. Merck silica gel (230–400 mesh) and $CHCl_3$ as a solvent were used for flash column chromatographic separations. Melting points were obtained on a Mel-Temp (Buchi SMP 20) capillary melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP-1025 with KBr pellets. ^{1}H-NMR spectra were obtained with a Bruker AC 80 MHz spectrometer and TMS was used as an internal standard. Elemental analyses were carried out with a Perkin-Elmer model 240-C apparatus. The results of the elemental analyses (C, H, N) were within ±0.4% of the calculated amounts.

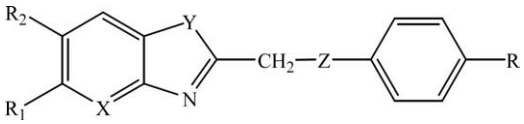
*4.1.1. Synthesis of methyl 3-amino-4-hydroxybenzoate (**9a**) and methyl 3,4-diaminobenzoate (**9b**)*

For the esterification, dry HCl treated over 30 ml MeOH until the weight increased 10%, and the compounds **8a** or **b** (3 g, 19.6 or 19.7 mmol) were added to the mixture and heated under reflux with stirring for 4 h [39]. The mixture was dissolved by adding water and the crude product **9a** or **b** was precipitated after the reaction mixture was neutralized with K_2CO_3. The precipitate was filtered, washed with water



Scheme 2

Table 2
Antimicrobial activity results (MIC, μg/ml) of the newly (**5–7**) and previously (**10–12**) [26,27] synthesized compounds with the standard drugs



							Microorganisms [a]						
							Gram-positive			Gram-negative			Fungus
Compound number	X	Y	Z	R	R_1	R_2	*S.a.*	*S.f.*	*B.s.*	*E.c.*	*K.p.*	*P.a.*	*C.a.*
5a	CH	O	O	H	H	H	25	50	12.5	50	25	50	25
5b	CH	O	O	H	NO_2	H	25	50	25	25	25	50	25
5c	CH	O	O	H	Cl	H	25	50	6.25	50	25	25	25
5d	CH	O	O	H	H	NO_2	25	100	12.5	50	25	50	25
5e	CH	O	O	H	$COOCH_3$	H	25	50	25	50	50	50	25
5f	CH	O	O	Cl	H	H	50	50	50	50	25	50	25
5g	CH	O	O	Cl	NO_2	H	50	50	25	25	25	50	25
5h	CH	O	O	Cl	H	NO_2	50	50	12.5	50	25	50	25
5i	CH	O	O	Cl	Cl	NO_2	50	50	12.5	50	25	50	25
5j	CH	O	O	Cl	$COOCH_3$	H	50	50	25	50	50	50	25
5k	CH	O	S	H	H	H	50	50	25	50	25	50	12.5
5m	CH	O	S	H	NO_2	H	50	50	12.5	25	25	50	12.5
5n	CH	O	S	H	H	NO_2	50	50	6.25	50	25	50	12.5
5o	CH	O	S	H	Cl	NO_2	50	50	25	50	25	25	12.5
5p	CH	O	S	H	$COOCH_3$	H	50	50	50	50	50	50	12.5
6a	CH	S	O	H	H	H	3.12	50	25	50	25	25	50
6b	CH	S	O	Cl	H	H	6.25	50	25	50	25	25	50
6c	CH	S	S	H	H	H	6.25	50	12.5	50	25	25	25
7a	CH	NH	O	H	H	H	25	50	25	50	25	50	25
7b	CH	NH	O	H	Cl	H	25	50	25	50	25	25	12.5
7c	CH	NH	O	H	NO_2	H	25	50	50	50	25	50	12.5
7d	CH	NH	O	H	CH_3	H	50	50	50	50	25	50	25
7e	CH	NH	O	H	$COOCH_3$	H	25	50	50	50	50	50	25
7f	CH	NH	O	Cl	H	H	50	50	25	50	25	25	25
7g	CH	NH	O	Cl	Cl	H	50	50	25	50	25	25	12.5
7h	CH	NH	O	Cl	CH_3	H	50	50	50	50	25	50	25
7i	CH	NH	O	Cl	$COOCH_3$	H	50	50	50	50	50	50	25
7j	CH	NH	S	H	H	H	50	50	12.5	50	25	50	12.5
7k	CH	NH	S	H	NO_2	H	50	50	25	25	25	50	12.5
7m	CH	NH	S	H	$COOCH_3$	H	50	50	25	50	50	50	12.5
7n	CH	NH	NH	H	H	H	50	100	6.25	50	25	50	25
7o	CH	NH	NH	H	CH_3	H	50	100	12.5	50	25	50	25
7p	CH	NH	CH_2	H	Cl	H	25	50	12.5	25	25	25	25
10a	CH	O	O	H	CH_3	H	25	25	25	25	25	25	25
10b	CH	O	O	H	H	CH_3	25	50	25	50	25	50	25
10c	CH	O	O	H	Cl	NO_2	25	50	12.5	50	25	25	25
10d	CH	O	O	Cl	Cl	H	50	50	12.5	50	25	25	25
10e	CH	O	O	Cl	CH_3	H	50	50	25	50	12.5	50	50
10f	CH	O	O	Cl	H	CH_3	50	50	25	50	25	50	50
10g	CH	O	S	H	Cl	H	50	50	6.25	50	25	25	12.5
10h	CH	O	S	H	CH_3	H	25	25	25	25	25	25	25
10i	CH	O	S	H	H	CH_3	50	50	12.5	50	25	50	25
11a	N	O	O	H	H	H	50	50	25	50	50	50	12.5
11b	N	O	O	Cl	H	H	100	50	25	50	50	50	12.5
12a	CH	NH	S	H	Cl	H	50	50	12.5	50	25	25	12.5
12b	CH	NH	S	H	CH_3	H	50	50	25	50	25	50	25
12c	CH	NH	CH_2	H	CH_3	H	25	50	12.5	50	25	50	12.5
Ampicillin							1.56	1.56	1.56	12.5	25	>200	–
Amoxycillin							1.56	1.56	1.56	3.12	12.5	>200	–
Tetracycline							1.56	1.56	1.56	3.12	3.12	50	–
Streptomycin							3.12	100	50	1.56	1.56	100	–
Clotrimazol							–	–	–	–	–	–	6.25
Haloprogin							–	–	–	–	–	–	3.12

[a] Abbreviations: *E.c., Escherichia coli; K.p., Klepsiella pneumoniae; P.a., Pseudomonas aeruginosa; S.a., Staphylococcus aureus; S.f., Streptococcus faecalis; B.s., Bacillus subtilis; C.a., Candida albicans*.

and recrystallized from aqueous EtOH and dried under vacuum over a night. **9a**: 76.5% yield; mp 109 °C (111 °C) [40], **9b**: 76% yield; mp 104 °C (108 °C) [41].

*4.1.2. General method for the synthesis of substituted benzoxazoles (**5a–p**), benzothiazoles (**6a–c**), 2-substituted-5-carboxymethyl-benzimidazoles (**7e, 7i, 7m**), method A*

A mixture of corresponding carboxylic acids **1a/b/** or **c** (5 mmol) and appropriate 4- and/or 5-disubstituted 2-aminophenols 2, or methyl 3-amino-4-hydroxybenzoate **9a** or 2-aminothiophenol **3** or methyl 3,4-diaminobenzoate **9b** (6.9 mmol) was heated under reflux with stirring at various temperatures and time in 15 ml PPSE. At the end of the reaction period, the mixture was taken to 30 ml dichloromethane and neutralized with 50 ml 1 N NaOH solution. The organic layer was separated and the aqueous solution extracted with 3 × 25 ml portions of CH_2Cl_2. The combined extracts were dried on Na_2SO_4, filtered and the solvent was removed with rotary evaporator. The residue was purified by flash chromatography, eluting with $CHCl_3$ and the obtained product was recrystallized.

*4.1.3. General method for the synthesis of substituted benzimidazoles (**7a–p**), method B*

A mixture of corresponding carboxylic acids **1a/b/c/d** or **e** (10 mmol) and appropriate 5-substituted-2-phenylendiamines **4** (10 mmol) was boiled under reflux with stirring for various time in 15 ml 6 N HCl. At the end of the reaction period, the mixture was neutralized with excess of $NaHCO_3$. The collected precipitate washed with water, dried in vacuum, purified by flash chromatography, eluting with $CHCl_3$ and recrystallized.

4.2. Microbiology

For determining both the antibacterial and the antifungal activity, the synthesized compounds and the control drugs were dissolved in absolute ethanol (0.8 mg/ml) [42]. Further dilutions were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/ml concentrations. The MIC were determined by using the method of twofold serial dilution technique [9,26,42,43]. 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 experiments and found inactive in culture medium. All the compounds were tested for their in vitro growth inhibitory activity against different bacteria and a fungus *C. albicans* RSKK 628. The origin of bacterial strains was *S. aureus* ATCC 6538, *S. faecalis* ATCC 10541, *B. subtilis* ATCC 6033 as Gram-positive and *E. coli* ATCC 10536, *K. pneumoniae* ATCC 52211, *P. aeruginosa* RSKK 355 as Gram-negative bacteria. The RSKK strains 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 Pharmacy, Ankara University.

4.2.1. Antibacterial assay

The cultures were obtained in Mueller-Hinton broth (Difco) for all the bacteria after 24 h of incubation at 37 ± 1 °C. Testing was carried out ion Mueller-Hinton broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^5 CFU/ml. A set of tubes containing only inoculated broth was kept as control. After incubation for 24 h at 37 ± 1 °C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/ml. Every experiment in the antibacterial assay was replicated twice in order to define the MIC values.

4.2.2. Antifungal assay

The yeast *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 twofold serial dilution technique was applied. The final inoculum size was 104 CFU/ml. A set of tubes containing only inoculated broth was kept as control. After incubation for 48 h at 25 ± 1 °C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/ml. Every experiment in the antifungal assay was replicated twice in order to define the MIC values.

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