Journal of Current Chemical & Pharmaceutical Sciences

J. Curr. Chem. Pharm. Sc.: 4(2), 2014, 54-64
ISSN 2277-2871

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 1,2,3-TRIAZOL-*N*-ARYLIDENE ACETOHYDRAZIDE INCORPORATING BENZIMIDAZOLE RING MOIETY AS POTENTIAL ANTIMICROBIAL AGENTS

BAHAA G. M. YOUSSIF[a], SAMIA GALAL ABDEL-MOTY[a] and IBRAHIM M. SAYED[b]

[a]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, ASSIUT – 71526, EGYPT
[b]Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, ASSIUT, EGYPT

(*Received : 03.03.2014; Accepted : 15.03.2014*)

ABSTRACT

Two series of new 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl]*N*`-(arylmethylidene) acetohydrazides (**4-14**) and 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl]*N*`-(α-arylethylidene) acetohydrazides (**15-20**) were prepared by the reaction of 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl]acetohydrazide (**3**) and the appropriate (un) substituted benzaldehyde or acetophenones. The purity of all new compounds was checked by TLC and elucidation of their structures was confirmed by IR, ^{1}H NMR, and mass spectrometry along with elemental microanalyses. All the target compounds were evaluated for their possible antimicrobial activity. Most of the tested compounds showed moderate to good antibacterial activity against most of the bacterial strains used in comparison with ciprofloxacin as a reference drug. The most active compounds were **(7)**, **(13)**, **(15)**, and **(20)**. Results of antifungal activity revealed that all compounds showed a good and potent antifungal activity in comparison to fluconazole as a reference drug. Compounds **(8)**, **(9)**, **(13)** and **(14)** were the most active compounds.

Key words: 1,2,3-Triazole, Benzimidazole, Antibacterial activity, Antifungal activity.

INTRODUCTION

The benzimidazole nucleus, which is a useful structure for research and development of new pharmaceutical molecules, has received much attention in the last decade. Due to their antimicrobial activities, new benzimidazoles have been synthesized and investigated for medical applications[1]. As resistance to antimicrobial drugs is widespread; there is an increase necessity for the identification of novel structures which could lead to the design of new, potent and less toxic antimicrobial agents. Numerous attempts have been made to develop new structural prototypes to search for more effective antimicrobials. The benzimidazoles still remain one of the most versatile classes of compounds against microbes and, therefore, are useful substructures for further molecular exploration. They exhibit a range of biological activities. Specifically, this ring system is present in numerous antioxidants[2,3], antiparasitic[4,5], antimicrobial[6-9], anthelmintic[10,11], antiproliferative[12], antiinflammatory[13,14], anticonvulsant[15], antineoplastic[16,17],

Available online at www.sadgurupublications.com
*Author for correspondence; E-mail: miar_ab_bahaa@yahoo.com

antihypertensive[18], and anti HIV agents[19]. Owing to the immense importance and varied biological activities exhibited by the benzimidazoles, efforts have been made periodically to generate libraries of these compounds and screen them for their potential biological activities. In addition, it is well documented that the triazole nucleus is associated with a variety of pharmacological activities. It displays pronounced antimicrobial[20,21], anti-inflammatory[22] and analgesic[23] activities. The effectiveness of the benzimidazole and triazole moieties towards various microbes prompted the synthesis of some new benzimidazole derivatives bearing the triazole nucleus and the screening of their potential antimicrobial activities.

EXPERIMENTAL

Materials and method

Reagents used for synthesis were purchased from Sigma-Aldrich (Gillingham-Dorest, UK) and Merck (Schuchardt, Germany). All solvents were obtained from commercial suppliers and used without further purification. The starting materials 2-(prop-2-ynylthio)-1*H*-benzimidazole **(1)**[24] and ethyl-2-azidoacetate[25] were prepared according to reported procedures.

Measurement

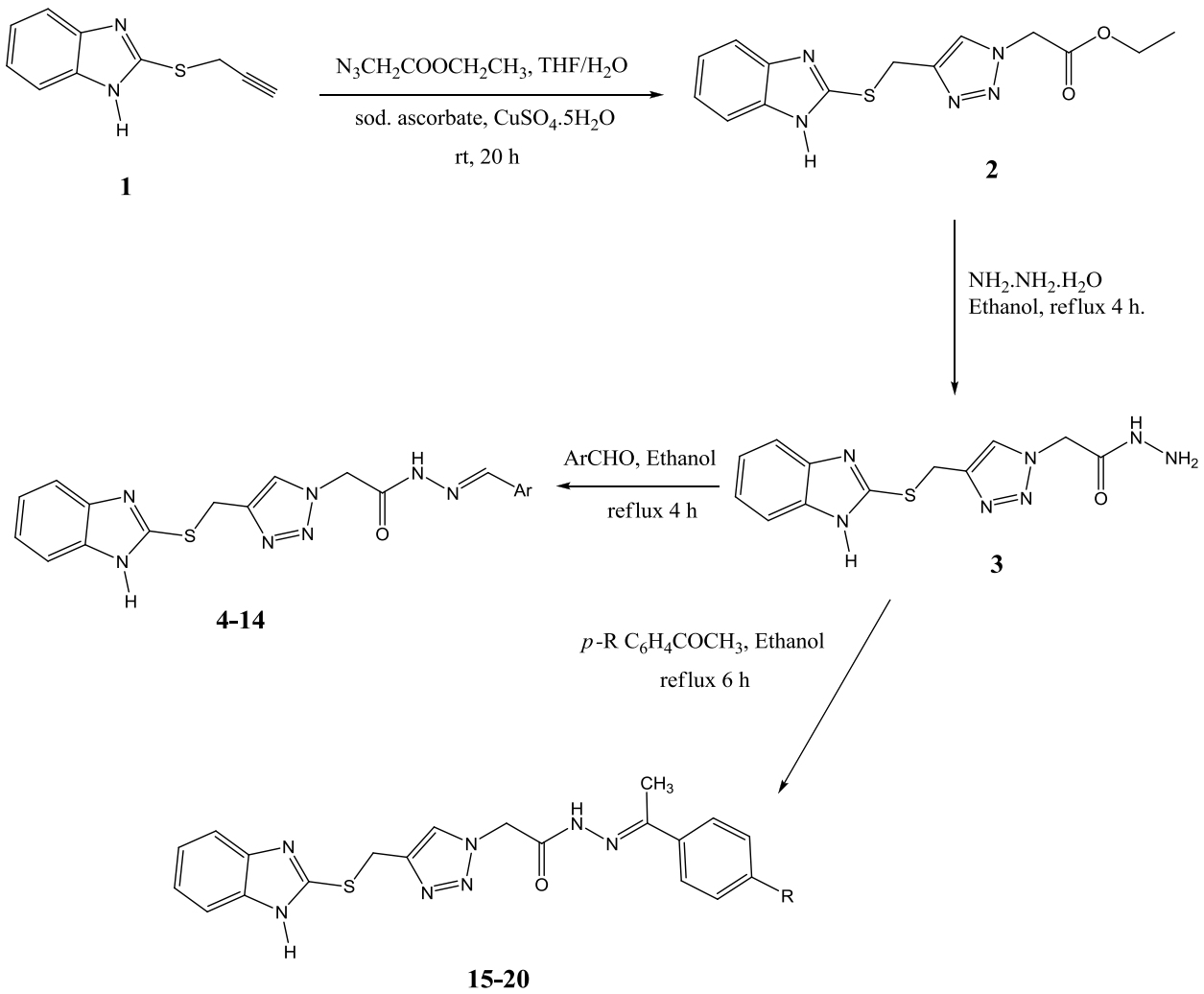
Melting points were determined on an electro thermal melting point apparatus (Stuart Scientific, model SMP3, England, UK), and were uncorrected. Pre-coated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for TLC monitoring of reactions. The developing solvent systems of $CHCl_3/CH_3OH$ (9.5:0.5 *V/V*) were used and the spots were detected at 254 nm wavelength using ultraviolet lamp (Spectroline, model CM-10, USA). The target compounds were crystallized from ethanol unless otherwise specified. IR spectra (KBr discs) were recorded on a Shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University, Assiut. ^{1}H NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using DMSO-d_6, unless otherwise specified, as a solvent, and deuterium oxide was used for the detection of exchangeable protons. Mass spectra were recorded with JEOL JMS600 mass spectrometer (JEOL, Tokyo, Japan) at Faculty of Pharmacy, Assiut University, Assiut and at the unit of Microanalysis, Faculty of Science, Cairo University, Cairo. Elemental microanalyses were performed on a Perkin-Elmer 240 elemental analyzer (Perkin-Elmer, USA) at the unit of Microanalysis, Faculty of Science, Cairo University, Cairo.

Synthesis of ethyl 2-[4-((1H-benzimidazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl] acetate (2)

To a solution of compound (**1**) (1.88 g, 10.0 mmol) in a mixture of THF/H_2O [2:1 (v/v), 15 mL], ethyl-2-azidoacetate (1.55 g, 12.0 mmol), $CuSO_4.5H_2O$ (0.12 g, 0.5 mmol) and sodium ascorbate (0.20 g, 1.0 mmol) were added. The mixture was stirred at room temperature for 20 h. the residue was extracted with $CHCl_3$ (3 x 10 mL), the organic phase was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The crude product was crystallized from ethanol to afford pure compound as a white solid **Scheme 1**, Table 1.

Synthesis of 2-[4-((1H-benzimidazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl] acetohydrazide (3)

To a solution of ethyl 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl]acetate (**2**) (3.17 g, 10.0 mmol) in absolute ethanol (40 mL), hydrazine hydrate 99% (0.75 g, 15.0 mmol) was added. The reaction mixture was refluxed for 6 h, and then cooled. The precipitated product was filtered, washed with cold ethanol, dried, and crystallized from ethanol as white crystals **(Scheme 1)** (Table 1).



Ar = C_6H_5, *p*-BrC_6H_4, *p*-ClC_6H_4, *m*-BrC_6H_4, *m*-ClC_6H_4, *p*-FC_6H_4, *p*-HOC_6H_4, *p*-$CH_3C_6H_4$, *p*-$CH_3OC_6H_4$, *p*-isopropylC_6H_4, *p*-dimethylaminoC_6H_4.

R = H, Br, Cl, F, CH_3, CH_3O.

Scheme 1: Synthetic route of compounds (2), (3), (4-14) and (15-20)

Table 1: Analytical data and elemental analysis of compounds (2-14)

Compd. No.	Ar	Yield (%)	M.P. (°C)	Molecular formula (Mol. wt.)	Microanalysis Calculated/found		
					C%	H%	N%
2	–	92	186-188	$C_{14}H_{15}N_5O_2S$ (317.09)	52.98 52.70	4.76 4.53	22.07 22.70
3	–	84	206-208	$C_{12}H_{13}N_7OS$ (303.09)	47.51 47.21	4.32 4.20	32.32 32.52
4	C_6H_5	89	196-198	$C_{19}H_{17}N_7OS$ (391.44)	58.30 58.18	4.38 4.33	25.05 25.09

Cont…

Compd. No.	Ar	Yield (%)	M.P. (°C)	Molecular formula (Mol. wt.)	Microanalysis Calculated/found		
					C%	H%	N%
5	*p*-BrC_6H_4	93	201-203	$C_{19}H_{16}BrN_7OS$ (470.34)	48.52 48.27	3.34 3.17	20.85 20.77
6	*p*-ClC_6H_4	91	198-199	$C_{19}H_{16}ClN_7OS$ (425.89)	53.58 53.50	3.79 3.53	23.02 22.67
7	*m*-BrC_6H_4	90	199-201	$C_{19}H_{16}BrN_7OS$ (470.34)	48.52 48.35	3.34 3.30	20.85 20.37
8	*m*-ClC_6H_4	89	197-199	$C_{19}H_{16}ClN_7OS$ (425.89)	53.58 53.28	3.79 3.57	23.02 22.85
9	*p*-FC_6H_4	91	201-203	$C_{19}H_{16}FN_7OS$ (409.44)	55.74 55.52	3.94 3.80	23.95 23.82
10	*p*-HOC_6H_4	88	207-209	$C_{19}H_{17}N_7O_2S$ (407.44)	56.01 55.77	4.21 4.33	24.06 24.54
11	*p*-$CH_3C_6H_4$	87	185-187	$C_{20}H_{19}N_7OS$ (405.47)	59.24 59.02	4.72 4.52	24.18 24.02
12	*p*-$CH_3OC_6H_4$	86	195-197	$C_{20}H_{19}N_7O_2S$ (421.47)	56.99 56.90	4.54 4.22	23.26 23.16
13	*p*-IsopropylC_6H_4	84	191-193	$C_{22}H_{23}N_7OS$ (433.52)	60.95 60.75	5.35 5.45	22.62 22.42
14	*p*-DimethylaminoC_6H_4	83	192-194	$C_{21}H_{22}N_8OS$ (434.51)	58.05 57.72	5.10 5.14	25.79 25.99

Synthesis of 2-[4-((1H-benzimidazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl]N`-(arylmethyl-idene) acetohydrazides (4-14)

To a suspension of 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl] acetohydrazide **(3)** (0.60 g, 2.0 mmol) in ethanol (10 mL) and the appropriate aryl aldehyde (2.0 mmol), 2 drops of glacial acetic acid were added. Then the reaction mixture was heated under reflux for 6 h. The reaction mixture was cooled and the precipitated product was filtered, washed with cold ether and crystallized from ethanol. Yields, m.p., elemental analyses data are listed in Table 1.

Synthesis of 2-[4-((1H-benzimidazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl]N`-(α-arylethyl-idene) acetohydrazides (15-20)

To a suspension of 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl] acetohydrazide (**3**) (0.60 g, 2.0 mmol) in ethanol (10 mL) and the appropriate acetophenone derivative (2.0 mmol), 2 drops of glacial acetic acid were added. Then the reaction mixture was heated under reflux for 8 h, cooled and the formed precipitate was filtered, dried and crystallized from DMF/H_2O [7:4 (v/v)]. Yields, m.p., elemental analyses data are listed in Table 2.

Table 2: Analytical data and elemental analysis of compounds (15-20)

Compd. No.	R	Yield (%)	M.P. (°C)	Molecular formula (Mol. wt.)	Microanalysis Calculated/found		
					C%	H%	N%
15	H	89	196-198	$C_{20}H_{19}N_7OS$ (405.47)	59.24 58.90	4.72 4.78	24.18 23.92
16	Br	91	201-202	$C_{20}H_{18}BrN_7OS$ (484.37)	49.59 49.27	3.75 3.72	20.24 19.94
17	Cl	90	198-200	$C_{20}H_{18}ClN_7OS$ (439.92)	54.60 54.61	4.12 4.53	22.29 22.37
18	F	90	191-193	$C_{20}H_{18}FN_7OS$ (423.46)	56.73 56.35	4.28 4.30	23.15 23.37
19	CH_3	89	182-184	$C_{21}H_{21}N_7OS$ (419.50)	60.12 60.28	5.05 5.57	23.37 23.85
20	CH_3O	91	185-187	$C_{21}H_{21}N_7O_2S$ (435.50)	57.92 57.76	4.86 4.80	22.51 22.69

Biological screening

Antibacterial activity

Organisms and culture conditions

The antibacterial activity of the entire target compounds **(3)**, **(4-14)** and **(15-20)** was investigated *in vitro* at the Department of Microbiology and Immunology, Faculty of Medicine, Assiut University. The title compounds were tested against *Staphylococcus aureus* (gram positive cocci) and *Escherichia coli* as a representative of gram negative bacilli (clinical isolates obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University) using agar cup diffusion method[26] for susceptibility screening, and two-fold dilution method[27] for MIC determination. Ciprofloxacin was used as reference antibiotic, and DMSO was used as a solvent control.

38 g of Mueller-Hinton agar medium (MH) (Hi-Media, M 001) were added to 1 L of distilled water, heated to boiling to dissolve the ingredients completely, and sterilized by autoclaving at 121°C for 30 min. High density inocula were made by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard unit with average turbidity 10^8 CFU mL^{27}. The sterile Petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50ºC) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using sterile cork borer. 100 µL of the tested compounds (20 mg/mL in DMSO), the blank solvent, ciprofloxacin (20 mg/mL in DMSO) was added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 h and then the average diameters of the inhibition zones were measured in millimeters. Results are displayed in Table 3.

Table 3: Inhibitory zone diameter (in mm) of compounds (3-20) and Ciprofloxacin

Compd. No.	Gram-positive bacteria	Gram-negative bacteria
	Staph. aureus	***E. coli***
3	12	20
4	14	22
5	14	20
6	18	22
7	20	32
8	20	24
9	26	22
10	16	20
11	18	22
12	20	24
13	30	30
14	18	22
15	20	28
16	26	20
17	20	20
18	20	20
19	18	22
20	20	28
Ciprofloxacin	40	40

Antifungal activity

Organisms and culture conditions

The used Sabouraud Agar (SA) media were prepared in the Department of Microbiology and Immunology, Faculty of Medicine, Assiut University. The test compounds **(3)**, **(4-14)** and **(5-20)** were evaluated for their antifungal activity *in vitro*, in comparison to fluconazole as a reference drug using the standard agar cup diffusion method[27] against a pathogenic fungal species *Candida albicans* (clinical isolate obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University).

Spore suspension in sterile distilled water was prepared from 7 days old culture of the test fungi growing on Sabouraud's dextrose broth (30 mL) media in 100 mL conical flasks. The final spore concentration was nearly 5 × 104 spores/mL. About 15 mL of the growth medium was introduced on sterilized Petri dishes of 10 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer and was filled with the solutions of the test compounds **(3)**, **(4-14)** and **(5-20)** and fluconazole (100 µmol/mL in DMSO). In addition, other cavities were impregnated with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28 ± 2°C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are displayed in Table 4.

Table 4: Inhibitory zone diameter (in mm) of compounds (3-20) and **Fluconazole**

Compd. No.	*Candida albicans*
3	19
4	22
5	26
6	20
7	24
8	30
9	26
10	24
11	20
12	24
13	30
14	28
15	26
16	20
17	26
18	24
19	22
20	21
Fluconazole	40

The minimum inhibitory concentrations (MICs)

The MIC values were determined using two fold-dilution method[28]. The test compounds **(3)**, **(4-14)** and **(5-20)** were diluted with DMSO to prepare a series of descending concentration down to 0.02 mg/mL. Diluted solutions were similarly assayed as mentioned before and the least concentration (below which no activity) was recorded. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC. MIC was obtained as antilog[29] and the results are cited in Table 5.

Table 5: Antimicrobial activity of tested compounds expressed as *MIC* in µmol mL^{-1}

Compd. No.	*Staph. aureus*	*E. coli*	*Candida albicans*
3	NT[a]	12.50	12.50
4	NT	12.50	12.50
5	NT	12.50	6.25
6	25.00	12.50	12.50
7	25.00	3.12	6.25

Cont…

Compd. No.	*Staph. aureus*	*E. coli*	*Candida albicans*
8	18.00	6.25	3.12
9	12.50	12.50	6.25
10	NT	12.50	6.25
11	25.00	12.50	12.50
12	25.00	6.25	6.25
13	12.50	3.12	3.12
14	25.00	12.50	3.12
15	25.00	6.25	6.25
16	18.00	12.50	12.50
17	18.00	12.50	6.25
18	25.00	12.50	6.25
19	25.00	12.50	12.50
20	25.00	6.25	6.25
Ciprofloxacin	**1.75**	**1.75**	**NT**
Fluconazole	**NT**	**NT**	**1.75**

[a]Not tested

RESULTS AND DISCUSSION

The key intermediate 2-(prop-2-ynylthio)-1*H*-benzimidazole; compound (**1)** was prepared according to a reported procedure and its structure was confirmed by matching its physical and spectral data with the reported one[24].

Treatment of compound (**1)** with ethyl-2-azidoacetate in a mixture of THF/H_2O in presence of sodium ascorbate and copper sulfate afforded a new key intermediate; ethyl 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl]acetate; compound **(2)**, which when refluxed with hydrazine hydrate yielded 2-[4-((1*H*-benzimidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl]acetohydrazide; compound **3 (Scheme 1)**.

IR spectrum of compound **(2)** showed NH band at 3440 cm^{-1} while IR spectrum of compound **(3)** showed bands at 3420, 3250 and 3160 cm^{-1} due to NH and $NHNH_2$ functions. Also IR spectra of compounds **(2)** and **(3)** showed bands at 1729 and 1652 cm^{-1} due to carbonyl function derived from ester and hydrazide structures, respectively. ^{1}H NMR spectrum of compound **(2)** showed a triplet signal at δ 1.10-1.50 ppm and quartet at 4.00-4.40 ppm corresponding to C_2H_5 moiety, singlet signals at 4.70 (-SCH_2), 5.40 (-NCH_2) and 8.10 (-CH triazole) ppm, in addition to a broad singlet at 11.87 corresponding to benzimidazole-NH group (exchangeable with D_2O). ^{1}H NMR spectrum of compound **(3)** showed new signals derived from hydrazide structure appeared at 3.98 ($NHNH_2$) and 8.90 ($NHNH_2$) ppm integrating for two protons and one proton, respectively (exchangeable with D_2O).

The target compounds; **(4-14)** and **(15-20)**, were synthesized by condensation of compound **(3)** with aryl aldehydes or substituted acetophenones, respectively (**Scheme 1**). The structures of compounds **(4-14)** and **(15-20)** were confirmed by IR and ^{1}H NMR, MS as well as elemental analyses. The IR spectra of

compounds **(4-14)** showed bands in the 3480-3135 cm^{-1} regions for the NH groups. The C=O groups of compounds **(4-14)** absorbed in the 1690-1656 cm^{-1} regions while, IR spectra of compounds **(15-20)** showed bands in the 3590-3155 cm^{-1} regions for the NH groups. The C=O groups of compounds **(15-20)** absorbed in the 1674-1671 cm^{-1} regions.

^{1}H NMR spectra of compounds **(4-14)** and **(15-20)** displayed additional signals due to the aromatic ring derived from aldehyde or acetophenone moieties in the aromatic region, and the signal belonging to NH_2 group of the hydrazide structure disappeared.

^{1}H NMR spectra of compounds **(4-14)** are characterized by some general features such as the presence of a singlet signals at 4.65-4.70 ($-SCH_2$), 5.70-5.80 ($-NCH_2$), 7.95-8.15 (-CH triazole), 8.00-8.30 (-N=CH), 11.20-11.40 (-CONH) ppm, in addition to a broad singlet at 11.90-12.10 corresponding to benzimidazole-NH group (exchangeable with D_2O).

The IR spectrum of compound **(6)** shows a characteristic bands at 3434 and 3215 cm^{-1} due to NH groups, band at 1686 of C=O group , and bands at 1604, 1582, 1545, 1512, and 1458 cm^{-1} corresponding to C=N and C=C functions. It also showed a band at 829 cm^{-1} characteristic of *p*-disubstituted benzene ring. Mass spectrum of compound **(6)** revealed the molecular ion peak M^+ at m/z 425.1 (100%) corresponding to the molecular mass of this compound. Also, the spectrum showed a peak at M^++2 (m/z 427.1, 36.1) corresponding to $CH^{37}ClNOS$.

^{1}H NMR spectrum of compound **(13)** showed a triplet signal at δ 1.33 ppm and multiplet at 2.7-3.2 ppm corresponding to isopropyl moiety, singlet signals at 4.65 ($-SCH_2$), 5.70 ($-NCH_2$) and 7.90 (-CH triazole) ppm, in addition to a broad singlet at 12.10 corresponding to benzimidazole-NH group (exchangeable with D_2O). Mass spectrum of compound **(13)** revealed the molecular ion peak M^+ at m/z 433.2 (3.7%) corresponding to the molecular mass of this compound.

^{1}H NMR spectrum of compound **(20)** showed a singlet signal at δ 2.25 ppm (CCH_3), singlet signal at 3.8 ppm corresponding to methoxy moiety, singlet signals at 4.65 ($-SCH_2$), 5.70 ($-NCH_2$) and 7.90 (-CH triazole) ppm, in addition to a broad singlet at 12.10 corresponding to benzimidazole-NH group (exchangeable with D_2O). Mass spectrum of compound **(20)** revealed the molecular ion peak M^+ at m/z 435.2 (12.7%) corresponding to the molecular mass of this compound.

Antimicrobial screening

Antibacterial activity

The synthesized compounds **(3)**, **(4-14)**, and **(5-20)** were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus* as a representative of Gram positive strains and *Escherichia coli* as a Gram negative strain using ciprofloxacin as a reference drug. The result revealed that most of the newly synthesized compounds exhibited promising antibacterial activity comparable to ciprofloxacin against the test organisms (Table 3).

First of all, the hydrazide derivative, compound **(3)**, was found to exhibit antibacterial activity nearly 55% that of ciprofloxacin against *E.Coli* (*MIC* = 12.5 μmol mL^{-1}), however it showed only 30% activity of ciprofloxacin against *Staphylococcus aureus* (*MIC* = 25 μmol mL^{-1}).

Compounds **(4-14)** were found to exhibit pronounced antibacterial activity which ranged from 35-75% that of standard drug against *Staphylococcus aureus* and 50-80% that of ciprofloxacin against *E.Coli* (*MIC* values of 3.125-12.5 μmol mL^{-1}). It is worthy-mentioning that compound **(13)** showed the highest

activity against *Staphylococcus aureus* (75% activity, *MIC* = 12.5 μmol mL^{-1}) while compound **7** was the most active derivative against *E.Coli* (80% activity, *MIC* = 3.125 μmol mL^{-1}).

Furthermore, compounds **(15-20)** exhibited moderate to good activity against *E.Coli* and their activity was 50-70% that of ciprofloxacin (*MIC* values of 6.25-12.5 μmol mL^{-1}), but they showed weak to moderate activity (45-65% that of ciprofloxacin) against *Staphylococcus aureus* (*MIC* ranged from 12.5-25 μmol mL^{-1}) and that compounds **(15)** and **(20)** were the most active compounds against *E. coli* as they showed 70% that of fluconazole (*MIC* = 6.25 μmol mL^{-1}) while compound **(16)** showed the highest activity against *Staphylococcus aureus* (65% that of ciprofloxacin, *MIC* = 18 μmol mL^{-1}).

Antifungal activity

All the synthesized compounds **(3)**, **(4-14)**, and **(5-20)** were tested as potential antifungal agents against *Candida albicans* using fluconazole as a reference drug (Table 4).

The results revealed that all the tested compounds have potent antifungal activity against the test organism with *MIC* values of 3.125-12.5 μmol mL^{-1}. Compound **(3)** showed activity 48% that of fluconazole (*MIC* = 12.5 μmol mL^{-1}). Moreover, further derivatization of compound **(3)** with different (un)substituted benzaldehyde, compounds **(4-14)** and acetophenones, compounds **(15-20)** affords compounds with improved antifungal activity against *Candida albicans*, showing activity 50-75% that of fluconazole. Compounds **(8)** and **(13)** displayed the higher antifungal activity among the other derivatives as they showed 75% activity of fluconazole (*MIC* = 3.125 μmol mL^{-1}).

CONCLUSION

In conclusion, several 1,2,3-triazol-*N*-arylidene acetohydrazide incorporating benzimidazole ring moiety derivatives were synthesized starting with 2-(prop-2-ynylthio)-1*H*-benzimidazole. A microbiological study was undertaken to evaluate the effect of the synthesized compounds on different bacterial and fungal strains. The results of the preliminary testing of the antibacterial activity of the final compounds revealed that the majority of the synthesized compounds show varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-negative bacteria was higher than against the Gram-positive bacteria. The triazole derivatives **(7)**, **(13)**, **(15)**, and **(20)** displayed the highest activity. Results of antifungal activity revealed that all compounds showed a good and potent antifungal activity and that compounds **(8), (9), (13)** and **(14)** were the most active ones.

REFERENCES

1. K. F. Ansari, C. Lal and R. K. Khitoliya, J. Serb. Chem. Soc., **76(3)**, 341-352 (2011).
2. G. Ayhan-Kilcigil, C. Kus, E. D. Ozdamar, B. Can-Eke and M. Iscan, Arch. Pharmacal. Res., **34**, 607-6011 (2007).
3. Z. Ates-Alagoz, C. Kus and T. Coban, J. Enzyme Inhibit. Med. Chem., **20**, 325-331 (2005).
4. G. Navarrete-Vázquez, R. Cedillo, A. Hernández-Campos, L. Yépez, F. Hernädez-Luis, J. Valdez, R. Morales, R. Cortés, M. Hernández and R. Castillo, Bioorg. Med. Chem. Lett., **11,** 187-190 (2001).
5. F. Hernández-Luis, A. Hernández-Campos, R. Castillo, G. Navarrete-Vázquez, O. Soria-Arteche, M. Hernández-Hernández and L. Yépez-Muli, Eur. J. Med. Chem., **45**, 3135-3141 (2010).
6. Y. Özkay, Y. Tunal, H. Karaca and I. Işıkdağ, Eur. J. Med. Chem., **45**, 3293-3298 (2010).
7. N. S. Pawar, D. S. Dalal, S. R. Shimpi and P. P. Mahulikar, Eur. J. Pharm. Sci., **21,** 115-118 (2004).

8. K. G. Desai and K. R. Desai, Bioorg. Med. Chem., **14,** 8271-8279 (2006).

9. O. O. Guven, T. Erdogan, H. Goker and S. Yildiz, Bioorg. Med. Chem. Lett., **17**, 2233-2236 (2007).

10. A. T. Mavrova, P. S. Denkova, Y. A. Tsenov, K. K. Anichina and D. I. Vutchev, Bioorg. Med. Chem., **15**, 6291-6297 (2007).

11. A. T. Mavrova, D. Vuchev, K. Anichina and N. Vassilev, Eur. J. Med. Chem., **45**, 5856-5861 (2010).

12. L. Garuti, M. Roberti, M. Malagoli, T. Rossi and M. Castelli, Bioorg. Med. Chem. Lett., **10**, 2193-2195 (2000).

13. H. T. Le, I. B. Lemaire, A. K. Gilbert, F. Jolicoeur, N. Leduc and S. Lemaire, J. Pharmacol. Exp. Ther., **309**, 146-155 (2004).

14. P. A. Thakurdesai, S. G. Wadodkar and C. T. Chopade, Pharmacol. Online, **1**, 314-329 (2007).

15. A. Chimirri, A. De Sarro, G. De Sarro, R. Gitto and M. Zappala, Farmaco, **56**, 821-826 (2001).

16. S. Ram, D. S. Wise, L. L. Wotring, J. W. McCall and L. B. Townsend, J. Med. Chem.**, 35**, 539-547 (1992).

17. A. A. M. Abdel-Hafez, Arch. Pharmacal Res., **30**, 678-684 (2007).

18. K. Kubo, Y. Inada, Y. Kohara, Y. Sugiura, M. Ojima, K. Itoh, Y. Furukawa, Y. K. Nishikawa and T. Naka, J. Med. Chem., **36**, 1772-1764 (1993).

19. A. Rao, A. Chimirri, E. De Clercq, A. M. Monforte, P. Monforte, C. Pannecouque and M. Zappala, Farmaco, **57**, 819-823 (2002).

20. B. G. Mohammed, M. A. Hussein, A. M. Abdel-Alim and M. Hashem, Arch. Pharmacal Res., **29**, 26-33 (2006).

21. N. N. Güllerman, H. N. Dogan, S. Rollas, C. Johansson and C. Celik, Farmaco, **56**, 953-958 (2001).

22. J. R. Maxwell, D. A. Wasdahl and A. C. Wolfson, J. Med. Chem., **27**, 1565-1570 (1984).

23. G. T-Zitouni, Z. A. Kaplancikli, K. Erol and F. S. Kilic, Farmaco, **54**, 218-223 (1999).

24. M. M. Heravi, A. Keivanloo, M. Rahimizadeh, M. Bakavoli and M. Ghassemzadeh, Tetrahedron Lett., **45**, 5747-5749 (2004).

25. L. Campbell-Verduyna, L. Mirfeizib, R. A. Dierckxb, P. H. Elsingab and B. L. Feringa, Chem. Commun., **16**, 2139-2141 (2009).

26. A. M. Clark, F. S. El-Feraly and W. S. Li, J. Pharm. Sci., **70**, 951-952 (1981).

27. A. Felten, B. Grandly, P. H. Lagrange and I. Casin, J. Clin. Microbiol., **40**, 2766-2771 (2002).

28. A. C. Scott, Laboratory Control of Antimicrobial Therapy, in, J. G. Collee, J. P. Duguid, A. G. Fraser and B. P. Marmion, (Eds.). Mackie & McCartney Practical Medical Microbiology, Churchill Livingstone, Edinburgh (1989) pp. 161-181.

29. W. Hewitt, Microbiological Assay, An Introduction to Quantitative Principles and Evaluation, Academic Press, New York (1977) pp. 17-69.