SYNTHESIS OF BENZIMIDAZOLES IN THE PRESENCE OF NANO-$TiCl_4.SiO_2$ AS ANTIFUNGAL AGENTS AND TAUTOMERISM THEORETICAL STUDY OF SOME PRODUCTS

LEILA ZAMANI[1], BI BI FATEMEH MIRJALILI[1], KAMIAR ZOMORODIAN[2]*, MANSOOR NAMAZIAN[1], SOGHRA KHABNADIDEH[3], EHSAN FAGHIH MIRZAEI[3,4]

[1]Department of Chemistry, College of Science, Yazd University, Yazd, P. O. Box 8915813149, Iran.
[2]Center of Basic Researches in Infectious Diseases and Department of Medical Mycology and Parasitology School of Medicine, Shiraz University of Medical Sciences, Post code 71348-45794, Shiraz, Iran
[3]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, I. R. Iran.
[4]Department of Medicinal Chemistry, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
** corresponding author: zomorodian@sums.ac.ir, zomorodian2@yahoo.com*

Abstract

Pathogenic fungi are associated with diseases ranging from simple dermatosis to life-threatening infections, particularly in immunocompromised patients. During the past two decades, resistance to established antifungal drugs has increased dramatically and has become extremely important to identify novel antimicrobial compounds. The purpose of this study was to synthesize some new benzimidazole derivatives and to evaluate their activity against some species of *Candida*, *Aspergillus* and dermatophytes. Benzimidazoles have been synthesized in the presence of nano-$TiCl_4.SiO_2$ as a reusable and efficient catalyst. The stability of different tautomers of some products has been investigated using standard *ab initio* calculations. The antimicrobial activities of the synthetic compounds have been tested against the fungi by Broth microdilution method as recommended by Clinical and Laboratory Standards Institute (CLSI). Inhibition studies showed that some of the tested compounds, in particular 2-(2,3-dihydroxyphenyl)-1*H*-5-Nitro-benzimidazole exhibited strong antifungal activities against all tested fungi at concentrations of less than 32 µg/mL while some of them only inhibited the growth of dermatophytes or *Aspergillus* species. These results suggest that the derivatives should be further investigated for possible use in antimicrobial products.

Rezumat

Ciupercile patogene sunt asociate cu o gamă largă de afecțiuni, de la simple dermatoze la infecții letale, în mod special la pacienții imunocompromiși. În ultimile două decenii, rezistența la medicamentele antifungice cunoscute a crescut în mod dramatic și a devenit extrem de importantă identificarea de noi compuși antimicrobieni. Scopul acestui studiu a fost sinteza unor derivați noi de benzimidazol și evaluarea activității lor asupra

unor specii de *Candida*, *Aspergillus* şi dermatofiţi. Benzimidazolii au fost sintetizaţi în prezenţa catalizatorului nano-$TiCl_4SiO_2$. Stabilitatea diferiţilor tautomeri a unora dintre produşi a fost investigată folosind calcule standard *ab initio*. Activitatea antimicrobiană a compuşilor sintetici a fost testată pe speciile menţionate prin metoda microdiluţiei *Broth* aşa cum este recomandat de Institutul de Standarde Clinice şi de Laborator. Studiile de inhibiţie au arătat că unii dintre compuşii testaţi, în mod special 2-(2,3-dihidroxifenil)-1*H*-5-Nitro-benzimidazol a arătat o activitate antimicotică puternică împotriva tuturor tulpinilor testate, la concentraţii mai mici de 32 µg/mL în timp ce alţi compuşi doar au inhibat creşterea dermatofiţilor şi a speciilor de *Aspergillus*. Aceste rezultate sugerează că derivaţii ar trebui investigaţi în continuare pentru posibila folosire în produse antimicrobiene.

Keywords: Benzimidazoles, Nano-$TiCl_4.SiO_2$, Antifungal.

Introduction

Fungal infections cause a wide range of symptoms from minor skin problems like dermatophytosis to life threatening invasive infections [1-3]. As the consequence of modern lifestyle, some clinical forms of dermatophytoses such as *Tinea pedis* are more common today than ever before [4]. Additionally, in parallel with the development of advanced therapeutic methods such as organ transplantation and chemotherapy as well as growing numbers of immonocompromised patients, the incidence of invasive fungal infections has increased dramatically in recent years [2, 3, 5]. Among these infections, candidiasis and aspergillosis are well known infections associated with increased rate of hospital stay and a considerable mortality rate in the hospitalized patients [3, 5]. On the other hand, the emergence of resistance to current antifungal drugs among fungal pathogens increased in the last two decades [6-9]. These resistant strains cause failure in treatment and enhance mortality risks, and sometimes contribute to complications. Unlike antibacterial antibiotics, the variety of antifungal drugs is restricted due to the similarity of structure and metabolism of eukaryotic fungal cells to those of mammalian cells. Hence, the discovery of antifungal agents that possess selective toxicity against the eukaryotic fungal cell remains an important scientific challenge. Considering the limited diversity of antifungal agents and recent resistance of fungi to the known antifungal drugs, the development of new bioactive compounds effective against resistant strains is highly needed.

Benzimidazoles and their derivatives demonstrate a large range of biological properties, their based-on-drugs are classified depending on the substituent pattern in the benzimidazole nucleus. Addition of small substituent into the 2- or 5-postition of benzimidazoles caused antihelminthic activity. Alternatively, addition of a bulky 2-substituent into the these drugs increased their healing activities against peptic ulcer.

Benzimidazole cores exist in vitamin B_{12}, purine based DNA [10], 2-aryl benzimidazole also shows activities such as anti-HIV [11], anti-viral [12], anti-tumor [13]. The common protocols for synthesis of benzimidazoles are the reaction between an *o*-phenylendiamine and a carboxylic acid, nitrile, amidate, orthoester or aldehyde in the presence of acidic catalyst and then aerobic oxidation [14-19]. Nano-$TiCl_4.SiO_2$ [20, 21] as an efficient and reusable acidic catalyst is synthesized *via* reaction of nano-silica gel with $TiCl_4$ in chloroform at room temperature. Following our investigations on solid acids in organic synthesis [22, 23], we have applied nano-$TiCl_4.SiO_2$ as an efficient catalyst for synthesis of benzimidazole derivatives *via* reaction of aldehydes with o-phenylenediamines which may elicit antifungal activity.

Materials and Methods

The chemicals were used from Merck Company without any additional purification. The products were characterized by Fourier transform – infrared spectrometry attenuated total reflectance (FT-IR ATR), nuclear magnetic resonance (^{1}H-NMR), and it was performed a comparison of their physical properties with those reported in the literature. FT-IR (ATR) spectra were registered on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avanes) NMR was used to record the ^{1}H NMR spectra. The absorption of titanium solutions was determined by an atomic absorption spectrometer.

Molecular Docking

The ligands were drawn in the Hyperchem 8.0. The geometry was optimized through the molecular dynamic method AMBER and semi-empirical method PM3. 14-α-demethylase protein bound with 4-phenyl-1*H*-imidazole (PDB code 1E9X) was obtained from Protein Data Bank and the atomic coordinates were used. All molecules of water were removed, along with other heteroatoms, to leave only hem molecule and the enzyme itself. Autodock 4.2 was used to perform dockings using the Lamarckian genetic algorithm, each starting from random initial positions. The active site of 1E9X was enclosed in a box with a grid spacing of 0.375 A˚. A population size of 250 and 150 cycles of calculation was used for the search, with the maximum of 2.5 million energy evaluations. The results of this dockings were clustered using an all-atom RMSD cutoff 2.0 A˚. The top scoring results of all compounds as well as clotrimazole were taken as indicative of the most appropriate binding mode for the ligand.

General procedure for the synthesis of benzimidazoles derivatives

A mixture of *o*-phenylenediamine (1 mmol), aldehyde (1 mmol) and 50% nano-$TiCl_4.SiO_2$ (0.05 g) was heated at 60 ˚C. The progress of the

reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, hot ethanol was added to the mixture and filtered to separation of in-soluble catalyst. By adding water to the filtrate, benzimidazole derivative was obtained. The compounds can be re-crystallized with hot ethanol to achieve highly pure products. All the products were identified by comparison of their physical and spectral data with those of authentic samples.

Computational details

Standard density functional theory calculations [24, 25] and *ab initio* molecular orbital theory [26, 27] were completed using the Gaussian 03 [28] software. Molecular geometries of all the species were optimized at the B3LYP/6-31G (d) level of theory [24, 25]. The nature of each stationary point was found *via* B3LYP/6- 31G (d) frequency calculations. The optimized geometries have been used for further calculations. MP2/G3MP2 large level of theory has been used for calculation of free energies together with thermochemical data calculated at recommended level of theory of B3LYP/6-31G (d) [26]. The MP2/G3MP2 large level of theory has been selected considering the size of molecules and hardware limitations. Nonetheless, the results of this method are reliable for the calculation of relative energies [29].

Determination of antifungal activities

Microorganisms

The antifungal activities of the synthetic compounds against nine American Type Culture Collection (ATCC) strains of fungi, including *Candida albicans* (ATCC 10261), *C. tropicalis* (ATCC 750), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 4344), *C. dubliniensis* (ATCC), *Cryptococcus neoformans* (ATCC), *Aspergillus flavus* (ATCC) and *A. fumigatus* (ATCC) as well as two clinical isolates of yeasts identified by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) [30, 31] were determined. Moreover, the inhibitory activities of the mentioned compounds against dermatophytes (*Trichophyton mentagrophytes*, *Microsporum gypseum* and *Epidermophyton flocossum*) which were identified by morphological and physiological tests were also examined in this study. The susceptibility of all clinical isolates of fungi against selected antibiotics was examined by microdilution and disk diffusion methods [32, 33]. The reference antifungal compounds fluconazole (Sigma, St. Louis, MO, USA), for yeasts and *Aspergillus* species, and griseofulvin (Sigma), for dermatophytes, were used as standard drugs.

Determination of minimum inhibitory concentration (MIC)

MICs were determined using the Broth microdilution method recommended by the CLSI with some modifications [32, 33]. Briefly, for

determination of antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1–1024 μg/mL) were prepared in 96-well microtiter plates using Rawell Park Memorial Institute (RPMI-1640 medium) (Sigma, St. Louis, MO, USA) buffered with morpholine propane sulfonic acid (MOPS) (Sigma). Stock inoculums were prepared by suspending three colonies of the examined yeast in 5 mL sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standard at 530 nm wavelength (this yields stock suspension of 1-5 × 10^6 cells/mL). For moulds (*Aspergillus* spp. and dermatophytes), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with Tween-20. The collected conidia were transferred in sterile saline and their turbidity was adjusted to OD=0.09-0.11 that yields 0.4-5 × 10^6 conidia/mL. Working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for moulds and yeasts, respectively. Working inoculums (0.1 mL) were added to the microtiter plates, which were incubated in a humid atmosphere at 30ºC for 24–48 h. Uninoculated medium (200 μL) was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without antibiotics or the synthetic compounds) were also included. The growth in each well was compared with that of the growth in the control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥ 95 % growth reduction compared with the growth in the control well. Each experiment was performed in triplicate.

In addition, media from the wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC). MFCs were determined as the lowest concentration yielding no more than 4 colonies, which corresponds to a mortality of 98% of the microbes in the initial inoculums.

Results and Discussion

Chemistry

The reaction of *o*-phenylenediamine (1 mmol) with 4-nitrobenzaldehyde (1 mmol) was investigated for the optimization of the reaction conditions (Figure 1). The best results were obtained using 0.05 g of 50% nano-$TiCl_4.SiO_2$ at 60 ºC under solvent free conditions (Table I, Entry XIV). To examine the reusability of nano-$TiCl_4.SiO_2$, after each run, the product was dissolved in $CHCl_3$ and filtered. The catalyst residue was washed with chloroform and reused. (Table I, Entries XV and XVI). The catalyst was reusable although a gradual decline was observed for its activity.

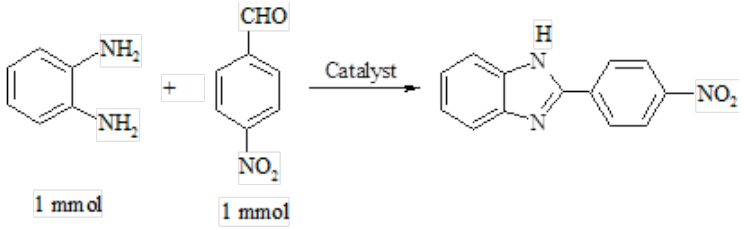


Figure 1

The condensation reaction between 4-nitrobenzaldehyde with *o*-phenylenediamines

Table I

Synthesis of 2-(4-nitrophenyl)-benzimidazole in various conditions[a]

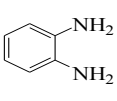
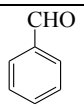
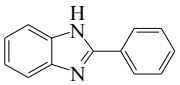
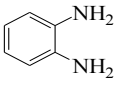
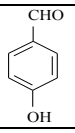
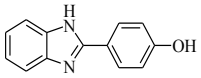
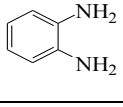
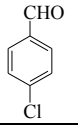
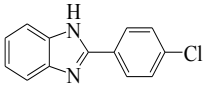
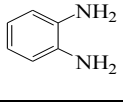
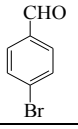
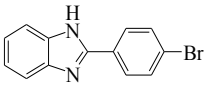
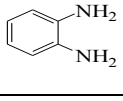
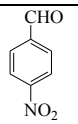
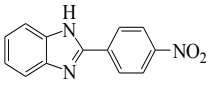
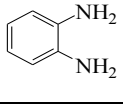
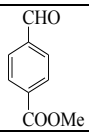
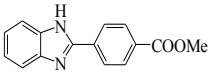
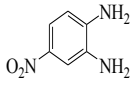
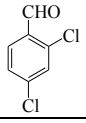
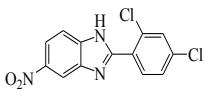
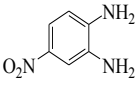
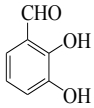
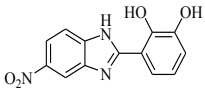
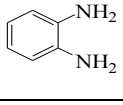
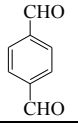
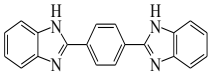
Entry	Catal./mol% (g)	Time (min)	Solvent/ T°C	Yield[a](%)	Ref
I	30%$TiCl_4.SiO_2$ (0.05)	10	Solvent-free/60˚C	70	-
II	50%$TiCl_4.SiO_2$ (0.05)	10	Solvent-free/60˚C	90	-
III	70%$TiCl_4.SiO_2$ (0.05)	10	Solvent-free/60˚C	92	-
IV	50%$TiCl_4.SiO_2$ (0.05)	30	EtOH/ reflux	70	-
V	50%$TiCl_4.SiO_2$ (0.05)	30	EtOAc/ reflux	80	-
VI	50%$TiCl_4.SiO_2$ (0.025)	15	Solvent-free/60˚C	83	-
VII	50%$TiCl_4.SiO_2$ (0.05)	10	Solvent-free/60˚C	90	-
VIII	50%$TiCl_4.SiO_2$ (0.07)	7	Solvent-free/60˚C	91	-
IX	50%$TiCl_4.SiO_2$ (0.1)	5	Solvent-free/60˚C	94	-
X	50%$TiCl_4.SiO_2$ (0.1)	60	MM/Solvent free[c]	50	
XI	50%$TiCl_4.SiO_2$ (0.1)	30	Sonication/EtOAc[d]	40	
XII	50%$TiCl_4.SiO_2$ (0.1)	10	MW/Solvent free[e]	70	
XIII	Nano-50%$TiCl_4SiO_2$ (0.07)	5	Solvent-free/60˚C	98	
XIV	Nano-50%$TiCl_4SiO_2$ (0.05)	5	Solvent-free/60˚C	96	
XV	Nano-50%$TiCl_4SiO_2$ (0.05), 2nd run	5	Solvent-free/60˚C	75	-
XVI	Nano-50%$TiCl_4SiO_2$ (0.05), 3rd run	10	Solvent-free/60˚C	72	-
XVII	CAN (5 mol%)	2h	PEG 400/50˚C	98	34
XVIII	Silica sulfuric acid	1h	Ethanol/ r.t	90	35
XIX	$Me_2S^+BrBr^-$/50	4 h	CH_3CN/ r.t	91	36
XX	TFA (30 mol%)	25	H_2O/Ethanol:1/2/r.t	100	37
XXI	75%Fe/CeO_2–ZrO_2 nano fine particles (0.015 g)	2h	Ethanol/ r.t	92	38
XXII	Air	4h	DMF/100˚C	91	39

[a]The molar ratio of 4-nitrobenzaldehyde: o-phenylenediamine is equal to 1:1
[b]Isolated yield.
[c]Using mixer mill (MM 400) in 25 Hz frequency.
[d]Using BANDELIN Sonopulse HD 3200 Ultrasonic apparatus with power equal to 20 KHz.
[e]Using microwave oven Kenwood, 1300W
TFA = trifluoracetic acid, DMF = dimethyl formamide, PEG = polyethyleneglycol

Consequently, several aromatic aldehydes were subjected to the condensation reaction with *o*-phenylenediamines to form 2-substituted benzimidazoles in the presence of 50% nano-$TiCl_4.SiO_2$ at 60 °C under solvent free condition (Figure 2 and Table II).

Table II

Synthesis of benzimidazole derivatives in the presence of nano-$TiCl_4.SiO_2$[a]

Compund	Amine	Aldehyde	Product	M.P. °C (Lit) Ref.	Yield (%)[b]/ Time (min)
E1				287-288 (292)[40]	95/5
E2				249-252 (253–255)[38]	92/6
E3				290-191 (287–289)[40]	88/5
E4				248-252	91/7
E5				311-312 (310–312)[38]	90/7
E6				193-194	82//8
E7				211-213	98/4
E8				201-204	98/4
E9				>300 (>300)[38]	87/6

[a]The reaction conditions: *o*-phenylenediamine (1.0 mmol): aldehydes (1.0 mmol): nano-$TiCl_4.SiO_2$ (0.05g), 60 ºC under solvent free.

[b]Isolated yield.

M.P. = melting point

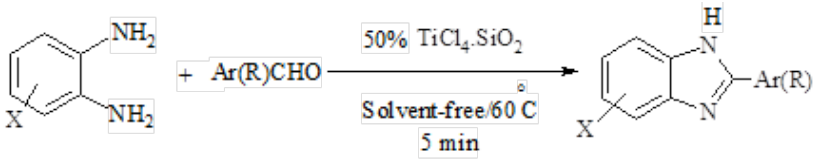


Figure 2
The condensation reaction between aromatic aldehydes with *o*-phenylenediamines

Based on a recent study [39], we have proposed a similar mechanism for the synthesis of benzimidazoles, as shown in Figure 3.

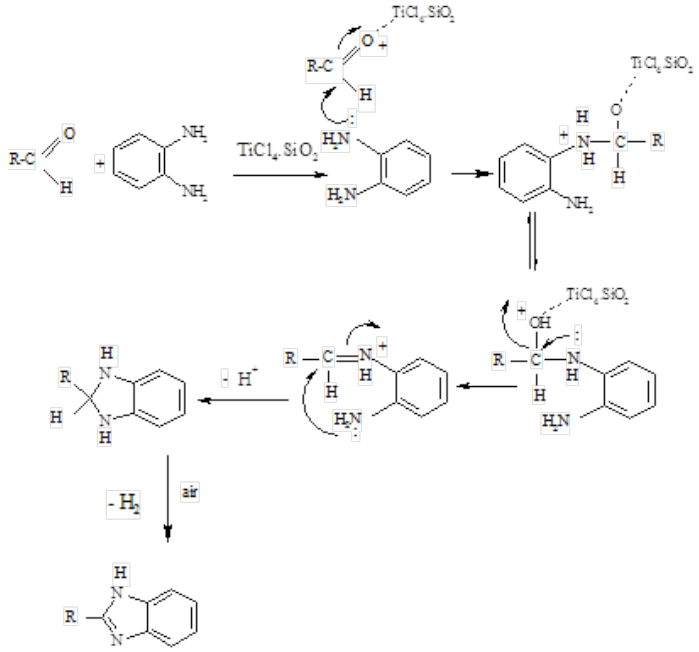


Figure 3
Proposed mechanism for the synthesis of benzimidazoles

Spectroscopy data:

2-phenyl-1H-benzimidazole (E1). Yellow solid, FT-IR: ν_{max} (ATR, neat, cm^{-1}): 1462 (C=C stretch), 1277 (C-N bend), 743 (C-H bend), 703 (C-H bend); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 7.29 (m, 2H), 7.5 (m, 3H), 7.66 (brs, 2H), 8.075 (2H, dd, J=7.4, J=2), 10 (brs, 1H, N-H).

(4-Hydroxyphenyl)-1H-benzimidazole (E2). Yellow solid, FT-IR: ν_{max} (ATR, neat, cm^{-1}): 3401 (O-H stretch), 1609 (C=N stretch), 1425 (C=C stretch), 1126 (C-N bend), 758 (C-H bend); ^{1}H NMR (DMSO-d_6, ppm): δ 9.98 (s, 1H, N-H), 7.98(d, J = 8.4, 2H), 7.5(m, 2H), 7.38(m, 2H), 6.9 (d, J =

8.4, 2H), 3.5 (br s, O-H); ^{13}C-NMR (DMSO-d_6, ppm): 160.36, 151.61,137.92, 129.11, 123.01, 119.6, 116.35, 114.86.

(4-Chlorophenyl)-1H-benzimidazole (E3). Orange solid, FT- IR: ν_{max} (ATR, neat, cm^{-1}): 1429 (C=C stretch), 1273 (C-N bend), 746 (=C-H); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.32 (brs, 2H), 7.42 (m, 3H), 7.52 (d, J=7.6, 1H), 7.83 (brs, 1H), 8.47 (d, J=7.6, 2H), 10.3 (brs, N-H) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 112.16, 119.55, 122.15, 123.21, 127.91, 130.82, 131.68, 132.55.

(4-Bromophenyl)-1H-benzimidazole (E4). Yellow solid, FT- IR: ν_{max} (ATR, neat, cm^{-1}): 1599 (C=N stretch), 1458 (C=C stretch), 1231 (C-N bend), 747 (C-H bend); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 7.20 (brs, 2H), 7.6 (brs, 2H), 7.8 (d, J=7.2, 2H), 8.1 (d, J=7.2, 2H), 13.1 (s, 1H, N-H).

(4-Nitrophenyl)-1H-benzimidazole (E5). Red solid, FT-IR: ν_{max} (ATR, neat, cm^{-1}): 1604 (C=N stretch), 1515 (NO_2 stretch), 1434 (C= C stretch), 1314 (NO_2 stretch), 1102 (C-N bend), 746 (C-H bend), 710 (C-H bend); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 7.26 (brs, 2H), 7.64 (brs, 2H), 8.41 (m, 4H), 13.65 (s, 1H, N-H);^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 123.46, 124.73, 127.81, 136.45, 148.22, 149.43.

(4-Methylacetatphenyl)-1H-benzimidazole (E6). Yellow solid, FT-IR: ν_{max} (ATR, neat, cm^{-1}): 1719 (C=O ester), 1610 (C=N stretch), 1434 (C=C stretch), 1279 (C-N stretch), 1105 (C-O stretch), 750 (C-H bend).

(2,4-Chlorophenyl)-1H-5-Nitro-benzimidazole (E7). Orange solid, FT-IR: ν_{max} (KBr, cm^{-1})**:** 3428 (N-H stretch), 1629(C=N stretch), 1459 (C=C stretch), 1107 (C-N bend), 803 (C-H bend).

(*2,3-Dihydroxyphenyl)-1H-5-Nitro-benzimidazole (E8).* Red solid, FT- IR: ν_{max} (ATR, neat, cm^{-1}) : 3391 (O-H), 1603(C=N stretch), 1459 (C=C stretch), 1338 (NO2 stretch), 1264 (C-N bend); ^{1}H-NMR (400 MHz, $(CD_3)_2CO$, ppm): 6.9 (t, J=7.6, 1H), 6.98 (d, J=8.8, 1H), 7.04 (d, J=7.6, 1H), 7.2 (d, J=7.6, 1H), 7.898 (s, 1H), 7.9 (d, J=8.4, 1H), 8.04 (s, O-H), 8.960 (s, 1H), 12.515 (s, 1H, N-H).

4-Dibenzimidazolyl-benzene (E9). Yellow solid, IR: ν_{max} (ATR, neat, cm^{-1}): 3067 (C-H stretch), 1442 (C=C stretch), 1279 (C-N bend), 735 (C-H bend); ^{1}H NMR (acetone-d_6, ppm): 12.9 (s, 1H, N-H), 8.42 (s, 4H), 7.68 (br d, 2H), 7.56 (br d, 2H), 7.23 (br s, 4H).

Modeling

All the compounds as well as clotrimazole were docked into the active site of 14a-demethylase, which was obtained from Protein Data Bank (1E9X) using Autodock 4.2. These new benzimidazole compounds were characterized by a docking mode in the active site of the cytochrome P450 14-a-sterol demethylase. The synthesized compounds also have drug-like

properties. These benzimidazoles have a molecular weight ranging from 194 to 310 and their log p varies between 2.52 and 4.54 (Table III).

Table III

Docking results of synthesized compounds into the active site of MT-CytP51 (1E9X)

Entry	Log p	Molecular weight (g/mol)	Final docked energy (Kcal/mol)
E1	2.86	194	-7.00
E2	2.47	210	-7.58
E3	3.42	228	-7.45
E4	3.69	280	-7.63
E5	3.30	239	-11.37
E6	2.68	252	-8.31
E7	4.54	308	-11.50
E8	2.52	271	-11.50
E9	3.69	310	-9.34

Nitro benzimidazoles (compounds E5, E7, E8) had more negative docking energy in comparison with the other benzimidazoles. Compound E8 which had exhibited strong inhibitory activities against all of the tested fungi also had the greatest negative docking energy as well as the lowest log p. In other compound there is not a strong correlation between antifungal activity and free docking energies.

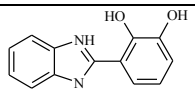
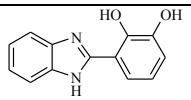
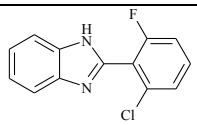
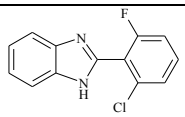
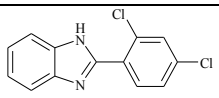
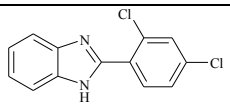
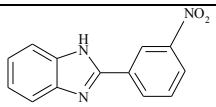
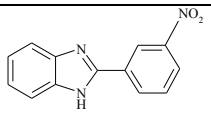
Computational details

The difference between the stability of these tautomers might be significant as a result of the intramolecular hydrogen bonding. We expect that cis isomer is much more stable for electronegative groups such as F, OH and Cl. We have calculated the stability of these two isomers using the calculation of Gibbs free energies at MP2/GTMP2 large level of theory in conjunction with B3LYP/6-31G(d) frequencies. The results of this level of theory are trustworthy if we calculate relative free energies. Benzimidazoles have two tautomers as shown in Figure 4, so that the hydrogen located on N1 can be in cis or trans positions relative to substituted X.

Table IV presents the calculated relative Gibbs free energies of the studied species in the absence of solvent interference. As we have expected, the cis conformers are significantly stable for the substituent of OH, F and Cl (Entries I, III and V). For the case of OH, the cis tautomer is -27 kJ/mol more stable than trans tautomer. For X= Cl and F, their stability differences are -14.7 and -9.8 kJ/mol, respectively. The position of X' was not of our concern and we ignored its role in these stabilities, though we are aware that it might have an interference. For cis and trans tautomers of 2-(3-nitrophenyl)-1*H*-benzimidazole, X=NO_2, as shown in entries of VII and VIII in Table IV, we did not observe any extra stability for cis tautomer because of the distance of the substituent with the hydrogen located on N1. It means that there is no significant interaction between these two groups.

Table IV

Absolute and relative Gibbs free energy, ΔG^0, in kJ/mol of the benzimidazoles tautomers[a]

Ent.	Compounds	ΔG^0	
		Absolute	Relative
I		-759.87672	-27.0
II		-759.86543	0.0
III		-1167.85892	-9.8
IV		-1167.85722	0.0
V		-1732.06912	-14.7
VI		-1732.06350	0.0
VII		-813.83684	0.0
VIII		-813.83684	0.0

[a]calculated at MP2/GTMP2Large level of theory in conjunction with B3LYP/6-31G(d) frequencies.

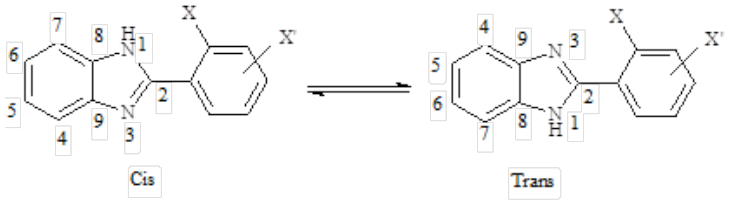


Figure 4
Tautomers of benzimidazoles

Antifungal activities of the synthetic compounds

Table V summarizes the inhibitory activities of the synthetic compounds and clotrimazole against the tested fungi. By comparing MIC values of the synthetic compounds, E8 exhibited strong inhibitory activities

against all of the tested fungi (Geometric mean MICs = 5.5 μg/mL, range MICs = >1 - 64 μg/mL) followed in activity by E1 and E2 respectively. Although E3, E4 and E5 showed no antifungal activities against the examined *Candida* and *Aspergillus* strains, they inhibited the growth of the tested dermatophytes at concentrations ranging from 16 μg/mL to 128 μg/mL. No antifungal activities against the fungi were found at examined concentrations by E6 and E7. Besides the fungistatic properties, E1, E2 and E8 exhibited fungicidal activities against the examined fungi at concentrations ranging from 4 to ≤ 512 μg/mL. In addition, the growth of azole-resistant standard and clinical isolates of *Candida* was inhibited by E1, E2 and E8 at concentrations of 2-512 μg/mL. Of the synthetic compounds, E8 exhibited the best inhibitory and fungicidal activities against *C. neoformance* with MICs comparable to fluconazole.

Comparing the antifungal activities of the synthetic compounds based on variation of substitutions on 2, 3 and 4-position of phenyl ring, we found that the base compound E1 exhibited a better antifungal activity against the tested fungi than the other compounds, except E8. Replacement of hydrogen with hydroxyl residue in 4-position of phenyl ring of E2 reduced its antifungal activity compared to E1, while it exhibited a better activity against the azole-resistant strains than the base compound, E1. In addition, replacement of hydrogen with Cl, Br or NO_2 at the 4-position of phenyl ring results in a considerable decrease of their antifungal activities against the tested fungi except dermatophyte species. Moreover, replacement of hydrogen with ester at the 4-position of phenyl ring provided E6 which showed no fungistatic and fungicidal activities against the examined fungi. This might be probably due to a lower solubility of E6 than the compounds E1-5 in aquatic media. Of the compounds with NO_2 in 5-position of benzimidazole cycle E7 and E8, replacement of two Cl molecules at position of 2 and 4 of phenyl ring with two hydroxyl groups at position of 2 and 3 of phenyl ring would result in significant enhancement of the inhibitory activity. Of the examined synthetic compounds, E2 and E8 were both effective against azole-resistant strains *of C. albicans* at concentrations ranging from 1-64 μg/mL, suggesting that the modes of action of these compounds are distinct from the examined antibiotics. All of the tested compounds successfully inhibited the growth of dermatophytes at concentrations ranging from >1 - 512 μg/mL, except E7. Of these compounds, E1 and E8 showed the best inhibitory effects against dermatophytic fungi with MICs comparable to those of griseofulvin.

Table V

Minimum inhibitory and fungicidal concentrations of the synthetic compounds (µg/mL) against the examined fungi

		E1		E2		E3		E4		E5		E6		E7		E8		E9		C	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Filamentous Fungi	*A. fumigatus (ATCC 14110)*	32	256	64	512	>512	>512	512	>512	512	>512	>512	>512	>512	>512	16	512	512	>512	<1	<1
	A. flavus (ATCC 64025)	128	512	128	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	64	>512	>512	>512	<1	<1
Yeasts	*C. albicans (ATCC 10261)*	8	16	128	256	>512	>512	>512	>512	512	>512	>512	>512	>512	>512	8	256	256	>512	<1	<1
	C. glabrata (ATCC 90030)	16	32	64	256	4	>512	8	>512	512	>512	>512	>512	>512	>512	2	>512	256	>512	<1	<1
	C. tropicalis (ATCC 750)	64	256	64	128	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	8	256	>512	>512	<1	<1
	C. dubliniensis (CBS 8501)	16	32	32	64	512	>512	512	>512	>512	>512	>512	>512	>512	>512	2	8	512	>512	<1	<1
	C.parapsilosis (ATCC 4344)	16	32	64	128	512	>512	512	>512	>512	>512	>512	>512	>512	>512	32	128	512	>512	<1	<1
	C. krusei (ATCC 6258)	32	64	64	128	256	>512	256	>512	128	>512	>512	>512	>512	>512	1	64	256	512	32	128
	C. albicans (1014)	512	>512	64	256	512	>512	>512	>512	512	>512	512	>512	>512	>512	8	256	>512	>512	64	>256
	C. albicans (2303)	512	>512	64	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	4	256	512	>512	128	>256
	C. neoformans	4	16	32	128	128	>512	128	>512	128	>512	>512	>512	>512	>512	<1	16	256	>512	<1	<1
Dermatophytes	*T. mentagrophytes*	4	64	32	128	32	>512	16	>512	32	>512	256	>512	512	>512	2	256	128	>512	<1	128
	M. gypseum	4	16	32	64	128	>512	32	>512	128	>512	256	>512	>512	>512	8	128	>512	>512	<1	128
	E. floccosum	2	4	32	64	16	512	16	>512	64	>512	258	>512	>512	>512	<1	32	128	>512	<1	<1

Conclusions

In conclusion, we have demonstrated a simple method for the synthesis of benzimidazole using Nano-$TiCl_4.SiO_2$ as a reusable, eco-friendly, low-cost, and professional catalyst. Short reaction times, high yield, simplicity of operation and easy work-up are some adventages of this method. The relative stabilities of cis tautomers for the studied compound have been investigated and a significant stability has been found for ortho-OH substituent. Also these benzimidazole compounds were characterized by docking and it was also observed they have drug-like properties. Considering antifungal activity of some of the synthetic compounds especially against azole resistant strains, they might be good candidates for further *in vivo* studies in order to elucidate their effects and toxicity as novel antifungal drugs. In particular 2-(2,3-dihydroxyphenyl)-1*H*-5-Nitro-benzimidazole exhibited strong antifungal activities against all tested fungi.

Acknowledgements

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