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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SCHIFF BASES OF BENZIMIDAZOLE DERIVATIVES

Asma Eswayah[1]*, Rima Hermas[1], Souad Khaliel[2], Rehab R. Walli[3], Amal Belaid[1], Omran Fhid[1], Marwa Elmelady [1], Esraa Elhaddad[1], Fadilah Aleanizy[4] and Fulwah Alqahtani[4]

[1]Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.
[2]Department of Chemistry, Faculty of Science, University of Benghazi, Benghazi, Libya.
[3]Department of Biochemistry and Clinical Biochemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.
[4]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

***Corresponding Author: Asma Eswayah**
Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.

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ABSTRACT
A series of benzimidazole derivatives having imine linkage were synthesized by using *o*-phenylenediamine as starting material. In first step, *o*-phenylenediamine is treated with 4-amino benzoic acid to give 2-(4-aminophenyl) benzimidazole (1a) which was then treated with different substituted aldehydes to give benzimidazole containing Schiff bases (1-12). All the synthesized compounds have been characterized by physical and spectral analysis, and their antimicrobial activity was investigated by agar-well diffusion method. Only compound 11 have shown antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, while compounds 10 and 11 showed antifungal activity against *Candida albicans*. The minimal inhibitory concentration (MIC) for compounds 10 and 11 was determined by broth microdilution method. The other compounds were found to exhibit no activity against the bacterial and fungal organism tested.

KEYWORDS: 2-Substituted Benzimidazole, Schiff Base, Antibacterial Activity, Antifungal Activity.

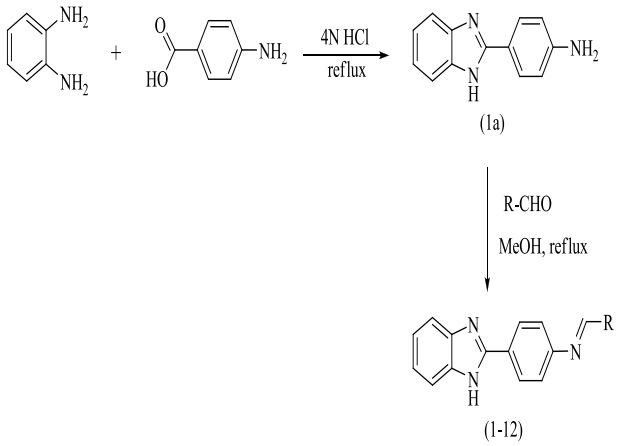
INTRODUCTION
Benzimidazole-containing compounds have been the aim of many researchers for many years because they constitute an important class of heterocyclic compounds exhibiting substantial chemotherapeutic activities such as antibacterial,[1,3] antifungal,[4,5] antitubercular[6] antiviral,[7] anthelmintic,[8] analgesic,[9,10] anti-inflammatory,[11,12] antioxidant,[13] anticancer,[14,15] antiulcer,[16] antihypertensive,[16,17] anticonvulsant,[18] antidepressant,[19] and antidiabetic properties.[20]

The compounds carrying imine or azomethine (–C=N–) functional group are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups and were first reported by Hugo Schiff.[21] Schiff bases are important class of compounds in medicinal and pharmaceutical field. They show biological properties include antibacterial, antifungal, antiviral, antitumor and antimalarial properties.[22,23]

The widespread interest in benzimidazole containing Schiff bases has prompted extensive studies for their synthesis and biological activities.[24-27] In the present work, we synthesized a series of benzimidazole-based Schiff bases in order to investigate their antibacterial activity against different strains of bacteria as well as antifungal activity against *Candida albicans.*

MATERIALS AND METHODS
All reagents and solvents were purchased from suppliers (Merck and Sigma Aldrich) and used without further purification unless otherwise stated. Reactions were monitored by TLC. TLC plates pre-coated with silica gel 60 F254 on aluminium (Merck KGaA) were used, being visualised by UV (254 or 365 nm). Infrared spectra were recorded on a Victor 22/Varian FT-IR spectrometer. Melting points were determined using Buchi B-540 melting point apparatus and are uncorrected.

Experimental Procedures
A series of 2-(4-aminophenyl) benzimidazole-based Schiff bases were synthesized from *o*-phenylenediamine and *p*-aminobenzoic acid to produce compound 1a. Furthermore, Schiff bases (1-12) were prepared from the reaction of compound 1a with various aromatic aldehydes (scheme 1).



Scheme 1: Synthesis of compounds 1a and 1-12

Procedure followed for synthesis

Preparation of 2-(4-aminophenyl) benzimidazole (1a)[28]

A mixture of equimolar quantities of *o*-phenylenediamine (0.01mol) and *p*-aminobenzoic acid (0.01mol) in 4N HCl (20 mL) was refluxed for 2 hrs. The mixture was then cooled to room temperature and filtered off. The resulting product was recrystallized from ethanol as greyish needles, yield 60%, ν (KBr) cm^{-1}: 3449, 3410 (N-H str.), 3083, 3041 (C-H str., Ar-H), 1609 (C=N str.), 1241 (C-N str.).

General procedure for the preparation of Schiff bases (1-12)[28]

A mixture of an aromatic aldehyde (0.015mol), compound 1a (0.01mol) in 20 mL of MeOH and few drops of glacial acetic acid was heated at reflux for 7-9 hours with stirring. The reaction mixture was then cooled to room temperature, and the solvent was concentrated in vacuum. The obtained product was filtered, dried and then recrystallized from a suitable solvent. Physical properties of Schiff bases (1-12) are listed in table 1.

Antimicrobial screening

The compounds are evaluated for their *in vitro* antimicrobial activity. Bacterial and fungal strains used in the antimicrobial evaluation are *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Klebsiella pneumonia* (ATCC 13883) and *Candida albicans* (ATCC 10231). The antimicrobial activity was carried out by the agar-well diffusion method.[29-32]

Sabouraud for *Candida albicans* and Mueller-Hinton for all the others was placed into Petri dish 90 mm. The agar plates were then inoculated with broth cultures diluted to 0.5 McFarland turbidity ($1.5.10^8$ cells/mL). The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface by using sterile cotton swab. Then, a hole with a diameter of 7 mm is punched aseptically with a small sterile glass tube, and a volume (50 µL) of the compound solution in DMSO at concentration of 1 mg/mL is introduced into the wells. Then, agar plates are incubated 24 hours at 37 ^oC for bacteria and at 35 ^oC for candida albicans. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.[29,33]

The minimum zone of inhibition (MZI) for ciprofloxacin (antibacterial), fluconazole (antifungal) was used as reference values (in millimeters). All experiments were conducted in triplicate and repeated if the results differed. All tested compounds having MZI larger than or equal to 14 mm were selected for MIC tests.

For the minimum inhibitory concentration MIC assays[33,34] a stock solution (1mg/mL) of the compounds shown antimicrobial activity was prepared in DMSO. Further, a serial dilution of test compounds was made in a liquid medium which is inoculated with broth cultures of 0.5 McFarland ($1.5.10^8$ cells/mL), to achieve concentrations ranging from 4 to 500 μg/mL.

Nutrient agar (for antibacterial) and Sabouraud liquid medium (for antifungal) were utilized as culture media. The tubes were incubated at 37^oC (antibacterial) or 35^oC (antifungal) for 24-48 h and then examined for the growth of the tested organisms or their absence. Ciprofloxacin and fluconazole were used as antibacterial and antifungal substances, respectively. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear, indicating that the bacterial or fungal growth was completely inhibited at this concentration.

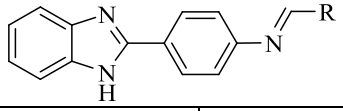
RESULTS AND DISCUSSION

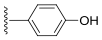
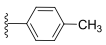
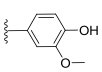
Chemistry

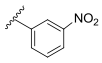
Our desired products were based on benzimidazole derivatives. It is possible to design a wide range of potential microbial inhibitors by replacing the hydrogen at various positions of the benzimidazole ring with different functional groups. However, the most accessible derivatives are those with substituents at the 1-, 2- and 5-positions.

In this study, 2-substituted benzimidazole derivatives (1-12), have been synthesized. The synthetic pathway for preparation of the desired compounds is shown in scheme 1. Compound 1a was obtained in 60% yield by refluxing of *o*-phenylenediamine and *p*-aminobenzoic acid in acidic medium (4N HCl). Reaction of 2-(4-aminophenyl) benzimidazole (1a) with different aromatic aldehydes in methanol under refluxing conditions gave the corresponding 2-substituted benzimidazole-based Schiff bases (1-12) (scheme 1). Their structural features along with IR spectral data and physicochemical parameters are shown in table 1.

Table 1: Structure and physicochemical properties of the compounds 1-12.



Comp. No.	R	Systematic name	M. Formula (M. Wt)	m.p. (°C)	Yield (%)	IR spectral data □□ cm^{-1})
1		4-(1*H*-benzimidazol-2-yl)-*N*-benzylidene aniline	$C_{20}H_{15}N_3$ (297.35)	185-187	25%	ν (cm^{-1}) = 3405 (N-H str.), 2913 (C-H str., Ar-H), 1681 (C=N imine), 1610, 1556 (C=C), 1286 (C-N)
2		4-((4-(1*H*-benzimidazol-2-yl)-phenylimino)methyl)phenol	$C_{20}H_{15}N_3O$ (313.35)	220-225	74%	ν (cm^{-1}) = 3389 (N-H), 2998 (C-H str., Ar-H), 1649 (C=N imine), 1599, 1578 (C=C), 1290 (C-N), 3120 (Ar-OH)
3		4-((4-(1*H*-benzimidazol-2-yl)-phenylimino)methyl)-*N*,*N*-dimethylaniline	$C_{22}H_{20}N_4$ (340.42)	175-178	49%	ν (cm^{-1}) = 3425 (N-H), 3012 (C-H str., Ar-H), 1610 (C=N imine), 1559, 1550 (C=C), 1285 (C-N), 2809 (Ar-N-CH_3)
4		4-(1*H*-benzimidazol-2-yl)-*N*-(4-chlorobenzylidene) aniline	$C_{20}H_{14}ClN_3$ (331.8)	180-183	41%	ν (cm^{-1}) = 3356 (N-H), 2997 (C-H str., Ar-H), 1608 (C=N imine), 1519 (C=C), 1285 (C-N), 840 (Ar-Cl)
5		4-(1*H*-benzimidazol-2-yl)-*N*-(4-methoxybenzylidene) aniline	$C_{21}H_{17}N_3O$ (327.38)	212-215	46%	ν (cm^{-1}) = 3369 (N-H), 3045 (C-H str., Ar-H), 1654 (C=N imine), 1598, 1581 (C=C), 1282 (C-N), 1202 (Ar-OCH_3)
6		4-(1*H*-benzimidazol-2-yl)-*N*-(4-methylbenzylidene) aniline	$C_{21}H_{17}N_3$ (311.38)	160-165	64%	ν (cm^{-1}) = 3412 (N-H), 3021 (C-H str., Ar-H), 1606 (C=N imine), 1506 (C=C), 1289 (C-N), 2918, 2859 (Ar-CH_3)
7		4-((4-(1*H*-benzimidazol-2-yl)phenylimino)methyl)-2-methoxyphenol	$C_{21}H_{17}N_3O_2$ (343.38)	120-123	62%	ν (cm^{-1}) = 3328 (N-H), 3038 (C-H str., Ar-H), 1689 (C=N imine), 1604, 1596 (C=C), 1269 (C-N), 1201 (Ar-OCH_3), 3319 (Ar-OH)
8		4-(1*H*-benzimidazol-2-yl)-*N*-(3-phenylallylidene) aniline	$C_{22}H_{17}N_3$ (323.39)	140-145	28%	ν (cm^{-1}) = 3416 (N-H), 2895 (C-H str., Ar-H), 1611 (C=N imine),

						1556, 1518 (C=C), 1284 (C-N)
9		4-(1*H*-benzimidazol-2-yl)-*N*-(furan-2-ylmethylene)aniline	$C_{18}H_{13}N_3O$ (287.32)	126-128	84%	ν (cm^{-1}) = 3448 (N-H), 2950 (C-H str., Ar-H), 1605 (C=N imine), 1512 (C=C), 1281 (C-N).
10		4-(1*H*-benzimidazol-2-yl)-*N*-(thiophene-2-ylmethylene)aniline	$C_{18}H_{13}N_3S$ (303.38)	132-135	47%	ν (cm^{-1}) = 3484 (N-H), 3051 (C-H str., Ar-H), 1660 (C=N imine), 1610, 1589 (C=C), 1289 (C-N), 687 (C-S-C)
11		2-((4-(1*H*-benzimidazol-2-yl)phenylimino)methyl)phenol	$C_{20}H_{15}N_3O$ (313.35)	176-180	35%	ν (cm^{-1}) = 3527 (N-H), 2995 (C-H str., Ar-H), 1621 (C=N imine), 1600, 1572 (C=C), 1283 (C-N), 3404 (Ar-OH)
12		4-(1*H*-benzimidazol-2-yl)-*N*-(3-nitrobenzylidene) aniline	$C_{20}H_{14}N_4O_2$ (342.35)	120-125	67%	ν (cm^{-1}) = 3376 (N-H), 3020, 2962 (C-H str., Ar-H), 1631 (C=N imine), 1603, 1592 (C=C), 1269 (C-N), 1500, 1399 (Ar-NO_2)

Microbiology

Preliminary values for growth inhibition of different microorganisms treated with the synthesized benzimidazole derivatives are shown in tables 2 and 3.

For the tested compounds, compound 11 was only the one that shows antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, while compounds 10 and 11 showed antifungal activity against *Candida albicans* (table 2). The minimum inhibitory concentration (MIC) was done for compounds 10 and 11 (table 3).

Table 2: The in vitro antimicrobial activity of the synthesized compounds and the control drugs (1mg/mL).

Compound No.	Microorganisms, growth inhibition zone of microbes in mm				
	Escherichia coli	Pseudomonas aeruginosa	Klebsiella pneumonia	Staphylococcus aureus	Candida albicans
1a	-----	-----	-----	-----	-----
1	-----	-----	-----	-----	-----
2	-----	-----	-----	-----	------
3	-----	-----	-----	-----	-----
4	-----	-----	-----	-----	-----
5	-----	-----	-----	-----	-----
6	-----	-----	-----	-----	-----
7	-----	-----	-----	-----	-----
8	-----	-----	-----	-----	-----
9	----	-----	-----	-----	----
10	----	-----	-----	-----	16
11	14	-----	16	15	28
12	-----	-----	-----	-----	-----
Ciprofloxacin	35	31	33	30	-----
Fluconazole					32
DMSO	-----	-----	-----	-----	-----

"----" indicates no significant inhibitory effect (<6 mm).

Table 3: Minimum inhibitory concentration (MIC) of selected compounds.

Organism	Compound	MIC (µg/mL)	MIC (µM)
Escherichia coli	11	62.5	183
Staphylococcus aureus	11	250	730
Klebsiella pneumona	11	125	365
Candida albicans	10	125	399
	11	62.5 (24h) 125 (48h)	183 365

MIC: minimum inhibitory concentration.

CONCLUSION

In conclusion, The synthesis of new 2-substituted benzimidazole derivatives was herein successfully achieved in appreciable yields. The newly synthesized benzimidazole derivatives are evaluated for their antibacterial and antifungal effects using agar-well diffusion method. We could observe marked effects for compound 11 and compound 10.

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