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Efficient and simple synthesis of novel 1,2,3-triazolyl-linked benzimidazolone, molecular docking and evaluation of their antimicrobial activity

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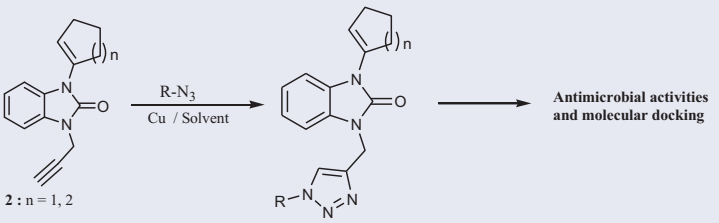
ABSTRACT

In this study, a novel series of 1,2,3-triazolyl-benzimidazolone derivatives have been synthesized by click reaction of azides with benzimidazolones **2a–b**. The latter compounds were prepared with excellent yields (85–97%), the structures of products were determined by spectral analysis. Then, the X-rays crystallographic analysis of compound **7a** revealed the self-assembling properties. The new heterocycles were evaluated for their *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacteria and against fungi strains. The most tested synthesized compounds showed potent antibacterial and antifungal activities against all tested strains. The compound **6c** was found to be the most active, particularly, against *Aspergillus niger* and *Penicillium* sp. with the same MIC and MBC of 0.0625 mg/mL. Furthermore, *in silico* molecular docking studies stipulated a sign of a good correlation between experimental activity and calculated binding affinity. According to the docking results, compound **6d** showed minimum binding energy and can be considered as a good antimicrobial agent.

GRAPHICAL ABSTRACT



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Introduction

The benzimidazolone is a heterocyclic compound found in many synthetic and natural products. The benzimidazole derivatives are of great biological interest such as cytotoxic,[1] antibacterial agents,[2,3] antitumor inhibitors,[4] and antinociceptive activity.[5] These compounds are important building blocks in the agrochemicals, inhibitors, pharmaceuticals, herbicides, pigments and fine chemicals.[6] In addition, benzimidazolone compounds play an important role as a progesterone receptor antagonist,[7] and other pharmacological agents.[8,9] For instance, it has been reported in recent literatures that benzimidazolone derivatives linked to the triazolic nucleus residue on the aromatic nitrogen are considered as effective inhibitors in various biological activities.[10,11] A number of methods have been reported for the synthesis of benzimidazol-2-ones and its derivatives. These methods comprise the condensation of ortho-phenylenediamine with carbonyl compounds including ketoester derivatives.[12,13]

The 1,2,3-triazole has been the subject of considerable researches mainly due to their usefulness in pharmacological properties and in derivatives synthetic organic chemistry. The heterocyclic such as 1,2,3-triazole derivatives has been shown to possess a variety of interesting biological activities, including antibacterial,[14–17] anti-inflammatory agents,[18] anti-allergic,[19] antifungal and anticancer activities.[20–22]

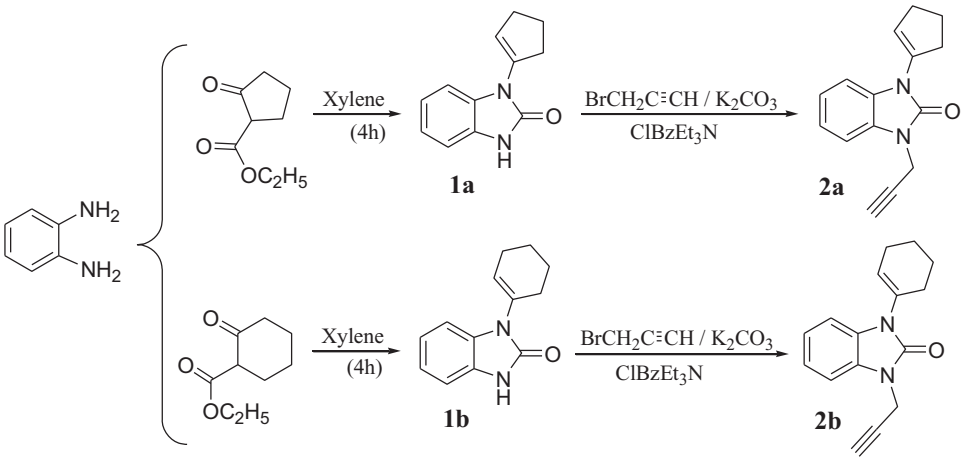
One of the most popular methods to prepare 1,2,3-triazoles is the cycloaddition of terminal alkynes with azide derivatives via the Huisgen reaction. This reaction is a regioisomeric mixture of the 1,4- and 1,5-disubstituted.[23] In the next, Sharpless et al. who developed this reaction,[24] showed that the use of copper (I) as catalyst leads to the formation of a single compound with a high regioselectivity and excellent yield allowing, exclusively, the 1,4-regioisomer.

Herein, we describe the synthesis of new triazole compounds, linked to the benzimidazolone ring via 1,3-dipolar cycloaddition reaction of azide derivatives and N-propargylbenzimidazol-2-ones **2a–b** catalyzed by CuI. The variation of dipoles aims to evaluate their effect on the yield of the cycloaddition product as well as on the regioselectivity of the reaction. Furthermore, the antimicrobial activities of the obtained benzimidazolone derivatives **1a–b**, **2a–b** and the 1,2,3-triazolyl-benzimidazolones **4a–b**, **6a–e**, **7a–e** and **9a–b**, were assessed *in vitro* against the Gram-negative and Gram-positive bacteria. The newly synthesized compounds **4a–b**, **6a**, **6c–e**, **7a**, **7c–e** and **9a–b** were screened for their *in vitro* antifungal activities against *Aspergillus niger*, *Penicillium* sp. and *Rhodotorula mucilaginosa*, also against fungi isolated in the Laboratory. Finally, selected candidates were subjected to molecular docking investigations.

Results and discussion

Chemistry

The benzimidazolones **1a** and **1b** were obtained by condensation of orthophenylenediamine with ethyl 2-oxocyclopentanecarboxylate and ethyl 2-oxocyclohexanecarboxylate, respectively, in xylene according to the method described by Rossi et al.[12] Propargylated benzimidazolone **2a–b** were also synthesized by reaction of benzimidazolone **1a–b** with propargyl bromide as described in the literature (Scheme 1).[13,25]



Scheme 1. Synthesis of benzimidazolone derivatives **2a–b**.

Next, the resulting compounds **2a–b** with terminal acetylene reacted with the azide derivatives (**3**, **5** and **8**) under standard "click chemistry" conditions using the copper-catalyzed azide-alkyne cycloaddition reaction in dry anhydrous acetonitrile under reflux, in each case, were prepared in order to give the new ring 1,4-substituted with 1,2,3-triazole (**4**, **6**, **7** and **9**) connected via benzimidazolone nucleus with excellent yields.

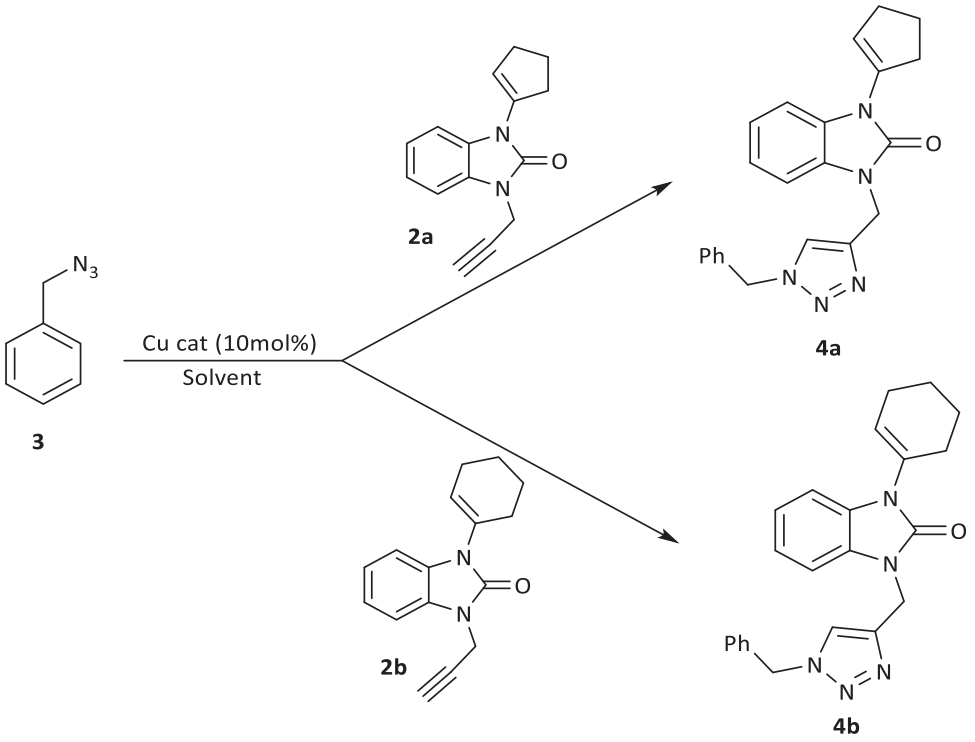
The structures of all synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR and HRMS spectra. The structure of cycloadduct **7a** was confirmed by X-ray diffraction analysis.

Synthesis of N-propargyl-benzimidazolones 2a-b

The synthetic strategy to prepare the different title compounds **2a–b** is illustrated in Scheme 1. The benzimidazolones **1a–b** were prepared by refluxing orthophenylenediamine with different cyclic ketoesters in xylene according to the method described by Rossi et al.[12] Then, the dipolarophiles **2a–b** were synthesized (85–83%) on reaction of compound **1a–b** with propargyl bromide, in the presence of potassium carbonate and benzyltriethylammonium chloride ($ClBzEt_3N$) (2% mol). The reaction was conducted using a procedure with a blending of N,N-dimethylformamide and acetonitrile (3:2 v/v) (Scheme 1). It should be noted that this dipolarophile contains a dipolarophile site C≡C capable of reacting with the azide derivatives.

The structures of all the compounds were determined by analytical and spectroscopic analyses. The known compounds **1a–b** have been identified based on their spectroscopic data reported in the literature.[12]

The ^{1}H NMR spectrum of product **1a** is characterized by the presence of three methylene groups at 2.15–2.95 ppm and a multiplet at 5.90 ppm assigned to the ethylene proton of the cyclopentenyl group. The ^{13}C NMR reveals, in particular, five signals at 22.24, 30.36, 30.45 and 122.69 ppm assigned to the three methylene groups and to the ethylene carbon (CH=) of the cyclopentenyl and one signal at 154.91 ppm attributed to the carbonyl group. The ^{1}H and ^{13}C NMR spectra of compound **2a** reveal besides the signals typical of the skeleton of the compound **1a** and those of the propargyl group.



Scheme 2. Synthesis of triazolyl-benzimidazolones **4a–b**.

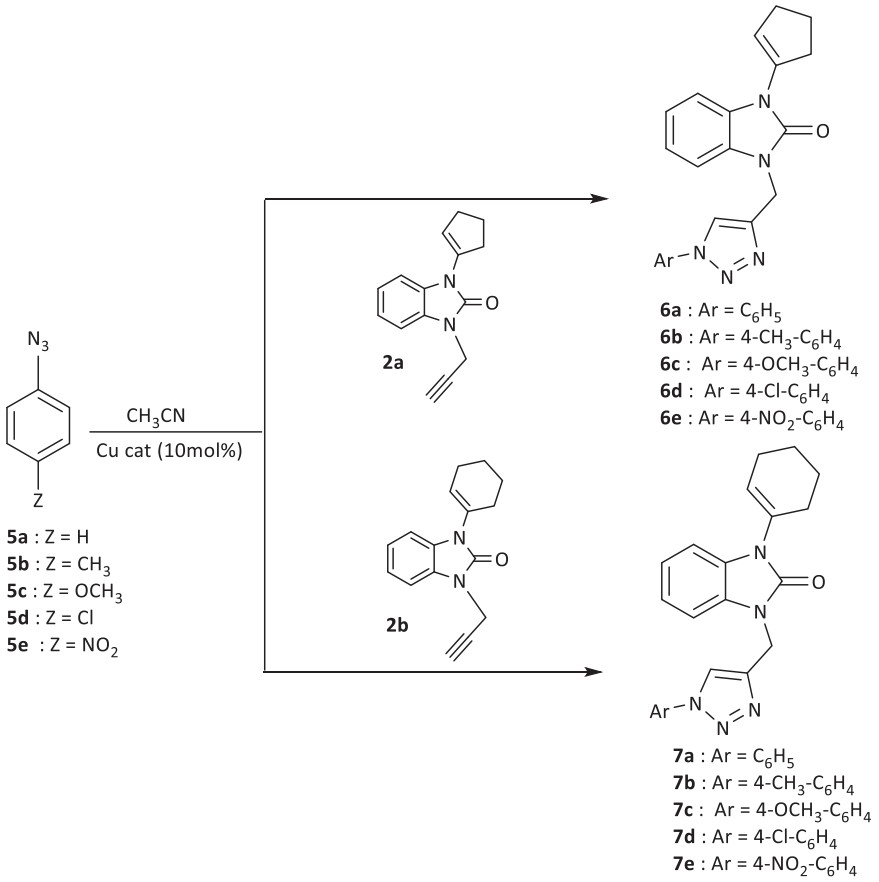
Synthesis of azide derivatives (3, 5 and 8)

The benzylazide **3** was conveniently synthesized from a known literature procedure using sodium azide and the corresponding benzylbromide at reflux in N,N-dimethylformamide.[26] In the next, similar azide **5a–e** were prepared from aniline derivatives via the reported method described by Noelting.[20,27] It consists first in preparing the hydrochloride of the amine from the parasubstituted aniline and the concentrated hydrochloric acid. The hydrochloride is subsequently diazotized with a solution of sodium nitrite. Ethyl azidoacetate **8** was prepared using sodium azide and the corresponding ethyl 2-bromoacetate at reflux in acetone.[28]

Coupling reactions

New propargylated benzimidazolones **2a–b** were subjected to copper (I)-catalyzed azide alkyne cycloaddition with various organic azides to yield triazolyl-benzimidazolones in very high yield. The benzimidazolones **2a–b** with terminal alkyne were reacted with organic azides in the presence of copper iodide in acetonitrile to afford the triazolyl-benzimidazolones **4**, **6**, **7** and **9** in very good yield.

The synthesis of new targets 1,2,3-triazolyl-benzimidazolones **4a–b** were prepared in excellent yield (88–92%) by 1,3-dipolar cycloaddition of benzylazide **3** with N-propargyl-benzimidazolones **2a–b** beneath conditions copper(I)-catalyzed alkyne-azide cycloaddition gave the new 1,2,3-triazolyl-benzimidazolones corresponding (Scheme 2).



Scheme 3. Synthesis of triazolyl-benzimidazolone derivatives 6a-e and 7a-e.

The reaction is regioselective in the presence of copper (I) as a catalyst because Cu (I) as a catalyst strongly activates the terminal acetylenes toward 1,3-dipole in azide to give the desired 1,4-disubstituted triazolyl-benzimidazole derivatives **4a–b** in good yields via click chemistry. This regioselectivity is in good agreement with the literature.[29,30]

The structures of products **4a–b** were confirmed by spectral data and high-resolution mass.

In the ^{1}H NMR we observed mainly a proton H5'-triazolic at 7.77 ppm, and two singlets at 5.03 and 5.33 ppm assigned to the CH_2 group. In the ^{13}C NMR spectrum, the regioselectivity is proved by the chemical shift of the triazolic C-5′ at 122.73 ppm. The mass spectrometry analysis of all cycloaddition compounds also confirms the proposed structures.

For the synthesis of compounds **6a–e** and **7a–e**, the reaction sequences are represented in Scheme 3 and were synthesized via the condensation of alkylated benzimidazolones **2a–b** with arylazide derivatives **5a–e** in anhydrous acetonitrile at reflux. The Thin Layer Chromatography examination of the reactions mixtures indicated the presence of only one regioisomer, resulting from the thermal cycloaddition of the 1,3-dipoles and the acetylene group. All the cycloaddition reactions were then performed in the presence of copper powder as a catalyst (CuI, 10 mol %) using the same protocol given above.

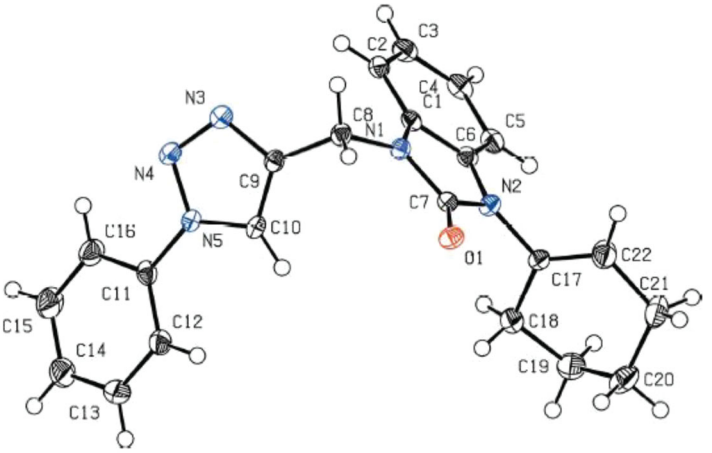


Figure 1. X-ray crystal structure of compound **7a**.

All structures of synthesized triazole compounds were completely identified by NMR (^{1}H and ^{13}C) and high-resolution mass spectrometry. Another piece of evidence of our structural assignment comes from the X-ray structural determination[31] performed on **7a**, confirming unambiguously the stereochemistry of the products. Two independent molecules are present in the Crystal system, of the Triclinic, P1 space group. This structure contains two planar: the cyclohexenyl ring (C17/C18–C22), displays a half chair conformation and its main plan is inclined to the benzimidazole ring system by 78.75 (12)°. The 1,2,3-triazole ring (*N*3/*N*4/*N*5/C10/C9) is almost normal to the plan of the benzimidazolone ring system, making a dihedral angle of 88.36 (8)°. The molecular structure of single crystal **7a** is illustrated in Figure 1.

On the other hand, the target products **9a–b** were synthesized performing a reaction as outlined in Scheme 4. The key step involved in the synthesis of 1,2,3-triazolyl-benzimidazolone derivatives **6** and **7** corresponded to the Copper (I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC), also known as click reaction,[25,32] the action of ethyl azidoacetate **8** on N-propargyl-benzimidazolones **2a–b**. The heterocyclic compounds **9a–b** were obtained with synthetically important yields between 88% and 85%. In general, reactions took less than an hour for their completion.

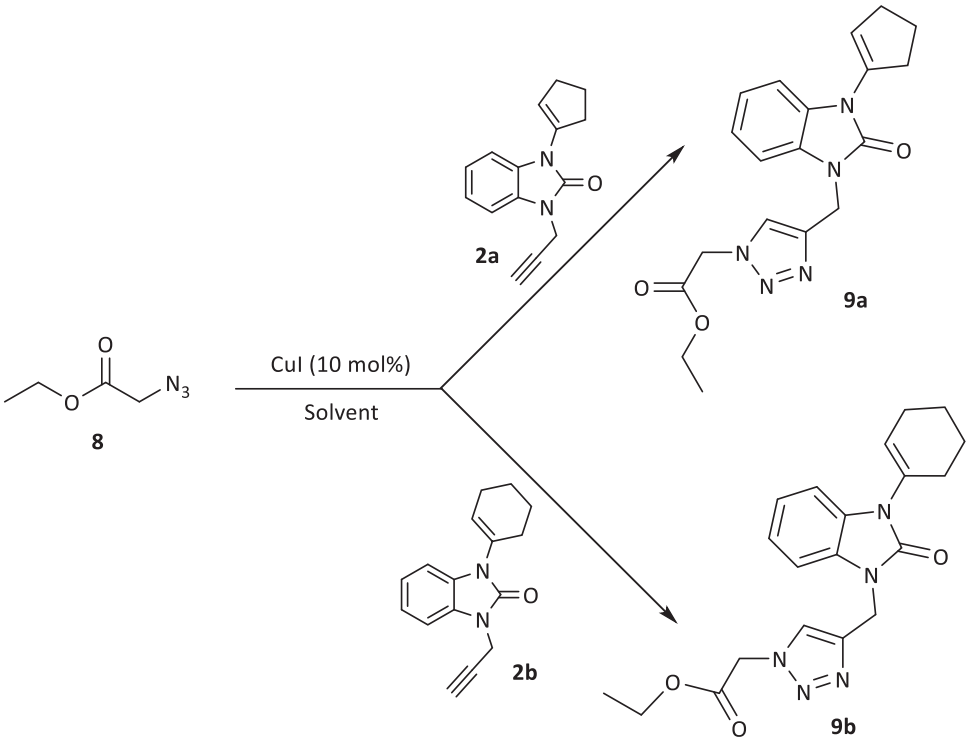
Structures of all these new regioisomers were determined based on their analytical and spectral data. The ^{1}H NMR spectra of the compounds **9a–b** exhibited a singlet at 7.75, and 7.62 ppm respectively, due to triazolyl-benzimidazolone protons in position 5′. The ^{13}C NMR spectra of **9a–b** exhibited a signal at 122.69 and 124.62 ppm respectively, corresponding to C-5′, thus confirming the regioselectivity of reaction.

Biology

Antimicrobial activity

Microbial strains

The synthesized compounds **1a–b**, **2a–b**, **4a–b**, **6a–e**, **7a–e** and **9a–b** were evaluated against Gram-negative bacteria: *Pseudomonas aeruginosa* (P. *aeruginosa*) (ATCC 7966), and *Klebsiella pneumonia* (*K. pneumonia*) (ATCC 13314), Gram-positive



Scheme 4. Synthesis of triazolyl-benzimidazolone derivatives 9a-b.

bacteria: *Micrococcus luteus* (*M. luteus*) (ATCC 10240) and *Bacillus subtilis* (*B. subtilis*) (ATCC 9524), and against the following fungi: *A. niger, Penicillium* sp. and *Rhodotorula mucilaginosa*. The antimicrobial activity was carried out using standard serial dilution method according to NCCLS guidelines.[33] Gentamycin and Fluconazole were used as a standard drug for antibacterial and antifungal analysis, respectively. Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of all compounds were determined.

Antibacterial activity screening of compounds 1a–b, 2a–b, 4a–b, 6a–e, 7a–e and 9a–b

The results of the inhibition zone noted for a series of synthesized products against several bacteria are illustrated in Table 1. The inhibition zone of strains ranged from 10.1 ± 0.1 mm to 18.1 ± 0.6 mm for Gram-positive bacteria and from 7.1 ± 0.3 mm to 13.2 ± 0.7 mm for Gram-negative bacteria. The highest inhibition zone was obtained in cases of **4b** and **7d** against *M. luteus* (18.1 mm) and *P. aeruginosa* (13.2 mm) respectively. The noted difference in the diameter inhibition between Gram-negative and Gram-positive was related to the cell structure of bacteria, particularly, to the presence of a membrane composed by phospholipids and lipopolysaccharides for Gram-negative bacteria.[34]

The preliminary *in vitro* antibacterial screening indicated that the majority of synthesized heterocycles possess an inhibition against the most tested bacteria except **1a–b**,

Table 1. Inhibition-zone diameters for compounds **1a–b**, **2a–b**, **4a–b**, **6a–e**, **7a–e** and **9a–b**.

	Gram-negative		Gram-positive		Fungi		Yeast
Microorganisms test	*M. luteus*	*B. subtilis*	*K. pneumoniae*	*P. aeruginosa*	*Aspergillus niger*	*Penicillium* sp.	*Rhodotorula mucilaginosa*
1a	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
1b	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
2a	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
2b	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
4a	12.8 ± 0.8	11.6 ± 0.7	11.1 ± 0.3	9.4 ± 0.4	22.9 ± 0.3	23.4 ± 0.7	22.1 ± 0.7
4b	18.1 ± 0.6	13.7 ± 0.5	12.6 ± 0.1	8.4 ± 0.6	25.6 ± 1.1	24.3 ± 0.1	21.7 ± 0.4
6a	14.3 ± 0.6	10.2 ± 0.4	12.3 ± 0.7	11.2 ± 0.8	23.1 ± 1.1	21.6 ± 0.4	21.1 ± 0.6
6b	12.2 ± 0.2	13.6 ± 0.3	NA	NA	19.7 ± 0.9	18.6 ± 0.8	16.3 ± 0.4
6c	14.7 ± 0.9	11.7 ± 0.4	10.3 ± 0.1	8.5 ± 0.3	27.8 ± 1.2	26.4 ± 0.8	26.7 ± 0.5
6d	12.3 ± 0.5	12.1 ± 0.9	10.9 ± 0.4	9.2 ± 0.7	22.1 ± 1.3	24.8 ± 0.7	20.7 ± 0.8
6e	13.3 ± 0.7	12.2 ± 0.7	9.6 ± 0.2	7.1 ± 0.3	27.3 ± 1.6	24.7 ± 0.5	23.8 ± 0.7
7a	11.1 ± 0.2	10.2 ± 0.7	8.6 ± 0.1	7.4 ± 0.1	23.3 ± 1	22.1 ± 1.3	19.3 ± 0.7
7b	NA	NA	13,1 ± 1.2	12,4 ± 0.1	21.3 ± 1.1	22.9 ± 0.7	18.1 ± 0.3
7c	12.7 ± 0.3	9.4 ± 0.2	8.6 ± 0.6	8.1 ± 0.5	26.5 ± 1.2	24.3 ± 1.2	23.9 ± 1.2
7d	14.6 ± 0.8	12.7 ± 0.1	13.2 ± 0.7	13.2 ± 0.6	24.3 ± 0.9	26.3 ± 0.9	19.3 ± 0.7
7e	13.9 ± 1.1	12.1 ± 0.3	10.3 ± 0.9	9.7 ± 0.7	24.6 ± 0.2	27.4 ± 0.4	17.9 ± 05
9a	11.3 ± 0.4	12.3 ± 0.2	10.3 ± 0.7	8.1 ± 0.2	21.3 ± 0.4	22.7 ± 0.4	21.9 ± 0.6
9b	14.2 ± 0.9	10.1 ± 0.1	NA	NA	26.3 ± 1.2	19.3 ± 0.2	18.9 ± 0.9
Gentamycin	**26.1 ± 0.5**	**20.6 ± 0.4**	**NA**	**14.9 ± 0.9**	**NT**	**NT**	**NT**
Fluconazole	**NT**	**NT**	**NT**	**NT**	**29.7 ± 0.3**	**30.4 ± 0.7**	**28.1 ± 0.8**

The values represent the average (standard deviations) for triplicate analyses.
NA: Not active; NT: Not tested.

2a–b against all tested bacteria (Table 2). *M. luteus* was the most sensitive to synthesized compounds **4a–b**, **6a–e**, **7a–e** and **9a–b** compared to the other bacteria. Moreover, 1,2,3-triazole derivatives **6d**, **7d** with p-chloro substituted benzene ring and **9b** with ethyl acetate group were the most active against Gram-positive bacteria (M. *luteus*) with MIC equal to 0.125 mg/mL. After that, the product **9a** has the lowest value of MBC (0.125 mg/mL) against *P. aeruginosa* bacteria.

In fact, the hydrophobic compounds with high partition coefficients have the capability to cross membranes in biological systems (lipophilicity). In addition, many of the proteins have hydrophobic amino acids that can bind with molecules with lipophilic character. As a result, this later plays an important role in producing antimicrobial activities.[35] The lipophilicity of the compounds expressed as Clog P, explains the main predictor for the activity. Moreover, the lipophilic power of products increased with increasing Clog P. The octanol/water partition coefficient Clog P, a measure of hydrophobicity/lipophilicity, was calculated using ChemDraw Ultra 16.0 software integrated with Cambridgesoft Software (Cambridgesoft Corporation). The results obtained are given in Table 3. In a comparison of compounds **6a–e**, we found that the triazole **6d** has the highest value of Clog P, which explains that this compound was the most active. In addition, products **7d** and **9b** were the most potent and had the highest Clog P in comparison with **7a–e** and **9a**.

Antifungal activity of compounds 4a–b, 6a–e, 7a–e and 9a–b

The results of the inhibition zone noted for a series of synthesized products against fungi are summarized in Table 1. The inhibition zone of strains ranged from 18.6 ± 0.8 mm to 27.8 ± 1.2 mm for fungi and 16.3 ± 0.4 mm to 26.7 ± 0.5 mm for yeast (including disks diameters of 6 mm). The tested product's effects were more pronounced against *A. niger*, the most potent compound was **6c** with inhibition zones of 27.8 ± 1.2 mm close to that of Fluconazole.

Then, the minimum inhibitory potential of 1,4-disubstituted 1,2,3-triazole-based benzimidazolone derivatives was evaluated *in vitro* against fungal strains: *A. niger* and *Penicillium* sp. The results were compared with the standard antibiotic (Fluconazole), the MIC and MFC values in mg/mL were estimated and summarized in Table 2. The synthesized 1,2,3-triazolyl-benzimidazolone derivatives **4a–b**, **6a–e**, **7a–e** and **9a–b** showed better activity against the fungal strain *A. niger* and *Penicillium* sp. In summary, the compound **6c** with methoxy-group at the para position of phenyl ring and cyclopentenyl in position 3 of benzimidazolone has been found to be a good inhibitor compared to other products against *A. niger* and *Penicillium* sp. with MIC and MCF values in order to 0.0625 mg/mL. It is noted that the heterocycles **7c–d** and **9a–b** had MIC at 0.0625 mg/mL against these two fungi.

The noted antifungal activity may be due to the chemical structure of the synthesized compounds as already studied by Hoshi and coworkers.[36] This study showed the importance of the structure-activity relationship (SAR) for the fungicidal activity of several synthesized compounds. Based on this study, the presence of a methoxy-group linked to phenyl at para position and a ring cyclopentenyl in position 3 of benzimidazolone may be the reason for the strong activity noted in the case of the compound **6c**. The obtained results showed the importance of 1,2,3-triazole as an alternative antifungal

Table 2. Minimal inhibitory concentrations (MIC) and Minimal bactericidal/fungicidal (MBC/MFC).

Compounds test		Gram-positive		Gram-negative		Fungi		Yeast
		M. luteus	*B. subtilis*	*K. pneumoniae*	*P. aeruginosa*	*Aspergillus niger*	*Penicillium* sp.	*Rhodotorula mucilaginosa*
4a	MIC	0.25	1	0.5	2	0.125	0.25	0.5
	MBC	0.5	0.5	1	2	0.25	0.5	0.5
4b	MIC	0.25	0.5	0.25	1	0.125	0.125	0.5
	MBC	1	2	1	4	0.5	0.5	1
6a	MIC	0.25	0.25	0.5	1	0.125	0.125	0.25
	MBC	0.5	0.5	1	1	0.25	0.25	0.25
6b	MIC	0.5	1	**NT**	**NT**	0.5	0.5	1
	MBC	1	2	**NT**	**NT**	1	1	2
6c	MIC	0.25	0.5	1	1	0.0625	0.0625	0.125
	MBC	0.5	0.5	1	2	0.0625	0.0625	0.25
6d	MIC	0.125	0.25	0.5	1	0.125	0.125	0.25
	MBC	0.5	0.5	1	2	0.25	0.25	0.25
6e	MIC	0.25	0.25	0.5	1	0.125	0.125	0.25
	MBC	0.25	0.5	0.5	2	0.25	0.25	0.5
7a	MIC	0.5	1	1	2	0.125	0.125	0.5
	MBC	1	2	2	4	1	1	1
7b	MIC	NT	NT	0.5	1	0.125	0.125	0.5
	MBC	NT	NT	2	2	1	1	1
7c	MIC	0.5	1	1	1	0.0625	0.0625	0.125
	MBC	1	2	1	2	0.5	0.5	0.5
7d	MIC	0.125	0.25	0.5	1	0.0625	0.0625	0.125
	MBC	0.5	0.5	1	2	0.25	0.25	0.25
7e	MIC	0.25	0.5	0.5	1	0.125	0.125	0.25
	MBC	0.25	0.5	0.5	2	0.25	0.25	0.5
9a	MIC	0.25	0.5	1	1	0.0625	0.0625	0.125
	MBC	0.5	1	2	0.125	0.25	0.0625	0.125
9b	MIC	0.125	1	**NT**	**NT**	0.0625	0.0625	0.125
	MBC	0.5	2	**NT**	**NT**	0.5	0.5	0.5
Gentamycin	MIC	**0.03**	**0.03**	**NT**	**0.03**	**NT**	**NT**	**NT**
	MBC	**0.03**	**0.03**	**NT**	**0.03**	**NT**	**NT**	**NT**
Fluconazole	MIC	**NT**	**NT**	**NT**	**NT**	**0.025**	**0.025**	**0.025**
	MBC	**NT**	**NT**	**NT**	**NT**	**0.025**	**0.025**	**0.025**

The values represent the average (standard deviations) for triplicate analyses.
NT: Not tested.

Table 3. Calculated C log P of compounds **4a–b**, **6a–e**, **7a–e** and **9a–b**.

Compounds	4a	4b	6a	6b	6c	6d	6e	7a	7b	7c	7d	7e	9a	9b
C log P	3.61	4.17	4.06	4.56	4.29	4.95	4.17	4.62	5.12	4.85	5.51	4.73	2.47	3.03

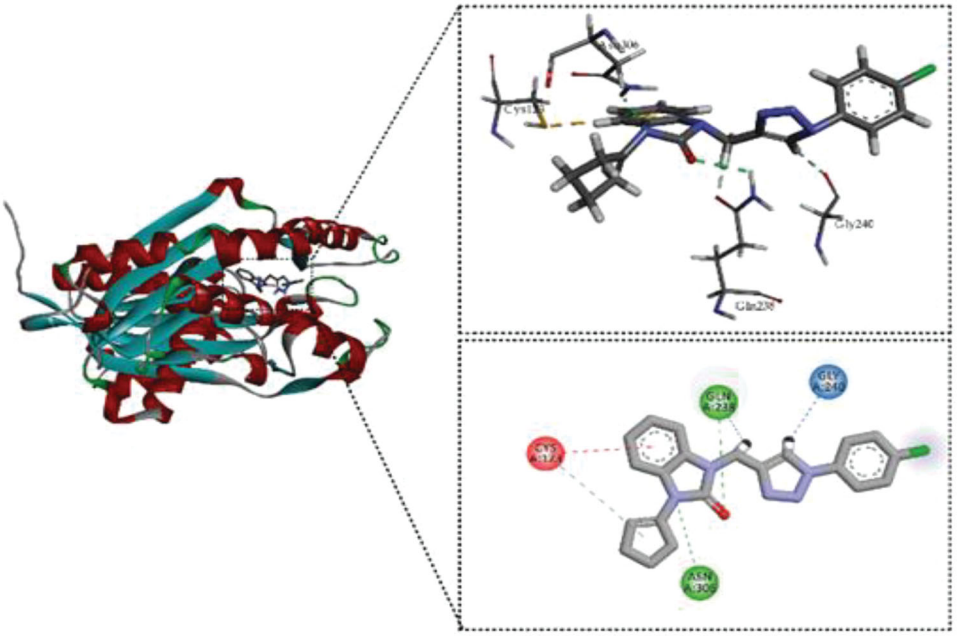


Figure 2. Compound **6d** from docking results in *M. luteus*.

drug. Hence, those results may need to be integrated, particularly, in the pharmaceutical industry. Besides, for the antibacterial activity, the results of the biological activity showed that compounds bearing the p-chloro substituted benzene ring in the triazole moiety significantly increase the activity. The SAR showed that the p-chloro had great influence on the activity, and the electron-withdrawing-chloro ring was more favorable than electron-donating groups' moieties existing in the other derivatives. Furthermore, the enhancement of the antibacterial activity might due to the introduction of the ethyl acetate substituent as well. Among all derivatives, only compounds **9a** and **9b** showed good inhibitory effect are having this substituent.

Molecular docking

Fabh from the *M. luteus* protein was used as a target for antimicrobial activity of the synthesized compounds as potential inhibitors. The *in silico* active pocket of this protein involved in binding with **6d**, **7d** and **9b** selected compounds are presented in Figures 2–4, respectively. Docking of these molecules with the enzyme showed that all of the inhibitors can bind to one or more amino-acids in the active site. Theoretical binding affinity of **6d**, **7d**, and **9b** compounds were −6.8, −5.2 and −5.6 kcal/mol, respectively. Among all docked molecules, compound **6d**, which has the highest value of Clog P, revealed the least binding energy in comparison with **7d** and **9b** docked compounds,

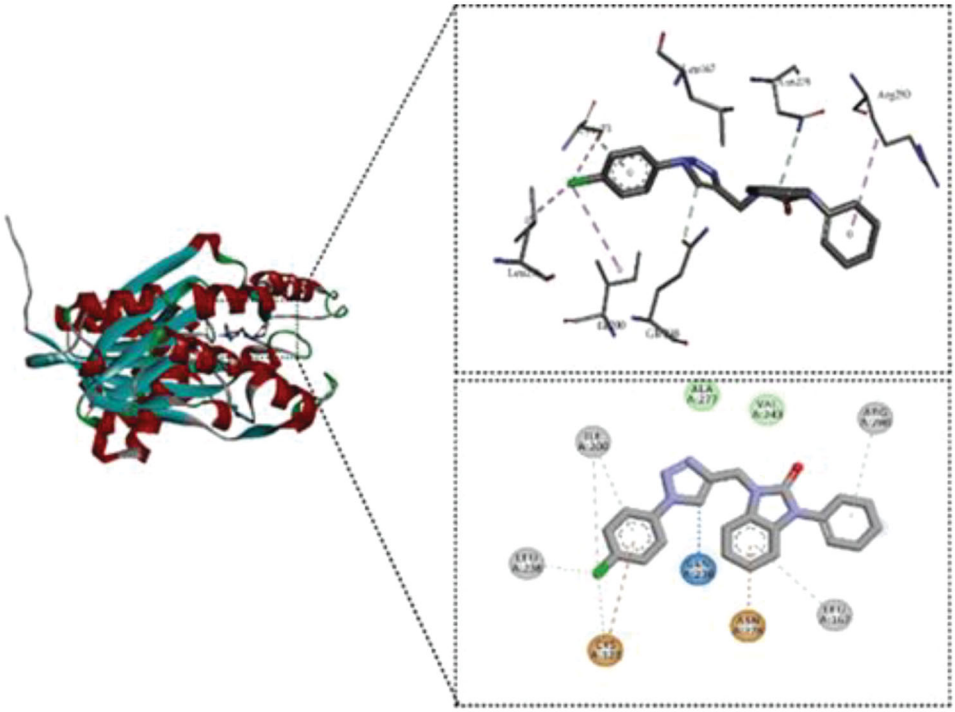


Figure 3. Compound **7d** from docking results in *M. luteus*.

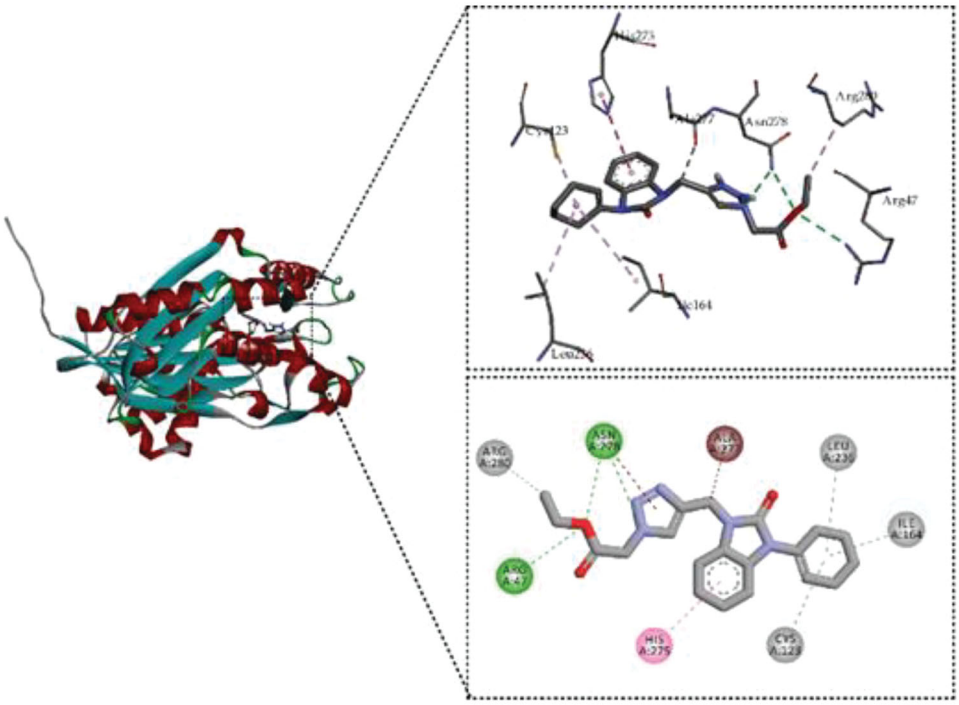


Figure 4. Compound **9b** from docking results in *M. luteus*.

thus suggesting that it may be considered as a good inhibitor of the *M. luteus* target and subsequently a good antimicrobial agent. This compound (i.e., **6d**) formed two conventional hydrogen bonds with GLN238 and ASN306 residues as well as a π-Sulfur interaction with the CYS123 amino-acid by its indole moiety. These findings are in accordance with experimental results.

Experimental

Melting points were taken in an open capillary tube on a Buchi 510 apparatus and are uncorrected. Spectra were recorded with the following instruments: ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker spectrometer with chemical shift values given in part per million (ppm) relative to TMS (0.00 ppm). High-Resolution Mass Spectral (HRMS) data were obtained on a Triple TOF^{TM} 5600 LC/MS/MS System, (AB SCIEX). The ionization mode used in mass spectra was Ion Spray Voltage (ISVF): 5500. Column chromatography was carried out using E-Merck silica gel 60-F254. The reaction progress was monitored by Thin Layer Chromatography (TLC) using, silica gel 60-F254, and the spots were detected with UV light (254 or 365 nm. The structure of all the compounds was determined by analytical and spectroscopic methods and by comparison with data of the structural related compounds reported in the literature. Reagents and solvents were purified in the usual way.

General procedure for the preparation of benzimidazolone 1a–b

A mixture of orthophenylenediamine (2.5 g, 23 mmol), ethyl 2-oxo-cyclopentanecarboxylate (4.25 g, 26 mmol) or ethyl 2-oxo-cyclohexanecarboxylate (4.4 g, 26 mmol) in xylene (100 mL) was refluxed for 4 h. The benzimidazolones were isolated by column chromatography on silica gel using hexane/ethyl acetate (6/4) as eluent. The heterocycles **1a–b** were obtained with a yield of 94% and 92% respectively. The product **1a** was obtained as white solid in 94% yield. Mp: 160–162 °C (ethanol) (Lit.: 157–159 °C[12]). IR (KBr, ν (cm^{-1}), 3413 (imidazole-NH), 1602 (C = O). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 1.95–3.01 (3 m, 6H, 3CH_2-cyclopentenyl), 5.90–6.09 (m, 1H, =CH-cyclopentenyl), 6.89–7.25 (m, 4H, H-Ar), 10.77 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 22.26, 30.53, 32.08 (3 C, 3CH_2-cyclopentenyl), 109.62, 109.87, 121.27, 121.64 (4 C, CH-Ar), 123.62 (1 C, =CH-cyclopentenyl), 128.42, 130.06, 135.92 (3 C, =C–), 154.91 (1 C, C = O). HRMS (*m/z*): $[M + H]^+$ *m/z* calcd for $C_{12}H_{13}N_2O$: 201.1022, found: 201.1020.

General procedure synthesis for the 1,2,3-triazole derivatives 4a–b, 6a–e, 7a–e and 9a–b

To a solution of compounds **2a** or **2b** (4 mmol) and azide derivatives (4.5 mmol) in acetonitrile (20 mL), copper powder as catalyst (10 mol %) was added. The reaction process was monitored at reflux for 1 h. After the reaction was finished, the solvent was evaporated. The residue was solubilized with dichloromethane and was then washed with water. The organic layer was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. The compounds were purified by column

chromatography eluting with hexane/ethyl acetate mixture (4/6: v/v). The isolated products were recrystallized to afford the pure cycloadduct in good yield. The product **4a** was obtained as a white solid in 88% yield. Mp: 140–142 °C (ethanol). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 2.00–2.89 (3 m, 6H, $3CH_2$-cyclopentenyl), 5.15 (s, 2H, N–CH_2), 5.45 (s, 2H, C_6H_5–CH_2–N), 5.92–5.93 (m, 1H, C = CH, H-cyclopentenyl), 7.05–7.41 (m, 9H, H-Ar), 7.55 (s, 1H, H-triazolic). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 22.23, 30.43, 24.69, 32.05 (3 C, $3CH_2$-cyclopentenyl), 36.41 (N–CH_2), 54.23 (1 C, C_6H_5–CH_2–N), 122.71 (N–CH = C, C-triazolic), 127.71 (1 C, CH = C, C-cyclopentenyl), 108.75, 109.56, 121.58, 121.92, 122.55, 128.16, 128.79, 129.02 (9 C, CH = C, C–Ar), 128.85, 129.11, 134.31, 135.99, 143.51 (5 C, =C–), 152.63 (1 C, C = O). HRMS (*m/z*): $[M + H]^+$ calcd for $C_{22}H_{22}N_5O$: 372.1819, found: 372.1831.

Conclusion

We have synthesized a new series of 1,2,3-triazolyl-benzimidazolones through click reaction under environmentally benign conditions between propargylated benzimidazolone and organic azides with good yields and high purity. These 1,3-dipolar cycloaddition reactions of azide derivatives with propargyl-benzimidazolones **2a–b** are completely regioselective and chemoselective. In all cases, only one type of regioisomer was obtained. Our results are in good agreement with those of Sharpless et al.[24] The use of copper (I) as catalyst leads to the formation of a single compound with a high regioselectivity and excellent yield allowing, exclusively, the 1,4-regioisomer. The synthesized compounds **1a–b**, **2a–b**, **4a–b**, **6a–e**, **7a–e** and **9a–b** were evaluated for the antibacterial against Gram-positive and Gram-negative bacteria and as antifungal activity against fungi and yeasts. The *in vitro* assay revealed that compound **6c** (MIC 0.0625 mg/mL, MBC 0.0625 mg/mL) was the most antibacterial and antifungal. The results obtained in this study clearly demonstrate that compounds derived from 1,2,3-triazolyl-benzimidazolone, exhibited potent antimicrobial activity. Hence, the effect of those compounds may be further investigated, for the evaluation of their activity against other pathogenic strains or against enzymes involved in several diseases. According to *in silico* studies, among all synthesized compounds, compound **6d** displays minimum binding energy and may be considered as a good inhibitor of Fabh from the *M. luteus* target that is in accordance with experimental findings.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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