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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 2-[4-(4-substitutedbenzamido/phenylacetamido) phenyl]benzothiazoles as antimicrobial agents

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Abstract A new series of 2-[4-(4-substitutedbenzamido/phenylacetamido)phenyl] benzothiazole derivatives (**6a–k**) were synthesized and evaluated for antibacterial and antifungal activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* with their drug-resistant isolates and a yeast *Candida albicans*. Microbiological results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms at minimum inhibitory concentration (MIC) values between 100 and 6.25 μg/ml. Compounds **6d** and **6k** exhibited significant antibacterial activity showing 6.25 μg/ml MIC values against drug-resistant *S. aureus* and *P. aeruginosa* isolates, respectively.

Keywords Benzothiazole · Antibacterial activity · Antifungal activity · Multi-drug resistance

Introduction

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria, and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotic drugs. The hospital-acquired infections are resistant to the most powerful antibiotics available, methicillin and vancomycin. These drugs are reserved to treat only the most intractable infections to slow development of resistance to them (Fridkin and Gaynes, 1999). So, there is still need for the new classes of antimicrobial agents.

The compounds which possess benzothiazole nucleus in their structure are involved in research aimed at evaluating new chemotherapeutically active agents, such as antimicrobial (Trapani *et al.*, 1994; Yalçin *et al.*, 1992; Yildiz-Oren *et al.*, 2004; Küçükbay and Durmaz, 1997), a topical carbonic anhydrase inhibitor (Kalina *et al.*, 1988), a cyclooxygenase inhibitor (Paramashivappa *et al.*, 2003), antitubercular (Koc *et al.*, 2002; Katz, 1953), anti-nematode (Surin, 1995), a dual inhibitor of thromboxane A_2 synthetase and 5-lipoxygenase (Komatsu and Minami, 1995), a selective and reversible inhibitor of monoamine oxidase type A (MAO-A) (Kagaya *et al.*, 1996), antiallergic (Ager *et al.*, 1988), multi-drug resistance cancer cell activities with inhibiting activity on eukaryotic topoisomerase II enzyme in cell-free system (Pinar *et al.*, 2004; Temiz-Arpaci *et al.*, 2005; Tekiner-Gulbas *et al.*, 2006), and antitumor agents (Shi *et al.*, 1996; Hutchinson *et al.*, 2002; Chua *et al.*, 1999).

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 (Shi *et al.*, 1996). Among them, lysylamide of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (Structure 1) had been selected for phase 1 clinical evaluation (Hutchinson *et al.*, 2002).

In the last years, we reported the synthesis of several 2-substitutedbenzothiazole derivatives as the antimicrobial agents (Yalçin *et al.*, 1992; Yildiz-Oren *et al.*, 2004) as seen in Structure 2. According to these studies, the compounds were found to have inhibitory effect with minimum inhibitory concentration (MIC) value of 3.12–50 μg/ml

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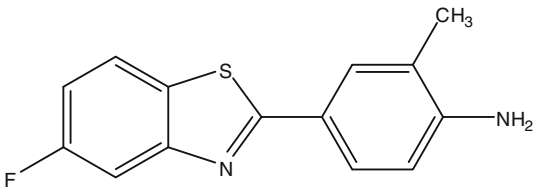


Structure 1

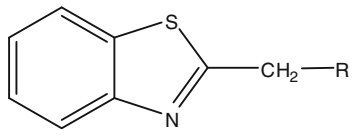
against some of Gram-positive, Gram-negative bacteria, and *Candida albicans* as yeast. Among the tested compounds, 2-(phenoxymethyl)benzothiazole was found as the most active derivative at a MIC value of 3.12 μg/ml against the tested *S. aureus* (Yildiz-Oren *et al.*, 2004). Moreover, the same compound was found very potent as an eukaryotic topoisomerase II inhibitor exhibiting a better inhibitor activity than reference drug etoposide (Pinar *et al.*, 2004; Temiz-Arpaci *et al.*, 2005; Tekiner-Gulbas *et al.*, 2006).

The goal of outset of this research is to develop new effective antimicrobial agents possessing benzothiazole nuclei in their structure. Herein, we have described the synthesis a series of 2-[4-(4-substitutedbenzamido/phenylacetamido)phenyl]-benzothiazole derivatives (Structure 3) as a new class of synthetic antimicrobial agents along with their in vitro antimicrobial activity tested against some Gram-negative, Gram-positive bacteria, and the drug resistance isolates as well as the yeast *C. albicans*. The tested compounds are synthesized as possessing phenyl acetamide or benzamide moiety holding different substituents on position R showing various physicochemical properties, such as electron donating, electron withdrawing, and steric effects to be able to discuss the effect of the substituent on the activity.

Experimental procedures

Chemistry

The chemicals were purchased from the commercial venders and were used without purification. The reactions were monitored and the purity of the products was checked by thin layer chromatography (TLC). Silicagel HF254 chromatoplates (0.3 mm) were used for TLC and the solvent systems were *n*-hexane:ethyl acetate (2:1) for 2-(4-aminophenyl)benzothiazole (**3**) and chloroform:methanol (20:1) for compounds **6a–k**. Melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by FT/IR-420 in KBr disks. ^1H-NMR spectra were obtained with a Varian Mercury 400 High Performance Digital FT-NMR-400 MHz spectrometer in d_6-DMSO, tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out with CHNS-932 (LECO) apparatus. The results (C, H, N) were within ±0.4% of the calculated values. Mass analysis was obtained by Waters 2695 Alliance ZQ Micromass LC–MS working with ESI apparatus. All of the synthesized compounds are original except **6f** (Dilworth *et al.*, 2007).



R = cyclohexylmethyl, phenylmethyl, phenoxy, *p*-chlorophenoxy, phenylmercaptoxy

Structure 2

General procedure for the synthesis of 2-(4-aminophenyl)benzothiazole (**3**)

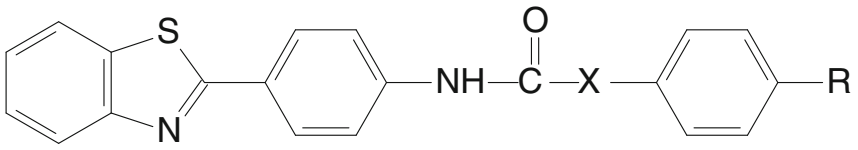
2-(4-Aminophenyl)benzothiazole (**3**) was prepared by heating 1 mmol *o*-aminothiophenol (**1**) with 1 mmol *p*-aminobenzoic acid (**2**) in 2.4 g polyphosphoric acid and stirring for 4 h (Scheme 1). After then, the residue was poured into ice–water mixture and neutralized with excess of 10% NaOH solution. The precipitate was boiled with activated charcoal in ethanol and then, filtered and recrystallized in ethanol (M.p.: 155–157°C) (Hein *et al.*, 1957; Stevens *et al.*, 1995).

General procedure for the synthesis of compounds (**6a–k**)

The final products, 2-[4-(4-substitutedbenzamido/phenylacetamido)phenyl]benzothiazole derivatives (**6a–k**) were synthesized by heating thionyl chloride (0.3 ml) and appropriate carboxylic acid (**4**) (1 mmol) in benzene (0.5 ml) at 80°C for 3 h, and then excess thionyl chloride was removed in vacuo (Scheme 2). The residue was dissolved in ether (1 ml) and the solution was added during 1 h to a stirred, ice-cold mixture of 2-(4-aminophenyl) benzothiazole (1 mmol), sodium bicarbonate (2 mmol), diethylether (1 ml), and water (1 ml) (Yalcin *et al.*, 1997). The mixture was stirred overnight at room temperature and then filtered. After then, the precipitate was washed with water, 2N HCl, and water. Ethanol was used for recrystallization and crystals are dried in vacuo.

*2-[4-(4-Fluorobenzamido)phenyl]benzothiazole (**6a**)*

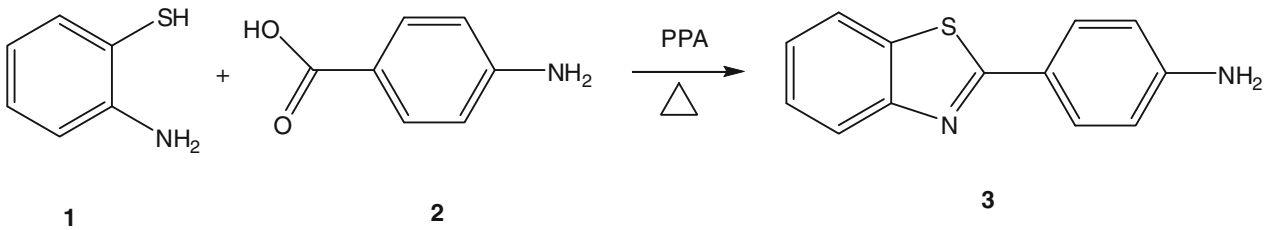
Yield: 27% mp 259–261°C. IR (cm^{-1}): 3351, 3039, 1653 (Amide I), 1602–1589, 1534 (Amide II),1502, 1483, 1434, 1232, 970–600, 728–620. ^1H NMR (400 MHz, $CDCl_3$):



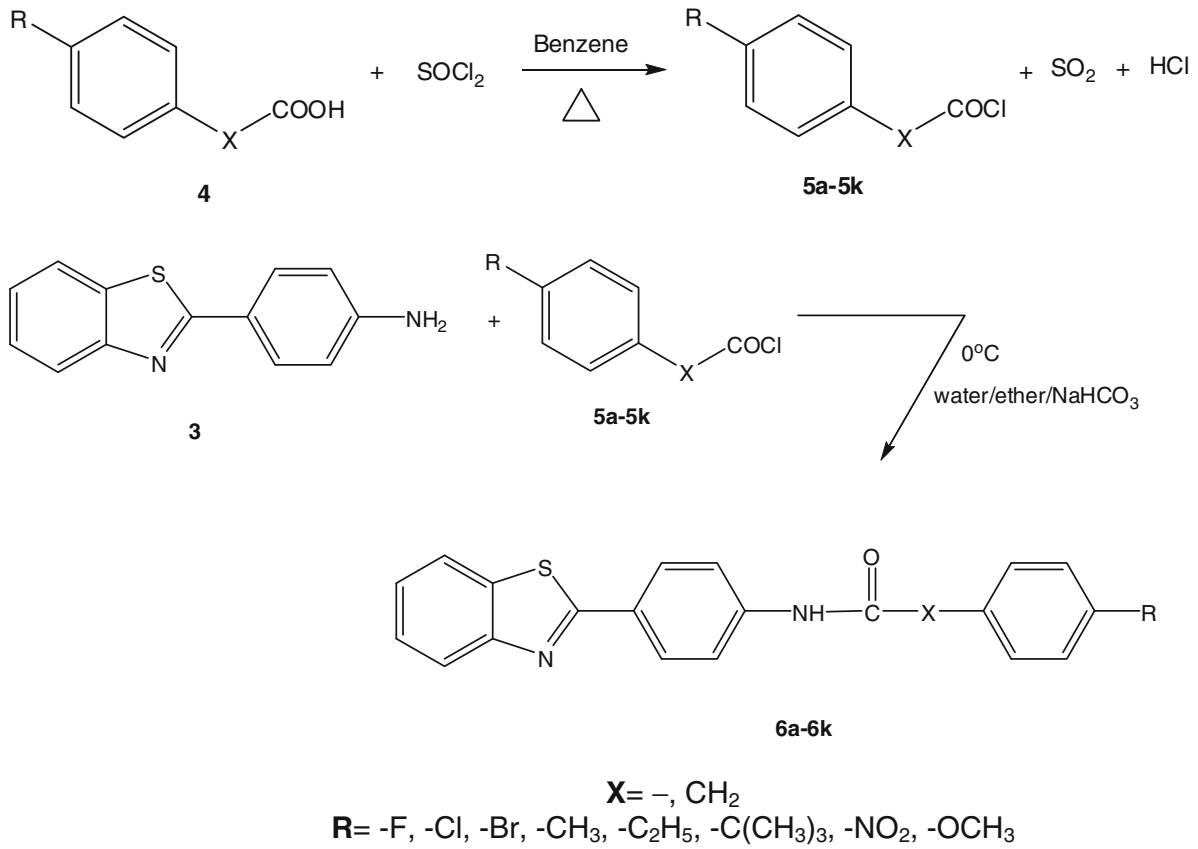
X= –, -CH_2-

R= -F, -Cl, -Br, -CH_3, -C_2H_5, -$C(CH_3)_3$, -NO_2, -OCH_3

Structure 3



Scheme 1 The synthesis of 2-(4-aminophenyl)benzothiazole (**3**)



X= –, CH_2

R= -F, -Cl, -Br, -CH_3, -C_2H_5, -$C(CH_3)_3$, -NO_2, -OCH_3

Scheme 2 The synthesis of 2-[4-(4-substitutedbenzamido/phenylacetamido)phenyl]-benzothiazole derivatives (**6a–k**)

7.258 (t, 2H), 7.305 (t, 1H), 7.399 (t, 1H), 7.853–8.001 (m, 8H), 10,45 (s, 1H). ESI (+) *m/e*: 348,88 (M^+, 68%), 349,16 (M^+ + H, 100%). Anal. Found: C, 68.89; H, 3.775; N, 8.171; S, 9.166. Calcd. for $C_{20}H_{13}FN_2OS$: C, 68.95; H, 3.761; N, 8.041; S, 9.204.

*2-[4-(4-Chlorobenzamido)phenyl]benzothiazole (**6b**)*

Yield: 35% mp 278–280°C. IR (cm^{-1}): 3358, 3054–2918, 1653 (Amide I), 1591, 1532 (Amide II), 1511, 1481, 1434, 1092, 969–606, 728–620. ^1H NMR (400 MHz, $CDCl_3$):

7.458 (t, 1H), 7.549 (t, 1H), 7.648 (dd, 2H), 8.002–8.058 (m, 5H), 8.122 (d, 2H), 8.153 (s, 1H), 10.628 (s, 1H. ESI (+) *m/e*: 364.88 (M^+, 61%), 365.09 ($M^+ + H$, 100%), 367,06 ($M^+ + H + 2$, 29%). Anal. Found: C, 66.17; H, 3.592; N, 7.818; S, 8.809. Calcd. for $C_{20}H_{13}ClN_2OS$: C, 65.84; H, 3.591; N, 7.678; S, 8.789.

*2-[4-(4-Bromobenzamido)phenyl]benzothiazole (**6c**)*

Yield: 32% mp 283–285°C. IR (cm^{-1}): 3364, 2923–2852, 1657 (Amide I), 1587, 1530 (Amide II), 1517, 1478, 1434, 1072, 966–607, 731–620. ^1H NMR (400 MHz, $CDCl_3$): 7.457 (t, 1H), 7.548 (t, 1H), 7.787 (dd, 2H), 7.955 (dd, 2H), 8.002–8.057 (m, 3H), 8.108–8.154 (m, 3H), 10.642 (s, 1H). ESI (+) *m/e*: 409.06 (M^+, 91%), 411.06 ($M^+ + 2$, 91%). Anal. Found: C, 57.42; H, 3.239; N, 6.769; S, 7.820. Calcd. for $C_{20}H_{13}BrN_2OS$–0.4 HOH: C, 57.67; H, 3.340; N, 6.726; S, 7.700.

*2-[4-(4-Ethylbenzamido)phenyl]benzothiazole (**6d**)*

Yield: 43% mp 238–240°C. IR (cm^{-1}): 3347, 3052, 2957, 2872, 2924, 2851, 1656 (Amide I), 1591, 1529 (Amide II), 1514, 1479, 1434, 967–622, 727–622. ^1H NMR (400 MHz, $CDCl_3$): 1.229 (t, 3H), 2.706 (q, 2H), 7.402 (d, 2H), 7.457 (t, 1H), 7.549 (t, 1H), 7.937 (d, 2H), 8.018–8.156 (m, 6H), 10.510 (s, 1H). ESI (+) *m/e*: 359.16 ($M^+ + H$, 100%). Anal. Found: C, 72.88; H, 4.995; N, 7.840; S, 8.596. Calcd. for $C_{22}H_{18}N_2OS$–0.2 HOH: C, 72.98; H, 5.122; N, 7.737; S, 8.857.

*2-[4-(4-tert-Butylbenzamido)phenyl]benzothiazole (**6e**)*

Yield: 37% mp 228–230°C. IR (cm^{-1}): 3385, 3060, 2954, 1656 (Amide I), 1604–1585, 1529 (Amide II),1500, 1482, 1434, 967–580, 728–622. ^1H NMR (400 MHz, $CDCl_3$): 1.334 (s, 9H), 7.452 (t, 1H), 7.544–7.587 (m, 3H), 7.929 (d, 2H), 8.009–8.054 (m, 3H), 8.098–8.150 (m, 3H), 10.510 (s, 1H). ESI (+) *m/e*: 387.17 ($M^+ + H$, 100%). Anal. Found: C, 74.36; H, 5.642; N, 7.239; S, 8.074. Calcd. for $C_{24}H_{22}N_2OS$: C, 74.58; H, 5.737; N, 7.248; S, 8.296.

*2-[4-(4-Nitrobenzamido)phenyl]benzothiazole (**6f**) (Dilworth et al., 2007)*

Yield: 25% mp 296–298°C. IR (cm^{-1}): 3350, 2919–2851, 1653 (Amide I), 1599–1588, 1526 (Amide II), 1489, 1477, 1434, 1347, 968–603, 713–620. ^1H NMR (400 MHz, $CDCl_3$): 7.460 (t, 1H), 7.551 (t, 1H), 8.013–8.061 (m, 3H), 8.130–8.151 (m, 3H), 8.228 (d, 2H), 8.400 (d, 2H), 10.873 (s, 1H). ESI (+) *m/e*: 376.12 ($M^+ + H$, 100%). Anal. Found: C, 63.51; H, 3.477; N, 10.970; S, 8.161. Calcd. for $C_{20}H_{13}N_3O_3S$–0.2 HOH: C, 63.38; H, 3.564; N, 11.087; S, 8.461.

*2-[4-(4-Fluorophenylacetamido)phenyl]benzothiazole (**6g**)*

Yield: 56% mp 218–220°C. IR (cm^{-1}): 3269, 3028, 2919, 2850, 1664 (Amide I), 1608, 1532 (Amide II),1511, 1484, 1434, 1218, 971–610, 727–625. ^1H NMR (400 MHz, $CDCl_3$): 3.695 (s, 2H), 7.159 (t, 2H), 7.376 (t, 2H), 7.425 (t, 1H), 7.518 (t, 1H), 7.798 (d, 2H), 8.010 (d, 1H), 8.043 (d, 2H), 8.112 (d, 1H), 10.529 (s, 1H). ESI (+) *m/e*: 363.17 ($M^+ + H$, 100%). Anal. Found: C, 68.99; H, 4.125; N, 7.731; S, 8.688. Calcd. for $C_{21}H_{15}FN_2OS$–0.1 HOH: C, 69.25; H, 4.206; N, 7.691; S, 8.804.

*2-[4-(4-Chlorophenylacetamido)phenyl]benzothiazole (**6h**)*

Yield: 58% mp 229–231°C. IR (cm^{-1}): 3305, 3049, 2923, 2850, 1659 (Amide I), 1601, 1544 (Amide II), 1532, 1484, 1433, 1091, 973–585, 721–620. ^1H NMR (400 MHz, $CDCl_3$): 3.708 (s, 2H), 7.369 (m, 4H), 7.420 (t, 1H), 7.514 (t, 1H), 7.798 (d, 2H), 8.008 (d, 1H), 8.044 (d, 2H), 8.104 (d, 1H), 10.549 (s, 1H). ESI (+) *m/e*: 379.01 ($M^+ + H$, 100%), 381.15 ($M^+ + H + 2$, 44%). Anal. Found: C, 66.14; H, 3.982; N, 7.460; S, 8.206. Calcd. for $C_{21}H_{15}ClN_2OS$–0.1 HOH: C, 66.25; H, 4.025; N, 7.359; S, 8.423.

*2-[4-(4-Bromophenylacetamido)phenyl]benzothiazole (**6i**)*

Yield: 55% mp 224–226°C. IR (cm^{-1}): 3320, 3047, 2924, 2851, 1658 (Amide I), 1601, 1544 (Amide II),1532, 1483, 1433, 1071, 964–608, 721–620. ^1H NMR (400 MHz, $CDCl_3$): 3.689 (s, 2H), 7.303 (d, 2H), 7.428 (t, 1H), 7.501–7.538 (m, 3H), 7.798 (dd, 2H), 8.011 (d, 1H), 8.044 (dd, 2H), 8.115 (d, 1H), 10.536 (s, 1H). ESI (+) *m/e*: 423.07 (M^+, 80%), 425.02 ($M^+ + 2$, 100%). Anal. Found: C, 59.65; H, 3.618; N, 6.653; S, 7.516. Calcd. for $C_{21}H_{15}BrN_2OS$: C, 59.58; H, 3.572; N, 6.618; S, 7.575.

*2-[4-(4-Methylphenylacetamido)phenyl]benzothiazole (**6j**)*

Yield: 64% mp 227–229°C. IR (cm^{-1}): 3252, 3052, 3180, 3108, 2924, 2853, 1659 (Amide I), 1598, 1541 (Amide II), 1515, 1480, 1433, 962–592, 720–619. ^1H NMR (400 MHz, $CDCl_3$): 2.282 (s, 3H), 3.646 (s, 2H), 7.145 (d, 2H), 7.242 (d, 2H), 7.439 (t, 1H), 7.531 (t, 1H), 7.810 (d, 2H), 8.023 (d, 1H), 8.053 (d, 2H), 8.125 (d, 1H), 10.497 (s, 1H). ESI (+) *m/e*: 359.16 ($M^+ + H$, 100%). Anal. Found: C, 73.57; H, 4.972; N, 7.832; S, 8.809. Calcd. for $C_{22}H_{18}N_2OS$: C, 73.71; H, 5.061; N, 7.815; S, 8.946.

*2-[4-(4-Methoxyphenylacetamido)phenyl]benzothiazole (**6k**)*

Yield: 28% mp 198–199°C. IR (cm^{-1}): 3327, 3032, 2963, 2927, 2910, 2832, 1670 (Amide I), 1608–1584, 1527 (Amide II), 1513, 1485, 1437, 1244, 1032, 970–593, 733–620. ^{1}H NMR (400 MHz, $CDCl_3$): 3.606 (s, 2H), 3.720 (s, 3H), 6.891 (d, 2H), 7.259 (d, 2H), 7.425 (t, 1H), 7.518 (t, 1H), 7.794 (d, 2H), 8.009 (d, 1H), 8.037 (d, 2H), 8.112 (d, 1H), 10.459 (s, 1H). ESI (+) *m/e*: 375.11 ($M^+ + H$, 100%). Anal. Found: C, 70.29; H, 4.891; N, 7.390; S, 8.384. Calcd. for $C_{22}H_{18}N_2O_2S$–0.1 HOH: C, 70.23; H, 4.876; N, 7.445; S, 8.522.

Microbiology

Microorganisms

Klebsiella pneumoniae isolate, which has an extended spectrum beta lactamase enzyme (ESBL), *Pseudomonas aeruginosa* isolate (gentamicin-resistant), *Escherichia coli* isolate, which has an extended spectrum beta lactamase enzyme (ESBL), *Bacillus subtilis* isolate (resistant to ceftriaxon), *Staphylococcus aureus* isolate [methicillin-resistant (MRSA)], *K. pneumoniae* RSKK 574 (Refik Saydam National Public Health Agency Culture Collection), *P. aeruginosa* ATCC 25853 (American Type Culture Collection), *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231.

Methods

Standard strains of *K. pneumoniae* RSKK 574, *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, and clinical isolates of these microorganisms resistant to various antimicrobial agents were included in the study. Resistance was determined by Kirby Bauer Disk Diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (2006a) in the clinical isolates.

Standard powders of ampicillin trihydrate, gentamycin sulfate, rifampicin, ofloxacin, fluconazole, and amphotericin B were obtained from the manufacturers. Stock solutions were dissolved in dimethylsulfoxide (ofloxacin), pH 8 phosphate buffer saline (PBS) (ampicillin trihydrate) methanol (rifampicin), and distilled water (gentamicin sulfate, fluconazole, and amphotericin B). Newly synthesized compounds (**6a–k**) were dissolved in 80% DMSO–20% EtOH.

Bacterial isolates were subcultured in Mueller–Hinton Agar (MHA) plates and incubated over night at 37°C and *C. albicans* was subcultured in Sabouraud dextrose agar (SDA) plates at 35°C for 24–48 h. The microorganisms were passaged at least twice to ensure purity and viability.

The solution of the newly synthesized compounds (**6a–k**) and standard drugs were prepared at 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.78, 0.39, 0.19, 0.095, 0.047, and 0.024 μg/ml concentrations, in the wells of microplates by diluting in Mueller–Hinton broth (MHB).

Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S16 (2006b). The bacterial suspensions used for inoculation were prepared at 10^5 CFU/ml by diluting fresh cultures at MacFarland 0.5 density (10^7 CFU/ml). Suspensions of the bacteria at 10^5 CFU/ml concentration were inoculated to the twofold diluted solution of the compounds. There were 10^4 CFU/ml bacteria in the wells after inoculations. MHB was used for diluting the bacterial suspension and for twofold dilution of the compound. 80% DMSO–20% EtOH, methanol, DMSO, PBS, pure microorganisms, and pure media were used as control wells. A 10 μl bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37°C and MIC endpoints were read after 24 h of incubation.

Candida albicans was subcultured in SDA plates, and incubated at 35°C for 24–48 h before antifungal susceptibility testing. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered pH 7 with 3-[*N*-morpholino]-propansulfonic acid (MOPS) and culture suspensions were prepared through the guideline of CLSI M27-A3 (2006c). Yeast suspensions were prepared according to McFarland 0.5 density and a working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension (2.5×10^3 CFU/ml). A 10 μl yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35°C and MIC end points were read after 48 h of incubation.

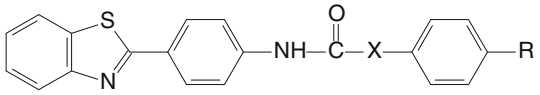
All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and MICs are reported in Table 1.

Results and discussion

Chemistry

The synthetic pathways for preparation of the target compounds are shown in Schemes 1 and 2. The final compounds were easily obtained in two steps. In the first, 2-(4-aminophenyl)benzothiazole as a starting material was performed by condensing of appropriate aminophenols and suitable acids in polyphosphoric acid (Chua *et al.*, 1999; Hein *et al.*, 1957; Stevens *et al.*, 1995) (Scheme 1). In the second reaction, an amidification was done. For this reaction, 2-(4-aminophenyl)benzothiazole was treated with

Table 1 The antibacterial and antifungal activity (MIC in μg/ml) of the newly synthesized compounds (**6a–k**) with the control drugs



Compounds	X	R	K.p.	K.p.[a]	P.a.	P.a.[b]	E.c.	E.c.[c]	B.s.	B.s.[d]	S.a.	S.a.[e]	C.a.
6a	–	F	25	50	25	25	50	50	50	50	50	25	25
6b	–	Cl	50	25	25	25	50	50	50	50	50	12.5	25
6c	–	Br	50	50	25	25	50	50	50	100	50	25	25
6d	–	C_2H_5	25	50	25	25	25	50	50	50	50	6.25	12.5
6e	–	$C(CH_3)_3$	25	50	25	25	25	50	50	100	50	12.5	12.5
6f	–	NO_2	50	50	25	25	25	50	50	50	50	12.5	12.5
6g	CH_2	F	50	50	25	25	25	50	50	25	100	50	25
6h	CH_2	Cl	50	50	25	25	50	50	50	50	50	50	25
6i	CH_2	Br	50	50	12.5	25	25	50	50	50	50	25	12.5
6j	CH_2	CH_3	25	50	25	25	50	50	50	50	50	25	25
6k	CH_2	OCH_3	12.5	50	25	6.25	12.5	25	12.5	50	50	12.5	12.5
Ampicillin trihydrate			–	–	–	–	3.125	400	0.78	0.78	0.78	–	–
Gentamycin sulfate			0.39	6.25	1.562	50	0.78	12.5	0.39	0.19	0.78	6.25	–
Rifampicin			–	–	–	–	–	–	0.19	3.125	0.0225	1.562	–
Ofloxacin			0.19	3.125	6.25	50	0.19	6.25	0.19	3.125	0.19	1.562	–
Fluconazol			–	–	–	–	–	–	–	–	–	–	0.78
Amphotericin B			–	–	–	–	–	–	–	–	–	–	0.78

K.p. Klebsiella pneumoniae RSKK 574, P.a. Pseudomonas aeruginosa ATCC 25853, E.c. Escherichia coli ATCC 25922, B.s. Bacillus subtilis ATCC 6633, S.a. Staphylococcus aureus ATCC 25923, C.a. Candida albicans ATCC 10231

[a] *K. pneumoniae isolate,* [b] *P. aeruginosa isolate,* [c] *E. coli isolate,* [d] *B. subtilis isolate,* [e] *S. aureus isolate*

suitable carboxylic chloride obtained by treating suitable carboxylic acid with thionyl chloride to get compounds **6a–k** as given in Scheme 2. The structures were supported by spectral data. The IR, ^1H-NMR, mass spectra, and elemental analysis results are in agreement with the proposed structures.

According to the spectroscopic data of the final compounds the IR showed characteristic C=O stretching bands in the 1,653–1,670 cm^{-1} (amide I) and 1,530–1,500 cm^{-1} (amide II) regions, respectively. Besides, C=N stretching bands were observed in the 1,489–1,532 cm^{-1} region. In the ^1H-NMR spectra of the compounds **6a–k**, the signal of NH proton was observed at 10.459–10.873 ppm as a singlet band; benzylic CH_2 protons were observed at 3.606–3.708 ppm as a singlet band for compounds **6g–k**. Aromatic methyl proton appeared at 2.282 ppm (for compound **6j**) as a singlet band, as well. Besides, all the aromatic protons were observed at the expected regions. On the other hand, mass spectra of the compounds showed $M^+ + H$ peaks, since the electrospray ionization method was employed, in accordance with their formulas.

In vitro antibacterial and antifungal activity

All the newly synthesized 2-[4-(4-substituted-benzamido/phenylacetamido)phenyl]-benzothiazole derivatives (**6a–k**) were in vitro tested for antibacterial activity against *K. pneumoniae* RSKK 574, *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *K. pneumoniae* isolate, which has an extended spectrum beta lactamase enzyme (ESBL), *E. coli* isolate (ESBL), *P. aeruginosa* isolate (gentamicin-resistant) as Gram-negative bacteria, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *B. subtilis* isolate (resistant to ceftriaxon), *S. aureus* isolate [meticilline-resistant (MRSA)] as Gram-positive bacteria, and the antifungal activity was evaluated against *C. albicans* ATCC 10231. The standard agents, ampicillin trihydrate, gentamycin sulfate, rifampicin, and ofloxacin for antibacterial activity and fluconazole and amphotericin B for antifungal activity were also screened under identical conditions for quality control and comparison. The MIC values were determined by microdilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (2006b, c).

All the observed in vitro antimicrobial activity results of the tested compounds are given in Table 1. The synthesized compounds were found showing an antibacterial activity at MIC values between 6.25 and 50 μg/ml for Gram-negative bacteria. Among the tested compounds, 2-[4-(4-methoxyphenylacetamido)phenyl]benzothiazole (**6k**) was found as the most potent derivative at a MIC value of 6.25 μg/ml against the screened drug-resistant enteric Gram-negative rod *P. aeruginosa* isolate providing three-fold higher potency than the compared standard drugs, gentamycin sulfate and ofloxacin. It revealed that the compound having phenyl acetamide moiety by holding a methoxy group on position R (**6k**) was performed twofold better activity against drug-resistant *P. aeruginosa* isolate than the other tested compounds. Besides, the derivatives **6a–j** exhibited onefold better activity than the reference drugs. However, all the compounds displayed lower antibacterial activity against *P. aeruginosa* ATCC 25853 with MIC values between 12.5 and 25 μg/ml than the compared control drugs, gentamycin sulfate and ofloxacin. Moreover, the compound **6k** also indicated a better inhibitory effect than the other tested compounds against *K. pneumoniae* and *E. coli.*

According to Table 1, the synthesized compounds showed a broad spectrum of activity with MIC values 100–6.25 μg/ml against some Gram-positive bacteria, such as *S. aureus, B. subtilis*, and their drug-resistant isolates. Most of the compounds were found as showing one or twofold dilutions more antibacterial activity against the screened drug-resistant *S. aureus* isolate compared to the nonresistant *S. aureus* ATCC 25923 strain. The compound, 2-[4-(4-ethylbenzamido)phenyl]benzothiazole (**6d**), displayed the most potent inhibitory effect against the drug-resistant *S. aureus* isolate with MIC value of 6.25 μg/ml. In addition, derivative **6d** showed the same activity compared to gentamycin sulfate and displayed lower antibacterial activity than the standard drugs, rifampicin and ofloxacin against *S. aureus* isolate.

Although, derivative **6k** exhibited only significant activity with MIC value of 12.5 μg/ml but less active than the tested reference drugs ampicillin trihydrate, gentamicin sulfate, rifampicin, and ofloxacin against *B. subtilis*.

Moreover, all of the synthesized compounds exhibited a moderate antifungal activity for *C. albicans* with MIC values between 12.5 and 25 μg/ml. The derivatives **6d–f**, **6i**, and **6k** displayed onefold better potent inhibitory effect with MIC value of 12.5 μg/ml than the other synthesized compounds. It can be concluded that the compounds holding ethyl, *ter*-butyl or nitro groups instead of fluorine, chlorine, and bromine atoms on position R of phenyl ring at benzamido moiety play a role for increasing the potency against *C. albicans*. However, none of the newly synthesized compounds showed better antifungal activity against *C. albicans* than the compared standard drugs, fluconazol and amphotericin B.

According to the antimicrobial results given in Table 1, it reveals that the binding a phenylacetamide moiety at position 2 holding a methoxy group on position R in the benzothiazole nuclei is important for increasing the potency against Gram-negative bacteria especially against the drug-resistant *P. aeruginosa* isolate. On the other hand, the compound having a benzamide moiety at position 2 was found more effective for increasing potency against the drug-resistant Gram-positive *S. aureus* isolate.

These observations provide some predictions to design further antimicrobial active compounds before their synthesis following with QSAR and molecular modeling studies.

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