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Synthesis of novel *N*-substituted benzyl *N*-(1,3-benzothiazol-2-yl) acetamides and their *in vitro* antibacterial activities

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Abstract: The novel Schiff bases **3a**–**d** were synthesized by reacting 6-methyl-2-aminobenzothiazole and different substituted benzaldehydes. Afterwards, the obtained Schiff bases were reduced with $NaBH_4$ to form amine compounds **4a**–**d**. In the final step, reaction of the amine with chloroacetyl chloride gave the novel amide derivatives **5a**–**d**. The structures of the all novel synthesized compounds were characterized by FT-IR, ^1H-NMR, ^{13}C-NMR, ESI MS, HETCOR, 2D (^1H–^1H) COSY spectra and elemental analyses. The antimicrobial activities of the novel synthesized compounds, were tested against some Gram-positive and Gram-negative bacterial as well as fungal species and the results were discussed.

Keywords: Schiff base; benzothiazole-2-yl-amide; acylation; antibacterial activity; minimum inhibitory concentration.

INTRODUCTION

Compounds containing the azomethine group (–CH=N–) have been known as Schiff bases. Schiff bases can be prepared by the condensation reaction of a primary amine with an active carbonyl group. Schiff bases contain active azomethine groups (–CH=N–), so they are compounds widely studied. In some studies, Schiff bases have been reported to exhibit various antibacterial, antifungal, herbicidal and clinical activity.[1–3] Heterocyclic compounds are organic compounds that contain at least one heteroatom other than carbon such as sulfur, nitrogen, oxygen and phosphorus, which are within a cyclic carbon structure, noting that these atoms are either outside or inside the ring. Heterocyclic compounds are the most important class of organic compounds as they have excellent activity against many diseases.[4–6] Among these heterocyclic compounds are Schiff bases. For this reason, many heterocyclic Schiff base derivatives have

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been synthesized and reported to show biological activities such as fungicide, antibiotic, pesticide,[7,8] cytotoxic,[9] anticonvulsants,[10] anticancer[11] and antifungal activities.[12]

Benzothiazole derivatives are bicyclic heterocycles which act as a weak base formed in the benzene ring fused with 4- and 5-positions for the thiazole rings and these exhibit wide ranges of chemical activities. Compounds with a thiazole nucleus in their structure have been widely studied because this five-membered aromatic ring containing sulfur and nitrogen atoms plays a vital role in the structure of various drugs, including the antineoplastic agents thiazofurin and dasatinib.[13] Similarly substituted *N*-benzothiazol-2-yl-amides exhibit a wide variety of biological properties such as ubiquitin ligase inhibitors,[14a] antitumor,[14b] anti rotavirus,[14c] the adenosine receptor,[14d–e] and the nuclear hormone receptor.[14e] In particular, some benzothiazoles substituted at the 2-position with a benzoyl amino moiety showed antibacterial, antifungal and antitubercular activity.[14f]

In our present work, we report on: *i*) the synthesis of four novel benzothiazole derivated Schiff bases in varied solvents such as ethanol, methanol, tetrahydrofuran, toluene, acetonitrile and benzene as well as ethyl lactate; *ii*) obtaining novel amine compounds by reduction of Schiff bases, *iii*) synthesis of the corresponding novel amide, *iv*) spectroscopic characterization of the compounds obtained and *v*) the evaluation of their antimicrobial activity. Ethyl lactate is an important monobasic ester. It is a clear to slightly yellow liquid, and is found naturally in small quantities in a wide variety of foods, including wine, chicken, and some fruits. Traditional synthesis methods of Schiff bases have used petroleum-derived solvents such as toluene, which are often toxic. Ethyl lactate is an environmentally benign solvent with effectiveness comparable to petroleum-based solvents. Ethyl lactate has interesting properties such as being a solvent that is non-corrosive, non-carcinogenic, non-teratogenic, biodegradable and does not harm the ozone layer. Moreover, ethyl lactate forms a suitable solution with water for the synthesis of Schiff base. Additionally, this green solvent provides many advantages, such as a catalyst-free protocol, short reaction times, simple operation and processing of products without the need for chromatography.

EXPERIMENTAL

General procedures

Melting point: Gallenkamp apparatus. IR spectra: Perkin Elmer Precisely Spectrum 100 FT-IR spectrophotometer; in KBr; $\tilde{v}$ in cm^{-1}. ^{1}H-NMR, ^{13}C-NMR, 2D (^{1}H–^{1}H) COSY and HETCOR spectra: Bruker DPX FT NMR (500 MHz) and (125 MHz) spectrometer; in TMS (DMSO-d_6); δ in ppm relative to Me_4Si as the internal standard, J in Hz. ESI MS: (LS/MS-APCl) AGILENT 1100 MSD spectrometer; at 100 eV; in m/z. Elemental analyses: Elementary Analsensysteme GmbH varioMICRO CHNS (Turkish Technical and Scientific Research Council Laboratories, Ankara, Turkey). TLC was performed on pre-coated silica gel plates (Merck 60, F$_{254}$, 0.25 mm). Organic solvents used were at HPLC grade or were purified by the standard procedure. All reagents were of commercial quality or were purified before use.

The spectroscopic data and spectra of the novel synthesized compounds **3a**–**5d** are given in the Supplementary material to this paper.

Bacterial and yeast strains obtained from American Type Culture Collection (ATTC; Rockville, MD, USA), Northern Regional Research Laboratory (NRRL; USDA; Peoria, IL, USA) were employed in this work. They included gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* NRRL-B 4378) and gram-negative bacteria (*Escherichia coli* NRRL-B 3008, *Pseudomonas aeruginosa* NRRL-B 2679). The following 2 fungal strains were also tested: *Candida albicans* NRRL-Y 12983 and *Candida parapsilosis* NRRL-Y 12696.

Synthetic procedures

Method A for the synthesis of N-*[(2-methylphenyl) methylidene]-6-methyl-1,3-benzothiazol-2-amine (3a)*.[9,15] A solution of 2-amino-6-methyl benzothiazole (**1**, 30 mmol) and *O*-methyl benzaldehyde (**2a**, 28.7 mmol) in ethanol (100 μl) were refluxed for 2 h at 75 °C. The mixture was allowed to stand at room temperature overnight and then concentrated. The residue was washed with *n*-hexane (2×100 μl) and filtered off then it was hydrolyzed in water and extracted with ethyl acetate (EtOAc, 4×50 μl). After drying over with anhydrous Na_2SO_4 and evaporation, crystalline product (**3a**, 70 %) was obtained and recrystallized from dichloromethane (DCM). Compound **3d** was prepared using the same method described above.

Method B for the synthesis of N-*[2-methoxyphenylmethylidene]-6-methyl-1,3-benzothiazol-2-amine (3b)*.[9] The solution of 2-amino-6-methyl benzothiazole (**1**, 5 mmol), *O*-methoxy benzaldehyde (**2b**, 6 mmol) and acetic acid (1 μl) in ethanol (25 μl) were heated for 30 min until no starting amine remained. After waiting for a while at room temperature, a precipitate formed, filtered, washed with diethylether, and the resulting product was crystallized in dichloromethane (**3b**, 76 %).

Modified method C for the synthesis of N-*[2-hyroxyphenylmethylidene]-6-methyl-1,3-benzothiazol-2-amine (3c)*.[16] A mixture of 2-amino-6-methyl benzothiazole (**1**, 5 mmol) the *O*-hydroxy benzaldehyde (**2c**, 6 mmol) in ethyl lactate–water system (3 μl, 70 vol. % ethyl lactate in water) was stirred at room temperature for 4 min. After completion of the reaction, as indicated by TLC, the reaction mixture was left overnight. The formed precipitate was isolated by filtration and washed with water to furnish pure *N*-[(2-hyroxyphenyl)methylidene]-6-methyl-1,3-benzothiazol-2-amine derivatives (**3c**) in excellent yields (90 %), with no need of purification.

General procedure for the synthesis of N-*(2-Methylbenzyl)-6-methyl-1,3-benzothiazol-2-amine (4a)*.[17,18] *N*-[2-methylphenylmethylidene]-6-methyl-1,3-benzothiazol-2-amine (**3a**, 2.5 g, 9.3 mmol) was dissolved in methanol (75 μl). Then, $NaBH_4$ was added to the stirred solution at room temperature until the solution became colourless. Cold water was added to the solution to precipitate the products. The precipitates were recrystallized from methanol to obtain amine derivative (**4a**, 87 %). Compounds **4b**–**d** were prepared using the same method described above.

*General procedure for the synthesis of 2-chloro-*N-*[6-methyl-1,3-benzothiazol-2-yl]-N-[2-methylbenzyl] acetamide (5a)*.[19] Chloracetyl chloride (0.148 μl×10, 1.86 mmol) was added to the novel amine compound (**4a**, 0.50 g, 1.86 mmol) in dry dichloromethane (DCM, 50 μl) in the presence of triethylamine (1 μl, 7.10 mmol). The mixture was then heated to reflux for 2 h and evaporated in vacuo. The residue was hydrolyzed in water (10 ml) and extracted with dichloromethane (5×10 μl). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated in vacuo. The crystalline was recrystallized from chloroform/dichloromethane to obtain (**5a**, 0.48 g, 76 %). Compounds **5b**–**d** were prepared using the same method as described above.

Biological activity

The standardized agar well diffusion method was used to determine the activity of the synthesized compounds against sensitive organisms.[20] These organisms were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria, *Candida parapsilosis* as fungus strains. Mueller–Hinton broth (MHB) is used to determine the susceptibility of bacteria to sulphonamides by the tube dilution method. In this study, the bacterial and yeast cultures were incubated in MHB at 35–37 °C until they were visibly turbid. The density of these cultures was adjusted to a turbidity equivalent to that of the 0.5 McFarland standard with sterile saline. Bacterial and yeast cell suspensions were finally diluted, respectively, to 5×10^5 and 10^4 CFU/µl for use in the assays. Mueller–Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungi were sterilized in a flask and cooled to 45–50 °C, distributed to the sterilized petri dishes (9 cm). The entire surfaces of the MHA plates and SDA plates were inoculated with the bacteria and fungi by spreading them with a sterile swab dipped into the adjusted suspensions. Six wells, each 6 mm in diameter, were cut out of the agar and 20 µl of the compounds. The inoculated petri dishes were incubated at 37 °C for 24 h. The results were expressed in terms of the diameter of the inhibition zone. Penicillin and chloramphenicol were used as positive controls for bacteria, fluconazole as a positive control for fungi. All assays were performed in duplicate.[21,22]

*Minimum inhibitory concentration (*MIC*)*

MIC was determined by the micro dilution method using a 96 well plate according to NCCLS[20] and 100 µl of MHB was placed in each well. Then, the stock solutions of compounds were dissolved in DMSO and transferred into first well, and serial dilutions were performed so that concentrations in the range of 625–5000 µg/ml were obtained. The inoculums were adjusted to contain approximately 10^5 CFU/ml bacteria and 10^4 CFU/ml fungi, as described previously. 100 µl of the inoculums was added to all wells and the plates were incubated at 37 °C for 24 h. *MIC* values were detected by adding 20 µl of 0.5 % triphenyl tetrazolium chloride (TTC) aqueous solution. The *MIC* value was taken as the lowest concentration of the compounds that inhibited any visible bacterial and fungi growth, as indicated by TTC staining after incubation.[20] Penicillin, chloramphenicol and fluconazole were used again as the reference antibiotic control.

RESULTS AND DISCUSSION

Synthesis and characterization

The title compounds **3a**–**5d** were synthesized according to the process described in Fig. 1. The structures of all the compounds **3a**–**5d** were established on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR, HETCOR, 2D(^{1}H–^{1}H) COSY and MS spectra and elemental analyses. In all compounds, the assignment of individual proton signals in the ^{1}H-NMR spectra was based on $J_{\rm HH}$ coupling constant values and confirmed by ^{1}H–^{1}H COSY and HETCOR spectra.

The novel Schiff bases **3a**–**d** were synthesized according to the route shown in Fig. 1. Among these compounds, only compound **3c** was synthesized by an alternative method other than that shown in Fig. 1. To synthesize Schiff base **3c**, various solvents such as ethanol, methanol, tetrahydrofuran, toluene, acetonitrile, benzene and ethyl L-lactate were used. For this purpose, it was aimed to increase the efficiency of the Schiff base, reduce the reaction time and obtain pure product without using purification techniques (Table I). Traditional synthesis methods of Schiff bases use petroleum-derived solvents such as toluene, which are often toxic. However, ethyl lactate is a green solvent and forms a suitable solution with water for the synthesis of Schiff base. In addition, it was observed that Schiff base was formed in high yield when some catalysts, such as scandium (III) triflate, ytterbium (III) triflate, were added to the medium with ethyl L-lactate solvent. For compound **3c**, in addition to the synthesis method of other compounds, we synthesized the Schiff base using ethyl L-lactate as the green solvent. As a result of our experiment on the **3c** compound for trial purposes, Schiff base **3c** was obtained with good yield (Table I, entry 7). Additionally, the reaction was complete within four minutes at room temperature and the imine was pure enough to avoid the necessity for recrystallization or other solvent insensitive isolation or purification procedures.

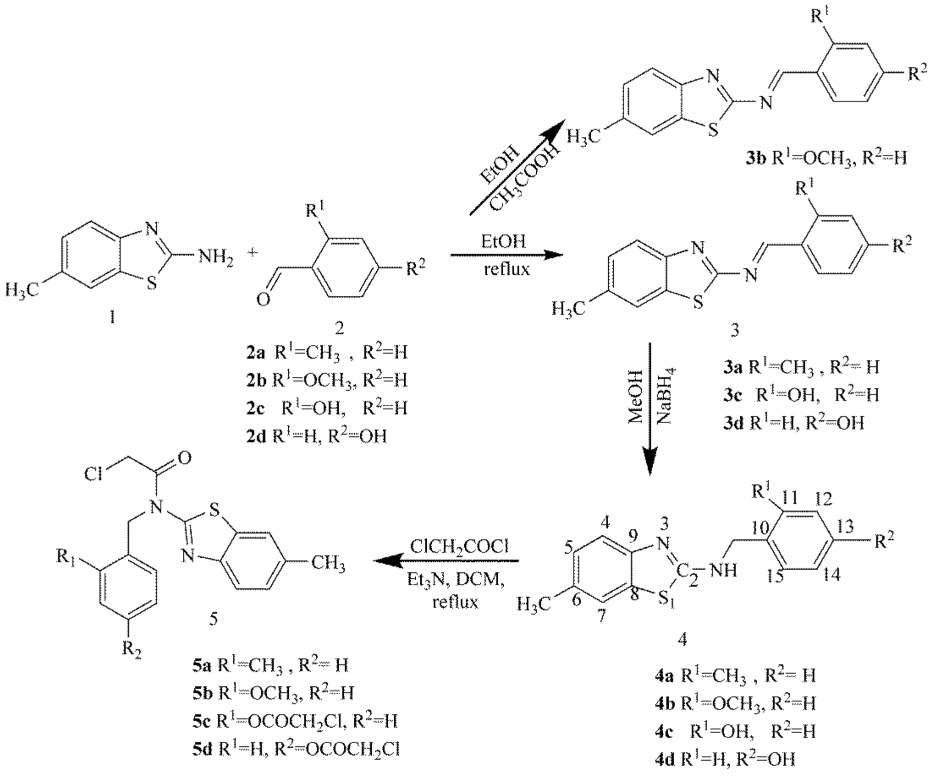

Fig. 1. General scheme of synthetic procedure for compounds **3a–5d.**

TABLE I. Reaction conditions for the synthesis of compound **3c**

No.	Solvent	Time	Yield[a], %
1	Acetonitrile	24 h	12[b]
2	Benzene	24 h	25[b]
3	Ethanol	2 h	85[b]
4	Ethyl lactate	4 min	95[d]
5	Methanol	24 h	c[b]
6	THF	24 h	c[b]
7	Toluene	24 h	35[b]

[a]Isolated yield; [b]all the reactions were carried out at reflux; [c]no reaction; [d]the reaction was carried out at room tempurature

The structures of compounds **3a**–**d** were established from their FTIR, ^{1}H-NMR, ^{13}C-NMR, COSY and HETCOR (also labeled COSY C single bond H) spectra. The FTIR spectra of Schiff bases **3a**–**d** showed a strong band at 1610, 1615, 1620 and 1618 cm^{-1}, attributed to azomethine ν(CH=N) (IR spectra of compounds **3a**–**5d** are given in the Supplementary material). The absence of band around 1730 and 3330 cm^{-1} due to carbonyl stretching and NH_2 stretching of 2-aminobenzothiazole and aldehyde respectively indicates the condensation of aromatic aldehyde and aromatic amines. ^{1}H-NMR spectra also confirmed the proposed stoichiometry and structure of compounds **3a**–**d** (^{1}H-NMR spectra of compounds **3a**–**5d** are given in the Supplementary material). In the ^{1}H-NMR spectra of compounds **3a**–**d**, the chemical shift of the aromatic protons was observed within the δ 6.96–8.12 ppm region of spectrum. The hydroxyl protons for compounds **3c** and **3d** showed as a broad signal within the δ 10.5–11.5 ppm. The observation of the OH proton of compound **3c** at δ 11.5 ppm is due to the hydrogen bond of the imine nitrogen, whereas the resonance of the hydroxide proton of compound **3d** was observed at δ 10.5 ppm due to the absence of hydrogen bond. The imine proton in compounds **3a**–**3d** was observed as a singlet in the range of δ 9.02–9.45 ppm. Among the novel compounds, the imine proton in compound **3c**, which resonates in the downfield at δ 9.45 ppm. This is due to the hydrogen bond between the imine nitrogen of the phenyl ortho substituent hydroxide group. However, the imine proton of compound **3d** was observed at δ 9.02 ppm, which was expressed as the increase of electron density around the imine due to the n→π conjugation effect of hydroxy oxygen. Singlets of benzothiazole substituent methyl protons were observed at δ 2.45, 2.46, 2.44 and 2.40 ppm for compounds **3a**–**d**, respectively. Two sharp singlets were also observed at δ 2.65 and 3.96 ppm for methyl protons (CH_3–Ar) and methoxy protons (Ar–OCH_3) for compound **3a** and **3b**, respectively. The signal for the remaining six protons appeared at 7.14–8.11 ppm, which was assigned to aromatic protons. For compound **3b**, a triplet at 7.14 ppm, doublet at 7.24 ppm and another doublet at 7.83 ppm were assigned for aromatic protons H14, H12 and H4, respectively. In addition, H5, H13 and H15 protons of the aromatic rings resonate as doublet of doublet at 7.35 ppm, doublet of triplet at 7.66 ppm and doublet of doublet at 8.11 ppm, correspondingly. Similar signals were observed in the ^{1}H-NMR spectra of compounds **3a**, **3c** and **3d** (the numbering of the protons of the compound is given in Fig. 1). This assignment was additionally substantiated by ^{1}H–^{1}H COSY analysis (^{1}H–^{1}H COSY spectra are given in the Supplementary material). In the ^{1}H–^{1}H COSY spectrum of compound **3b**, the two signals in the compound, the doublet of the doublet, are assigned as belonging to the H5 proton and the H13 proton. The H5 proton coupled first to the H4 proton, then to the H7 proton long-range coupling. On the other hand, the H15 proton interacts with both the H14 proton and the H13 proton, giving the signal of the doublet of the doublet. Similarly, the H13 coupled to the H14 proton, the H12 proton, and the H15 proton, giving the triplet of the doublet (Fig. S-6 of the Supplementary material). The HETCOR spectrum compound **3b** clearly showed that there were no hydrogen atoms bonded to C2, C6, C8, C9, C10 and C11 as expected. The correlations between C4 and H4, C7 and H7, C5 and H5 in the benzothiazole ring and C12 and H12, C14 and H14, C15 and H15, C13 and H13 in the phenyl ring were also clearly observed in the HETCOR spectrum (Fig. S-7 of the Supplementary material). The ^{13}C-NMR spectrum of compounds **3a**–**d** showed 16 signals corresponding to the 16 carbon atoms present in the molecule as shown in Fig. 1. In the ^{13}C-NMR spectrum, signals belonging to the imine carbon were observed at 164, 161, 166 and 166.5 ppm for compounds **3a**–**d** (Figs. S-12 and S-14 of the Supplementary material). Due to the presence of the carbon–nitrogen π-bond, the signal of the azomethine carbon was observed in the downfield region. The 13 signals observed at 117.88–150.54 ppm were also marked as belonging to aromatic carbons, for compound **3a**. Similar signals were observed in the ^{13}C-NMR spectra of compounds **3b–d.** The ESI mass spectrum of compound **3b** showed a molecular ion peak at *m*/*z* 283.1 ($C_{16}H_{14}N_2SO + H^+$) and fragment ions at *m*/*z* 223.1 ($C_{13}H_7N_2S + H^+$), 165.1 ($C_{12}H_7N + H^+$), Fig. S-8 of the Supplementary material.

Reduction of **3a**–**3d** with $NaBH_4$ in methanol at room temperature occurred easily to give the corresponding amine derivative **4a**–**d** as the only product in good yields 87–93 % (Fig. 1). The structures of **4a**–**d** were clearly assigned as amine compounds by FT-IR, ^{1}H-NMR, ^{13}C-NMR, HETCOR, COSY and ESI MS spectra and elemental analyses. The FT-IR spectral data of the amines **4a**–**d** showed medium intensity absorption bands at 3412, 3430, 3409 and 3434 cm^{-1}, respectively, which were attributed to the ν(NH) stretching vibration. This assignment was further supported by the disappearance of the absorption band that was assigned to the azomethine proton. The ^{1}H-NMR spectra of the compounds **4a**–**d** revealed a fine triplet peak at δ 8.25–8.30 ppm for the –NH– group (^{1}H-NMR spectra of compounds **3a**–**5d** are given in the Supplementary material). The signals of the methylene protons for the –CH_2–NH– group were detected in the region expected in the range of δ 4.47–4.55 ppm as a doublet. The spectra of **4c** and **4d** show a singlet at δ 9.75 and δ 9.30 ppm due to the hydrogen of the hydroxyl group. In the ^{1}H-NMR spectrum of compound **4b**, methoxy and methyl protons were observed at δ 2.31 and 3.83 ppm as singlets. The four doublets at δ 7.02 and 7.25 ppm indicated the H12 and H4, H5 and H15 protons, respectively. The signals observed at δ 6.92 and 7.29 ppm as a triplet and at δ 7.46 ppm as a singlet may be assigned to protons H14, H13 and H7, respectively (Figs. S-21 and S-22 of the Supplementary material). Aromatic protons of compounds **4a**, **4c** and **4d** showed similar characteristics as those discussed in compound **4b**. In the ^{13}C-NMR spectrum of **4b**, carbon atoms of the phenyl and benzothiazole ring were observed at δ 111.06, 118.18, 120.62, 121.32, 126.79, 126.96, 128.46, 128.81, 130.44, 130.97, 150.83, 157.32, 166.00 ppm. The methyl, methoxy and methylene carbon atoms were observed at δ 21.22, 42.89 and 55.82 ppm, respectively (Fig. S-23 of the Supplementary material). Similar signals were observed in the ^{13}C-NMR spectra of compounds **4a**, **4c** and **4d**. In the HETCOR spectrum of compound **4b**, the signals of aromatic carbon atoms were observed at 111.06, 118.18, 120.62, 121.32, 126.79, 126.96, 128.46, 128.81, 130.44, 130.97, 150.83, 157.32 and 166.00 ppm, respectively (Figs. S-26 and S-27 of the Supplementary material). The mass spectrum of compound **4b** shows a molecular ion peak at *m*/*z* 285.0 (10.2 %) of $M^+ + 1$.

The substituted amine compounds **4a**–**d** were reacted with chloroacetyl chloride in the presence of triethylamine to provide the corresponding amides **5a** and **5b** and esters **5c** and **5d** in good yields 76–82 % (Fig. 1). The structures of compounds **5a**–**d** were established from FTIR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analyses. The FT-IR spectra of the ester products depicted ν(N–C=O) bands within the range of 1668–1663 cm^{-1} and ν(OC=O) bands at 1775–1762 cm^{-1}, which supported the assigned structures **5c** and **5d.** In the FT-IR spectra of the obtained amides **5a** and **5b**, carbonyl amide absorption bands appeared at 1698 and 1683 cm^{-1}, respectively. The ^{1}H-NMR spectrum of compound **5b** exhibited four doublets at δ 6.92, 7.08, 7.25 and 7.63 ppm due to H12, H15, H5 and H4 protons, respectively. In addition, H13 proton and H14 proton of the phenyl ring resonated as triplet at 6.87 and 7.29 ppm, correspondingly. The one singlet at 7.81 ppm was assigned for H7 proton (Figs. S-42–S44 of the Supplementary material). Aromatic protons of compounds **5a**, **5c** and **5d** showed similar characteristics to those discussed in compound **5b**. Three sharp singlets were also observed at δ 2.40, 3.53 and 4.75 ppm for the methyl protons (CH_3––benzothiazole), the methoxy protons (Ar–OCH_3) and the methylene protons (–NCH_2–), respectively. Another singlet at δ 5.43 ppm was assigned for –N(CO)––CH_2Cl group. The triplet originating from the NH protons at δ 8.22 ppm in compound **4b** was not detected in compound **5b**. In the ^{1}H-NMR spectrum of compounds **5c** and **5d**, unlike compounds **5a** and **5d**, another singlet at δ 4.80 and 4.76 ppm was assigned to the –N(CO)CH_2Cl group. The molecular ion mass of compound **5a** was observed as m/z 345 in ESI MS. Ion peaks at m/z 309 and 267 showed that firstly the chlorine atom and then the CH_2ClCO– group were ionized from the ionic mass of the molecule (Fig. S-40 of the Supplementary material). The ^{13}C-NMR spectrum of compounds **5a** and **5b** had a signal at 168 and 167.86 (N–C=O–) as expected, leading us to predict that the product is an amide compound. The ^{13}C-NMR spectrum of compounds **5c** and **5d** could not be obtained despite many attempts.

Antimicrobial activity studies

The antibacterial activity of the twelve novel synthesized compounds **3a**–**5d** was tested against a range of Gram-positive and Gram-negative bacteria and two fungal species in the range of *MIC* values of 625–5000 µg/µl. As shown in Table II, these organisms were *S. aureus*, *S. epidermidis* and *B. subtilis* as Gram-positive bacteria and *E. coli* and *P. aeruginosa* as Gram-negative bacteria, *C. parapsilosis* as fungus strains. Among all compounds tested, **3b**–**d** and **4b** and **4c** showed activity against the bacteria at different *MIC* values (625–5000 µg/µl). However, compounds **3a**, **4a** and 4**b** and **5a**–**c** were not active against the bacteria tested. According to preliminary results, among these compounds, compound **3c** showed activity against Gram-negative bacteria *S. aureus* ATCC 6538, *P. aeruginosa* NRRL-B 2679 with a *MIC* value of 625 µg/µl. Whereas, compounds **3b**–**d** exhibited activity against *C. parabsilosis* NRRL-Y 12696 at a *MIC* of 1250 µg/µl, while compound **4c** showed activity against gram-negative *P. aeruginosa* NRRL-B 2679 and *C. parapsilosis* NRRL-Y 12696 fungus strains with *MIC* values ranging from 2500 to > 50000 µg/µl. Moreover, compounds **3b**–**d** and **4c** showed antifungal activity on *C. parapsilosis* at an inhibition zone of 8 to 14 mm and *MIC* value (1250–2500 µg/µl), but other new compounds such as **3a**, **4a**, **4b** and **5a**–**d** were inactive. According to these results, we could see that among all the compounds, the compound with the ortho substituent hydroxy group, where only the imine group was present, was more active. For example, compound **3c** was slightly more effective compared to compounds **3b** and **3d.** We also found that the scaffold containing the imine group retained antibacterial activity, and the presence of a hydroxy substituent group, especially at the ortho position, increased the activity. Recent reports demonstrated the role of *C. parapsilosis* in the etiopathogenesis of otitis, arthritis, endocarditis, endophtalmitis, meningitis, wound infections, denture stomatitis, reproductive system infections in women, vaginitis, cervicitis and salpingitis.[24] The reason for that different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall.[25] Among the compounds we synthesized, only compounds **3b**–**d** and **4c** showed antifungal activity against *C. parapsilosis* in an inhibition zone of 8–14 mm. This is due to the activity of the imine and amine groups and the substituted electron donating OCH_3 and OH in the phenyl ring. Therefore, the biological activity of compounds **3–d** and **4c** depends on the substituted groups. Depending on the substituent groups in the compounds, the activity is OH > OCH_3 > CH_3. In addition, none of the new compounds synthesized were effecttive on other bacteria and fungi such as *S. epidermidis*, *E. coli*, *B. subtilis* and *C. albicans*.

TABLE II. Antibacterial and antifungal activities of compound **3b**–**4c** as inhibition zone diameters (mm) and *MIC* (µg/µl); P = peniciline G; C = chloramphenicol; F = fluconazole

Microorganism	**3b**	**3c**	**3d**	**4b**	**4c**	P	C	F
S. aureus ATCC 6538	–	625	–	–	–	62.5	7.8	–
P. aeuroginosa NRRL-B 2679	–	625	–	–	5000<	62.5	62.5	–
C. parabsilosis NRRL-Y 12696	1250	1250	1250	–	2500	–	–	125

For the further determination of the antibacterial spectrum of our compounds, the most promising agent **3c** was tested against to commonly used antimicrobial agent (Table III). Among all novel compounds, **3c** showed the highest inhibitory activity against the gram-negative bacteria *S. aureus* and *P. aeruginosa* (*MIC* = 625 µg/µl) compared to the reference drugs (peniciline G: *MIC* = 62.5 µg/µl, chloramphenicol: *MIC* = 7.8 µg/µl, respectively). *S. aureus* bacteria have been reported to be the most common cause of bloodstream, skin and soft tissue, and respiratory tract infections. This pathogen is among those that cause severe infections in patients.[23] *P. aeruginosa* is frequently associated with infections of the urinary and respiratory tract in humans. *P. aeruginosa* infections are also common in patients receiving treatment for severe burns or other traumatic skin damage and in people suffering from cystic fibrosis.[20] Compounds **3b–d** revealed the highest *MIC* (1250 µg/µl) against standard *C. parabsilosis*, while the reference compounds peniciline G and chloramphenicol were inactive.

TABLE III. Microbial activity of novel synthesized compounds (**3b**–**5c**); P – peniciline G; C – chloramphenicol; F – fluconazole; inactive = inhibition < 6mm; slightly active = inhibition zone 6–9 mm; moderately active = inhibition zone 9–12 mm and highly active = inhibition zone > 12 mm

Microorganism	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**5a**	**5b**	**5c**	P	C	F
S. aureus ATCC 6538	–	10	–	–	–	–	–	–	–	–	21	24	–
S. epidermidis ATCC 12228	–	–	–	–	–	–	–	–	–	–	24	22	–
E. coli NRRL-B 3008	–	–	–	–	–	–	–	–	–	–	20	26	–
P. aeruginosa NRRL-B 2679	–	9	–	–	–	8	–	–	–	–	36	32	–
B. subtilis NRRL-B 4378	–	–	–	–	–	–	–	–	–	–	36	30	–
C. albicans NRRL-Y 12983	–	–	–	–	–	–	–	–	–	–	–	–	22
C. parapsilosis NRRL-Y 12696	14	10	10	–	–	8	–	–	–	–	–	–	25

CONCLUSION

In this study, compound **3c**, one of the novel synthesized Schiff bases, was synthesized in the green solvent ethyl L-lactate medium. This synthesis method has many advantages over the traditional methods used, which are given as follows: the reaction was completed in four minutes and resulted in a 95 % yield, the product formed was not crystalline and required no further purification, the solvent used as a green solvent is not hazardous to the environment, non-corrosive, non-carcinogenic, non-teratogenic and biodegradable, showing an important benefit of our method. In this work, the antimicrobial activity of the novel synthesized was researched. The results showed that some of the novel compounds tested showed significant antimicrobial activity. Microbes that have gained resistance to drug therapy are an increasing public health problem. While there are a few really effective antifungal preparations currently available for the treatment of systemic mycoses, the efficiency of existing drugs is rather limited. The present study has clearly indicated that compound **3c** could be a promising new source of an antibacterial and antifungal agent. The results obtained from ethyl L-lactate synthesis method and activity studies could contribute to the literature.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12631, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ *N*-СУПСТИТУИСАНИХ БЕНЗИЛ *N*-(1,3-БЕНЗОТИАЗОЛ-2-ИЛ)-АЦЕТАМИДА И ЊИХОВА *IN VITRO* АНТИБАКТЕРИЈСКА АКТИВНОСТ

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Нове Шифове базе **3а**–**д** синтетисане су у реакцији 6-метил-2-аминобензотиазола са различитим супституисаним бензалдехидима. Добијене Шифове базе су редуковане помоћу $NaBH_4$ да би се формирали одговарајући амини **4а**–**д**. У последњем кораку, у реакција амина и хлороацетил-хлорида добијени су нови деривати амида **5а**–**д**. Структуре свих нових синтетизованих једињења су окарактерисане FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI MS, HETCOR, 2D (^{1}H-^{1}H) COSY спектрима и елементалним анализама. Да би се истражила антимикробна активност нових синтетизованих једињења, они су тестирани против неких Грам-позитивних и Грам-негативних бактеријских и гљивичних врста, а добијени резултати су даље дискутовани.

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