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Research Article

Microwave-Assisted Synthesis and Antimicrobial Activity of Some Novel Isatin Schiff Bases Linked to Nicotinic Acid via Certain Amino Acid Bridge

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The coupling reaction of nicotinic acid with certain L-amino acid methyl esters including valine, leucine, and phenylalanine was done by the use of acid chloride method. The products were reacted with hydrazine hydrate 99% to give the corresponding hydrazides that were reacted with indoline-2,3-dione (isatin) to get Schiff bases under the application of microwave irradiation technique. These novel compounds were characterized by means of their FT-IR, ^{1}H NMR, and mass spectral data. Additionally, the specific optical rotation and elemental analysis were measured. The *in vitro* antimicrobial activity of the synthesized compounds was evaluated by agar diffusion method. The compounds showed a strong antimicrobial inhibitory activity. Most of the test compounds possessed a broad spectrum of activities having MIC values ranging from 50 μg/mL to 500 μg/mL.

1. Introduction

Nicotinic acid (pyridine-3-carboxylic acid), also known as niacin and vitamin B3, is found in various plants and animals; also, it has vital roles in such biological processes as production of energy [1]. Nicotinic acid derivatives and its isomers have antibacterial, antioxidant, anti-inflammatory, anticarcinogenic, and antitubercular activities [2], signal transduction, regulation of gene expression [3], and involvement in the synthetic pathway of lipids [4]. On the other hand, some of new heterocyclic and peptide derivatives have been studied with respect to antivirus [5], anti-inflammatory [6], enzymatic peptide [7], and antimicrobial activities [8, 9]. Also, there are several successful publications of microwave-assisted solid phase peptide synthesis of various unnatural biopolymers such as peptoids, pseudopeptides, small peptides [10], phosphopeptides [11], difficult peptides [12], β-peptide libraries [13], and glycopeptides [14].

It is as well known that isatin is an endogenous compound identified in humans and that its biological properties include a range of actions in the brain; it offers protection against certain types of infections [15]. Isatin constitutes an important class of bioactive compounds exhibiting caspase inhibitor [16, 17], antiproliferative [18], and antibacterial activities [19]. Furthermore, novel inhibitors of mycobacterium tuberculosis, prepared from indoline-2,3-dione (isatin) derivatives by the use of microwave irradiation technique, have been reported [20].

Due to the importance of nicotinic acid derivatives, amino acids, peptides, and isatin as bioactive compounds and

in continuation of our previous works in heterocyclic and peptide chemistry [21–26], the aim of this study is to prepare novel antimicrobial compounds that have crucial importance in the way of overcoming the remarkable adaptability of the bacteria.

2. Experimental

2.1. Chemistry

2.1.1. General. The organic solvents and the chemicals used in this part were obtained from Sigma (USA) and Fluka (Switzerland) chemical companies and the used amino acids are of L-configuration. Microwave irradiations were carried out using a domestic microwave oven LG-MS-2044 W/OO, with frequency of 2450 MHz and operating at 420 watts of the total power. Infrared (IR) spectra were recorded as KBr disks using the Perkin Elmer FT-IR Spectrum BX Apparatus located at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Melting points were determined in opened glass capillary tubes with an "Electrothermal" Digital Melting Point Apparatus (model: IA9100) and are uncorrected. Elemental microanalysis for carbon, hydrogen, and nitrogen was measured at the Micro-analytical Unit, National Research Centre (Cairo, Egypt), and was found within the acceptable limits of the calculated values (±0.4%). Specific optical rotations were measured at the National Research Centre (Cairo, Egypt) with "A. KRUSS, OPTRONIC, P8000" polarimeter (Germany), in a 1 dm length for the observation tube, in methanol as a solvent. NMR spectra were scanned in DMSO-d_6 on a Bruker NMR spectrophotometer operating at 500 MHz for ^{1}H at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Chemical shifts are expressed in δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants (J) are expressed in Hz. D_2O was added to confirm the exchangeable protons. High resolution mass spectra were measured on a JEOL M Station JMS-700 system at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Analytical thin layer chromatography (TLC) was performed on silica gel aluminum sheets, 60 F_{254} (E. Merck).

*2.1.2. Synthesis of Esters (**3a–c**).* These compounds were prepared by the reaction of acid chloride **1** with amino acid esters **2a–c** according to the reported method [27].

*2.1.3. Synthesis of Hydrazide Derivatives (**4a–c**).* Hydrazine hydrate 99% (0.8 mL, 16 mmol) was added to a methanolic solution (10 mL) of **3a–c** (1 mmol). The reaction mixture was refluxed for 10 h, after which the solvent was evaporated under reduced pressure. The obtained residue was triturated with ether, filtered off, and crystallized to afford the corresponding hydrazides (**4a–c**). The physical properties and spectral data of compounds *N*-(1-hydrazinyl-3-methyl-1-oxobutan-2-yl)nicotinamide (**4a**) and *N*-(1-Hydrazinyl-1-oxo-3-phenylpropan-2-yl)nicotinamide (**4c**) were identical with those reported in [28].

*N-(1-Hydrazinyl-4-methyl-1-oxopentan-2-yl)nicotinamide (**4b**).* Yield (66%); m. p. 182–185°C; $-[\alpha]_D^{25} = -80$ (c = 0.02, MeOH); IR (KBr) ν_{max}/cm^{-1} 3413–3276 (NH), 1685 (C=O), 1593 (C=N); ^{1}H NMR (DMSO-d_6) δ 0.87–0.92 (m, 6H, $2CH_3$), 1.52-1.53 (m, 1H, -C<u>H</u>$(CH_3)_2$), 1.64–1.69 (m, 2H, -CH_2), 4.31 (br. s, 2H, D_2O exchangeable, NH_2 hydrazide), 4.50-4.51 (m, 1H, -NH-C<u>H</u>-CO), 7.5 (s, 1H, pyridine H5), 8.23 (s, 1H, pyridine H4), 8.71 (s, 2H, D_2O exchangeable, NH amide + NH hydrazide), 9.04 (s, 1H, pyridine H6), 9.29 (s, 1H, pyridine H2); HR-MS: 250.3587. Anal. Calcd. for $C_{12}H_{18}N_4O_2$ (250.2969): C (57.58), H (7.25), N (22.38). Found: C (57.54), H (7.24), N (22.35).

*2.1.4. Synthesis of Isatin Hydrazones (**6a–g**) Using Microwave Radiation.* A solution of isatin **5a–c** (1 mmol) and hydrazide **4a–c** (1 mmol) in ethanol (15 mL) was prepared. Few drops of glacial acetic acid were added and whole reaction mixture was irradiated under microwave irradiation at 420 watts (~ 110°C) for 5–10 minutes. The reaction mixture was cooled. Then, the solid separated on cooling was filtered, washed with cold ethanol, dried, and recrystallized from the appropriate solvent to obtain hydrazones **6a–g**.

*N-(1-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazinyl)-3-methyl-1-oxobutan-2-l)nicotinamide (**6a**).* Yield (69%); m. p. 210–214°C; $-[\alpha]_D^{25} = -48$ (c = 0.01, MeOH); IR (KBr) ν_{max}/cm^{-1} 3410 (NH), 1690 (C=O), 1618 (C=N); ^{1}H NMR (DMSO-d_6) δ 0.98–1.04 (m, 6H, $2CH_3$), 1.90-1.91 (m, 1H, -C<u>H</u>$(CH_3)_2$), 4.41–4.47 (m, 1H, -NH-C<u>H</u>-CO), 5.40-5.41 (m, 1H, -NH-C<u>H</u>-CO), 6.86–8.08 (m, 5H, Ar-H), 8.64 (s, 1H, Ar-H), 8.93 (s, 1H, D_2O exchangeable, NH amide), 9.17–9.40 (m, 2H, Ar-H), 11.60 (s, 1H, D_2O exchangeable, NH isatin), 12.57 (s, 1H, D_2O exchangeable, NH hydrazide), 13.39 (s, 1H, D_2O exchangeable, NH hydrazide); HR-MS: 399.1098. Anal. Calcd. for $C_{19}H_{18}ClN_5O_3$ (399.11): C (57.07), H (4.54), N (17.52). Found: C (57.04), H (4.58), N (17.50).

*N-(4-Methyl-1-oxo-1-(2-(2-oxoindolin-3-ylidene)hydrazinyl) pentan-2-yl)nicotinamide (**6b**).* Yield (93%); m. p. 220–222°C; $-[\alpha]_D^{25} = -132$ (c = 0.03, MeOH); IR (KBr) ν_{max}/cm^{-1} 3414 (NH), 1700 (C=O), 1641 (C=N); ^{1}H NMR (DMSO-d_6) δ 0.92–1.10 (m, 6H, $2CH_3$), 1.62–1.92 (m, 3H, -C<u>H</u>$(CH_3)_2$ + -CH_2), 4.64–4.66 (m, 1H, -NH-C<u>H</u>-CO), 5.53–4.54 (m, 1H, -NH-C<u>H</u>-CO), 6.93–7.56 (m, 5H, Ar-H), 8.28 (s, 1H, Ar-H), 8.76 (s, 1H, D_2O exchangeable, NH amide), 8.98–9.24 (m, 2H, Ar-H), 11.29 (s, 1H, D_2O exchangeable, NH isatin), 12.52 (s, 1H, D_2O exchangeable, NH hydrazide), 13.57 (s, 1H, D_2O exchangeable, NH hydrazide); HR-MS: 379.9433. Anal. Calcd. for $C_{20}H_{21}N_5O_3$ (379.41): C (63.31), H (5.58), N (18.46). Found: C (63.30), H (5.59), N (18.49).

*N-(1-(2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazinyl)-4-methyl-1-oxopentan-2-yl)nicotinamide (**6c**).* Yield (88%); m. p. 178–180°C; $-[\alpha]_D^{25} = -120$ (c = 0.01, MeOH); IR (KBr) ν_{max}/cm^{-1} 3413–3233 (NH), 1701 (C=O), 1639 (C=N); ^{1}H NMR (DMSO-d_6) δ 0.93–1.10 (m, 6H, $2CH_3$), 1.63–1.92 (m,

3H, -CH(CH$_3$)$_2$ + -CH$_2$), 4.66-4.67 (m, 1H, -NH-CH-CO), 5.54-5.55 (m, 1H, -NH-CH-CO), 6.94–7.56 (m, 4H, Ar-H), 8.29 (s, 1H, Ar-H), 8.76 (s, 1H, D$_2$O exchangeable, NH amide), 9.00–9.26 (m, 2H, Ar-H), 11.41 (s, 1H, D$_2$O exchangeable, NH isatin), 12.45 (s, 1H, D$_2$O exchangeable, NH hydrazide), 13.52 (s, 1H, D$_2$O exchangeable, NH hydrazide); HR-MS: 458.1546. Anal. Calcd. for $C_{20}H_{20}BrN_5O_3$ (458.31): C (52.41), H (4.40), N (15.28). Found: C (52.39), H (4.43), N (15.26).

*N-(1-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazinyl)-4-methyl-1-oxopentan-2-yl)nicotinamide (**6d**).* Yield (90%); m. p. 194–196°C; $-[\alpha]_D^{25} = -70$ (c = 0.01, MeOH); IR (KBr) ν_{max}/cm^{-1} 3413–3232 (NH), 1700 (C=O), 1641 (C=N); ^{1}H NMR (DMSO-d_6) δ 0.93–1.10 (m, 6H, 2CH$_3$), 1.62–1.92 (m, 3H, -CH(CH$_3$)$_2$ + -CH$_2$), 4.66-4.67 (m, 1H, -NH-CH-CO), 5.54–4.55 (m, 1H, -NH-CH-CO), 6.90–7.68 (m, 4H, Ar-H), 8.30 (s, 1H, Ar-H), 8.78 (s, 1H, D$_2$O exchangeable, NH amide), 9.00–9.26 (m, 2H, Ar-H), 11.42 (s, 1H, D$_2$O exchangeable, NH isatin), 12.43 (s, 1H, D$_2$O exchangeable, NH hydrazide), 13.51 (s, 1H, D$_2$O exchangeable, NH hydrazide); HR-MS: 413.0183. Anal. Calcd. for $C_{20}H_{20}ClN_5O_3$ (413.86): C (58.04), H (4.87), N (16.92). Found: C (58.01), H (4.83), N (16.95).

*N-(1-Oxo-1-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-3-phenylpropan-2-yl)nicotinamide (**6e**).* Yield (84%); m. p. 218–220°C; $-[\alpha]_D^{25} = -110$ (c = 0.03, MeOH); IR (KBr) ν_{max}/cm^{-1} 3413–3228 (NH), 1702 (C=O), 1621 (C=N); ^{1}H NMR (DMSO-d_6) δ 3.08–3.33 (m, 2H, -CH$_2$), 4.90-4.91 (m, 1H, -NH-CH-CO), 5.67–4.68 (m, 1H, -NH-CH-CO), 7.11–7.58 (m, 10H, Ar-H), 8.19 (s, 1H, Ar-H), 8.72 (s, 1H, D$_2$O exchangeable, NH amide), 9.01–9.60 (m, 2H, Ar-H), 11.35 (s, 1H, D$_2$O exchangeable, NH isatin), 12.61 (s, 1H, D$_2$O exchangeable, NH hydrazide), 13.55 (s, 1H, D$_2$O exchangeable, NH hydrazide); HR-MS: 413.1841. Anal. Calcd. for $C_{16}H_{15}N_5$ OS (413.43): C (66.82), H (4.63), N (16.96). Found: C (66.78), H (4.61), N (16.99).

*N-(1-(2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)nicotinamide (**6f**).* Yield (81%); m. p. 200–204°C; $-[\alpha]_D^{25} = -105$ (c = 0.01, MeOH); IR (KBr) ν_{max}/cm^{-1} 3410–3230 (NH), 1700 (C=O), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ 3.07–3.39 (m, 2H, -CH$_2$), 4.90-4.91 (m, 1H, -NH-CH-CO), 5.64–4.65 (m, 1H, -NH-CH-CO), 6.86–7.96 (m, 9H, Ar-H), 8.17 (s, 1H, Ar-H), 8.76 (s, 1H, D$_2$O exchangeable, NH amide), 9.04–9.41 (m, 2H, Ar-H), 11.48 (s, 1H, D$_2$O exchangeable, NH isatin), 12.47 (s, 1H, D$_2$O exchangeable, NH hydrazide), 13.48 (s, 1H, D$_2$O exchangeable, NH hydrazide); HR-MS: 491.0701. Anal. Calcd. for $C_{16}H_{15}N_5$ OS (492.32): C (56.11), H (3.69), N (14.23). Found: C (56.10), H (3.65), N (14.26).

*N-(1-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)nicotinamide (**6g**).* Yield (80%); m. p. 226–230°C; $-[\alpha]_D^{25} = -20$ (c = 0.01, MeOH); IR (KBr) ν_{max}/cm^{-1} 3413–3280 (NH), 1741–1692 (C=O), 1620 (C=N); ^{1}H NMR (DMSO-d_6) δ 3.07–3.39 (m, 2H, -CH$_2$), 4.90-4.91 (m, 1H, -NH-CH-CO), 5.64–4.65 (m, 1H, -NH-CH-CO), 6.93–7.54 (m, 9H, Ar-H), 8.18 (s, 1H, Ar-H), 8.73 (s, 1H, D$_2$O exchangeable, NH amide), 9.0–9.40 (m, 2H, Ar-H), 10.97 (s, 1H, D$_2$O exchangeable, NH isatin), 11.40 (s, 1H, D$_2$O exchangeable, NH hydrazide), 11.70 (s, 1H, D$_2$O exchangeable, NH hydrazide); HR-MS: 447.4478. Anal. Calcd. for $C_{23}H_{18}ClN_5O_3$ (447.87): C (61.68), H (4.05), N (15.64). Found: C (61.69), H (4.02), N (15.67).

2.2. Biological Study

2.2.1. Samples Preparation. All samples were dissolved in dimethyl sulfoxide (RFCL Limited, New Delhi, India) (DMSO) at 10 mg/mL concentration, in comparison with different standard antibiotics as shown in Table 1. Antibiotic discs of streptomycin (S) (10 μg) and tetracycline (TE) (30 μg) were used as positive control for bacteria, neomycin (N) (30 μg) and nystatin (NY) (100 μg) were used for fungi, and sterilized paper discs without compounds or antibiotics were used as negative controls for both bacteria and fungi. The experiment was performed in triplicate.

2.2.2. Antimicrobial Activity. The ability to inhibit the growth of Gram-positive and Gram-negative bacteria, yeasts, and filamentous fungi was observed using an overlay method [29].

2.2.3. Antimicrobial Assay

(1) Strains Used. The common pathogenic and food spoilage microorganisms were selected for their relevance in bakery products and other foods: the Gram-positive bacteria were *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria was *Escherichia coli*, yeasts such as *Candida albicans*, and fungi was *Aspergillus niger*.

(2) Media Used. The bacteria were slanted on nutrient agar (Merck, Darmstadt, Germany), yeast was slanted and mentioned onSabaroud's agar medium (Lab M., Bury, Lancashire, UK), and the fungi were slanted and mentioned on the Potato Dextrose Agar medium (Lab M Limited, Bury, Lancashire, UK). Mueller-Hinton agar (Lab M., Bury, Lancashire, UK) following the manufacturer's instructions was used for the assay.

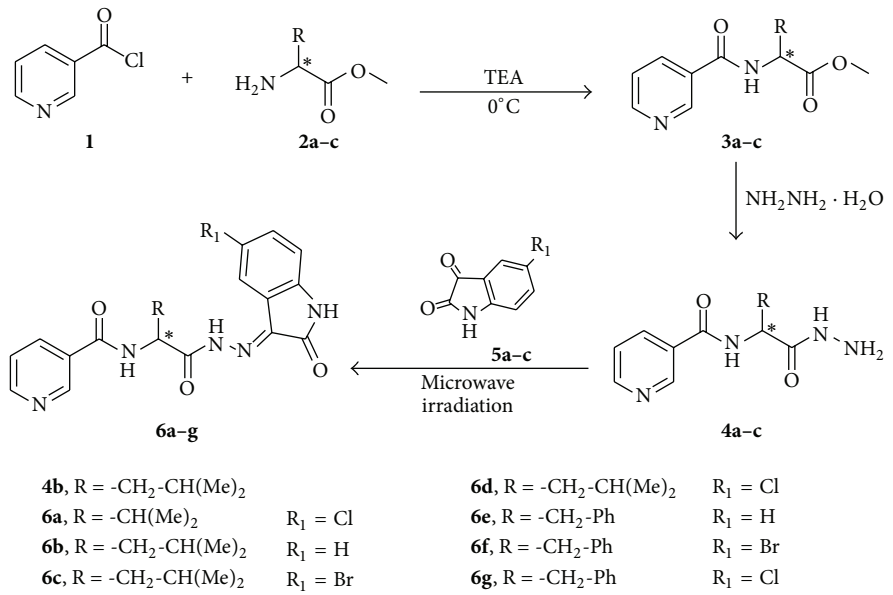
(3) Bioassay. The antibacterial screening was performed by the well diffusion agar method described by Moosdeen et al. [30]. The organisms were streaked in radial patterns on the agar plates. Plates were incubated under aerobic conditions at 37°C and 28°C for 24 h and 48 h for bacteria and fungi, respectively. In order to obtain comparable results, all prepared solutions were treated under the same conditions. All tests were performed for three replicates. Plates were examined for evidence of antimicrobial activities, represented by a zone of inhibition of microorganism's growth around the holes, and diameters of clear zones were expressed in millimeters [31].

(4) Determination of Minimum Inhibitory Concentration (MIC). The *in vitro* minimum inhibitory concentration (MIC) of the synthesized compounds was determined by agar well diffusion method. Nutrient broth medium was used to

Table 1: Antimicrobial activity of the synthesized compounds at 10 mg/mL.

Comp. number	Bacteria			Fungi	
	Gram-positive		Gram-negative	Unicellular	Filamentous
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*Aspergillus niger*
4b	15.13 ± 0.1768	00.00 ± 0.0000	00.00 ± 0.0000	11.125 ± 0.1768	00.00 ± 0.0000
6a	30.18 ± 0.2475	22.12 ± 0.1626	25.125 ± 0.1768	30.125 ± 0.1768	00.00 ± 0.0000
6b	18.17 ± 0.2404	15.10 ± 0.1414	15.125 ± 0.1768	15.10 ± 0.1414	15.16 ± 0.2263
6c	23.18 ± 0.2475	23.18 ± 0.2475	20.175 ± 0.2475	20.115 ± 0.1626	00.00 ± 0.1768
6d	20.10 ± 0.1414	24.08 ± 0.1060	20.175 ± 0.2475	20.17 ± 0.2404	00.00 ± 0.0000
6e	25.20 ± 0.2828	30.13 ± 0.1768	30.15 ± 0.2121	25.175 ± 0.2475	00.00 ± 0.0000
6f	27.18 ± 0.2475	32.10 ± 0.1410	32.13 ± 0.1768	25.10 ± 0.1414	00.00 ± 0.0000
6g	15.13 ± 0.176	15.00 ± 0.0000	15.10 ± 0.1414	00.00 ± 0.0000	14.10 ± 0.1414
S* = 10 μg	14.00 ± 0.0000	00.00 ± 0.0000	12.00 ± 0.0000	00.00 ± 0.0000	00.00 ± 0.0000
TE* = 30 μg	18.00 ± 0.0000	00.00 ± 0.0000	23.5 ± 2.1213	00.00 ± 0.0000	00.00 ± 0.0000
N* = 30 μg	00.00 ± 0.0000	00.00 ± 0.0000	00.00 ± 0.0000	16.00 ± 1.4142	15.00 ± 0.0000
NS* = 100 μg	00.00 ± 0.0000	00.00 ± 0.0000	00.00 ± 0.0000	00.00 ± 0.0000	15.00 ± 0.0000

*S: streptomycin; TE: tetracycline; N: neomycin, and NS: nystatin.



Scheme 1: Synthetic routes for compounds **4b** and **6a–g**.

prepare different concentrations ranging from 50 μg/mL to 500 μg/mL by serial dilutions. Each prepared concentration in tubes was inoculated with 100 μL of each of the 10^6 cfu/mL bacterial and fungal strains and the assay was applied by agar well diffusion method. Blank nutrient broth was used as negative control. The plates were incubated aerobically at 37°C for 18 h to 24 h for bacterial strains and 25°C for 48 h for fungal strains. The lowest inhibition zone in the series dilution was taken as the MIC.

3. Results and Discussion

3.1. Chemistry. In the present work, we suggested the coupling of two heterocyclic compounds via certain amino acid as a bridge and studied the biological activities of these compounds as potent antimicrobial agents. The preparation and characterization of new compounds represented by the **4b** and **6a–g**, as well as the acid chloride method of coupling, was successfully applied as the method of choice for assembling the peptide bond. Herein, a series of chiral linear carboxamide derivatives incorporating peptide linkage have been prepared via the coupling of nicotinoyl chloride **1** with suited L-amino acid methyl esters **2a–c** including valine, leucine, and phenylalanine which produced the corresponding peptide methyl esters **3a–c**, respectively. Hydrazinolysis of esters **3a–c** with hydrazine hydrate 99% produced the hydrazide derivatives **4a–c,** respectively. The latter compounds were acidified and coupled with isatin derivatives **5a–c** in ethanol and irradiated successfully under microwave irradiation at 420 watts (~110°C) for 5–10 minutes to afford the corresponding Schiff bases **6a–g,** respectively; see Scheme 1. The results indicated that microwave technique

Table 2: MIC of different compounds against most of tested pathogens.

Comp. number	Inhibition zone diameters (mm)				MIC (μg/mL)			
	B. subtilis	*E. coli*	*S. aureus*	*C. albicans*	*B. subtilis*	*E. coli*	*S. aureus*	*C. albicans*
6e	11	11	11	11	50	50	50	50
6f	11	12	12	12	100	50	50	50
6a	11	11	11	12	500	75	500	500
6c	11	11	11	—	50	75	75	—
6d	11	11	—	—	75	50	—	—

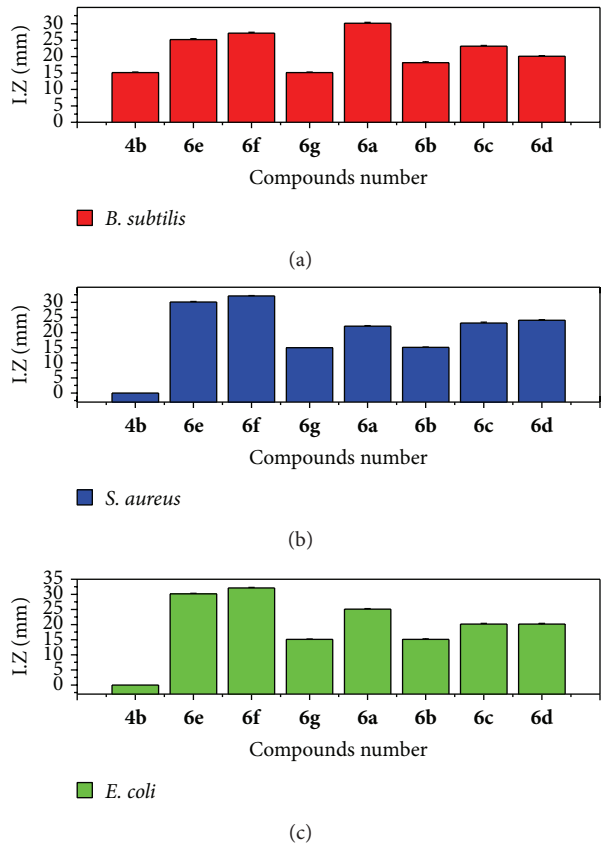


Figure 1: Antibacterial activity of the synthesized compounds at concentration of 10 mg/mL: (a) *B. subtilis*, (b) *S. aureus* and (c) *E. coli*.

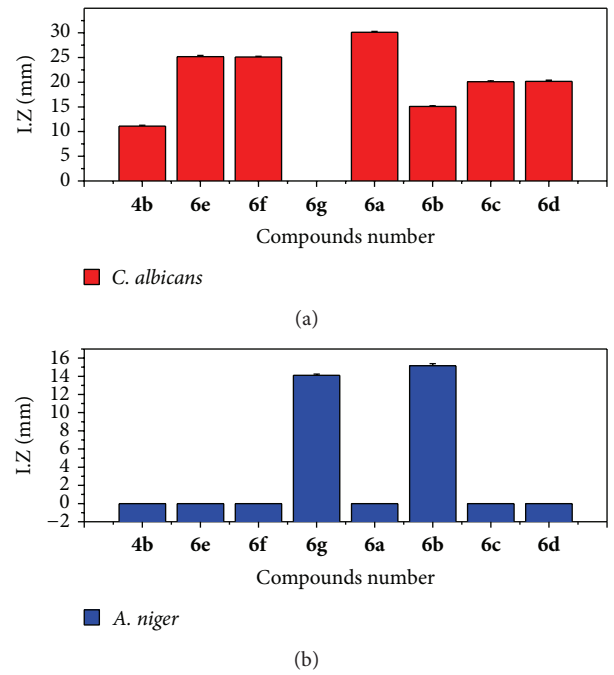


Figure 2: Antifungal activity of the synthesized compounds at concentration of 10 mg/mL: (a) *C. albicans* and (b) *A. niger*.

gave improved yield in less reaction time. So this confirms that the microwave (MW) synthesis has been shown to be an invaluable tool for medicinal chemistry and drug discovery applications since it often dramatically reduces reaction times, typically from days or hours to minutes or even seconds [32].

3.2. Biological Evaluations. The results in Table 1 showed that most of the compounds have a strong antibacterial inhibitory effect against most of tested pathogens. Most of the compounds showed a strong inhibitory effect against Gram-positive bacteria such as *Bacillus subtilis* and *S. aureus* except compound **4b,** which showed a negative inhibitory effect in comparison with the antibacterial standard streptomycin. The inhibitory zone diameters ranged from 15 mm to 30 mm and were illustrated in Figures 1(a) and 1(b). A Gram-positive bacterium infects the upper respiratory tract. So, these compounds can be used in the treatment of these pathogen groups. Also, a strong inhibitory effect (15 mm to 32 mm) was noticed against *Escherichia coli* as example of Gram-negative bacteria except compound **4b** which showed a negative inhibitory effect, illustrated in Figure 1(c).

The antifungal activities are presented in Figure 2, in case of unicellular fungi. Compounds **4b**, **6e**, **6f**, **6a**, **6b**, **6c,** and **6d** showed a strong antifungal effect against *Candida albicans*, while compound **6g** was characterized by negative antifungal effect; see Figure 2(a). In case of filamentous fungi, compounds **6b** and **6g** have the significant antifungal effect against *Aspergillus niger* while the other compounds did not show any inhibitory effect, compared to the antifungal standard,neomycin and nystatin (Figure 2(b)).

Aspergillus niger is one of the fungal pathogens that can infect the respiratory tract. *A. niger* is a causative agent of pulmonary diseases including aspergillosis, bronchial asthma, and acute allergic alveolitis. The fungus colonizes old tuberculosis or bronchiectatic cavities, in which it forms a large colony (aspergilloma); or it may actually invade the lung

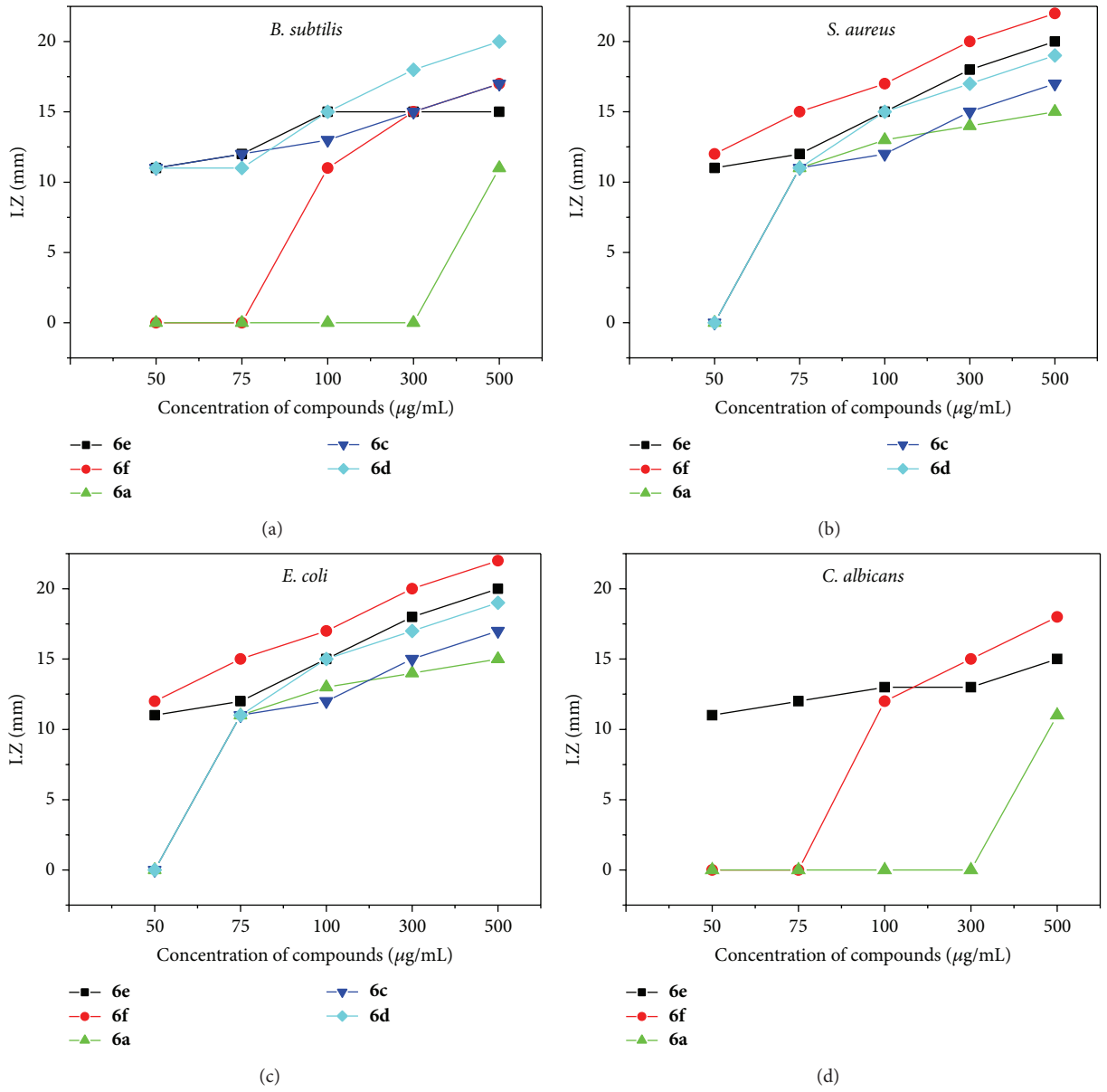


Figure 3: MIC of compounds against Gram-positive bacteria (*B. subtilis*) (a) and (*S. aureus*) (b), Gram-negative bacteria (*E. coli*) (c), and yeast (*C. albicans*) (d).

tissue to produce hemorrhagic and necrotizing pneumonia [33]. The minimum inhibitory concentrations (MIC) of the synthesized compounds are presented in Table 2 and Figures 3(a), 3(b), 3(c), and 3(d). The MIC was in the range from 50 μg/mL to 500 μg/mL based on the compounds tested. The MIC of compound **6e** was 50 μg/mL against all pathogens tested. The MIC of **6f** was 50 μg/mL against all strains except *Bacillus subtilis* which displayed MIC of 100 μg/mL. On the other hand, the MIC of **6c** was 50 μg/mL against *Bacillus subtilis*.

3.3. Structure Activity Relationship (SAR). The antimicrobial activity of compounds **6a**, **6c**, **6d**, **6e,** and **6f** demonstrated significant activities against all tested strains. The MIC was found in the following order: **6e** > **6f** > **6c** > **6d** > **6a**. Compound **6e** having unsubstituted isatin and phenylalanine as bridge was found to be the most active compound against all strains with MIC of 50 μg/mL. Compound **6f** having 5-bromoisatin and phenylalanine as bridge was found to have significant activity. From the antimicrobial activity data, structure activity relationship can be concluded as follows.

(1) The compounds with phenylalanine as a bridge weremore active than leucine and valine as a bridge.

(2) The compound with unsubstituted isatin and phenylalanine as bridge was the most active compound.

(3) The compound with 5-bromoisatin and phenylalanine as bridge displayed significant activity.

(4) The compound with 5-chloroisatin and valine as bridge displayed the least activity.

(5) The compounds with 5-chloro/5-bromoisatin and leucine as bridge displayed the moderate activity.

4. Conclusion

The coupling reaction of nicotinic acid with certain L-amino acid methyl ester was performed by the use of acid chloride method. The ester obtained was reacted with hydrazine hydrate 99% to give the corresponding hydrazide which was reacted with isatin and substituted isatin under the application of microwave irradiation to produce final compounds (**6a–g**). The synthesized compounds were characterized by spectroscopic data and checked for purity. The *in vitro* antimicrobial activity of the synthesized compounds was performed against Gram-positive bacteria (*Bacillus subtilis* and *S. aureus*), Gram-negative bacteria (*Escherichia coli*), yeast (*Candida albicans*), and filamentous fungi (*Aspergillus niger*). Most of the samples displayed significant antimicrobial activity. Compound **6e** having unsubstituted isatin and phenylalanine as bridge was found to be the most active compound against all strains with MIC of 50 μg/mL. Compound **6e** can be used as a lead compound for further development of more potent and broad spectrum antimicrobial agent.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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