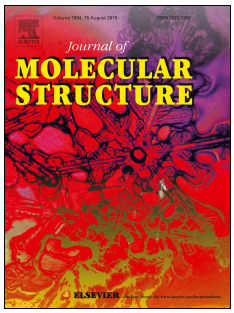


Design, synthesis, biological evaluation, molecular docking, DFT calculations and *in silico* ADME analysis of (benz)imidazole-hydrazone derivatives as promising antioxidant, antifungal, and anti-acetylcholinesterase agents

Imene Amine Khodja, Houssem Boulebd, Chawki Bensouici, Ali Belfaitah

PII: S0022-2860(20)30852-8

DOI: https://doi.org/10.1016/j.molstruc.2020.128527

Reference: MOLSTR 128527

To appear in: *Journal of Molecular Structure*

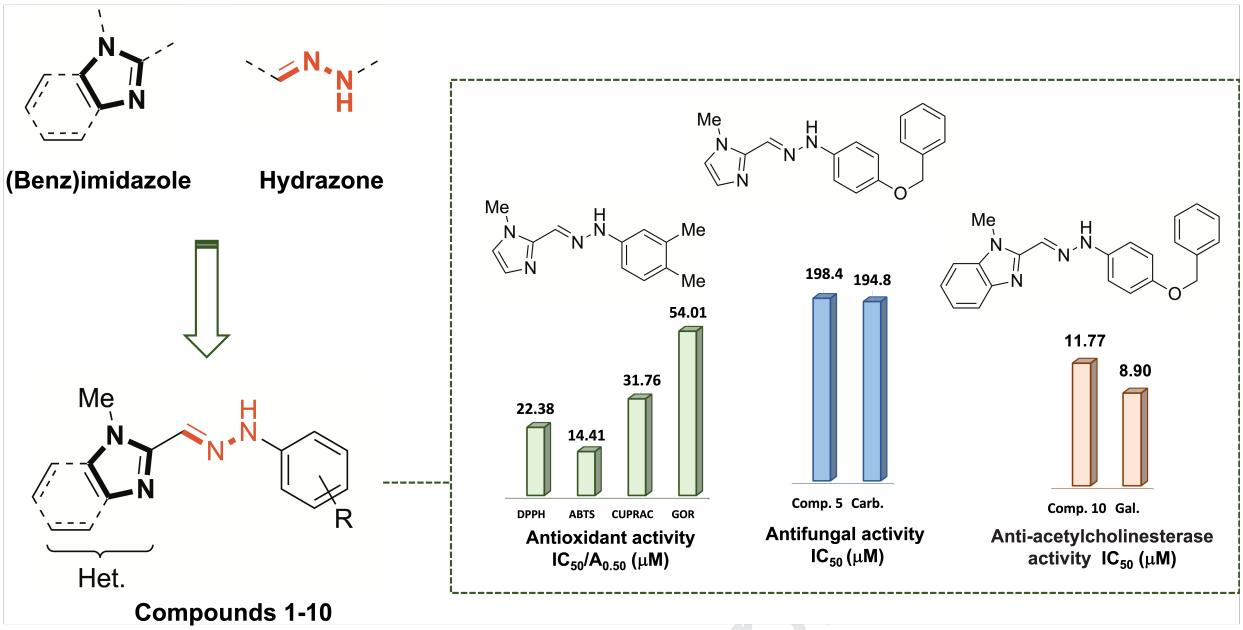
Received Date: 18 February 2020

Revised Date: 19 May 2020

Accepted Date: 25 May 2020

Please cite this article as: I.A. Khodja, H. Boulebd, C. Bensouici, A. Belfaitah, Design, synthesis, biological evaluation, molecular docking, DFT calculations and *in silico* ADME analysis of (benz)imidazole-hydrazone derivatives as promising antioxidant, antifungal, and anti-acetylcholinesterase agents, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.128527.

Imene Amine Khodja: Investigation; **Houssem Boulebd:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Writing – review and editing; **Chawki Bensouici:** Resources; **Ali Belfaitah:** Resources.



Design, synthesis, biological evaluation, molecular docking, DFT calculations and *in silico* ADME analysis of (Benz)imidazole-hydrazone derivatives as promising antioxidant, antifungal, and anti-acetylcholinesterase agents

Imene Amine Khodja[a], Houssem Boulebd[a,*], Chawki Bensouici[b], Ali Belfaitah[c]

[a] *Laboratory of Synthesis of Molecules with Biological Interest, University of Frères Mentouri Constantine 1, Constantine, Algeria.*
[b] *Centre de Recherche en Biotechnologie Ali Mendjli Nouvelle Ville, Constantine, Algérie*
[c] *Laboratoire des Produits Naturels d'Origine Végétale et de Synthèse Organique, Faculté des Sciences Exactes, Campus de Chaabat Ersas, Université des frères Mentouri-Constantine, Constantine 25000, Algeria*
**Corresponding author: E-mail: boulebd.houssem@umc.edu.dz*

Abstract

Ten hydrazone derivatives bearing a (benz)imidazole nucleus were designed, synthesized and evaluated for their antioxidant, antifungal, and anti-acetylcholinesterase activities. All the synthesized compounds (**1-10**) showed good to excellent antioxidant activity. Among them, compound **10** was found to be the best acetylcholinesterase inhibitor with an IC_{50} value comparable to that of the galantamine. Compound **5** was found to be the best antifungal agent against *Fusarium oxysporum* fungal strain when compared to the commercial fungicide carbendazim. DFT calculations, for representative molecules **1** and **6**, were also performed to investigate the antioxidant mechanisms, and it was found that SETPT (sequential electron transfer-proton transfer) is the most favorable mechanism in ethanol. Molecular docking studies of the most active compounds were carried out, and results showed reasonable binding modes in the active site of *Fusarium oxysporum* FGB1 enzyme and acetylcholinesterase. Finally, *in silico* predictions of ADME and pharmacokinetic parameters indicated that these compounds should have good oral bioavailability.

Keywords: (Benz)imidazole; Hydrazone; Acetylcholinesterase; Antioxidant activity; Docking study; DFT calculations.

1. Introduction

Nitrogen-containing heterocycles such as imidazole and benzimidazole exhibit diverse range of biological activities[1-5]. These heterocycles cover nearly all ranges of activities, such as anticancer[6], anti-HIV[7], antitubercular[8, 9], antihepatitis C[10], anti-inflammatory[11], antibacterial[12], antihypertensive[13], cholinesterase inhibitors[14], antioxidants[15], and antiprotozoal[16]. In the medical field, a great number of imidazole and benzimidazole-based compounds as clinical drugs have been extensively used to treat various types of diseases with high therapeutic potency. For examples (Fig. 1), Emedastine is used as H_1 receptor antagonist in eye drops to alleviate the symptoms of allergic conjunctivitis[17]. Clotrimazole is an antifungal drug used to treat some dermatophytes, yeasts, and dimorphic as well as filamentous fungi infections[18]. Carbendazim is a widely used, broad-spectrum benzimidazole fungicide[19]. Dacarbazine is chemotherapy agent used in the treatment of melanoma and Hodgkin's lymphoma[20]. Albendazol is an anthelmintic drug used for the treatment of a variety of parasitic worm infestations[21]. Losartan is an angiotensin II receptor antagonist used to treat hypertension[22].

On the other hand, the hydrazone functional group presents innumerous applications in medicinal chemistry[23], organic synthesis[24], supramolecular chemistry[25], dynamic combinatorial chemistry[26], and others[27]. In medicinal chemistry, compounds bearing this pharmacophore also exhibit a broad spectrum of biological activities including antimicrobial[28], antioxidant[29], antiproliferative[30], MAO Inhibitory[31], leishmanicidal[32], and antiproliferative[33]. Hydrazone functional group is present in numbers of commercialized drugs, Fig. 1 shows some examples. Isocarboxazide is a non-selective irreversible monoamine oxidase inhibitor (MAOI)[34]. Ferimzone is an antifungal drug used for the control of fungal diseases in rice[35]. Dihydralazine is a smooth muscle relaxant used to treat high blood pressure by acting as a vasodilator[36]. Mitoguazone is an anticancer agent used in chemotherapy[37]. Nifuroxazide is an antibiotic used to treat colitis and diarrhea[38]. Levosimendan is a calcium sensitizer used in the treatment of acute congestive heart failure.[39]

Our goal in this paper was to incorporate these two independently biologically active moieties into one molecule to generate compounds with new and/or enhanced biological activities. In this context, and as a continuation of our previous developments on this topic[40-44], we have designed and synthesized (Fig. 1) a series of hybrid compounds bearing imidazole or benzimidazole nucleus and hydazone moiety and evaluated them for their antioxidant, antifungal, and anti-acetylcholinesterase activities. To gain more insights into the

structure-activity relationship of the investigated compounds, some *in silico* studies such as DFT calculations, molecular modeling, and ADME predictions have been also performed and discussed.

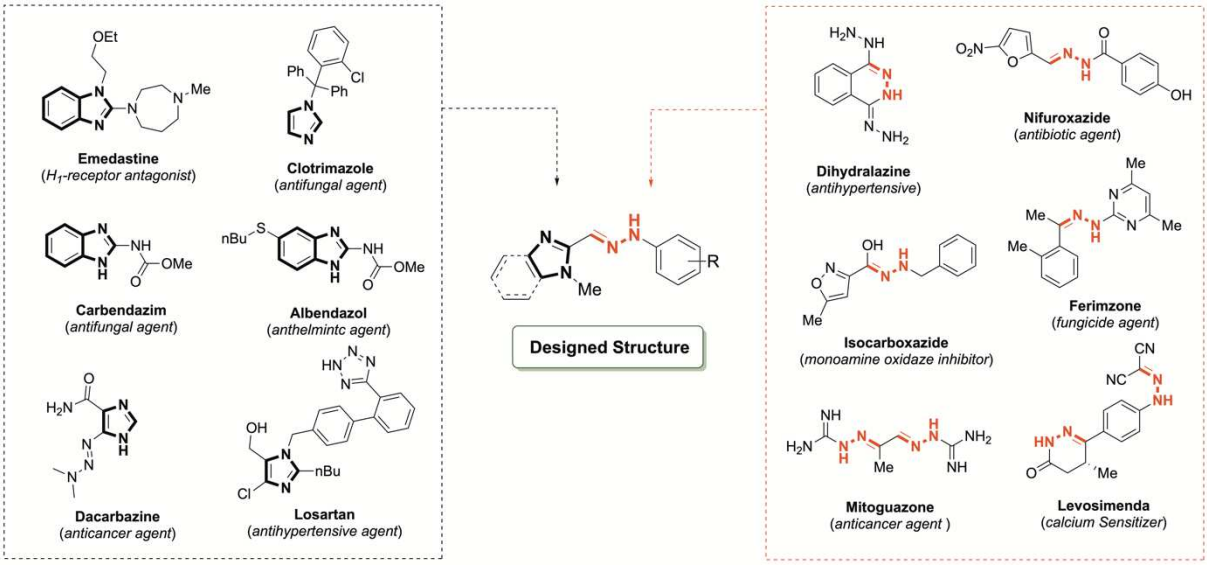


Fig. 1. Design of (benz)imidazole-phenylhydrazone hybrids.

2. Experimental section

2.1. Materials and instrumentation

The FTIR spectra were recorded with a JASCO FT/IR-6300typeA spectrometer and only significant absorption band frequencies are cited. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX250. The measurements of the diffracted intensities were recorded on APEX II diffractometer equipped with a two-dimensional detector Kappa CCD ($\lambda K\alpha$ = 0.71073 Å). Melting points were determined on a Kofler melting point apparatus. Commercial grade reagents were used as supplied (Alfa Aesar). The starting materials 1-methylbenzimidazole-2-carbaldehyde and 1-methylimidazole-2-carbaldehyde were prepared as described in our previous studies[41, 43].

2.2. General procedure for the synthesis of (benz)imidazole-hydrazone derivatives 1-10

The hydrazones **1-10** were prepared by a condensation reaction between phenylhydrazine derivatives and imidazole or benzimidazole carbaldehydes[45-47]. In a 25-mL Erlenmeyer flask, 1 mmol of 1-methylbenzimidazole-2-carbaldehyde or 1-methylimidazole-2-carbaldehyde and 1 mmol of phenylhydrazine derivative (phenylhydrazine, 4-methoxyphenylhydrazine, 3,4-dimethylphenylhydrazine, 4-chlorophenylhydrazine and (4-benzylphenyl)hydrazine) were dissolved in 3 ml of ethanol. The reaction mixture was stirred

at room temperature for 4h, and then filtered and air-dried. The resulting residue was then purified by recrystallization in a mixture of ethanol/DMSO to give the pure product.

(E)-1-méthyl-2-((2-phenylhydrazineylidene)methyl)-1H-imidazole (**1**). Yield: 97%. (yellow powder). Mp=164°C. IR: ν_{max} 1637 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.12 (s, 1H, NH_{Hyd}), 7.82 (s, 1H, CH_{Hyd}), 7.24 (t, *J* = 7.7 Hz, 2H, H_{arom}), 7.12 (s, 1H, H_{imid}), 7.08 (d, *J* = 7.7 Hz, 2H, H_{arom}), 7.00 (s, 1H, H_{imid}), 6.79 (t, *J* = 7.7 Hz, 1H, H_{arom}), 4.09 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 146.29, 144.67, 130.19, 128.55, 128.18, 124.95, 120.78, 113.39, 36.16.

(E)-2-((2-(4-methoxyphenyl)hydrazineylidene)methyl)-1-methyl-1H-imidazole (**2**). Yield: 76%. (yellow powder). Mp>260°C. IR: ν_{max} 1648 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.53 (s, 1H, NH_{Hyd}), 7.88 (s, 1H, CH_{Hyd}), 6.98-6.77 (m, 6H, H_{imid}, H_{arom}), 6.86 (t, *J* = 7.2 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.69 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 152.73, 142.79, 139.14, 128.53, 127.81, 123.92, 119.54, 114.74, 113.54, 112.98, 55.32, 35.24

(E)-2-((2-(3,4-dimethylphenyl)hydrazineylidene)methyl)-1-methyl-1H-imidazole (**3**). Yield: 88%. (orange powder). Mp=166°C. IR: ν_{max} 1651 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.12 (s, 1H, NH_{Hyd}), 7.08 (s, 1H, CH_{Hyd}), 6.54-6.10 (m, 6H, H_{imid}, H_{arom}), 3.74 (s, 3H, NCH_3), 1.49 (s, 3H, CH_3), 1.44 (s, 3H, CH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 143.32, 138.32, 131.07, 129.09, 125.03, 124.69, 124.40, 117.50, 114.85, 111.03, 35.80, 20.08, 18.95.

(E)-2-((2-(4-chlorophenyl)hydrazineylidene)methyl)-1-methyl-1H-imidazole (**4**). Yield: 79%. (yellow powder). Mp=248°C. IR: ν_{max} 1638 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.12 (s, 1H, NH_{Hyd}), 7.88 (s, 1H, CH_{Hyd}), 7.39-7.16 (m, 6H, H_{imid}, H_{arom}), 4.03 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 144.07, 143.55, 130.23, 126.77, 125.17, 123.06, 122.85, 121.24, 115.39, 104.76, 36.02.

(E)-2-((2-(4-(benzyloxy)phenyl)hydrazineylidene)methyl)-1-methyl-1H-imidazole (**5**). Yield: 95%. (yellow powder). Mp=148°C. IR: ν_{max} 1661 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.46 (s, 1H, NH_{Hyd}), 7.85 (s, 1H, CH_{Hyd}), 7.45-7.21 (m, 6H, H_{arom}), 6.95-6.84 (m, 5H, H_{arom}), 5.03 (s, 2H, CH_2), 3.91 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 151.76, 142.71, 139.28, 137.56, 128.75, 128.44, 127.88, 127.71, 123.95, 115.88, 112.90, 69.65, 35.24

(E)-1-methyl-2-((2-phenylhydrazineylidene)methyl)-1H-benzo[d]imidazole (**6**). Yield: 92%. (yellow brown). Mp=252°C. IR: ν_{max} 1633 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.80 (s, 1H, NH_{Hyd}), 8.06 (s, 1H, CH_{Hyd}), 7.60 (t, *J* = 7.6 Hz, 2H, H_{arom}), 7.34-7.10 (m, 6H, H_{arom}), 6.86 (t, *J* = 7.2 Hz, 1H, H_{arom}), 4.13 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6):

δ 148.34, 144.37, 142.68, 136.81, 129.40, 128.89, 122.72, 121.93, 119.91, 119.57, 118.84, 113.05, 112.39, 109.98, 32.12.

(E)-2-((2-(4-methoxyphenyl)hydrazineylidene)methyl)-1-methyl-1H-benzo[d]imidazole (**7**). Yield: 80%. (yellow powder). Mp=162°C. IR: ν_{max} 1656 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.79 (s, 1H, NH_{Hyd}), 7.99 (s, 1H, CH_{Hyd}), 7.58 (t, *J* = 8.1 Hz, 2H, H_{arom}), 7.29-7.17 (m, 2H, H_{arom}), 7.05 (d, *J* = 7.4 Hz, 2H, H_{arom}), 6.93 (d, *J* = 7.4 Hz, 2H, H_{arom}), 4.12 (s, 3H, OCH_3), 3.72 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 153.3, 148.61, 142.73, 138.29, 136.81, 127.53, 122.53, 121.86, 118.71, 114.85, 113.45, 109.87, 55.29, 32.09.

(E)-2-((2-(3,4-dimethylphenyl)hydrazineylidene)methyl)-1-methyl-1H-benzo[d]imidazole (**8**). Yield: 87%. (brown powder). Mp=232°C. IR: ν_{max} 1653 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 10.78 (s, 1H, NH_{Hyd}), 8.00 (s, 1H, CH_{Hyd}), 7.58 (t, *J* = 8.8 Hz, 2H, H_{arom}), 7.30-7.18 (m, 2H, H_{arom}), 7.03 (t, *J* = 8.0 Hz, 1H, H_{arom}), 6.89-6.83 (m, 2H, H_{arom}), 4.12 (s, 3H, NCH_3), 2.21 (s, 3H, CH_3), 2.15 (s, 3H, CH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 148.50, 142.69, 142.34, 136.97, 136.78, 130.28, 127.88, 127.46, 122.55, 121.85, 118.72, 113.71, 109.88, 109.83, 32.04, 19.86, 18.65.

(E)-2-((2-(4-chlorophenyl)hydrazineylidene)methyl)-1-methyl-1H-benzo[d]imidazole (**9**). Yield: 86%. (yellow powder). Mp=240°C. IR: ν_{max} 1642 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 11.04 (s, 1H, NH_{Hyd}), 8.05 (s, 1H, CH_{Hyd}), 7.64-7.61 (m, 2H, H_{arom}), 7.35-7.09 (m, 6H, H_{arom}), 4.12 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 148.05, 143.30, 142.63, 136.78, 129.73, 129.20, 123.17, 122.83, 121.98, 118.91, 113.85, 110.04, 32.11.

(E)-2-((2-(4-(benzyloxy)phényl)hydrazineylidène)méthyl)-1-méthyl-1H-benzo[d]imidazole (**10**). Yield: 94%. (yellow powder). Mp=160°C. IR: ν_{max} 1658 (C=N) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-*d*6): δ 11.67 (s, 1H, NH_{Hyd}), 7.05 (s, 1H, CH_{Hyd}), 6.85-6.82 (m, 1H, H_{arom}), 6.71-6.67 (m, 1H, H_{arom}), 6.71-6.27 (m, 9H, H_{arom}), 5.98 (d, *J* = 8.6 Hz, 2H, H_{arom}), 4.01 (s, 2H, CH_2), 2.99 (s, 3H, NCH_3). ^{13}C NMR (62.5 MHz, DMSO-*d*6): δ 154.21, 137.20, 136.80, 133.09, 130.62, 128.47, 127.87, 127.78, 125.94, 125.03, 115.75, 115.64, 115.24, 113.32, 112.17, 69.55, 31.81.

2.3. *In vitro* antioxidant evaluation

2.3.1. DPPH free radical scavenging assay

The free radical-scavenging activity was determined spectrophotometrically by the DPPH assay[48]. In its radical form, $DPPH^{\bullet}$ absorbs at 517nm, but upon reduction by an antioxidant or a radical species its absorbance decreases. Briefly, a 0.1 mM solution of $DPPH^{\bullet}$ in ethanol

was prepared and 4 mL of this solution was added to 1 mL of sample solutions in ethanol at different concentrations (3.12, 6.25, 12.5, 25, 50, 100 and, 200 µM). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. BHT and BHA, under the same conditions as the samples and for each concentration, were used as antioxidant standards. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of the reference and sample obtained from the UV–visible spectrophotometer, respectively. The results were given as IC_{50} (µM) corresponding to the concentration of 50% of inhibition.

2.3.2. ABTS radical scavenging assay

The $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re et *al*[49], 10 µL aliquot of each tested sample at different concentrations (3.12, 6.25, 12.5, 25, 50, 100, and 200 µM) were added to 1.0 mL of diluted $ABTS^{\bullet+}$ solution. The $ABTS^{\bullet+}$ was generated by the reaction between 7mM ABTS in water and 2.45mM potassium persulfate, stored in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.703 ± 0.025 at 734 nm with ethanol which was used as a control. After 10 min, the absorbance was measured at 734 nm. BHT and BHA, under the same conditions as the samples and for each concentration, were used as antioxidant standards. The ABTS radical scavenging activity was calculated using the following equation:

$$\text{ABTS scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of the reference and sample obtained from the UV–visible spectrophotometer, respectively. The results were given as IC_{50} (µM) corresponding to the concentration of 50% of inhibition.

2.3.3. Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric reducing capacity of the compounds was determined by the CUPRAC method[50]. One millilitres of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 1 mL of ammonium acetate buffer at pH 7.0 and 1 mL of neocaproin solution (0.0075 M) were mixed to 0.5 mL of samples or standard of different concentrations solution (3.12, 6.25, 12.5, 25, 50, 100 and, 200 µM). The final volume of the mixture was adjusted to 4.1 mL by adding 0.6 mL

of distilled water. The resulting mixture was incubated for 1 h at room temperature, and then the absorbance of the solution was measured at 450 nm by the use of a spectrophotometer against blank and BHT and BHA as standards. The results were given as $A_{0.5}$ (µM) corresponding the concentration indicating 0.50 absorbance intensity.

2.3.4. Galvinoxyl free radicals (GOR) scavenging assay

The GOR scavenging activity was determined according to the method of Shi et al.[51]. 160 µL of 0.1 mM ethanolic solution of Galvinoxyl was added to 40 µL of different concentrations (3.12, 6.25, 12.5, 25, 50, 100, and 200 µM) of compounds **1-10** in ethanol. The absorbance was read at 428 nm after 120 min incubation in dark at room temperature. Galvinoxyl solution in ethanol was used as a control. BHT and BHA were used as antioxidant standards. The results were given as IC_{50} (µM).

2.4 *In vitro* antifungal evaluation

The antifungal activity of the synthesized hydrazones **1-10**, on the mycelium growth of the phytopathogenic agent (*fusarium oxysporum*), is determined by measuring the radial growth of the fungi on PDA medium (potato dextrose agar) containing the molecule to be tested. A volume of 1 mL of DMSO containing a different mass for each product for a concentration of 200µM was added to 75 mL of PDA medium at 60°C. Previously sterilized and then distributed in 3 petri dishes. Similarly, 1 mL of DMSO was added to 75 mL of PDA medium, and was considered as a positive control. The negative control contains the PDA medium without any other products.

Experimentally, a disk of 5 mm in diameter is taken from a young fungal culture and is deposited aseptically in the center of the petri dish containing the PDA medium and the molecule to be tested. The experiment is replicated 3 times for each treatment. After 6 days of incubation at 28°C, the mycelial growth of the phytopathogenic agents is measured at millimetric scale. Results were expressed as the percentage of growth inhibition of each compound with respect to the mean colony diameters of each fungus grown in control medium. The inhibition activity was expressed as a percentage and was calculated to the formula:

$$I = \frac{C - T}{C} \times 100$$

where I = inhibition rate in %; C = radial growth of phytopathogenic agent in mm on PDA medium with DMSO (control); T = the radial growth, in mm, of the phytopathogenic agent on PDA medium containing the molecule to be tested.

To identify the lowest inhibitory concentration, the test was repeated with concentrations of 800µM, 400µM, 200µM, 100µM, and 50µM. The same result was obtained which means that the efficiency threshold concentration can be lower.

2.5. Inhibition of acetylcholinesterase

AChE inhibitory activity was measured using quantitative colorimetric assay using a 96-well microplate reader according to the method described by Rhee et al[52] based on Ellman's method[53]. The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent: 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) to produce 2-nitrobenzoic-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. In this method, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of test solution at different concentrations (3.12, 6.25, 12.5, 25, 50, 100, and 200 µM) and 20 µL of AChE from *Electrophorus electricus* (5.32×10^{-3} units) solutions were mixed and incubated for 15 min at 25°C, and 10 µL of 0.5 mM (DTNB) were added. The reaction was then initiated by the addition of 10µL of acetylthiocholine iodide (0.71 mM). The hydrolysis of this substrates was monitored spectrophotometrically at a wavelength of 412 nm, every 5 min for 15 min in triplicate experiments. The results were given as IC_{50}(µM) and the percentage of inhibition was determined by the comparison of reaction rates of samples relative to the blank sample (methanol in phosphate buffers, pH 8) using the formula:

$$\text{Percentage of inhibition (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of the reference and sample obtained from the UV–visible spectrophotometer, respectively.

2.6. Docking study

In order to investigate the possible binding modes of compound **5** and **10** (the most active compounds) to the enzymes FGB1 (Guanine nucleotide-binding protein beta) and hAChE (human acetylcholinesterase), respectively, molecular docking studies were carried out with "Achilles" Blind Docking Server (http://bio-hpc.eu). Using a "blind docking" approach, the docking of the small molecule to the targets is done without a priori knowledge of the location of the binding site by the system[54]. Figures were drawn using the BIOVIA Discovery

Studio (https://3dsbiovia.com/). The ligand structures have been built and energy minimized using the program Gaussian09 [M06-2X/6-311++G(d,p)]. Due to the absence of X-ray crystal structure of FGB1 in Protein Data Bank, homology model was carried out to determine the 3D coordination of FGB1. Using SWISS-MODEL tools (https://swissmodel.expasy.org), Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (PDB ID: 3SN6)[55] was selected as the best template, which shown an identity of 67.46%. The modeled protein structure was checked and validated using the Ramachandran plot[56]. The coordinates of human AChE (PDB ID: 4EY6)[57], were obtained from the Protein Data Bank (PDB).

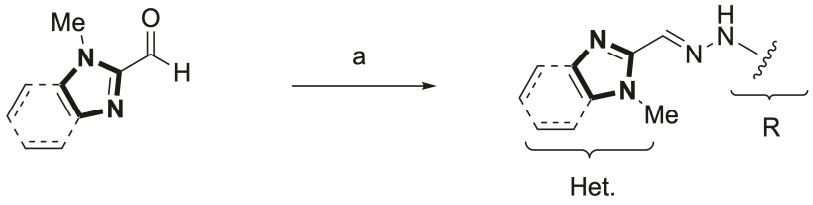
2.7. Computational details

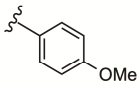
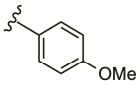
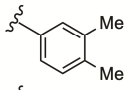
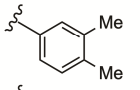
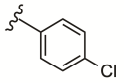
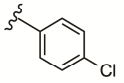
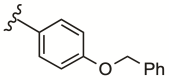
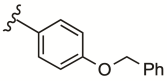
Density functional theory (DFT) calculations have been carried out using Gaussian 09 software [58]. The M06-2X functional[59] and the 6-311++G(d,p) basis set have been used for all calculations. The reliability of DFT/M06-2X method for calculations of reaction energies involving free radicals has been confirmed by previous studies [60, 61]. Solvent effects of ethanol was approximated by the Truhlar's SMD solvation model[62]. All the ground states were confirmed by vibrational frequency analysis (no imaginary frequency). Thermodynamic descriptors of the antioxidant mechanism (BDE, IP, PDE, PA, and ETE) have been calculated as described in our previous studies[44, 63-68].

3. Results and discussion

3.1. Synthesis

The (benz)imidazole-hydrazone derivatives **1-10** were synthesized as outlined in Scheme 1. The starting materials 1-methylimidazole-2-carbaldehyde and 1-methybenzimidazole-2-carbaldehyde were prepared as described in our previous studies[41, 43]. The reaction of an equimolar quantity of 1-methyl(benz)imidazole-2-carbaldehyde and appropriate phenylhydrazine derivative in ethanol at room temperature (4h), gave the corresponding imidazole or benzimidazole phenylhydrazone derivatives (**1-10**) in good to excellent yields (76-97%). Structures of all prepared compounds were confirmed by IR, ^{1}H NMR and ^{13}C NMR analysis and their data are reported in the experimental section. Among the synthesized compounds, the molecular structure of compound **6** was confirmed by single crystal X-ray diffraction analyses (Fig. 2). X-ray data has been deposited at the Cambridge crystallographic data center with the CCDC number 1984681. The obtained structure confirms the (*E*)-configuration of the hydrazone function.



Compound	Het.	R	Yield(%)	Compound	Het.	R	Yield(%)
1	Imidazole		97	**6**	Benzimid.		92
2	Imidazole		76	**7**	Benzimid.		80
3	Imidazole		88	**8**	Benzimid.		87
4	Imidazole		79	**9**	Benzimid.		86
5	Imidazole		95	**10**	Benzimid.		94

Scheme 1: Synthesis of (benz)imidazole-hydrazone derivatives **1-10**. *Reagents and conditions:* (a) EtOH, room temp., 4h.

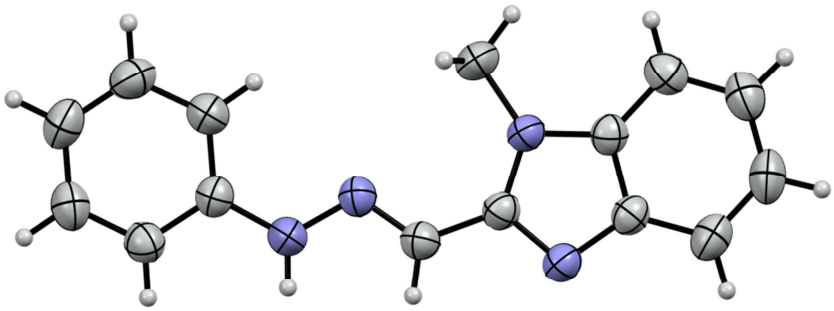


Fig. 2. ORTEP plot of the X-ray crystal structure of compound **6**. Displacement ellipsoids are drawn at the 40 % probability level.

3.2. Evaluation of biological and antioxidant properties

3.2.1. Evaluation of antioxidant activity

In vitro evaluation. The antioxidant activity of the synthesized compounds (**1-10**) was determined using DPPH, CUPRAC, ABTS and GOR assays. The IC_{50} and $A_{0.50}$ values were determined for all compounds and presented in Table 1.

Table 1. Determination of antioxidant activity of compounds **1-10** by DPPH, ABTS, CUPRAC, and GOR assays.

Compound	DPPH assay IC_{50} µM*	ABTS assay IC_{50} µM*	CUPRAC assay $A_{0.50}$ µM*	GOR assay IC_{50} µM*
1	41.7±2.2	14.8±0.3	31.5±2.3	30.9±0.9
2	51.8±2.6	14.8±1.0	32.8±1.6	30.6±0.4
3	22.4±1.2	14.4±0.6	31.8±1.7	54.0±1.0
4	59.9±1.2	7.3±0.8	17.8±0.4	31.4±0.5
5	47.8±2.1	22.4±0.2	49.5±0.4	29.9±0.6
6	82.4±3.4	36.0±1.6	20.1±0.8	33.8±0.7
7	55.2±0.9	3.2±0.3	24.1±0.9	27.0±0.2
8	60.4±1.9	<3.12	20.7±0.6	30.7±0.4
9	55.8±4.0	<3.12	27.7±1.9	25.2±0.5
10	40.4±0.9	3.6±0.1	41.9±1.1	33.0±0.5
BHT	70.8±6.6	7.2±1.7	53.4±4.8	29.3±0.2
BHA	26.0±1.9	8.2±0.4	16.5±0.9	48.5 ±0.1

* Values expressed are means±S.D. of three parallel measurements. (p<0.05).

In DPPH assay, all tested compounds have good antioxidant activity, compound **3** shows the lowest IC_{50} value among the synthesized hydrazones (22.4±3.2 µM) with higher antioxidant activity than that of the standard BHT (70.8±6.6 µM) and comparable to that of the standard BHA (26.0±1.7 µM). Except compound **6** (IC_{50}= 82.4±3.4 µM), all the prepared hydrazones exhibit better antioxidant activity (IC_{50} between 22.4±3.2 and 60.4±1.9 µM) than that of the standard BHT (70.8±6.6 µM). The effect of the heterocycle ring (imidazole or benzimidazole) on the antioxidant activity can be analyzed by comparing the activity of compounds **1-5** and compounds **6-10**. In general, the imidazole and benzimidazole derivatives

have comparable antioxidant activity, with the exception of compounds **1** and **6** (IC_{50}: 41.7±2.2 *vs* 82.4±3.4 μM) and compounds **3** and **8** (IC_{50}: 22.4±3.2 *vs* 60.4±1.9 μM).

In ABTS assay, all tested compounds exhibit also a high-antioxidant activity, the benzimidazole derivatives **7-10** are the best antioxidant agents ($IC_{50} \leq$ 3.6±0.1 μM) followed by compound **4** (IC_{50}= 7.3±0.8 μM). These compounds have higher or comparable antioxidant activity than that of the standards BHT and BHA (IC_{50}: 7.2±1.7 μM and 8.2±0.4 μM, respectively).

In CUPRAC assay, all the prepared hydrazones exhibit a better or comparable antioxidant activity ($A_{0.5}$ between 17.8±0.4 and 49.5±0.4 μM) than that of the standard BHT (53.4±4.8 μM). The best result was obtained with compound **4**, which shows an $A_{0.5}$ (17.8±0.4 μM) comparable to that of the standard BHA (16.5±0.9 μM), and approximately three times less than that of the standard BHT (53.4±4.8 μM).

Finally, In GOR assay, all compounds show a high antioxidant activity with IC_{50} values in the range of 25.2±0.5–54.0±1.0 μM. The best result was obtained with compound **9**, which shows an IC_{50} value (25.2±0.5 μM) two times less than that of the standard BHA (48.4 ±0.1 μM) and comparable to that of the standard BHT (29.3±0.2 μM). Except compounds **3**, all the synthesized hydrazones exhibit higher antioxidant activity (IC_{50} between 25.2±0.5 μM and 33.0±0.5 μM) than that of the standards BHA (48.4 ±0.1 μM) and comparable to that of the standard BHT (29.3±0.2 μM).

In summary, from the results of the antioxidant evaluation by DPPH, ABTS, CUPRAC and GOR assays the following observations can be derived:

- The studied assays for measuring antioxidant activity are not correlated with each other. This result can be explained by the different mechanisms of action of the assays.
- With the exception of the ABTS assay, the other assays have a small dynamic range in the data. This may also be an explanation for the non-correlation of the results.
- All the prepared hydrazone derivatives exhibit high antioxidant activity towards DPPH, ABTS, GOR and CUPRAC assays.
- In DPPH assay, compound **3** is the best antioxidant with higher antioxidant activity than that of the standards BHA and BHT (IC_{50} equal to that of the BHA and four times less than that of the BHT).
- In ABTS assay, compounds **7-10** are the best antioxidants with higher antioxidant activity than the standards BHA and BHT.

- In CUPRAC assay, the best results were obtained with compound **4** which shows higher antioxidant activity than that of the standards BHA and BHT ($A_{0.50}$ equal to that of the BHA and three times less than that of the BHT)
- In GOR assay, compound **7** is the best antioxidant with comparable or higher antioxidant activity than that of the standards BHA and BHT (IC_{50} equal to that of the BHT and two times less than that of the BHA).

DFT calculations. As described in our previous studies[44, 63-68], antioxidants scavenge free radicals through three main mechanisms, namely hydrogen atom transfer (HAT), sequential electron transfer proton transfer (SETPT), and sequential proton loss electron transfer (SPLET)[69-72]. These mechanisms are characterized by several thermodynamic descriptors such as BDE (bond dissociation enthalpy), IP (ionization potential), PDE (proton dissociation enthalpy), PA (proton affinity) and ETE (electron transfer enthalpy). HAT is characterized by BDE value, SPLET is characterized by IP and PDE values, and finally SETPT is characterized by PA and ETE values. The lower the values of the thermodynamic descriptors, the higher the antioxidant activity.

In order to have a better understanding of the antioxidant properties of the synthesized hydrazones, and in which mechanism they follow to scavenge free radicals, all the mentioned thermodynamic descriptors (BDE, IP, PDE, PA, and ETE) have been computed for compounds **1** and **6**, as representative compounds, using DFT method at M06-2X/6-311++G(d,p) level of theory. Since the experimental study has been performed in solution, the implicitly of ethanol has been also considered. The obtained results are presented in Fig. 3 and tabulated in Table S1 (supporting information). Analyzing of the obtained results show that both compounds **1** and **6** have comparable values of the thermodynamic descriptors. For example, the difference in BDE values is only about 1 kcal/mol. This indicates that compounds **1** and **6** have comparable radical scavenging activity. By comparing BDE, IP and PA values, it is clearly observed that PA is significantly lower than the other thermodynamic descriptors. For example, PA of compound **1** is about 30 kcal/mol lower than BDE and 45 kcal/mol lower than IP. Hence, it can be concluded that the SETPT mechanism is more favorable than the other mechanisms in ethanol. These results agree well with previous studies on phenolic compounds[70, 73, 74]

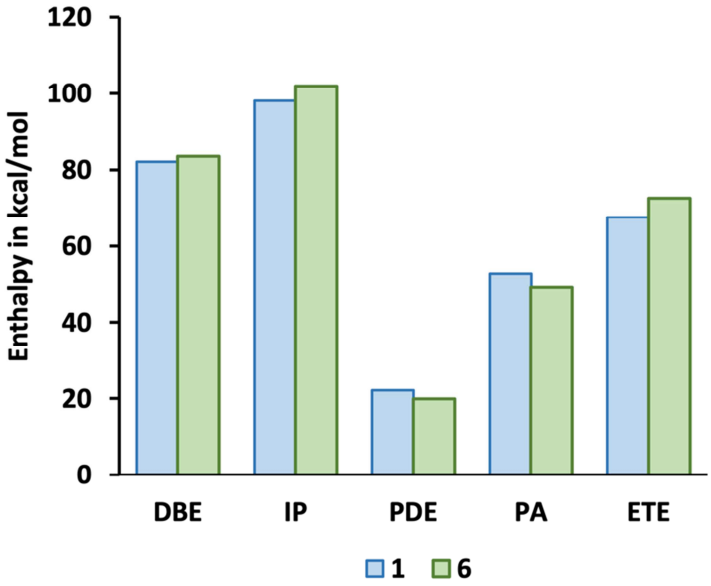


Fig. 3. Thermodynamic descriptors of the antioxidant mechanisms for compounds **1** and **6** calculated at M06-2X/6-311++G(d,p) level of theory in EtOH.

3.2.2. Evaluation of antifungal activity

In vitro evaluation. The antifungal activity of the synthesized hydrazones **1-10** was evaluated *in vitro* against *Fusarium oxysporum* fungal strain at a concentration of 200 µM using the benzimidazole derivative "carbendazim (car.)" as positive control. The genus *Fusarium* is a taxonomic group of filamentous, cosmopolitan fungi that can be both beneficial and harmful to humans, animals, and plants[75]. *Fusarium oxysporum* is the most important phytopathogen, which has been shown to infect a variety of food and ornamental plants[76, 77]. The obtained results of the evaluation of the antifungal activity are shown in Fig. 4.

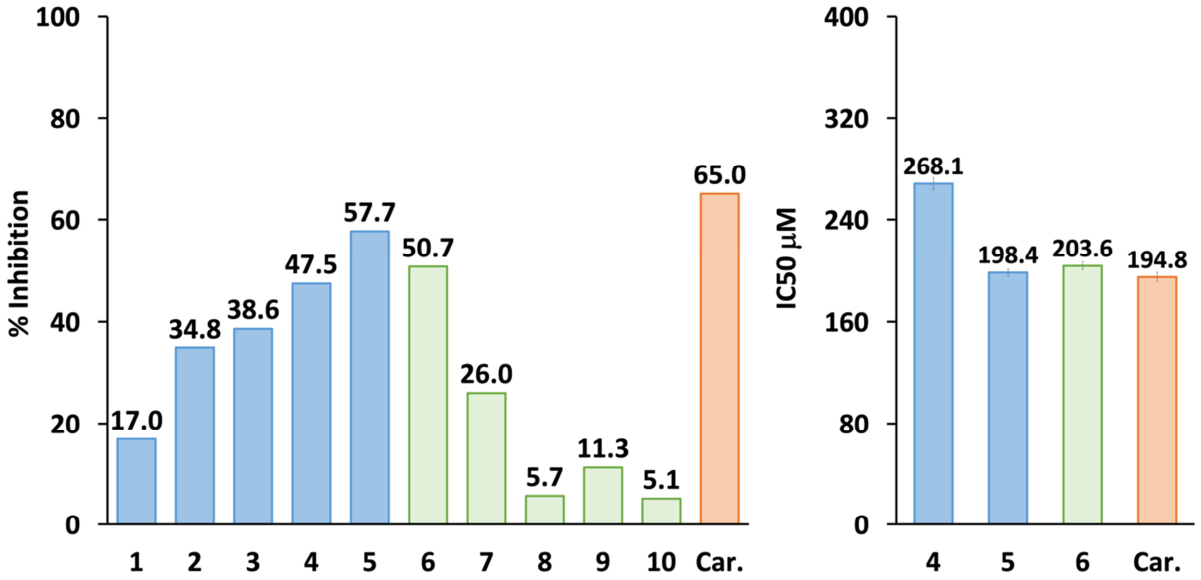


Fig. 4. Antifungal activity of compounds **1-10** against *Fusarium oxysporum* fungal strain. Carbendazim (Car.) was used as a positive control.

Examination of the obtained results reveals that the target compounds have different levels of antifungal activity against the fungi tested at 200 µM. The imidazole derivatives **1-5** (17.0-57.7%) are clearly more reactive than their benzimidazole analogues **6-10** (5.1-50.7%). The best results were obtained with imidazole derivatives **4** (R=Cl) and **5** (R=OCH_2Ph), and benzimidazole **6** (R=OMe), which displayed inhibitory rates of 47.5%, 57.7% and 50.7%, respectively. Compared to that of the commercial fungicide carbendazim, these compounds have good antifungal activity. Compounds **2**, **3**, and **7** were shown a moderate antifungal activity with inhibitory rates ranging from 26.0% to 38.6%. While, the other compounds (**1** and **8-10**) were displayed inhibitory rates ranging from 5.1% to 17.0%, reflecting a relatively low antifungal activity. Based on these results, we calculated the IC_{50} values for the most active compounds **4-6** and standard carbendazim using concentrations of 100 to 800 µM (Fig. 4). As can be seen from Fig. 4, the three studied compounds (**4-6**) were shown a good antifungal activity when compared to that of the commercial fungicide carbendazim. Compound **5** (198.4±1.7 µM) and, relatively, compound **6** (203.6±0.9 µM) have an IC_{50} value comparable to that of carbendazim (194.8±1.6 µM). These results indicate that compounds **4-6** are promising antifungal agents against *Fusarium oxysporum* fungal strain.

Molecular docking study. In order to rationalize the promising *in vitro* results obtained for the most active compound (**5**), molecular docking study was carried out with Guanine

nucleotide-binding protein beta (FGB1) as the target receptor[78]. FGB1 is one of the most important membrane proteins of *F. oxysporum* fungal and it is implicated in various biological processes, including gene expression, cellular function and metabolism such as cAMP level, heat resistance, colony morphology and conidia formation[79-81]. These biological processes make FGB1 a potential target to develop potent anti-*F. oxysporum* agents.

The molecular docking study of the interaction of the most active compound (**5**) as ligand and FGB1 as receptor was performed using Blind Docking Server. Due to the absence of X-ray crystal structure of FGB1 in Protein Data Bank, homology model was carried out to determine the 3D coordination of FGB1 (see Experimental section for details). To validate the approach used, we have also performed a molecular docking study for the standard carbendazim, and the obtained results for the most energetically favorable binding mode of this compound are tabulated in Table S2 in SI. Fig. 5 shows the most energetically favorable binding mode of compound **5** at FGB1 enzyme, and Table S3 in SI summarizes all the molecular docking binding interactions. The obtained results reveal that both compound **5** and carbendazim could favorably interacts with the enzyme FGB1 as can be concluded from their low binding energies of −8.30 kcal/mol and −6.80 kcal/mol, respectively. In this pose, compound **5** forms two hydrogen bonds between the NH of the hydrazone and the carbonyl groups of residues Gln214 and Ile169. It also forms three hydrophobic interactions with Leu209, Arg167, and Pro123 by means of the phenyl moieties. These results indicate that compound **5** could interact favorably with the FGB1 enzyme and forms stable complex, which could be considered as an explication of its antifungal activity.

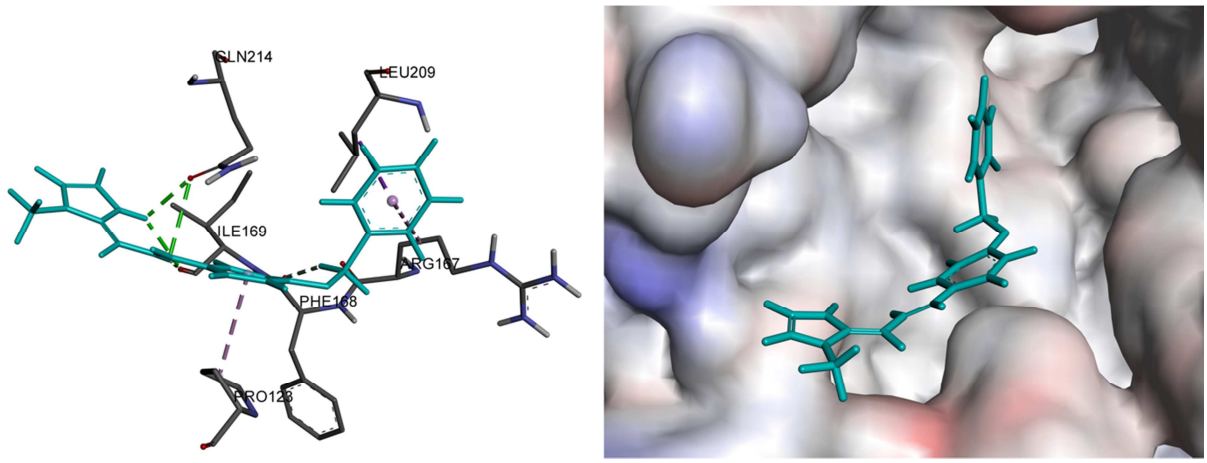


Fig. 5. Binding mode of compound **5** at the active site of FGB1 (template PDB ID: 3SN6).

3.2.3. Evaluation of acetylcholinesterase inhibitory activity

In vitro evaluation. Acetylcholinesterase (AChE) is an enzyme that catalyzes the hydrolysis reaction of acetylcholine to choline and acetic acid. This reaction is necessary to allow the cholinergic receptors to return to their resting state after activation. AChE is recognized as a primary target for symptomatic improvement of Alzheimer's disease[82]. The capacity of the synthesized hydrazones **1-10** to inhibit the enzyme AChE was evaluated using Ellman's assay[53]. Galantamine, used for mild Alzheimer' disease, was used as positive control. The results are expressed as IC_{50} values and are tabulated in Table 2. As can be seen, all the investigated imidazole-hydrazones **1-5** are weak AChE inhibitors with IC_{50} values > 200 µM. On the other hand, their benzimidazole analogues **6-10** are moderate to good inhibitors with IC_{50} values ranging from 11.8±0.1 µM to 61.8±1.7 µM. Compounds **9** and **10**, bearing respectively 4-Cl and 4-OCH_2Ph groups, are the best AChE inhibitors with IC_{50} values (11.8±0.1 µM for **9**, and 13.1±1.2 µM for **10**) comparable to that of the Galantamine (8.9±1.2 µM). Compound **7**, bearing 4-OMe group, is the least active AChE inhibitor with an IC_{50} value of 61.8±1.7 µM. Accordingly, hydrozones **6-10** could be considered as promising acetylcholinesterase inhibitors.

Table 2. IC_{50} values for the inhibition of AChE for compounds **1-10**. Values expressed are means±S.D. of three parallel measurements. (p<0.05).

Compound	AChE IC_{50} µM
1	>200
2	>200
3	>200
4	>200
5	>200
6	48.3 ± 1.5
7	61.8 ± 1.7
8	22.5 ± 0.9
9	13.1 ± 1.2
10	11.8 ± 0.1
Galantamine	8.9 ± 0.1

Molecular docking study. A docking study was performed in order to investigate the interaction modes of the most active compound (**10**) with human acetylcholinesterase (hAChE, PDB code: 4EY6). The most energetically favorable binding mode of compound **10** at the active site of hAChE is shown in Fig. 6, and all the molecular docking binding interactions are summarized in Table S4 in SI. As results, the most favorable binding mode of compound **10** is characterized by a binding energy of –9.00 kcal/mol. In this pose compound **10** is located in the PAS and no interactions with the catalytic active site (CAS) were found. As can be observed in Fig. 6, the hydrazone group of compound **10** forms two hydrogen bonds with residue Ser293. The benzimidazole nucleus and the phenyl moieties forms several π-π stacking with residues Gln291, Trp286, Tyr341 and Gnt604. Two hydrophobic interactions are also found with residues Leu289 and Trp286. Some of these residues are reportedly involved in ligand-receptor complexes of Tacrine, Galantamaine, Huperzine A, and Donepezil[57].

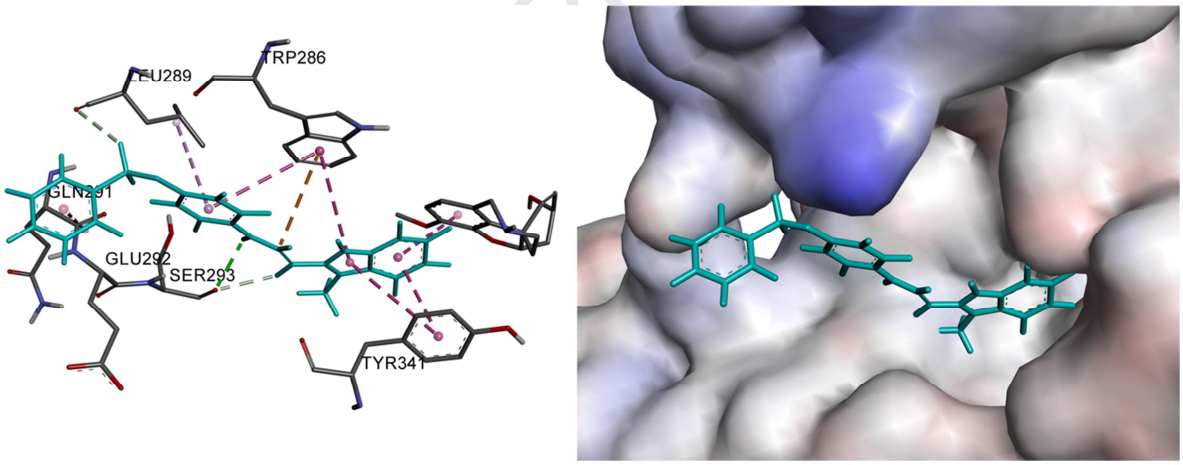


Fig. 6. Binding mode of compound **10** at the active site of hAChE (PDB ID: 4EY6).

3.4. ADME analysis

In drug development, it is well known that absorption, distribution, metabolism and excretion (ADME) properties of a molecule are one of the main reasons of its failure in clinical trials. In order to evaluate the ADME properties of the synthesized hydrazones **1**-**10**, their pharmacokinetic parameters were calculated by using Molinspiration online property calculation toolkit (available at: http://www.molinspiration.com.). All the obtained parameters are presented in Table 3. According to Lipinski's rule[83], in general, an orally active drug has no more than one violation of the following criteria: (i) No more than 5 hydrogen bond

donors (n-OHNH). (ii) No more than 10 hydrogen bond acceptors (n-ON). (iii) A molecular weight (MW) less than 500 D and (iv) An octanol-water partition coefficient (milogP) not greater than 5. As observed in Table 3, except compounds **8-10** which show one violation, all the hydrazones did not shown any violation of Lipinski's rule. In addition, the percentage of absorption of the compounds has been calculated and interesting values have been obtained for all the compounds (90.79-94.05%)[84]. These results suggested that the synthesized hydrazones have a good ADME parameters and can be considered as drug candidates.

The BBB (blood brain barrier) permeability is another important parameter, which affects the biological activity results. Drugs that specifically target the central nervous system, such as cholinesterase inhibitors, must cross the blood brain barrier. The BBB permeability of the synthesized Hydrazones was evaluated by using the CBLigand-BBB prediction server (available at: http://www.cbligand.org) and the obtained results are presented in Table 3. Accordingly, all the compounds were found to be BBB-positive, which is required for the acetylcholinesterase activity.

Table 3. *In silico* some physicochemical and pharmacokinetic parameters of the synthesized (benz)imidazole-hydrazone derivatives **1-10**

Comp.	miLogP	TPSA (^{o}A)	MW	nON	nOHNH	n-rotb	MV	%ABS	vio.	BBB
	<5		<500	<10	<5				<1	
1	2.92	42.22	200.25	4	1	3	188.89	94.05	0	+
2	2.98	51.45	230.27	5	1	4	214.43	90.79	0	+
3	3.75	42.22	228.30	4	1	3	222.01	94.05	0	+
4	3.60	42.22	234.69	4	1	3	202.42	94.05	0	+
5	4.58	51.45	306.37	5	1	6	286.08	90.79	0	+
6	4.43	42.22	250.31	4	1	3	232.88	94.05	0	+
7	4.49	51.45	280.33	5	1	4	258.42	90.79	0	+
8	5.25	42.22	278.36	4	1	3	266.00	94.05	1	+
9	5.11	42.22	284.75	4	1	3	246.41	94.05	1	+
10	6.08	51.45	356.43	4	1	6	330.07	90.79	1	+

Percentage of absorption (%ABS); Topological polar surface area (TPSA); Number of rotatable bonds (n-rotb); Molecular weight (MW); Molecular volume (MV); Logarithm of partition coefficient between n-octanol and water (miLogP); Number of hydrogen bond donors (n-OHNH); Number of hydrogen bond acceptors (n-ON); Lipinski's violation (vio);

4. Conclusion

In this paper, ten hydrazone derivatives bearing an imidazole or benzimidazole nucleus have been designed, synthesized and evaluated for their antioxidant, antifungal, and anti-

acetylcholinesterase activities. It was found that all the synthesized hydrazones are potent antioxidants. The study of the antioxidant mechanisms of representative molecules suggests that SPLET is the most favorable mechanism in ethanol. Among the investigated hydrazones, compounds **5** and **10** were shown good to excellent antifungal and anti-acetylcholinesterase activities, respectively. In addition, the docking results reveled that these compounds inhibited AChE and FGB1 enzymes through interactions including H-bonds, π-π stacking, and hydrophobic interaction. Finally, *in silico* ADME studies have demonstrated that these compounds have a good pharmacokinetic profile.

On the basis of our investigations, hydrazones **1-10** with antioxidant activity and some of them antifungal and anti-acetylcholinesterase activities, providing promising starting points for the design and development of new potent biological active compounds.

Acknowledgements

We would like to thank MESRS (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, Algeria) and DGRSDT (Direction Générale de la Recherche Scientifique et du développement Technologique, Algeria) for financial support, as well as the HPC resources of UCI-UFMC (Unité de Calcul Intesif) of the university Fréres Mentouri Constantine 1 for the computational resources used.

References

[1] B. Narasimhan, D. Sharma, P. Kumar, Biological importance of imidazole nucleus in the new millennium, Medicinal Chemistry Research 20(8) (2011) 1119-1140.
[2] L. Zhang, X.-M. Peng, G.L.V. Damu, R.-X. Geng, C.-H. Zhou, Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry, Medicinal Research Reviews 34(2) (2014) 340-437.
[3] L.D. Luca, Naturally Occurring and Synthetic Imidazoles: Their Chemistry and Their Biological Activities, Current Medicinal Chemistry 13(1) (2006) 1-23.
[4] B. Mariana, G. Mercedes, Imidazole and Benzimidazole Derivatives as Chemotherapeutic Agents, Mini-Reviews in Medicinal Chemistry 5(4) (2005) 409-424.
[5] F.A. Bassyouni, T.S. Saleh, M.M. ElHefnawi, S.I.A. El-Moez, W.M. El-Senousy, M.E. Abdel-Rehim, Synthesis, pharmacological activity evaluation and molecular modeling of new polynuclear heterocyclic compounds containing benzimidazole derivatives, Archives of Pharmacal Research 35(12) (2012) 2063-2075.
[6] A.T. Baviskar, C. Madaan, R. Preet, P. Mohapatra, V. Jain, A. Agarwal, S.K. Guchhait, C.N. Kundu, U.C. Banerjee, P.V. Bharatam, N-Fused Imidazoles As Novel Anticancer Agents That Inhibit Catalytic Activity of Topoisomerase IIα and Induce Apoptosis in G1/S Phase, Journal of Medicinal Chemistry 54(14) (2011) 5013-5030.
[7] P. Zhan, X. Liu, J. Zhu, Z. Fang, Z. Li, C. Pannecouque, E.D. Clercq, Synthesis and biological evaluation of imidazole thioacetanilides as novel non-nucleoside HIV-1 reverse transcriptase inhibitors, Bioorganic & Medicinal Chemistry 17(16) (2009) 5775-5781.

[8] J. Pandey, V.K. Tiwari, S.S. Verma, V. Chaturvedi, S. Bhatnagar, S. Sinha, A.N. Gaikwad, R.P. Tripathi, Synthesis and antitubercular screening of imidazole derivatives, European Journal of Medicinal Chemistry 44(8) (2009) 3350-3355.
[9] K. Gobis, H. Foks, K. Suchan, E. Augustynowicz-Kopeć, A. Napiórkowska, K. Bojanowski, Novel 2-(2-phenalkyl)-1H-benzo[d]imidazoles as antitubercular agents. Synthesis, biological evaluation and structure–activity relationship, Bioorganic & Medicinal Chemistry 23(9) (2015) 2112-2120.
[10] M.P. Windisch, S. Jo, H.-Y. Kim, S.-H. Kim, K. Kim, S. Kong, H. Jeong, S. Ahn, Z. No, J.Y. Hwang, Discovery of 2-iminobenzimidazoles as potent hepatitis C virus inhibitors with a novel mechanism of action, European Journal of Medicinal Chemistry 78 (2014) 35-42.
[11] K.C.S. Achar, K.M. Hosamani, H.R. Seetharamareddy, In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives, European Journal of Medicinal Chemistry 45(5) (2010) 2048-2054.
[12] N. Rani, A. Sharma, R. Singh, Imidazoles as Promising Scaffolds for Antibacterial Activity: A Review, Mini Reviews in Medicinal Chemistry 13(12) (2013) 1812-1835.
[13] Y. Zhang, J. Xu, Y. Li, H. Yao, X. Wu, Design, Synthesis and Pharmacological Evaluation of Novel NO-Releasing Benzimidazole Hybrids as Potential Antihypertensive Candidate, Chemical Biology & Drug Design 85(5) (2015) 541-548.
[14] A.S. Alpan, G. Sarıkaya, G. Çoban, S. Parlar, G. Armagan, V. Alptüzün, Mannich-Benzimidazole Derivatives as Antioxidant and Anticholinesterase Inhibitors: Synthesis, Biological Evaluations, and Molecular Docking Study, Archiv der Pharmazie 350(7) (2017) e1600351.
[15] B.F. Abdel-Wahab, G.E.A. Awad, F.A. Badria, Synthesis, antimicrobial, antioxidant, anti-hemolytic and cytotoxic evaluation of new imidazole-based heterocycles, European Journal of Medicinal Chemistry 46(5) (2011) 1505-1511.
[16] G. Ayhan-Kilcigil, C. Kus, T. Çoban, B. Can-Eke, M. Iscan, Synthesis and Antioxidant Properties of Novel Benzimidazole Derivatives, Journal of Enzyme Inhibition and Medicinal Chemistry 19(2) (2004) 129-135.
[17] L. Bielory, K.W. Lien, S. Bigelsen, Efficacy and tolerability of newer antihistamines in the treatment of allergic conjunctivitis, Drugs 65(2) (2005) 215-228.
[18] P.R. Sawyer, R.N. Brogden, K.M. Pinder, T.M. Speight, G.S. Avery, Clotrimazole: a review of its antifungal activity and therapeutic efficacy, Drugs 9(6) (1975) 424-447.
[19] Y.-S. Wang, Y.-J. Huang, W.-C. Chen, J.-H. Yen, Effect of carbendazim and pencycuron on soil bacterial community, Journal of hazardous materials 172(1) (2009) 84-91.
[20] L. Serrone, M. Zeuli, F.M. Sega, F. Cognetti, Dacarbazine-based chemotherapy for metastatic melanoma: thirty-year experience overview, Journal of experimental & clinical cancer research: CR 19(1) (2000) 21-34.
[21] J. Horton, Albendazole: a review of anthelmintic efficacy and safety in humans, Parasitology 121(S1) (2000) S113-S132.
[22] B.M. Brenner, M.E. Cooper, D. De Zeeuw, W.F. Keane, W.E. Mitch, H.-H. Parving, G. Remuzzi, S.M. Snapinn, Z. Zhang, S. Shahinfar, Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy, New England journal of medicine 345(12) (2001) 861-869.
[23] S. Rollas, S.G. Küçükgüzel, Biological activities of hydrazone derivatives, Molecules 12(8) (2007) 1910-1939.
[24] R. Lazny, A. Nodzewska, N,N-Dialkylhydrazones in Organic Synthesis. From Simple N,N-Dimethylhydrazones to Supported Chiral Auxiliaries, Chemical Reviews 110(3) (2010) 1386-1434.
[25] J.-M. Lehn, Constitutional dynamic chemistry: bridge from supramolecular chemistry to adaptive chemistry, Constitutional Dynamic Chemistry, Springer2011, pp. 1-32.

[26] Nguyen, R. Ivan Huc, Optimizing the reversibility of hydrazone formation for dynamic combinatorial chemistry, Chemical Communications (8) (2003) 942-943.
[27] R. Lygaitis, V. Getautis, J.V. Grazulevicius, Hole-transporting hydrazones, Chemical Society Reviews 37(4) (2008) 770-788.
[28] Y. Özkay, Y. Tunalı, H. Karaca, İ. Işıkdağ, Antimicrobial activity and a SAR study of some novel benzimidazole derivatives bearing hydrazone moiety, European Journal of Medicinal Chemistry 45(8) (2010) 3293-3298.
[29] G. Gürkök, T. Coban, S. Suzen, Melatonin analogue new indole hydrazide/hydrazone derivatives with antioxidant behavior: Synthesis and structure–activity relationships, Journal of Enzyme Inhibition and Medicinal Chemistry 24(2) (2009) 506-515.
[30] W.-Y. Liu, H.-Y. Li, B.-X. Zhao, D.-S. Shin, S. Lian, J.-Y. Miao, Synthesis of novel ribavirin hydrazone derivatives and anti-proliferative activity against A549 lung cancer cells, Carbohydrate Research 344(11) (2009) 1270-1275.
[31] Ö.D. Can, D. Osmaniye, Ü. Demir Özkay, B.N. Sağlık, S. Levent, S. Ilgın, M. Baysal, Y. Özkay, Z.A. Kaplancıklı, MAO enzymes inhibitory activity of new benzimidazole derivatives including hydrazone and propargyl side chains, European Journal of Medicinal Chemistry 131 (2017) 92-106.
[32] J.C. Coa, W. Castrillón, W. Cardona, M. Carda, V. Ospina, J.A. Muñoz, I.D. Vélez, S.M. Robledo, Synthesis, leishmanicidal, trypanocidal and cytotoxic activity of quinoline-hydrazone hybrids, European Journal of Medicinal Chemistry 101 (2015) 746-753.
[33] M. Demurtas, A. Baldisserotto, I. Lampronti, D. Moi, G. Balboni, S. Pacifico, S. Vertuani, S. Manfredini, V. Onnis, Indole derivatives as multifunctional drugs: Synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity of indole hydrazones, Bioorganic Chemistry 85 (2019) 568-576.
[34] J.E. Overall, L.E. Hollister, A.D. Pokorny, J.F. Casey, G. Katz, Drug therapy in depressions: Controlled evaluation of imipramine, isocarboxazide, dextroamphetamine amobarbital, and placebo, Clinical Pharmacology & Therapeutics 3(1) (1962) 16-22.
[35] K. Matsuura, Y. Ishida, T. Kuragano, K. Konishi, Development of a new fungicide, ferimzone, JOURNAL OF PESTICIDE SCIENCE-PESTICIDE SCIENCE SOCIETY OF JAPAN-JAPANESE EDITION- 19 (1994) 325-325.
[36] J.R. Wacker, B.K. Wagner, V. Briese, B. Schauf, L. Heilmann, C. Bartz, H. Hopp, Antihypertensive therapy in patients with pre-eclampsia: A prospective randomised multicentre study comparing dihydralazine with urapidil, European Journal of Obstetrics & Gynecology and Reproductive Biology 127(2) (2006) 160-165.
[37] D.P. Kelsen, R. Fein, C. Coonley, R. Heelan, M. Bains, Cisplatin, vindesine, and mitoguazone in the treatment of esophageal cancer, Cancer treatment reports 70(2) (1986) 255-259.
[38] E. Said, S.A. Zaitone, M. Eldosoky, N.M. Elsherbiny, Nifuroxazide, a STAT3 inhibitor, mitigates inflammatory burden and protects against diabetes-induced nephropathy in rats, Chemico-biological interactions 281 (2018) 111-120.
[39] A. Biolo, W.S. Colucci, M.M. Givertz, CHAPTER 38 - Inotropic and Vasoactive Agents in the Cardiac Intensive Care Unit, in: A. Jeremias, D.L. Brown (Eds.), Cardiac Intensive Care (Second Edition), W.B. Saunders, Philadelphia, 2010, pp. 470-478.
[40] H. Boulebd, L. Ismaili, H. Martin, A. Bonet, M. Chioua, J. Marco Contelles, A. Belfaitah, New (benz)imidazolopyridino tacrines as nonhepatotoxic, cholinesterase inhibitors for Alzheimer disease, Future Medicinal Chemistry 9(8) (2017) 723-729.
[41] H. Boulebd, S. Zama, B. Insaf, A. Bouraiou, S. Bouacida, H. Merazig, A. Romero, M. Chioua, J. Marco-Contelles, A. Belfaitah, Synthesis and biological evaluation of heterocyclic privileged medicinal structures containing (benz)imidazole unit, Monatshefte für Chemie - Chemical Monthly 147(12) (2016) 2209-2220.

[42] H. Boulebd, L. Ismaili, M. Bartolini, A. Bouraiou, V. Andrisano, H. Martin, A. Bonet, I. Moraleda, I. Iriepa, M. Chioua, Imidazopyranotacrines as Non-Hepatotoxic, Selective Acetylcholinesterase Inhibitors, and Antioxidant Agents for Alzheimer's Disease Therapy, Molecules 21(4) (2016) 400.
[43] M.I. Chouiter, H. Boulebd, D.M. Pereira, P. Valentão, P.B. Andrade, A. Belfaitah, A.M.S. Silva, New chalcone-type compounds and 2-pyrazoline derivatives: synthesis and caspase-dependent anticancer activity, Future Medicinal Chemistry 12(6) (2020) 493-509.
[44] I. Amine Khodja, H. Boulebd, Synthesis, biological evaluation, theoretical investigations, docking study and ADME parameters of some 1,4-bisphenylhydrazone derivatives as potent antioxidant agents and acetylcholinesterase inhibitors, Molecular Diversity (2020).
[45] M. Zhang, Z.-C. Dai, S.-S. Qian, J.-Y. Liu, Y. Xiao, A.-M. Lu, H.-L. Zhu, J.-X. Wang, Y.-H. Ye, Design, Synthesis, Antifungal, and Antioxidant Activities of (E)-6-((2-Phenylhydrazono)methyl)quinoxaline Derivatives, Journal of Agricultural and Food Chemistry 62(40) (2014) 9637-9643.
[46] R. Narang, B. Narasimhan, S. Sharma, A review on biological activities and chemical synthesis of hydrazide derivatives, Current medicinal chemistry 19(4) (2012) 569-612.
[47] X. Wang, Y.-F. Chen, W. Yan, L.-L. Cao, Y.-H. Ye, Synthesis and biological evaluation of benzimidazole phenylhydrazone derivatives as antifungal agents against phytopathogenic fungi, Molecules 21(11) (2016) 1574.
[48] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature 181(4617) (1958) 1199.
[49] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free radical biology and medicine 26(9-10) (1999) 1231-1237.
[50] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir, Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method, Journal of Agricultural and Food Chemistry 52(26) (2004) 7970-7981.
[51] H. Shi, N. Noguchi, E. Niki, Galvinoxyl method for standardizing electron and proton donation activity, Methods in Enzymology, Academic Press2001, pp. 157-166.
[52] I.K. Rhee, M. van de Meent, K. Ingkaninan, R. Verpoorte, Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining, Journal of Chromatography A 915(1) (2001) 217-223.
[53] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology 7(2) (1961) 88-95.
[54] I. Sánchez-Linares, H. Pérez-Sánchez, J.M. Cecilia, J.M. García, High-throughput parallel blind virtual screening using BINDSURF, BMC bioinformatics 13(14) (2012) S13.
[55] S.G.F. Rasmussen, B.T. DeVree, Y. Zou, A.C. Kruse, K.Y. Chung, T.S. Kobilka, F.S. Thian, P.S. Chae, E. Pardon, D. Calinski, J.M. Mathiesen, S.T.A. Shah, J.A. Lyons, M. Caffrey, S.H. Gellman, J. Steyaert, G. Skiniotis, W.I. Weis, R.K. Sunahara, B.K. Kobilka, Crystal structure of the β2 adrenergic receptor–Gs protein complex, Nature 477(7366) (2011) 549-555.
[56] G.T. Ramachandran, V. Sasisekharan, Conformation of polypeptides and proteins, Advances in protein chemistry, Elsevier1968, pp. 283-437.
[57] J. Cheung, M.J. Rudolph, F. Burshteyn, M.S. Cassidy, E.N. Gary, J. Love, M.C. Franklin, J.J. Height, Structures of Human Acetylcholinesterase in Complex with

Pharmacologically Important Ligands, Journal of Medicinal Chemistry 55(22) (2012) 10282-10286.
[58] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. aramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision E.01, Wallingford, CT, 2009.
[59] Y. Zhao, D.G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals, Theoretical Chemistry Accounts 120(1) (2008) 215-241.
[60] A. Galano, J.R. Alvarez-Idaboy, Kinetics of radical-molecule reactions in aqueous solution: A benchmark study of the performance of density functional methods, Journal of Computational Chemistry 35(28) (2014) 2019-2026.
[61] Y. Zhao, D.G. Truhlar, How Well Can New-Generation Density Functionals Describe the Energetics of Bond-Dissociation Reactions Producing Radicals?, The Journal of Physical Chemistry A 112(6) (2008) 1095-1099.
[62] A.V. Marenich, C.J. Cramer, D.G. Truhlar, Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions, The Journal of Physical Chemistry B 113(18) (2009) 6378-6396.
[63] H. Boulebd, Comparative study of the radical scavenging behavior of ascorbic acid, BHT, BHA and Trolox: Experimental and theoretical study, Journal of Molecular Structure 1201 (2020) 127210.
[64] H. Boulebd, DFT study of the antiradical properties of some aromatic compounds derived from antioxidant essential oils: C–H bond vs. O–H bond, Free Radical Research 53(11-12) (2019) 1125-1134.
[65] H. Boulebd, The role of benzylic-allylic hydrogen atoms on the antiradical activity of prenylated natural chalcones: a thermodynamic and kinetic study, Journal of Biomolecular Structure and Dynamics (2020) 1-10.
[66] H. Boulebd, Theoretical Insights into the Antioxidant Activity of Moracin T, Free Radical Research (2020).
[67] H. Boulebd, A. Mechler, N.T. Hoa, Q.V. Vo, Thermodynamic and Kinetic Studies of the Antiradical Activity of 5□Hydroxymethylfurfural: Computational Insights, New Journal of Chemistry (2020).
[68] H. Boulebd, I. Amine Khodja, M.V. Bay, N.T. Hoa, A. Mechler, Q.V. Vo, Thermodynamic and Kinetic Studies of the Radical Scavenging Behavior of Hydralazine and Dihydralazine: Theoretical Insights, The Journal of Physical Chemistry B (2020).
[69] B.N. Ames, M.K. Shigenaga, T.M. Hagen, Oxidants, antioxidants, and the degenerative diseases of aging, Proceedings of the National Academy of Sciences of the United States of America 90(17) (1993) 7915-7922.

[70] G. Wang, Y. Xue, L. An, Y. Zheng, Y. Dou, L. Zhang, Y. Liu, Theoretical study on the structural and antioxidant properties of some recently synthesised 2,4,5-trimethoxy chalcones, Food Chemistry 171 (2015) 89-97.
[71] L. Estévez, N. Otero, R.A. Mosquera, A Computational Study on the Acidity Dependence of Radical-Scavenging Mechanisms of Anthocyanidins, The Journal of Physical Chemistry B 114(29) (2010) 9706-9712.
[72] Y.-Z. Zheng, G. Deng, R. Guo, Z.-M. Fu, D.-F. Chen, Theoretical insight into the antioxidative activity of isoflavonoid: The effect of the C2=C3 double bond, Phytochemistry 166 (2019) 112075.
[73] Y. Shang, H. Zhou, X. Li, J. Zhou, K. Chen, Theoretical studies on the antioxidant activity of viniferifuran, New Journal of Chemistry 43(39) (2019) 15736-15742.
[74] Y.-Z. Zheng, G. Deng, Q. Liang, D.-F. Chen, R. Guo, R.-C. Lai, Antioxidant Activity of Quercetin and Its Glucosides from Propolis: A Theoretical Study, Scientific Reports 7(1) (2017) 7543.
[75] K.H. Bhuvanendra, S.A.C. Udaya, C. Nayaka, S.R. Kini, H.S. Shetty, H.S. Prakash, Biochemical characterization of Fusarium oxysporum f. sp. cubense isolates from India, African Journal of Biotechnology 9(4) (2010).
[76] S. Steinkellner, R. Mammerler, H. Vierheilig, Microconidia germination of the tomato pathogen Fusarium oxysporum in the presence of root exudates, Journal of plant interactions 1(1) (2005) 23-30.
[77] K.B. Lengeler, R.C. Davidson, C. D'Souza, T. Harashima, W.-C. Shen, P. Wang, X. Pan, M. Waugh, J. Heitman, Signal transduction cascades regulating fungal development and virulence, Microbiol. Mol. Biol. Rev. 64(4) (2000) 746-785.
[78] F. Abrigach, Y. Rokni, A. Takfaoui, M. Khoutoul, H. Doucet, A. Asehraou, R. Touzani, In vitro screening, homology modeling and molecular docking studies of some pyrazole and imidazole derivatives, Biomedicine & Pharmacotherapy 103 (2018) 653-661.
[79] S. Jain, K. Akiyama, T. Kan, T. Ohguchi, R. Takata, The G protein β subunit FGB1 regulates development and pathogenicity in Fusarium oxysporum, Current genetics 43(2) (2003) 79-86.
[80] J. Delgado-Jarana, A.L. Martínez-Rocha, R. Roldán-Rodriguez, M.I.G. Roncero, A. Di Pietro, Fusarium oxysporum G-protein β subunit Fgb1 regulates hyphal growth, development, and virulence through multiple signalling pathways, Fungal Genetics and Biology 42(1) (2005) 61-72.
[81] L. Guo, L. Yang, C. Liang, J. Wang, L. Liu, J. Huang, The G-protein subunits FGA2 and FGB1 play distinct roles in development and pathogenicity in the banana fungal pathogen Fusarium oxysporum f. sp. cubense, Physiological and Molecular Plant Pathology 93 (2016) 29-38.
[82] V.N. Talesa, Acetylcholinesterase in Alzheimer's disease, Mechanisms of ageing and development 122(16) (2001) 1961-1969.
[83] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Advanced drug delivery reviews 23(1-3) (1997) 3-25.
[84] Y.H. Zhao, M.H. Abraham, J. Le, A. Hersey, C.N. Luscombe, G. Beck, B. Sherborne, I. Cooper, Rate-limited steps of human oral absorption and QSAR studies, Pharmaceutical research 19(10) (2002) 1446-1457.

Highlights

- Ten hydrazone derivatives bearing (benz)imidazole nucleus were synthesized.
- Antioxidant, antifungal, and anti-AChE activities have been investigated.
- DFT calculations of the antioxidant mechanisms have been performed.
- *In silico* molecular docking and ADME studies have be carried out.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: