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ORIGINAL PAPER

Microwave-assisted synthesis of (3,5-disubstituted isoxazole)-linked benzimidazolone derivatives: DFT calculations and biological activities

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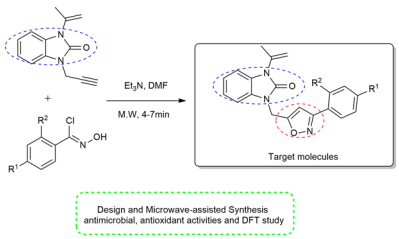
Abstract

The present work describes the synthesis, characterization, and biological activities of a novel series of isoxazole linked benzimidazolones. The structures of the target compounds and intermediates were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. Furthermore, all isolated molecules were evaluated for their antioxidant and antimicrobial activities. Some of these compounds were found to be the most potent antimicrobial agents. Moreover, most compounds exhibited significant scavenging activity on DPPH assay. Besides, the compounds' chemical reactivity was also assessed theoretically through Conceptual Density Functional Theory (CDFT) indices. The wB97X-D/cc-pVDZ calculation allowed to classify some derivatives as moderate-to-strong electrophiles and strong nucleophiles.

Graphic abstract



Keywords *N*-Isopropenyl benzimidazolone · Click chemistry · 3,5-Disubstituted isoxazole · Terminal alkyne · Antimicrobial · Antioxidant · DFT calculations

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Introduction

Among the various nitrogenic heterocycles, benzimidazolone derivatives are broadly distributed in many synthetic and natural products [1, 2]. A literature survey revealed that fused benzimidazolone is of great biological interest regarding its anti-HIV [3, 4], antibacterial [5], antituberculosis [6], antifungal [7], antimicrobial [8], and anticancer [9] activity. Droperidol [10], flibanserin [11], domperidone [12], and sumanriol [13] are examples of marketed drugs bearing benzimidazolone ring. On the other hand, isoxazole and its derivatives have attracted the attention of medicinal chemists due to their usefulness in pharmacology and in synthetic organic chemistry [14]. A good number of such heterocyclic scaffolds are found to possess a variety of interesting

biological activities. Among these activities are its antibacterial [15], antiviral [16], anti-inflammatory [17], antioxidant [18], anticancer [19, 20], anti-alzheimer [21], antileishmanial, antiglycation [22], and antifungal [23] activities. Figure 1 shows that some drugs based on isoxazole scaffold contain sulfisoxazole [24], valdecoxib [25], cloxacillin [26], and leflunomide [27]. Organic chemists generally use various methods for the synthesis of isoxazoles compounds. Among these methods, one can cite 1,3-dipolar cycloaddition, condensation, cycloisomerization, and direct functionalization [28, 29]. However, from a synthetic point of view, these methods are usually not general enough to produce a large library wide range of molecular architectures containing 3,5-disubstitued isoxazole heterocycles. In many cases, expensive and sensitive metallic catalysts or harsh reaction conditions are needed and the corresponding building blocks are usually prepared on a small scale and/or poor regioselectivity. Nowadays, milder methods like click chemistry are widely used in the preparation of these five heterocyclic compounds, under mild reaction conditions and with excellent properties such as high stability, excellent yield, and higher regioselectivity [30]. Previous reports in the literature suggest that the use of Cu-catalyzed in 1,3-dipolar cycloaddition reaction of nitrile oxides and alkynes significantly

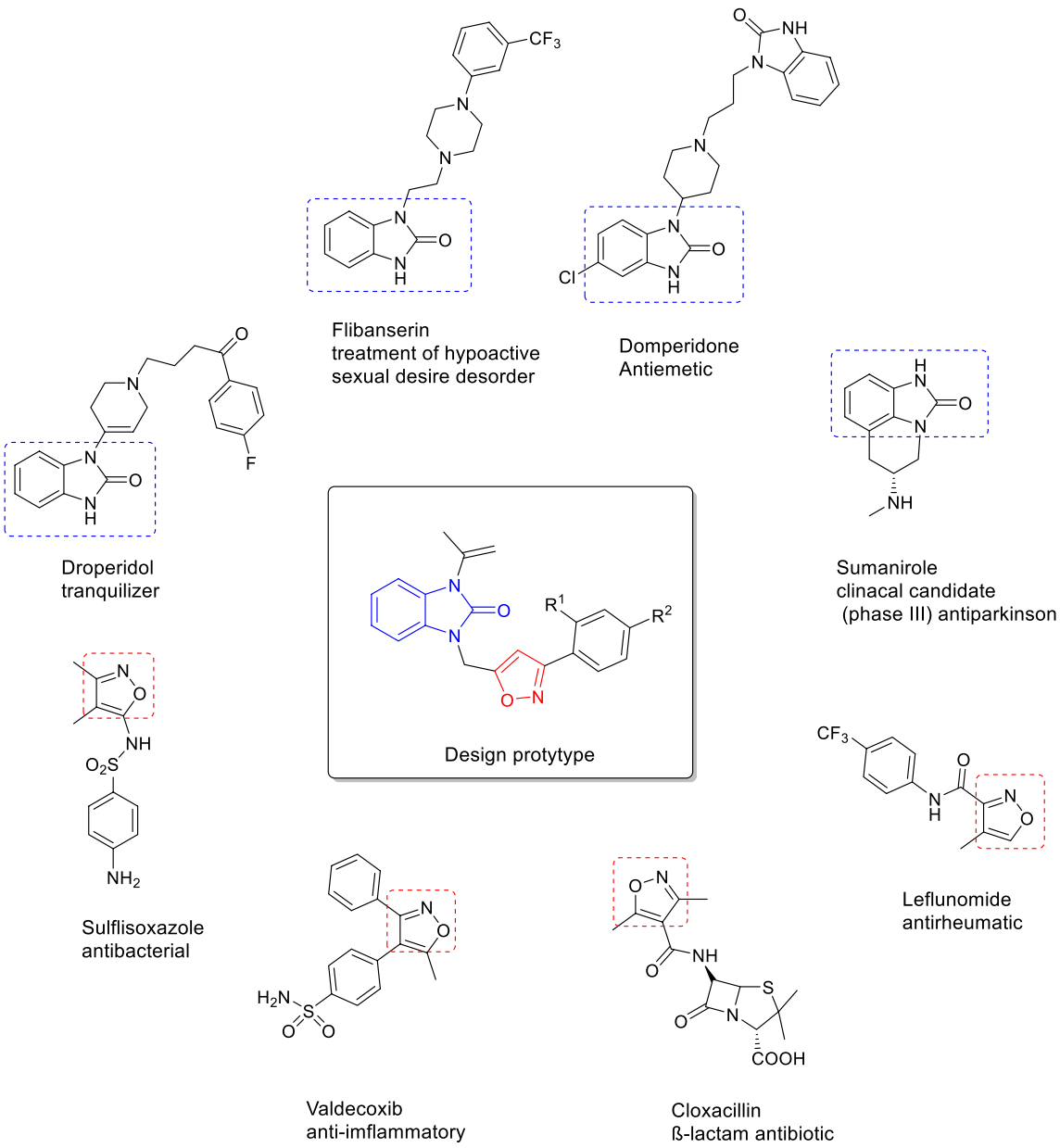


Fig. 1 Representative examples of biologically active drugs containing benzimidazolone and isoxazole derivatives

improved particularly concerning yields and regioselectivity (only 3,5-disubstituted regioisomer is formed) [31]. Another way to synthesize valuable target compounds in higher yields with shorter reaction times and under mild reaction conditions is the use of microwave-irradiation technique. To date, microwave-irradiation-assisted organic synthesis is dominant among green methods, due to its higher selectivity, higher yield, and operational simplicity, as compared to conventional synthesis routes which use minimization of a byproduct formation and reduced energy consumption for rapid synthesis of biologically active organic scaffolds [32].

The diverse biological properties of benzimidazolone and isoxazole derivatives along with the advantages of microwave-irradiation technology allowed us to synthesize new hybrid compounds by combining of benzimidazol-2-one moiety with isoxazole via 1,3-dipolar cycloaddition using various nitrile oxides. A regiospecific, simple, versatile copper(I)-catalyzed and microwave-assisted procedure was used to prepare a series of isoxazoles linked to benzimidazolone. The antimicrobial and antioxidant activities of the prepared compounds were evaluated. Conceptual Density Functional Theory (CDFT) (wB97X-D/cc-pVDZ) was applied to understand the chemical reactivity of the compounds **5a**, **5d**, **5f**, and **5h** (Fig. 1).

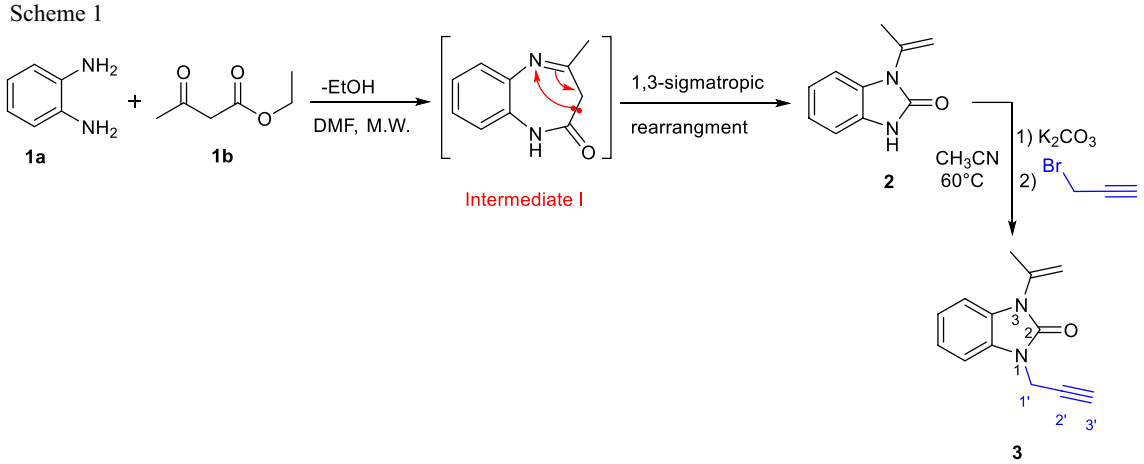
Results and discussion

Chemistry

In view of the above facts, we planned to prepare 3,5-disubstituted isoxazole linked to benzimidazol-2-one to study the antibacterial, antifungal, and antioxidant activities of these privileged heterocyclic compounds. To achieve this goal, it was necessary to first synthesize the *N*-isopropenyl benzimidazol-2-one. Generally, the condensation of o-phenylenediamine (**1a**) with ethyl acetoacetate (**1b**), in refluxed xylene gives 4-methyl-1,5-benzodiazepin-1-one, but by prolonging the reaction time and by thermal degradation of the diazepinic intermediate (**I**) gives *N*-isopropenylbenzimidazol-2-one (**2**) as a result of a [1, 3]-sigmatropic rearrangement [33, 34]. In this report, a microwave-assisted technique was developed for the preparation of **2** via condensation reaction of the starting materials **1a** and **1b** in DMF. The precursor *N*-isopropenylbenzimidazol-2-one was obtained with excellent yield (96%). Using the microwave-irradiation technique reduces the reaction time and increase yield. Mechanistically, the synthesis of the fragment **2** starts by condensation reaction between the reagents **1a** and **1b** to give the intermediate **I** (4-methyl-1,5-benzodiazepin-1-one) with the elimination of ethanol molecule [35]. Intramolecular rearrangement, type 1,3-sigmatropic, of the so formed intermediate led to *N*-isopropenyl benzimidazolone (**2**). The structure of **2** was ascertained on the basis of their ^{1}H and ^{13}C NMR spectral data and ES-HRMS. Subsequently, *N*-isopropenyl benzimidazol-2-one was propargylated by reaction with propargyl bromide and potassium carbonate K_2CO_3 at the reflux of acetonitrile to give the corresponding N_1-propargyl N_3-isopropenyl benzimidazol-2-one (**3**) in excellent yield (95%). The structure of the synthesized derivative **3** was evidenced by their NMR. In fact, the ^{1}H NMR spectrum of this scaffold **3** showed the absence of the singlet related to exchangeable proton (-N_1H-) at $\delta = 10.61$ ppm and the presence of doublet at 4.61 ppm ($J = 2.4$ Hz) attributable to methylene group protons (N_1-CH_2-) and a triplet at 2.23 ppm ($J = 2.4$ Hz) corresponding to the acetylenic proton (–C≡C–H). The ^{13}C NMR spectra of **3** displayed in particular three new signals at $\delta = 30.4$ ppm, 72.8 ppm, and 76.6 ppm are attributable to carbons $C_{1'}$, $C_{2'}$, and $C_{3'}$ of propargyl group, respectively, (Scheme 1).

Having established appropriate access to the intermediate key **3**, its triple-bond group made it a valuable key precursor to obtain the desired **5a**-**5k** hybrid compounds through the 1,3-dipolar cycloaddition reaction.

Scheme 1



Furthermore, this type of reaction depends on the substrate and reaction conditions (solvent, temperature, and amount of catalyst). At the outset of our studies, we considered the cycloaddition reaction between the *N*-hydroxy-4-methoxybenzenecarboximidoyl chloride (**4c**), *N*-hydroxy-4-chlorobenzenecarboximidoyl chloride (**4g**), and benzimidazol-2-one-alkyne **3** as model substrates under conventional heating and microwave irradiation in the presence or absence of CuI catalyst. The most suitable conditions of this study are shown in Table 1; Scheme 2.

Starting by uncatalyzed 1,3-dipolar cycloaddition reaction between alkyne **3** (1.4 mmol) and two equivalents of *N*-hydroxy-4-chlorobenzenecarboximidoyl chloride (**4g**) at 100 °C with the presence of Et_3N resulted in a mixture of two compounds: the 3,5-disubstituted isoxazole and unexpected product contained once 3,5-disubstituted isoxazoles and 3,5-disubstituted isoxazoline after 2 days (ratio ~ 78/22). The yield and the structures of the products were determinated from the ^{1}H NMR spectrum of the crude cycloadduct which showed the duplication of signals in the aromatic

Table 1 Optimization of the cyclization reaction[a]

Entry	Equiv. CuI[b]	Solvent	Equiv. Et_3N	Condition	Time/h	Formed isomer	Yield/%[d]
1	0	Toluene	2	100 °C	48	Mixture	35
2	0.1	DCM	2	rt	4	3,5-disubstituted	50
3	0.1	DCM	2	rt	8	3,5-disubstituted	60
4	0.1	DCM	2	rt	20	3,5-disubstituted	60
5	0.1	DMF	2	rt	20	3,5-disubstituted	52
6	0.1	*t*-BuOH/H_2O	2	rt	4	No reaction	0
7	0.1	THF/H_2O	2	rt	6	3,5-disubstituted	30
8	0.1	*t*-BuOH/H_2O	2	MW[c]	0.1	3,5-disubstituted	70
9	**0.1**	DMF	**2**	MW[c]	**0.1**	3,5-disubstituted	90

Bold in entry highlights the optimal reaction conditions:

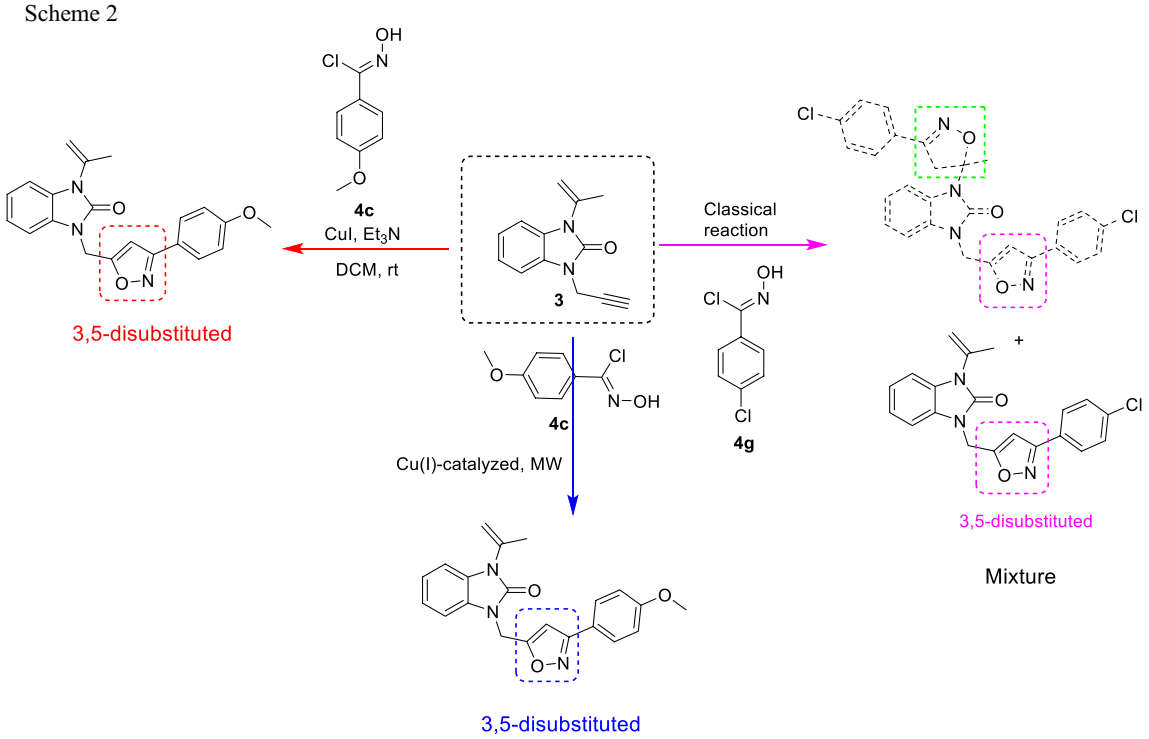
[a]Alkyne **3** (1.4 mmol) and 2 equiv of arylnitrile oxides **4c** or **4 g**

[b]Referred to the starting alkyne **3**

[c]The reaction was performed at 300 W

[d]Isolated yield after column chromatography based on the starting dipolarophile **3**

Scheme 2



region corresponding to the aromatic protons introduced by the phenyl ring of arylnitoloxide **4g** with the appearance of two doublets attributed to the methylene proton of isoxazoline and a singlet attributable to the proton of the isoxazole moiety (Table 1, entry 1). In this case, the Cu catalyzed reaction between the dipolarophile **3** with *N*-hydroxy-4-methoxybenzenecarboximidoyl chloride (**4c**) at room temperature offered specially 50% of the 3,5-rigioisomere isoxazole after 4 h in DCM (Table 1, entry 2). A marked improvement of the yield (up to 60%) was observed by prolonging reaction time for the formation of the desired product (Table 1, entry 3, entry 4) at room temperature. Using the same conditions in DMF at room temperature led to the isoxazole 3,5-disubstuted with a decrease in yield (Table 1, entry 5). Moreover, when we worked in mixed-solvent systems with the same conditions, there was no conversion even after 1 h. This may be explained by the poor solubility of substrate **4** in such solvent (Table 1, entry 6). Comparatively, the use of THF-H_2O as solvent with the same conditions resulted in the isoxazole 3,5-disubstuted with a yield of 30% (Table 1, entry 7). To compare the classical heating methods with the microwave-assisted synthesis method, the desired compound **5c** was synthesized under microwave irradiation. It is clear that in this case, much shorter reaction time and higher yield were achieved under microwave irradiation as compared with classical heating (Table 1, entry 8). Interestingly, the reaction under microwave conditions with the presence of 0.1 eq of catalyst CuI and triethylamine in DMF was the key to obtain the highest yield (90%) of the desired isoxazole 3,5-disubstuted (Table 1, entry 9). The optimal condition (Table 1, entry 9) was applied in a regiospecific approach between 1-(prop-1-en-2-yl)-3-(prop-2-yn-1yl)-benzimidazol-2-one (**3**) and various arylnitrile oxides **4a**-**4k** in the presence of CuI catalyst and Et_3N resulting in the formation of isoxazole derivatives **5a**-**5k** in excellent yields. All reactions were carried out under microwave irradiation (300 W) and completed within 4–7 min. The new compounds **5a**-**5k** were obtained in yields ranging from 82 to 95% (Table 2). This method is the simplest, the most regiospecific, giving the best yields at shorter reaction times than those reported in the literature (Scheme 3).

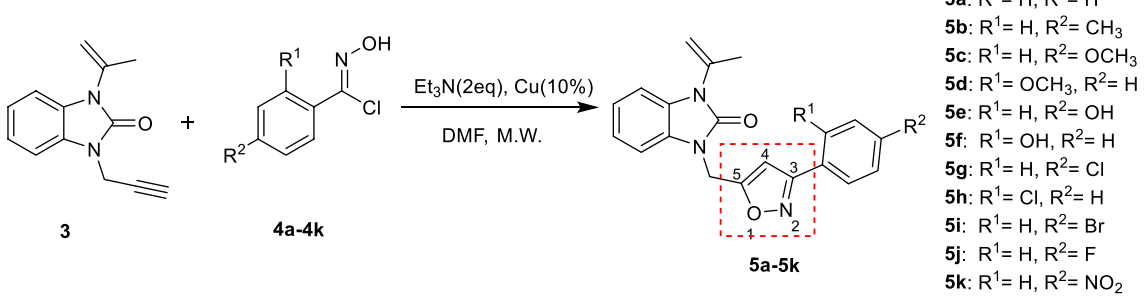
Table 2 One-pot synthesis of 3,5-disubstituted isoxazoles **5a**-**5k**

Entry	Compound	R^1	R^2	Time /min	Yield/%
1	**5a**	H	H	6	86
2	**5b**	H	CH_3	5	89
3	**5c**	H	OCH_3	4	90
4	**5d**	OCH_3	H	5	86
5	**5e**	H	OH	7	83
6	**5f**	OH	H	6	82
7	**5g**	H	Cl	5	88
8	**5h**	Cl	H	4	92
9	**5i**	H	Br	4	89
10	**5j**	H	F	6	84
11	**5k**	H	NO_2	7	95

The structures of the target compounds isoxazole-benzimidazolone **5a**-**5k** were evidenced by their spectral (^{1}H and ^{13}C NMR) and ES-HRMS data. In fact, the ^{1}H NMR spectra showed no signal related to acetylenic proton at 2.23 ppm. However, a new signal at 6.48–6.75 ppm (1H, s), corresponding to H_4 proton because of the proximity of the isoxazolinic oxygen, and the appearance of new characteristic signals attributable to the aromatic protons introduced by aromatic arylnitroloxide. The same features are appeared in ^{13}C NMR spectra, showing essentially the disappearance of the signal relating to terminal carbon of alkyne group at $\delta = 72.9$ ppm, and the appearance of a new signal of the tertiary carbon C_4 at 101.2–108 ppm after cycloaddition. Furthermore, all products exhibited a peak at 166.7–170 ppm, which is characteristic of the quaternary carbon C_5 of the isoxazole ring. These chemical values' shift clearly confirms the regiochemistry of the cycloaddition and are in line with the values reported in the literature [36, 37]. Further proof of our structural assignment stemming from mass spectrometry analysis is also in accordance with the proposed structure for the adducts.

For the regioisomer **5c**, the ^{1}H NMR spectra showed a new signal at $\delta = 6.48$ ppm attributable to the proton H_4 and signals in the aromatic region (6.90–7.65 ppm) corresponding to aromatic protons introduced by the phenyl ring of

Scheme 3



arylnitroloxide derivative **4c**. Particular characteristics are the ^{13}C NMR isoxazole ring resonances with three peaks at $\delta = 101.2$, 161.2, and 167.3 ppm for the isoxazole carbons C_4, C_5, and C_3. Also, mass spectrometry analysis corroborates the proposed structure for the compound **5c**.

Antibacterial activity

Benzimidazolone derivatives are widely known for their significant antibacterial activity [38–40]. In the present study, the synthesized compounds **5a**-**5k** were evaluated for their antibacterial activity. These compounds were tested at a concentration of 100 µg/cm^3 against three Gram-positive (*B. subtilis*, *E. faecalis*, and *S. aureus*) and three Gram-negative (*E. coli*, *P. aeruginosa*, and *S. typhi*) bacteria using well agar diffusion assay. The diameters of inhibition zones are shown in Fig. 2 and compared by standard antibiotic ampicillin. All compounds displayed strong activity against the most strains and the data revealed that the strains *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* were the most sensitive. Based on zones of inhibition results and a part from the nature of the substituents in the *para*- and/or *ortho*-positions of the terminal phenyl, the lowest activity was observed for the compounds **5a** and **5b**. The addition of the methoxy group (**5c** and **5d**) and halogen groups (**5g**, **5h**, **5i**, and **5j**) increased the activity. For example, **5d** derivative showed a zone inhibition diameter of 15 mm against *E. coli* and *S. aureus* bacteria, **5i** have a 19 mm^3 diameter of zone inhibition against *P. aeruginosa*, *E. coli*, and *S. aureus*. Interestingly, **5e**, **5f**, and **5k** compounds showed better antibacterial activity, which reached a value of 26 mm^3 against the strain *E. coli*. These values are higher than the standard drug tetracycline [39]. In addition, these compounds also contain a hydroxyl and nitro groups combined to the terminal phenyl, which may also be responsible for higher antibacterial activity of these species [41].

Antifungal activity

In vitro antifungal activity of the novel benzimidazolone derivatives against two fungal strains (*A. brasiliensis* and *A. fumigatus*) and two yeast strains (*C. albicans* and *C. neoformans*) were assessed using well agar diffusion. The diameters of inhibition zones observed with the well diffusion method are presented in Fig. 3. Overall, the solvent used to prepare the compound solutions (DMSO) did not show any inhibition against the tested organisms (negative control). It was also observed that the strain *C. neoformans* was the most resistant. In fact, all compounds exhibited low activity against this yeast strain with inhibition zones between 0 mm^3 and 2 mm^3. Interestingly, under identical experimental conditions **5e**, **5f**, **5g**, **5h**, **5i**, **5j**, **5k** showed higher inhibitory effect on the growth of fungi than **5a**, **5b**, **5c**, and **5d** molecules (Fig. 3). In other words, compounds possessing nitro, hydroxyl groups, and halogenated derivatives on the phenyl ring showed remarkable growth inhibition. These derivatives' inhibition zone showed between 12 mm^3 and 17 mm^3 in diameter against *A. fumigatus* and 11 mm^3 to 16 mm^3 against *C. albicans* and *A. brasiliensis*. Moreover, these compounds exhibited weaker antifungal activity than the standard drug fluconazole [42], which owns 24 mm^3 in diameter of zone inhibition against *C. albicans*. The literature survey revealed that the antifungal activity of these benzimidazolone derivatives is comparable to and, in some cases, even higher, than previously synthesized and studied analogous benzimidazolones [45].

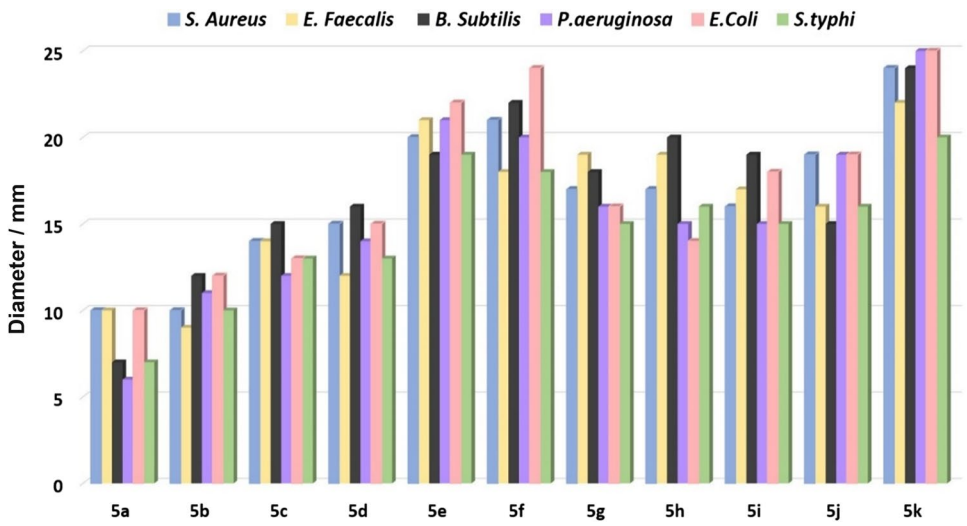


Fig. 2 In vitro antimicrobial activity

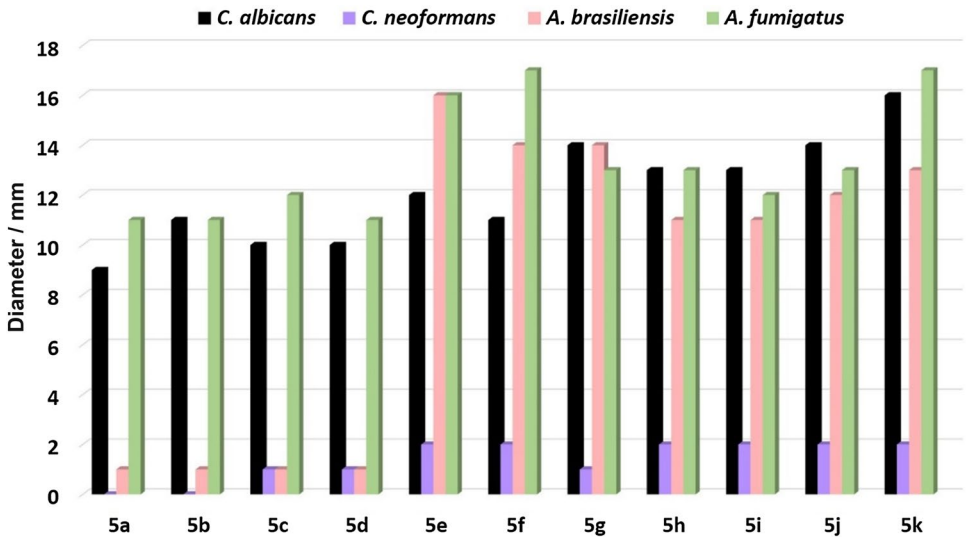


Fig. 3 In vitro antifungal activity

Antioxidant activity by DPPH radical scavenging method

The scavenging activities of the synthesized compounds **5a**-**5k**, and standard (vitamin C) against DPPH were evaluated. The antioxidant activity results (% RSA: percentage of radical scavenging activity) and IC_{50} values are shown in Fig. 4 and Table 3, respectively. It is evident from the results that free radical scavenging activity depends on the concentration and basic skeleton of the molecule as well as on the nature of substituents. % RSA values revealed that all compounds are found to be more active than the standard (compound **5a**). Highly significant antioxidant results (% RSA 73.08, 65.37, and 67.66) were expressed by the derivatives containing nitro and hydroxyl groups at different positions in the phenyl ring (compounds **5k**, **5e**, and **5f**). The presence of nitro group in any organic molecule in general confers significant biological activity [43, 44]. Moreover, the phenolic groups were the major complementary compounds of propolis that had beneficial effects as natural antioxidants and prevent oxidative damage of DNA caused by reactive oxygen species [45]. Among the halogenated derivatives (compounds **5g**, **5h**, **5i**, and **5j**), the fluoro group produced significant % RSA, i.e., 60.67 as compared to the bromo and chloro groups (% RSA 50.57 and ~ 49, respectively). In the methoxy derivatives, it was found that the compounds containing one methoxy group (compounds **5c** and **5d**) showed low level of antioxidant activity (% RSA 33.60 and 31.54). Similarly,

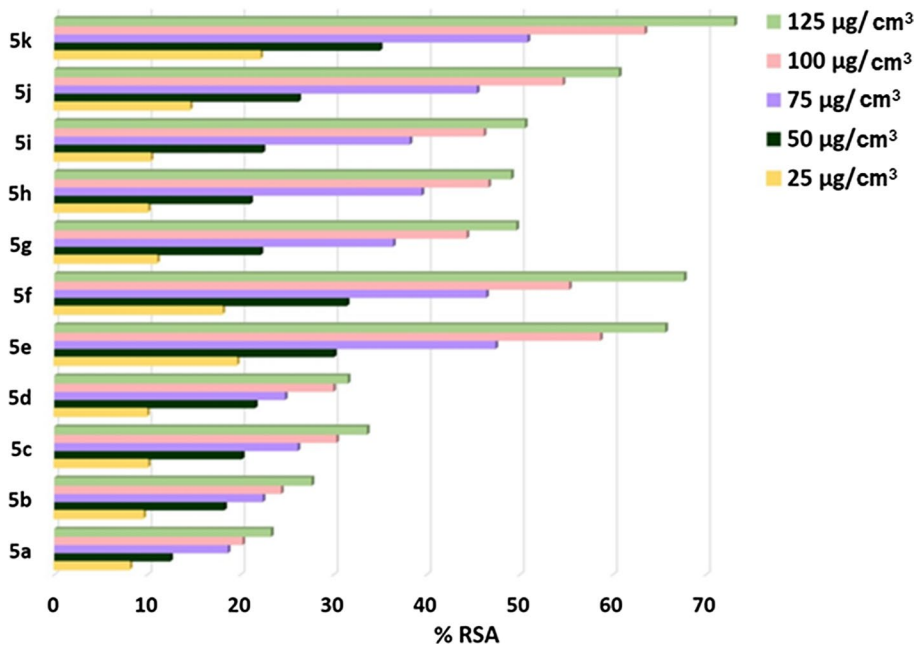


Fig. 4 DPPH radical scavenging activity of the benzimidazolone derivatives **5a**-**5k**

Table 3 IC_{50} values of benzimidazolone derivatives for DPPH scavenging assay. Vitamin C was used as standard

Compounds/standards	IC_{50}/mg cm^{-3}
5a	4.3185
5b	2.4267
5c	1.4601
5d	1.4673
5e	0.0244
5f	0.0256
5g	0.0625
5h	0.0673
5i	0.0609
5j	0.0408
5k	0.0196
Vitamin C	0.0078

compound **5b** showed low level of radical scavenging activity (% RSA 27.65).

The results of the radical scavenging were also expressed in terms of half-inhibition concentration (IC_{50}) which denotes the concentration required to scavenge 50% of DPPH radicals, such as the higher IC_{50} values represent lower antioxidant activity. These values are shown in Table 3 and compared to that of vitamin C. The obtained results are 2.426 for **5b**, ~ 1.46 for methoxy derivatives, ~ 0.06 for halogenated derivatives, ~ 0.0196 nitro group, and 0.0078 for vitamin C. Moreover, the nitro and hydroxyl groups were found to be more effective to inhibit the formation of DPPH radicals.

Computational analysis of chemical reactivity properties: a conceptual DFT study

The chemical reactivity of isoxazol-benzimidazolone compounds was investigated through conceptual density functional theory (CDFT) approach, referred to as Chemical Reactivity Theory [46, 47]. All calculations were accomplished at wB97X-D/cc-pVDZ level of theory. Gaussian 09, Rev D.01 software [48] was employed in conjunction with GaussView 6.0 [49] and AVOGADRO 1.2.0 molecular viewers [50]. The energy levels and surfaces of the highest occupied (HOMO) and the lowest unoccupied (LUMO) molecular orbitals are illustrated in Figs. 5, 6. The calculated four global reactivity descriptors are shown in Table 4.

As can be seen, the electron density distribution at HOMOs of four derivatives was delocalized on their benzimidazolone rings. On the other hand, LUMO isosurfaces have a relatively uniform electron density over the isoxazole ring. The predicted chemical potential (μ) ($\mu \approx 1/2(E_{LUMO} + E_{HOMO})$) for **5a** and **5h** was found to be −3.422 and −3.402 eV, respectively. While **5d** and **5f** exhibited relatively lower μ values with −3.247 and −3.277 eV, respectively. Additionally, the calculated chemical hardness (η) (donated as $\eta \approx (E_{LUMO} - E_{HOMO})$) values are comparable and range from 4.412 eV (**5h**) to 4.611 eV (**5a**).

The global electrophilicity index ($\omega = \mu^2/2\eta$) values were in the range of 1.146–1.311 eV. The higher electrophilicity power is shown for **5h** and the lower one is observed for **5d**. According to Domingo's electrophilicity scale [51], all studied molecules are classified as moderate-to-strong electrophiles. Furthermore, the global

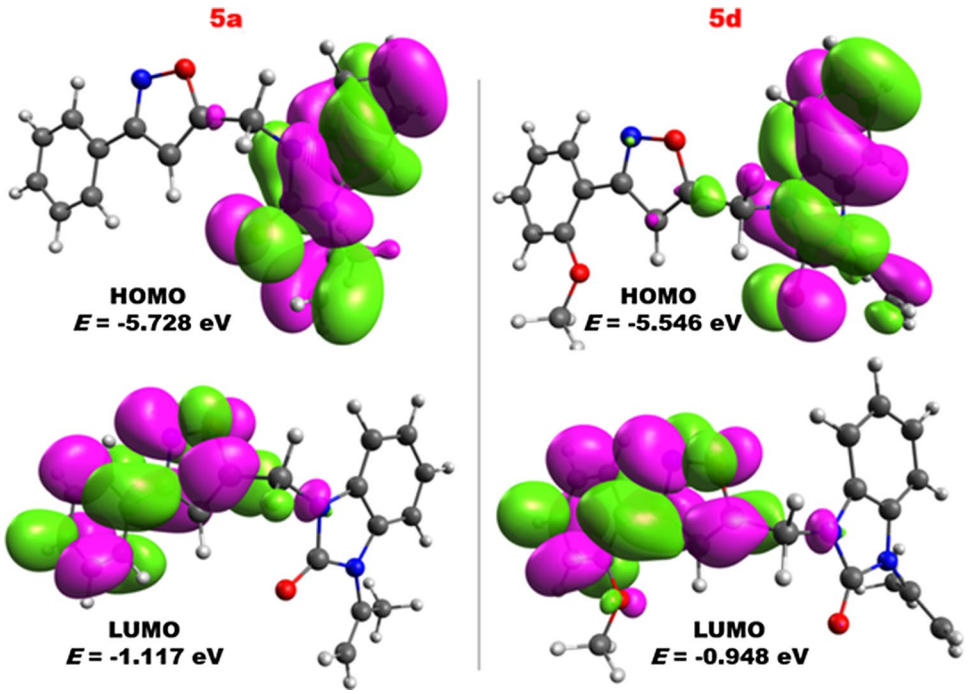


Fig. 5 Electron density distribution and energies of frontier molecular orbitals calculated at wB97X-D/cc-pVDZ level, for compounds **5a** and **5d**

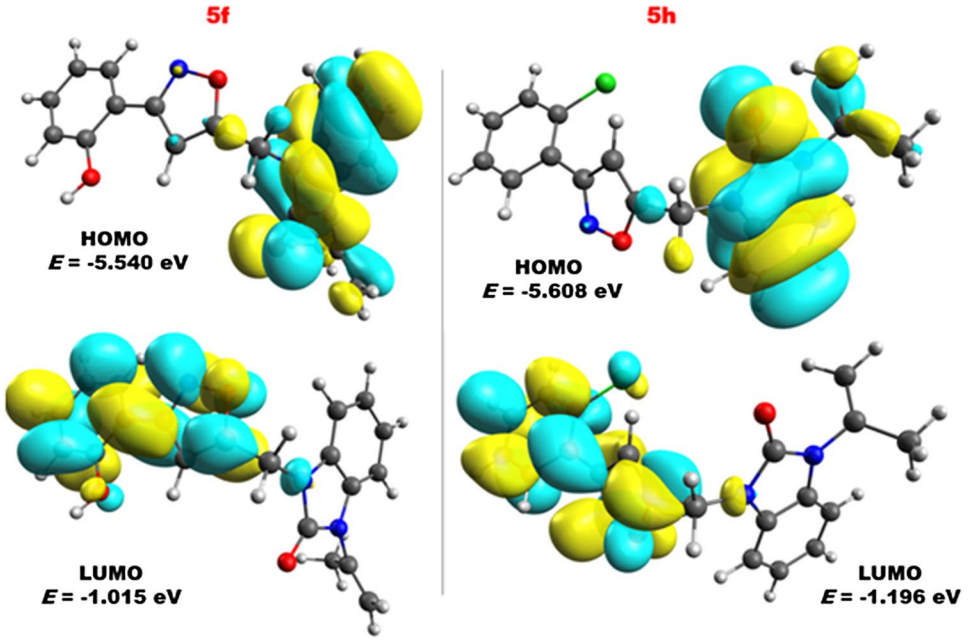


Fig. 6 Electron density distribution and energies of frontier molecular orbitals calculated at wB97X-D/cc-pVDZ level, for compounds **5f** and **5h**

Table 4 Chemical reactivity descriptors values (in eV) calculated at wB97X-D/cc-pVDZ level of theory

Compound	Electronic chemical potential (μ)	Chemical hardness (η)	Global electrophilicity (ω)	Global nucleophilicity (N)
5a	−3.422	4.611	1.269	3.392
5d	−3.247	4.598	1.146	3.574
5f	−3.277	4.525	1.186	3.580
5h	−3.402	4.412	1.311	3.512

nucleophilicity (N) is calculated based on the HOMO energies:$N = E_{HOMO} - E_{HOMO(TCE)}$, tetracyanoethylene (TCE) was taken as reference, because it behaves lower HOMO energy (−9.12 eV). Notably, from the nucleophilicity index scale, it may be seen that the title species are strong nucleophiles as they show nucleophilicity power larger than 3.00 eV [52] (Fig. 6).

Conclusion

In conclusion, a new series of benzimidazolone-linked 3,5-disubstitued isoxazoles was designed and synthesized via regiospecific 1,3-dipolar cycloaddition reaction catalyzed by CuI and microwave-assisted N_1-propargyl N_2-isopropenylbenzimidazol-2-one with some arylnitroloxides **4a**-**4k**. The easy process, short reaction time, and high yield of isolated products make this method attractive for the synthesis of potential biologically active molecules. All the newly prepared species **5a**-**5k** have been tested for their in vitro antimicrobial and antioxidant activities. Compounds **5g** and **5h** exhibited excellent antibacterial activity particularly against *P. aeruginosa*, *E. coli*, and *S. aureus* and showed the highest antifungal activity against *A. fumigatus*, *C. albicans*, and *A. brasiliensis*. Similarly, these three compounds displayed the most significant antioxidant activity. Theoretical DFT-based study has also been performed to explain the reactivity behaviors of the products **5a**, **5d**, **5f**, and **5h**. All studied molecules could be classified as moderate-to-strong electrophiles.

Experimental

All solvents were purified and dried using standard methods. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at room temperature in deuterated $CDCl_3$ and DMSO-d_6 on a Bruker AC-300 using residual non-deuterated solvent signal as internal reference. High-resolution mass spectra (HRMS) were obtained with Micromass LCT (ESI technique, positive mode) spectrometers. The microwave was a Biotage AB Initiator EXP EU with a maximum power of 800 W (2450 MHz). Coupling constants are given in Hz and all chemical shifts were reported as values (ppm). Signals multiplicities are using the following abbreviations: s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet), etc. Melting points were determined on a Büchi-510 apparatus using capillary tubes. Column chromatography purifications were performed on silica gel (40–63 µm). All reactions were monitored by TLC using aluminum sheets of sds silica gel 60 F254, 0.2 mm.

1,3-Dihydro-1-isopropenyl-2*H*-benzo[*d*]imidazol-2-one (2, $C_{10}H_{10}N_2O$) A mixture of 10 g *o*-phenylenediamine (**1a**, 0.092 mol) and 12 g ethyl acetoacetate (**1b**, 0.092 mol) was stirred in 15 cm^3 dry DMF. The reaction mixture was stirred under irradiation (300 W) for 5 min. The water and ethanol formed were removed progressively by extraction with ether diethylic. The organic phase was dried with $MgSO_4$ and concentrated. The mixture was then cooled, and the precipitated brown solid was collected by filtration, washed two times with ether, dried in the oven to give the *N*-isopropenylbenzimidazolone **2**. Yield: 15 g (96%); brown solid; m.p.: 122–124 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.18$ (s, 3H, CH_3), 5.24 (s, 1H, CH_2), 5.40 (d, 1H, $J = 1.2$ Hz, CH_2), 7.04–7.13 (m, 4H, H_{arom}), 10.67 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 109.2 (CH_{arom}), 110.1 (CH_{arom}), 114.1 (CH_2), 121.5 (CH_{arom}), 122.1 (CH_{arom}), 128.6 (Cq), 130.1 (Cq), 137.9 (Cq), 154.9 (C = O) ppm; HRMS (ESI): *m/z* calcd for $C_{10}H_{10}N_2NaO$ ($[M + Na]^+$) 197.0703, found 197.0704.

1,3-Dihydro-1-(prop-1-en-2-yl)-3-(prop-2-yn-1-yl)-2*H*-benzo[*d*]imidazol-2-one (3, $C_{13}H_{12}N_2O$) To a solution of 4 g N-isopropenylbenzimidazol-2-one (**2**, 23 mmol) in 100 cm^3 acetonitrile at 80 °C was added 1.53 cm^3 3-bromopropyne (1.1 mmol) and K_2CO_3 (46 mmol) for 12 h. The crude reaction was filtered through Celite and the filtrate was concentrate under reduced pressure to give a residue. The mixture obtained was purified by recrystallization from ether diethylic to obtain colorless crystals. Yield: 4.6 g (95%); brown solid; m.p.: 123–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.14$ (t, 3H, $J = 0.6$ Hz, CH_3), 2.22 (t, 1H, $J = 2.7$ Hz, $CH_2C{\equiv}\underline{CH}$), 4.61 (d, 2H, $J = 2.7$ Hz, *N*-CH_2), 5.12 (d, $J = 0.6$ Hz, 1H, CH_2), 5.27 (m, 1H, CH_2), 7.03–7.14 (m, 4H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 30.4 (CH_2), 72.8 ($\underline{C}{\equiv}CH$), 76.6 (CH), 108.7 (CH_{arom}), 109.3 (CH_{arom}), 113.4 (CH_2), 121.9 (CH_{arom}), 122 (CH_{arom}), 128.8 (Cq), 129.1. (Cq), 137.9 (Cq), 152.1 (C = O) ppm; HRMS (ESI): *m/z* calcd for $C_{13}H_{12}N_2NaO$ ($[M + Na]^+$) 235.0802, found 235.0804.

General procedure for the synthesis of compounds 5a-5k

At room temperature and stirring, to a mixture of dipolarophile **3** (0.7 mmol, 1 equiv), triethylamine (2 equiv, 2.8 mmol), and 0.1 equiv copper(I) iodide (CuI) in DMF, the appropriate hydroximyl chlorides **4a**-**4k** (2 equiv, 2.8 mmol) were added and the mixture was stirred under microwave irradiations at 300 W for 4–7 min. The crude reaction was diluted with water and then extracted with CH_2Cl_2 (3×20 cm^3). The organic layer was dried over Na_2SO_4. After removal of solvent in vacuum, the resulting residue was purified by column chromatography on silica gel and eluted with cyclohexane: ethyl acetate (from 9:1 to 7:3) to give the pure new cycloadducts **5a**-**5k** in 82–95% yield.

1,3-Dihydro-1-[(3-phenylisooxazol-5-yl)methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5a, $C_{20}H_{17}N_3O_2$) White solid; m.p.: 138–140 °C; yield: 200 mg (86%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.18$ (d, 3H, $J = 3$ Hz, CH_3), 5.15 (m, 3H, H_6, H_{7a}), 5.30 (d, 1H, $J = 3$ Hz,H_{7b}), 6.48 (s, 1H, $CH_{isoxazol}$), 7.02–7.06 (m, 4H, H_{arom}), 7.33–7.36 (m, 4H, H_{arom}), 7.66–7.69 (m, 1H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.7 (CH_2), 101.5 (C_4), 108.5 (CH_{arom}), 109.5 (CH_{arom}), 113.6 (CH_2), 122.2 (CH_{arom}), 122.2 (CH_{arom}), 122.3 (CH_{arom}), 127 (CH_{arom}), 127 (CH_{arom}), 128.8 (Cq), 129.1 (Cq), 129.2 (CH_{arom}), 129.2 (CH_{arom}), 130.4(Cq), 138 (Cq), 152.5 (C = O), 163 (C_5), 167.6(C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{17}N_3NaO_2$ ($[M + Na]^+$) 354.1208, found 354.1213.

1,3-Dihydro-1-(prop-1-en-2-yl)-3-[[3-(p-tolyl)isoxazol-5-yl]methyl]-2*H*-benzo[*d*]imidazol-2-one (5b, $C_{21}H_{19}N_3O_2$) Yellow solid; m.p.: 145–147 °C; yield: 215 mg (89%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.25$ (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 5.23 (m, 3H, H_6, H_{7a}), 5.38 (d, 1H, $J = 3$ Hz,H_{7b}), 6.53 (s, 1H, $CH_{isoxazol}$), 7.12–7.13 (m, 4H, H_{arom}), 7.21 (d, 2H, $J = 9$ Hz, H_{arom}), 7.64 (d, 2H, $J = 6$ Hz, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 21.6 (CH_3), 36.7 (CH_2), 101.4 (C_4), 108.5 (CH_{arom}), 109.5 (CH_{arom}), 113.6 (CH_2), 122.2 (CH_{arom}), 122.3 (CH_{arom}), 126 (CH_{arom}), 126.9 (CH_{arom}), 126.9 (CH_{arom}), 129 (Cq), 129.2 (Cq), 129.8 (CH_{arom}), 129.8 (Cq), 138 (Cq), 140.5 (Cq), 152.5 (C = O), 162.9 (C_5), 167.4 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_3NaO_2$ ($[M + Na]^+$) 368.1369, found 368.1369.

1,3-Dihydro-1-[[3-(4-methoxyphenyl)isoxazol-5-yl]methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5c, $C_{21}H_{19}N_3O_3$) Yellow solid; m.p.: 142–144 °C; yield: 230 mg (90%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.23$ (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 5.18–5.21 (m, 3H, H_6, H_{7a}), 5.38 (d, 1H, $J = 3$ Hz,H_{7b}), 6.48 (s, 1H, $CH_{isoxazol}$), 6.90 (d, 2H, $J = 9$ Hz, H_{arom}), 7.07–7.11 (m, 4H, H_{arom}), 7.65 (d, 2H, $J = 9$ Hz, 2H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.7 (CH_2), 55.5 (OCH_3), 101.2 (C_4), 108.5 (CH_{arom}), 109.5 (CH_{arom}), 113.6 (CH_2), 114.5 (CH_{arom}), 114.5 (CH_{arom}), 121.3 (CH_{arom}), 122.2 (CH_{arom}), 122.2 (CH_{arom}), 122.2 (CH_{arom}), 128.4 (Cq), 128.4 (Cq), 129.2 (Cq), 138 (Cq), 152.6 (C = O), 161.2 (Cq), 162.5 (C_5), 167.3 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_3NaO_3$ ($[M + Na]^+$) 384.1302, found 384.1304.

1,3-Dihydro-1-[[3-(2-methoxyphenyl)isoxazol-5-yl]methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one

(5d, $C_{21}H_{19}N_3O_3$) Yellow solid; m.p.: 140–142 °C; yield: 220 mg (86%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (d, 3H, $J = 0.6$ Hz, CH_3), 3.82 (s, 3H, OCH_3), 5.20 (m, 3H, H_6, H_{7a}), 5.35 (d, 1H, $J = 1.5$ Hz, H_{7b}), 6.75 (s, 1H, $CH_{isoxazol}$), 6.86 (d, $J = 9$ Hz, 1H, H_{arom}), 7.09–7.10 (m, 5H, H_{arom}), 7.30–7.33 (m, 1H, H_{arom}), 7.80–7.81 (m, 1H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.6 (CH_2), 56.1 (OCH_3), 108.6 (C_4), 109.4 (CH_{arom}), 109.4 (CH_{arom}), 112.9 (CH_2), 113.6 (CH_{arom}), 119.1 (CH_{arom}), 122.1 (CH_{arom}), 122.1 (CH_{arom}), 122.2 (CH_{arom}), 122.2 (CH_{arom}), 126 (Cq), 129.1 (Cq), 129.2 (Cq), 131.1 (Cq), 138 (Cq), 155.8 (C = O), 166.4 (C_5), 170 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_3NaO_3$ ($[M + Na]^+$) 384.1302, found 384.1303.

1,3-Dihydro-1-[[3-(4-hydroxyphenyl)isoxazol-5-yl]methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5e, $C_{20}H_{17}N_3O_3$) Brown solid; m.p.: 148–150 °C; yield: 220 mg (84%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H, CH_3), 5.18–5.21 (m, 3H, H_6, H_{7a}), 5.36 (d, 1H, $J = 1.2$ Hz, H_{7b}), 6.47 (s, 1H, $CH_{isoxazol}$), 6.91 (d, 2H, $J = 9$ Hz, H_{arom}), 7.07–7.11 (m, 4H, H_{arom}), 7.65 (d, 2H, $J = 9$ Hz, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.7 (CH_2), 102.2 (C_4), 108.5 (CH_{arom}), 109.5 (CH_{arom}), 113.6 (CH_2), 114.5 (CH_{arom}), 114.5 (CH_{arom}), 121.3 (CH_{arom}), 122.2 (CH_{arom}), 122.2 (CH_{arom}), 128.4 (CH_{arom}), 128.4 (Cq), 129(Cq), 129.1 (Cq), 138 (Cq), 152.4 (C = O), 161.3 (Cq), 162.6 (C_5), 167.3 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{17}N_3NaO_3$ ($[M + Na]^+$) 370.1163, found 370.1164.

1,3-Dihydro-1-[[3-(2-hydroxyphenyl)isoxazol-5-yl]methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5f, $C_{20}H_{17}N_3O_3$) White solid; m.p.: 144–146 °C; yield: 200 mg (82%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.23$ (s, 3H, CH_3), 5.22–5.23 (m, 3H, H_6, H_{7a}), 5.37 (d, 1H, $J = 1.2$ Hz, H_{7b}), 6.65 (s, 1H, $CH_{isoxazol}$), 6.89–7.01 (m, 1H, H_{arom}), 7.02–7.13 (m, 5H, H_{arom}), 7.28–7.42 (m, 2H, H_{arom}), 9.31 (s, 1H, OH_{alcool}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.2$ (CH_3), 36.3 (CH_2), 108 (C_4), 109.5 (CH_{arom}), 112.8 (CH_2), 113.5 (CH_{arom}), 116.8 (CH_{arom}), 119.8 (CH_{arom}), 122.1 (CH_{arom}), 122.2 (CH_{arom}), 122.3 (CH_{arom}), 128 (CH_{arom}), 128.6 (Cq), 129 (Cq), 131.7 (Cq), 137.7 (Cq), 144.6 (Cq), 154.7 (C = O), 166.1 (C_5), 166.7 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{17}N_3NaO_3$ ($[M + Na]^+$) 370.1163, found 370.1162.

1-[[3-(4-Chlorophenyl)isoxazol-5-yl]methyl]-1,3-dihydro-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5g, $C_{20}H_{16}ClN_3O_2$) White solid; m.p.: 153–155 °C; yield: 225 mg (88%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H, CH_3), 5.20–5.21 (m, 3H, H_6, H_{7a}), 5.36 (d, 1H, $J = 1.2$ Hz, H_{7b}), 6.51 (s, 1H, $CH_{isoxazol}$), 7.09–7.11 (m, 4H, H_{arom}), 7.37 (d, 2H, $J = 8.7$ Hz, H_{arom}), 7.67 (d, 2H, $J = 8.7$ Hz, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.2$ (CH_3), 36.4 (CH_2), 101.2 (C_4), 108.2 (CH_{arom}), 109.4 (CH_2), 109.4 (CH_{arom}), 113.4 (CH_{arom}), 122 (CH_{arom}), 122.1 (CH_{arom}), 122.1 (CH_{arom}), 127.1 (CH_{arom}), 128.1 (CH_{arom}), 128.7 (Cq), 129 (Cq), 129.2 (Cq), 136.2 (Cq), 137.7 (Cq), 152.3 (C = O), 161.8 (C_5), 167.7 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_3NaO_2$ ($[M + Na]^+$) 388.0822, found 388.0823.

1-[[3-(2-Chlorophenyl)isoxazol-5-yl]methyl]-1,3-dihydro-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (6h, $C_{20}H_{16}ClN_3O_2$) White solid; m.p.: 155–157 °C; yield: 235 mg (92%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H, CH_3), 5.20–5.22 (s, 3H, H_6, H_{7b}), 5.35 (d, 1H, $J = 1.2$ Hz, H_{7b}), 6.70 (s, 1H, $CH_{isoxazol}$), 7.09–7.14 (m, 4H, H_{arom}), 7.29–7.45 (m, 3H, H_{arom}), 7.63–7.67 (m, 1H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.3$ (CH_3), 36.6 (CH_2), 104.7 (C_4), 108.4 (CH_{arom}), 109.5 (CH_{arom}), 113.6 (CH_2), 122.1 (CH_{arom}), 122.2 (CH_{arom}), 127.2 (CH_{arom}), 128.1 (CH_{arom}), 129 (CH_{arom}), 129.1 (CH_{arom}), 130.5 (Cq), 131.1 (Cq), 131.1 (Cq), 131.1 (Cq), 138 (Cq), 152.4 (C = O), 161.5 (C_5), 166.7 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_3NaO_2$ ($[M + Na]^+$) 388.0822, found 388.0823.

1-[[3-(4-Bromophenyl)isoxazol-5-yl]methyl]-1,3-dihydro-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5i, $C_{20}H_{16}BrN_3O_2$) White solid; m.p.: 150–152 °C; yield: 256 mg (89%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H, CH_3), 5.20–5.21 (m, 3H, H_6, H_{7b}), 5.36 (d, 2H, $J = 2.4$ Hz, H_{7b}), 6.51 (s, 1H, $CH_{isoxazol}$), 7.09–7.12 (m, 4H, H_{arom}), 7.52–7.62 (m, 4H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.6 (CH_2), 101.3 (C_4), 108.4 (CH_{arom}), 109.6 (CH_{arom}), 113.6 (CH_2), 122.2 (CH_{arom}), 122.3 (CH_{arom}), 124.7 (CH_{arom}), 127.7 (CH_{arom}), 128.5 (CH_{arom}), 128.5 (CH_{arom}), 128.9 (Cq), 129.2 (Cq), 132.3 (Cq), 132.3 (Cq), 138 (Cq), 152.5 (C = O), 162 (C_5), 168 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}BrN_3NaO_2$ ($[M + Na]^+$) 432.0314, found 432.0318.

1-[[3-(4-Fluorophenyl)isoxazol-5-yl]methyl]-1,3-dihydro-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5j, $C_{20}H_{16}FN_3O_2$) White solid; m.p.: 153–155 °C; yield: 205 mg (84%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.15$ (s, 3H, CH_3), 5.19–5.20 (m, 3H, H_6, H_{7a}), 5.36 (d, 1H, $J = 2.4$ Hz H_{7a}), 6.50 (s, 1H, $CH_{isoxazol}$), 7.06–7.12 (m, 6H, H_{arom}), 7.68–7.74 (m, 2H, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.2$ (CH_3), 36.4 (CH_2), 101.9 (C_4), 108.2 (CH_{arom}), 109.3 (CH_{arom}), 113.4 (CH_2), 115.9 (CH_{arom}), 121.9 (CH_{arom}), 122.4 (CH_{arom}), 124.8 (CH_{arom}), 127.6 (CH_{arom}), 128.7 (CH_{arom}), 129 (Cq), 129.5 (Cq), 137.8 (Cq), 161.8 (C = O), 162.2 (Cq), 165.5 (C_5), 167.6 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}FN_4NaO_4$ ($[M + Na]^+$) 372.1117, found 372.1123.

1,3-Dihydro-1-[[3-(4-nitrophenyl)isoxazol-5-yl]methyl]-3-(prop-1-en-2-yl)-2*H*-benzo[*d*]imidazol-2-one (5k, $C_{20}H_{16}N_4O_4$) Brown solid; m.p.: 158–160 °C; yield: 250 mg (95%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H), 5.21–5.23 (m, 3H, H_6, H_{7a}), 5.36 (d, 1H, $J = 1.2$ Hz, H_{7b}), 6.62 (s, 1H, $CH_{isoxazol}$), 7.10–7.13 (m, 4H, H_{arom}), 7.92 (d, 2H, $J = 9$ Hz, H_{arom}), 8.27 (d, 2H, $J = 9$ Hz, H_{arom}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 36.6 (CH_2), 101.6 (C_4), 108.2 (CH_{arom}), 109.7 (CH_{arom}), 113.7 (CH_2), 122.3 (CH_{arom}), 122.4 (CH_{arom}), 122.4 (CH_{arom}), 122.4 (CH_{arom}), 127.9 (CH_{arom}), 127.9 (CH_{arom}), 128.8 (Cq), 129.2 (Cq), 134.8 (Cq), 137.9 (Cq), 148.9 (Cq), 152.4 (C = O), 161.1 (C_5), 168.8 (C_3) ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}N_5NaO_4$ ($[M + Na]^+$) 399.1065, found 399.1064.

Antibacterial screening

The antibacterial activities of the synthesized compounds **5a**-**5k** were evaluated by the agar well-diffusion assay technique against three Gram-positive bacteria, i.e., *B. subtilis*, *E. faecalis*, and *S. aureus*, and three Gram-negative bacteria, i.e., *E. coli*, *P. aeruginosa*, and *S. typhi*. These pathogenic strains were cultivated on nutrient agar at 37 °C for 24 h. Colonies were suspended in 10 cm^3 of physiological medium and mixed for 5 min, and suspensions were adjusted to 0.5 McFarland standard turbidity. One centimeter of bacterial suspension was spread over Muller Hinton Agar medium plates and incubated for 30 min at 37 °C. Using a punch, ≈ 6 mm-diameter wells were bored in the seeded agar plates and each test compound reconstituted in DMSO was added into the wells. DMSO was used as the control for all the test compounds. After holding the plates at room temperature for 2 h to allow diffusion of the compounds into the agar, the plates were incubated at 37 °C for 24 h. After 24 h, the diameters of the clear zone of inhibition surrounding the sample were measured in millimeters by digital caliper.

Antifungal activity

Fungal strains: two fungi strains, *A. fumigatus* and *A. brasiliensis*, were cultured on inclined Sabouraud agar in Falcon tube 15 cm^3 at 25 °C for 7 days (optimal temperature for their growing). The spore of fungal strain was suspended in peptone water and counted to have 106 spores/cm^3. Then, 1 ml of fungal suspension was spread over Sabouraud agar medium plates and incubated for 30 min at 25 °C. After that, 6 mm-diameter wells were dug in agar medium using sterile glassy borer. The synthesized compounds **5a**-**5k** were prepared in DMSO (1 mg/cm^3) and introduced into the respective wells, and one of the wells was supplemented with DMSO as control. These plates were placed in a 25 °C incubator for 5 days to allow fungal growth. After 5 days, the diameters of the clear zone of inhibition surrounding the sample were measured in centimeters by digital caliper.

Yeasts strains: two yeasts strains, *C. neoformans* and *C. albicans*, were cultured on Sabouraud agar at 37 °C (optimal temperature for bacteria growth) for 48 h. Then, pure colonies were suspended in 10 cm^3 of physiological medium and mixed well for 5 min, and suspensions were adjusted to 0.5 McFarland standard turbidity. One centimeter of yeast suspension was spread over Sabouraud agar medium plates and incubated for 30 min at 37 °C. Then, 6 mm^3-diameter wells were dug in agar medium using sterile glassy borer. The synthesized compounds **5a**-**5k** were prepared in DMSO (1 mg/cm^3) and introduced into the respective wells, and one of the wells was supplemented with DMSO as control. These plates were placed in a 37 °C incubator for 48 h to allow yeast growth. After 48 h, the diameters of the clear zone of inhibition surrounding the sample were measured in centimeters by digital caliper.

Antioxidant activity by DPPH method

The antioxidant activity of the benzimidazolone derivatives **5a**-**5k** was evaluated in vitro via their ability to scavenge DPPH radicals. In the experiments, different concentrations of our compounds were taken (prepared in DMSO) and 4.0 cm^3 of DPPH (0.004%) in methanol solution was added to our compounds' mixture. After 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm. DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition of DPPH } = (A_0 - A_1/A_0)\text{x}100,$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the presence of compounds. Vitamin C has been used as standards and their scavenging ability was compared with our results. IC_{50} (50% inhibition concentration) values of benzimidazolone derivatives and standard (vitamin C) were also calculated for comparison. IC_{50} value of the sample is the required concentration to scavenge 50% of DPPH free radicals.

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