Journal of Molecular Structure 1116 (2016) 102–108

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Synthesis, antifungal activity and docking study of 2-amino-4*H*-benzochromene-3-carbonitrile derivatives

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ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form
1 March 2016
Accepted 1 March 2016
Available online 7 March 2016

Keywords:
2-Amino-4*H*-benzochromenes
Nano-$TiCl_4.SiO_2$
Docking study
Synthesis
Antifungal
Antibacterial

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 made it crucial to identify novel antimicrobial compounds.

Here, we selected 12 new compounds of 2-amino-4*H*-benzochromene-3-carbonitrile drivetives (***C1-C12***) for synthesis by using nano-$TiCl_4.SiO_2$ as efficient and green catalyst, then nine of synthetic compounds were evaluated against different species of fungi, positive gram and negative gram of bacteria. Standard and clinical strains of antibiotics sensitive and resistant fungi and bacteria were cultured in appropriate media. Biological activity of the 2-amino-4*H*-benzochromene-3-carbonitrile derivatives against fungi and bacteries were estimated by the broth micro-dilution method as recommended by clinical and laboratory standard institute (CLSI). In addition minimal fangicidal and bactericial concenteration of the compounds were also determined.

Considering our results showed that compound 2-amino-4-(4-methyl benzoate)-4*H*-benzo[*f*]chromen-3-carbonitrile (***C9***) had the most antifungal activity against *Aspergillus clavatus, Candida glabarata, Candida dubliniensis, Candida albicans* and *Candida tropicalis* at concentrations ranging from 8 to $\leq$128 μg/mL. Also compounds 2-amino-4-(3,4-dimethoxyphenyl)-4*H*-benzo[*f*]chromen-3-carbonitrile (***C4***) and 2-amino-4-(4-isopropylphenyl)-4*H*-benzo[*f*]chromen-3-carbonitrile (***C3***) had significant inhibitory activities against *Epidermophyton floccosum* following 2-amino-4-(4-methylbenzoate)-4*H*-benzo[*f*]chromen-3-carbonitrile (***C9***), respectively.

Docking simulation was performed to insert compounds ***C3***, ***C4*** and ***C9*** in to CYP51 active site to determine the probable binding model.

1. Introduction

During the past two decades, resistance to established antimicrobial drugs has increased dramatically and it is serious public health problem in a wide range of infectious disease [1–3]. 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. In

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http://dx.doi.org/10.1016/j.molstruc.2016.03.002
0022-2860/

spite of a large number of antibiotics and chemotherapeutics available for medical use, the antimicrobial resistance created substantial medical need for new classes of antimicrobial agents. Design and synthesis of newer antimicrobials will always remain an area of immense significance [4,5]. Among the important pharmacophores responsible, the chromene moiety is an important structural shape of many synthetic compounds of biological and pharmaceutical interest such as cytotoxicity [6], antioxidant [7], antiplasmodial [8], antimalarial [9], antirhinovirus [10], antifungal [11] and antibacterial [12].

A particularly interesting group of chromenes are 2-amino-4*H*-benzo[*f*]chromenes which are generally prepared by reaction of malononitrile, aldehydes and naphthols in one-pot procedure. This multicomponent reaction has been catalyzed with K_2CO_3 [13], Mg/Alhydrotalcite [14], nano-sized magnesium oxide [15], Preyssler heteropolyacid [16], basic alumina [17], potassium phosphate tribasic trihydrate [18], cetyltrimethylammonium bromide (CTABr) [19], sodium carbonate [20], DBU [21], 3-butyl-1-methylimidazolium hexafluorophosphate, 1-methyl imidazoliumiodide [mim]Cl [22], Na_2CO_3 [23] and tetrabutylammoniumbromide (TBABr) [24].

However, each method has certain restrictions with regards to scope and reaction conditions; for example, longer reaction times, purification problems and harsh reaction conditions. To avoid these limitations, our studies towards the development of more capable methods accompanied with higher yields for the synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile in the presence of nano-$TiCl_4.SiO_2$. We previously described the design and synthesis of some organic reactions in princess of solid acid catalysts as a green, cheap and efficient technique [25–30].

Also, some of the synthesized compounds were evaluated for their antifungal and antibacterial activity.

The antibacterial activities of the above compounds were compared to Ampicillin as positive control and the antifungal activities of them were evaluated to Fluconazole.

2. Materials and methods

2.1. Chemistry

The chemicals were purchased from Merck and used without any additional purification. The products were characterized by FT-IR (ATR), ^{1}H NMR, and a comparison of their physical properties with those reported in the literature. FT-IR (ATR) spectra were acquired on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avance) nmr was used to record the ^{1}H NMR spectra. Spectrophotometer (UV/Vis biotek model UVIKONXL), Melting points were determined with a Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.), Gass Chromatography Mass Spectrometery (Agilent 7000 Series Triple Quad -MS, Made in USA).

2.2. Docking studies

Molecular vina docking studies were performed using PYRX software [Wolf LK, Chem Eng News. 2009 87: 31], the complex of enzyme Mycobacterium tuberculosis-CYP51 with Fluconazole (PDB ID: 1EA1) was obtained from Protein Data Bank (http://www.rcsb.org), Water molecules and cognate ligand were removed from the receptor. The chemical structures were drawn into computer using HyperChem software (Version 7, Hypercube Inc), then the semi-empirical AM1 method was used for geometry optimization and saved in pdb file format. Binding mode figures were generated with PYMOL.*3.3. General procedure for synthesis of 2-amino-4H-benzochromene-3-carbonitrile*.

A mixture of aldehyde (1 mmol), malononitrile (1mmole), naphthol (1 mmol) and nano-$TiCl_4.SiO_2$ (0.1 g) was stirred at 90 °C under solvent–free conditions. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the reaction mixture was dissolved to $CHCl_3$ and filtered. The catalyst residue was washed with acetone and reused. The product was triturated with 2 mL of cooled ethanol to give the solid product. The spectroscopy of the pure products has been shown in Table 1.

2.3. Determination of antifungal activities

2.3.1. Microorganisms

The antifungal activities of the synthetic compounds against some American Type Culture Collection (ATCC) strains of fungi, including *Aspergillus flavus (ATCC 64025), Aspergillus fumigatus (ATCC 14110), Aspergillus clavatus, Candida albicans (ATCC 1912), C. albicans (ATCC 1905), C. albicans (SUCC 2303), C. albicans (SUCC 625), Candida glabarata (ATCC 2192), C. glabarata (ATCC 863), C. glabarata (ATCC 2175), C. glabarata (ATCC 2175), Candida dubliniensis (ATCC 8501), C. dubliniensis (ATCC 7988), Candida tropicalis (SUCC 194), C. tropicalis (SUCC 611), C. tropicalis (ATCC 750), Cryptococcus neoformance (ATCC 9011),* as well as two clinical isolates of yeasts identified by PCR-RFLP were determined. Moreover, the inhibitory activities of the mentioned compounds against dermatophytes (*Trichophyton rubrum*, *Microsporum canis* 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 [31,32]

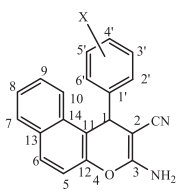
2.3.1.1. Determination of minimum inhibitory concentration. MICs were determined using the broth microdilution method recommended by the CLSI with some modifications [31,32]. Briefly, for determination of antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1–1024 μg/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with 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 standards at 530 nm wavelengths (this yields stock suspension of 1–5 × 106 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 × 106 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 30 °C 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.

3. Results and discussion

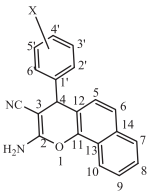
3.1. Chemistry

Our investigation is based on the development of heterogeneous catalyst for reducing risks to human and the environment. For finding the best reaction conditions, the reaction of benzaldehyde, malononitrile and 2-naphthol was examined under various states. According to the obtained results, the best result was obtained in

Table 1
Spectral data of 2-amino-4*H*-benzochromene-3-carbonitrile compounds.



(C1, C2, C3, C4, C6, C7, C8, C9, C10)



(C5, C11, C12)

Compound	Mp (C) Found reported[ref]	Spectral data
C1	273-274 273-275 [20]	FT-IR (neat, ATR) = 3429, 3336 (stretch, NH_2), 2181 (stretch, CN), 1638 (bending, NH_2), 1408, 1588 (stretch, C=C), 1261, 1024 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.8 (m, $2H_{7,10}$), 7.7 (d, J = 7.2 Hz, $1H_6$), 7.4 (m, $2H_{8,9}$), 7.29 (m, $1H_{4'}$), 7.26 (m, $2H_{3',5'}$), 7.20 (d, J = 7.2 Hz, $3H_{2',6',5}$), 5.26 (s, $1H_1$), 4.57 (s, 2H, NH_2), MS: (*m*/*z*, %), 297 (M^+, 10), 221 (100).
C2	217-219 -	FT-IR (neat ATR) = 3463, 3353 (stretch, NH_2), 2189 (stretch, CN), 1656 (bending, NH_2), 1588, 1410 (stretch, C=C),1526, 1348 (stretch, N=O), 1216, 1028 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 8.1 (s br, $1H_{2'}$), 8.08 (d, J = 8 Hz, $1H_{4'}$), 7.99 (d, J = 8.8 Hz, $1H_6$), 7.9 (m, $2H_{7,10}$), 7.6 (t, J = 8, $1H_{5'}$), 7.4 (d, J = 7.6 Hz, $1H_{6'}$), 7.4 (m, $2H_{8,9}$), 7.38 (d, J = 9.2, $1H_5$), 6.42 (s, 2H, NH_2), 5.6 (s, $1H_1$), MS: (*m*/*z*, %), 343 (M^+, 15), 221 (100).
C3	217-219 -	FT-IR (neat ATR) = 3423, 3335 (stretch, NH_2), 2190 (stretch, CN), 1647 (bending, NH_2), 1589, 1412 (stretch, C=C), 1233, 1038 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.81 (d, J = 8.4 Hz, $2H_{7,10}$), 7.72 (m, $1H_6$), 7.4 (m, $2H_{8,9}$), 7.26 (m, $1H_5$), 7 (s br, $4H_{2',3',5',6'}$), 5.23 (s, $1H_1$), 4.55 (s, 2H, NH_2), 2.83 (sept. J = 7.2 Hz, $1H_{isopropyl}$), 1.18 (d, J = 7 Hz, $6H_{isopropyl}$), MS: (*m*/*z*, %), 337 (30), 91 (100).
C4	190-192 -	FT-IR (neat ATR) = 3428, 3333 (stretch, NH_2), 2185 (stretch, CN), 1648 (bending, NH_2), 1589, 1403 (stretch, C=C), 1260, 1231, 1134, 1017 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.8 (d, J = 8.4, $2H_{7,10}$), 7.7 (m, $1H_6$), 7.4 (m, $2H_{8,9}$), 7.25 (d, J = 9.6 Hz,$1H_5$), 6.7 (d, J = 6.8, $2H_{5',6'}$), 6.66 (d, J = 8.4 Hz, $1H_{2'}$), 5.21 (s, $1H_1$), 4.6 (s, 2H, NH_2), 3.80 (s, $3H_{methoxy}$), 3.78 (s, $3H_{methoxy}$), MS: (*m*/*z*, %), 358 (M^+, 25), 221 (100).
C5	258-263 -	FT-IR (Neat) = 3401(stretch, NH_2), 2194 (stretch, CN), 1710 (stretch, C=O), 1664 (bending, NH_2), 1606, 1411(stretch, C=C), 1280, 1106 (stretch, C-O of ester), 1189, 1029 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.9 (d, J = 8.4 Hz, $2H_{3',5'}$), 7.8 (m, $2H_{7,10}$), 7.6 (m, $1H_6$), 7.4 (m, $2H_{8,9}$), 7.27 (t, J = 2.8 Hz, $3H_{2',6',5}$), 5.3 (s, $1H_4$), 4.65 (s, 2H, NH_2), 3.87 (s, $3H_{methy}$), MS: (*m*/*z*, %), 357 (M^+), 314 (60), 221 (100).
C6	238-240 232-234 [21]	FT-IR (neat ATR) = 3461, 3322 (stretch, NH_2), 2200 (stretch, CN), 1658 (bending, NH_2), 1587, 1404 (stretch, C=C), 1260, 1045 (stretch, C-O) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.83 (m, $2H_{7,10}$), 7.6 (d, J = 7.2, $1H_6$), 7.4 (m, $3H_{8,9,5}$), 7.25 (m, 1H3′), 7.04 (d, J = 8.4, $1H_{5'}$), 6.8 (d, J = 8.4, $1H_{6'}$), 5.84 (s, $1H_1$), 4.65 (s, 2H, NH_2), MS: (*m*/*z*, %), 366 (M^+, 5), 221 (100).
C7	271-273 259-261 [20]	FT-IR (neat ATR) = 3458, 3349 (stretch, NH_2), 2178 (stretch, CN), 1654 (bending, NH_2), 1589, 1409 (stretch, C=C), 1219, 1040 (stretch, C-O), 798 (bending C-H) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.8 (m, $2H_{7,10}$), 7.67 (d, J = 8 Hz, $1H_6$), 7.4 (m, $3H_{8,9,3'}$), 7.25 (m, $1H_{4'}$), 7.1 (m, $2H_{5',6'}$), 6.9 (d, J = 7.2 Hz, $1H_5$), 5.9 (s, $1H_1$), 4.62 (s, 2H, NH_2), MS: (*m*/*z*, %), 332 (M^+, 15), 221 (100).
C8	256-257 -	FT-IR (neat ATR) = 3434, 3328 (stretch, NH_2), 2185 (stretch, CN), 1649 (bending, NH_2), 1585, 1408 (stretch, C=C), 1227, 1024 (stretch, C-O), 798, 724 (bending C-H) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.9 (m, $3H_{7,10,6}$), 7.4 (m, $2H_{8,9}$), 7.3 (d, J = 8.8 Hz, $1H_5$), 6.94 (s, $1H_{2'}$), 6.69 (d, J = 8.4 Hz, $1H_{6'}$), 6.6 (d, J = 8 Hz, $1H_{5'}$), 6.14 (s, 2H, NH_2), 5.24 (s, 1H1), MS: (*m*/*z*, %), 326 (15), 221 (100).
C9	232-235 -	FT-IR (neat ATR) = 3379, 3313 (stretch, NH_2), 2195 (stretch, CN), 1707 (stretch, C=O), 1650 (bending, NH_2), 1590, 1404 (stretch, C=C), 1287, 1187 (stretch, C-O of ester), 1233, 1022 (stretch, C-O), 726 (bending C-H) cm^{-1},^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.9 (d, J = 8.4 Hz, $2H_{3',5'}$), 7.8 (m, $2H_{7,10}$), 7.6 (m, $1H_6$), 7.4 (m, $2H_{8,9}$), 7.27 (t, J = 2.8 Hz, $3H_{2',6',5}$), 5.3 (s, $1H_1$), 4.65 (s, 2H, NH_2), 3.87 (s, $3H_{methy}$), MS: (*m*/*z*, %), 357 (M^+), 314 (60), 221 (100).
C10	163-164 -	FT-IR (neat ATR) = 3392, 3346 (stretch, NH_2), 2221(stretch, CN), 1657 bending, NH_2), 1597, 1481 (stretch, C=C), 694 (bending C-H) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.3 (m, $9H_{6,7,8,9,10,3',4',5',6'}$), 7.1 (d, J = 6.8 Hz, $1H_{2'}$), 7.0 (d, J = 8 Hz 1H5), 5.0 (s, $1H_1$), 3.8 (s, 2H, NH_2), 3.05 (t, J = 7.6 Hz, $2H_{methylene}$), 2.7 (t, J = 8 Hz, $2H_{methylene}$), MS: (*m*/*z*, %), 326 (M^+, 15), 221 (100).
C11	211-214 -	FT-IR (Neat ATR) = 3344 (stretch, NH_2), 2194 (stretch, CN), 1667 1647 (bending, NH_2), 1573, 1398 (stretch, C=C), 1103 (stretch, C-O), 797 (bending C-H) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 8.18 (d, J = 8 Hz, $1H_7$), 7.79 (d, J = 7.6 Hz, $1H_{10}$), 7.5 (m, $3H_{6,8,9}$), 7.15(s br, $4H_{2',3',5',6'}$), 7.06 (d, J = 8.4, $1H_5$), 4.85 (s, $1H_4$), 4.72 (s, 2H, NH_2), 2.87(sept. J = 6.8 Hz, $1H_{isopropyl}$), 1.2 (d, J = 6.8 Hz, $6H_{isopropyl}$), MS: (*m*/*z*, %), 337 (30), 91 (100).
C12	222-224 -	FT-IR (Neat ATR) = 3343, 3331(stretch, NH_2), 2195(stretch, CN), 1667(bending, NH_2), 1601, 1404 (stretch, C=C), 1272, 1037 (stretch, C-O), 804(bending C-H) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 7.9 (m, $3H_{7,10,6}$), 7.4 (m, $2H_{8,9}$), 7.3 (d, J = 8.8 Hz, $1H_5$), 6.94 (s, $1H_{2'}$), 6.69 (d, J = 8.4 Hz, $1H_{6'}$), 6.6 (d, J = 8 Hz, $1H_{5'}$), 6.14 (s, 2H, NH_2), 5.24 (s, $1H_4$), MS: (*m*/*z*, %), 326 (15), 221 (100).

the presence of nano-$TiCl_4.SiO_2$ (0.1 g) under solvent free conditions (Table 2, Entry 8).

Then, scope of synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile using a wide range of aromatic aldehydes was investigated and it can be seen in Table 3 and Fig. 1. Clearly, the reactivity of aldehyde is the key factor for this one-pot protocol. Electron deficient aromatic aldehydes produced higher yields of production in a shorter time in comparison to the aldehayes that are rich in electrons.

Meanwhile, both aldehyde groups of terphthaldialdehyde reacted to malononitrile and 2-naphthol. The production was 4, 4′-(1, 4-phenylene)bis(2-amino-4*H*-benzo[*f*]chromene-3-carbonitrile) with 92% yield after 100 min at 90 °C (Scheme 1).

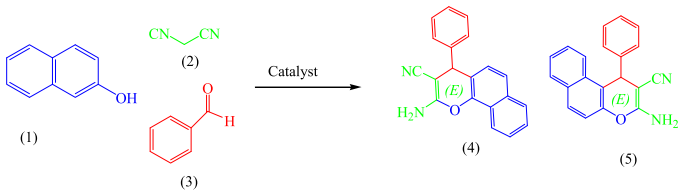
Trioxan as a source of formaldehyde reacted to 2-naphthol and malononitrile. It produced 2-amino-4*H*, 4*H* mixing at 90 °C (Scheme 2).

In reaction of 3-phenyl propionaldehyde as an aliphatic aldehyde with malononitrile and 2-naphthol, corresponding chromene product was obtained in 80% yield after 100 min at 90 °C. In this protocol, 4-(*N*,*N*-dimethylamino) benzaldehyde as an aldehyde with electron releasing group only reacted to malononitrile and produced benzylidinemalononitrile with 85% yield after 120 min mixing at 90 °C. Ketones such as, acetophenone did not produced any chromene derivatives in condensation with malononitrile and 2-naphthol. It only produced methylbenzylidinemalononitrile with 88% yield after 150 min at 90 °C.

3.2. Antifungal activities of the synthetic compounds

In this study, nine compounds (***C1-C9***) evaluated against fungi (Table 4). On the other hands, compounds ***C1, C2, C5, C6, C7*** and ***C8***

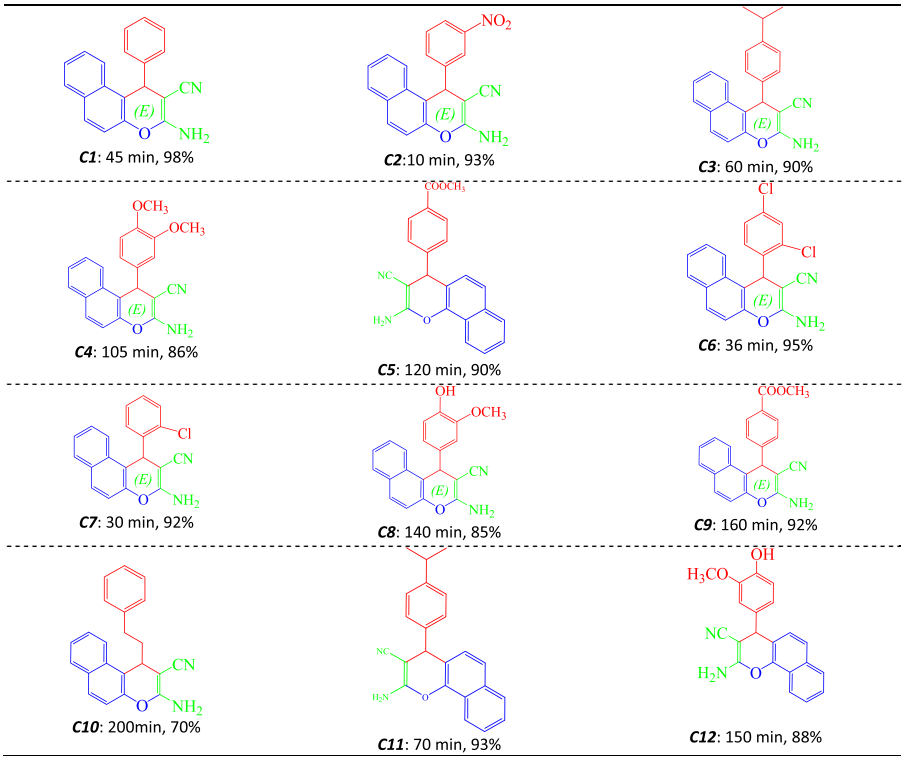
Table 2
Synthesis of 2-amino-4-phenyl-4*H*-benzo[f]chromen-3-carbonitrilein various conditions.[a]



Entry	Catalyst	Mol% (g)	ArOH	Condition	Solvent	Product [Yield(%)][ref]
1	Na_2CO_3	10	α-naphthol	Grinding	–	4 [92%] [23]
2	Na_2CO_3	10	β-naphthol	125 °C	–	5 [100%] [20]
3	TBABr	(0.8)	α-naphthol	MW	H_2O	4 [94%] [24]
4	K_2CO_3	10	β-naphthol	MW	–	4[92%] [13]
5	CTABr	(0.01 mL)	β-naphthol	Reflux –110 °C	H_2O	5 [80%] [19]
6	Mg/Al (HT)	50	α-naphthol	MW	–	4 [84%] [14]
7	γ-alumina.H_2O	–	α-naphthol	Reflux	H_2O	4 [96%] [17]
8	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	90 °C	–	5 [98%]-
9	Nano-$TiCl_4.SiO_2$	0.15	β-naphthol	90 °C	–	5 [98%]-
10	Nano-$TiCl_4.SiO_2$	0.05	β-naphthol	90 °C	–	5 [85%]-
11	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	80 °C	–	5 [90%]-
12	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	110 °C	–	5 [98%]-
13	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Reflux	Water	5 [70%]-
14	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Reflux	EtOH	5 [78%]-
15	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Reflux	EtOH: H_2O	5 [85%]-
16	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Reflux	EtOAc	5 [80%]-
17	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Reflux	*n*-Hexane	5 [80%]-
18	Nano-$TiCl_4.SiO_2$	0.1	β-naphthol	Sonication	EtOH: H_2O	5 [75%]-

[a] The molar ratio of benzaldehyde: naphthol: and malonitrile is 1: 1: 1.

Table 3
Preparation of 2-amino-4*H*-benzochromene-3-carbonitrile in the presence of nano-$TiCl_4.SiO_2$[a].



[a] A mixture of aldehyde (1mmol), malononitrile (1mmol), naphthol (1mmol), and nano-$TiCl_4.SiO_2$ (0.1 g) was stirred at 90 °C under solvent–free conditions

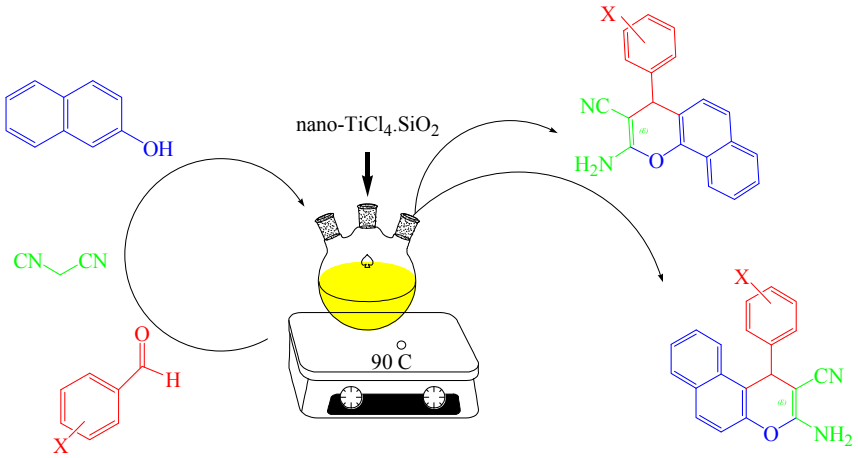
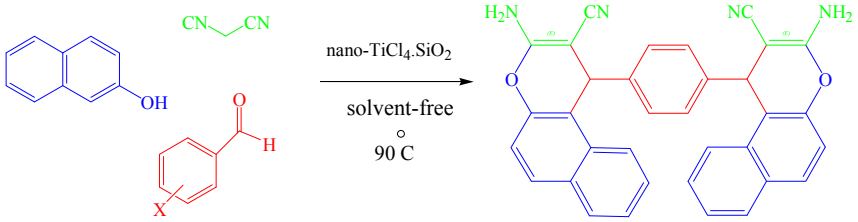


Fig. 1. Synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile.



Scheme 1. Synthesis of 4,4′-(1,4-phenylene)bis(2-amino-4*H*-benzo[f]chromene-3-carbonitrile).

showed no antifungal activities against examined Candida, *Aspergillus* strains and *dermatophytes*. Compound ***C9*** exhibited fungicidal activity against *A. clavatus, C. glabarata, C. dubliniensis, C. albicans,* and *C. tropicalis*. In comparing MIC values of the synthetic compounds, ***C4*** exhibited strong inhibitory activities against *Epidermophyton floccosum* followed in activity by ***C3*** and ***C9***, respectively.

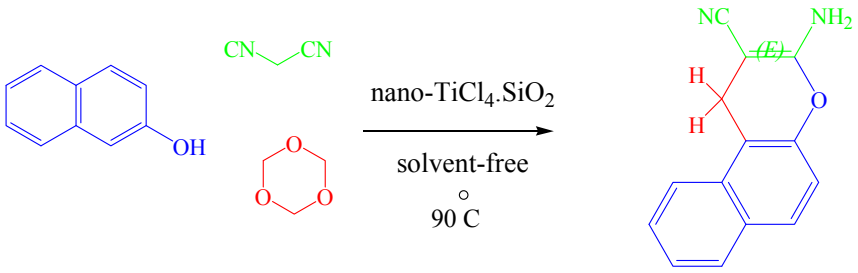
In this class, replacement of hydrogen with methoxy residue in para and meta-positions of phenyl ring of ***C4*** increased its antifungal activity against *E. floccosum* (at concentrations ranging from 1 to 32 μg/mL) compared to ***C1*** (Table 4).

In addition, isopropyl substitution at para position of the phenyl ring of ***C3*** improved of its antifungal activity against the tested *E. floccosum* at concentrations ranging from 2 to 8 μg/mL compared to ***C1*** (Table 3). Replacement of methyl ester at para position of phenyl ring provided ***C9*** would result in significant enhancement of the inhibitory activity against *E. floccosum* at concentrations ranging from 8 to 16 μg/mL. Also this compound showed good fungistatic and fungicidal activity against some species of Candida at concentrations ranging from 16 to 128 μg/mL (Table 4). This might be probably due to higher solubility of ***C9*** than the other compounds in aquatic media.

Also, in this survey, showed that *E. floccosum* was the most sensitive of the studied dermatophytes, that good inhibited by ***C4***, ***C3*** and ***C9*** compared to *T. rubrum* and *M. canis*.

None of the synthetic compounds had any effect on negative gram and positive gram of microorganisms except ***C1*** that it had inhibitory effects on *S. aureus* microorganism.

In comparison of the antifungal and antibacterial activities of the synthetic compounds based on variation of substitutions on ortho, meta and para position of phenyl ring, we found that the base compound ***C1*** exhibited a better antibacterial activity against



Scheme 2. Synthesis of 2-amino-4*H*,4*H*-benzo[*f*]chromen-3-carbonitrile.

Table 4
Result of Minimum inhibitory concentrations of the 2-amino-4*H*-benzochromene-3-carbonitrile drivatives (μg/mL) against fungi.

	Compounds	*C1*		*C2*		*C3*		*C4*		*C5*		*C6*		*C7*		*C8*		*C9*		*Fluconazolel*	
	Fungi(number of strains)	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC
Fungi	*A. flavus (ATCC 64025))*	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	A. fumigatus(ATCC 14110))	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	A. clavatus (ATCC)	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Dermatophytes	*C. glabarata (ATCC 863)*	1>	1>	>128	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. dubliniensis (ATCC 8501)	1>	1>	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. dubliniensis (ATCC 7988)	1>	1>	>128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. glabarata (ATCC 863)	1>	1>	>128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. albicans (ATCC 1912)	1>	1>	>128	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. glabarata (ATCC 2192)	1>	1>	>128	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. glabarata (ATCC 2175)	1>	1>	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. albicans (ATCC 1905)	1>	1>	>128	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. albicans (SUCC 2303)	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. albicans (SUCC 625)	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. tropicalis (SUCC 194)	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. tropicalis (SUCC 611)	1>	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	C. tropicalis (ATCC 750)	1>	1>	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Yeasts	*M. canis*	128	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	T. rubrum	128	1>	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
	E. floccosum	1>	1>	16	8	>128	>128	>128	>128	>128	>128	>128	>128	32	1	8	2	>128	>128	>128	>128

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration.

the tested *S. aureus* (positive garam microorganism) than the other compounds. We find Compound ***C9*** exhibited fungicidal activity against *A. clavatus, C. glabarata, C. dubliniensis, C. albicans*, and *C. tropicalis*. In comparing MIC values of the synthetic compounds, ***C4*** exhibited strong inhibitory activities against *E. floccosum* followed in activity by ***C3*** and ***C9***, respectively.

3.3. Docking studies

The rasemate compounds were tested but docking studies of binding mode of R and S isomer of compounds ***C3***, ***C4*** and ***C9*** were performed by PYRX to achieve better understanding on the mechanism, potency and guide additional structure–activity relationships (SAR). According to the related literature, there are no any experimental structural information available for the active site of *C. albicans* enzyme so the most close homology X-ray crystallographic structure of the *Mycobacterium tuberculosis* enzyme CYP51 (PDB ID:1EA1) were used [33,34]. Interactions of fluconazole in active site of CYP51 for validation and comparison were depicted in Fig. 2 (***a***). The R isomers have no effective interaction so there are comparisons between isomer S of compounds ***C3***, ***C4*** and ***C9*** and binding model of fluconazole. Fig. 2 (***b, c***, and ***d***) indicated the polar interaction with Arg 96, His 259 and Thr 260 into the binding pocket that accommodates well to pharmacophore residues. In summery these H-bond and hydrophobic interaction might be favor to the anti fungal activity. It seems that suitable substitute on para and meta site of compounds ***C3***, ***C4*** and ***C9*** orientated toward the front of the pocket of heme iron of CYP51, These interactions are analogous to interactions typically seen between the triazole ring of fluconazole and heme iron of CYP51.

We have demonstrated a simple method for the synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile by nano-$TiCl_4.SiO_2$ as efficient catalyst under solvent-free condition. The antibacterial and antifungal activities of nine synthetic compounds were investigated.

It is evident that synthesized compounds, ***C3***, ***C4*** and ***C9*** exhibited strong inhibitory activities against *E. floccosum* and thereby, these compounds can constituting promising antifungal both individually and in combined therapy.

A computer model of the interaction of compound ***C3***, ***C4*** and ***C9*** with the *Mycobacterium tuberculosis* enzyme CYP51 binding pocket was proposed. The model involved a favorable polar interaction that accommodated in complete pharmacophoric equivalency to the known anti-fungal activity binding mode.

4. Conclusion

We have demonstrated a simple method for the synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile by nano-$TiCl_4.SiO_2$ as efficient catalyst under solvent-free condition. The antibacterial and antifungal activities of nine synthetic compounds were investigated.

It is evident that synthesized compounds, ***C3***, ***C4*** and ***C9*** exhibited strong inhibitory activities against *E. floccosum*, these compounds can constituting promising antifungal both individually and in combined therapy.

A computer model of the interaction of compound ***C3***, ***C4*** and ***C9*** with the *Mycobacterium tuberculosis* enzyme CYP51 binding pocket was proposed. The model involved a favorable polar interaction that accommodated in complete pharmacophoric equivalency to the known anti-fungal activity binding mode.

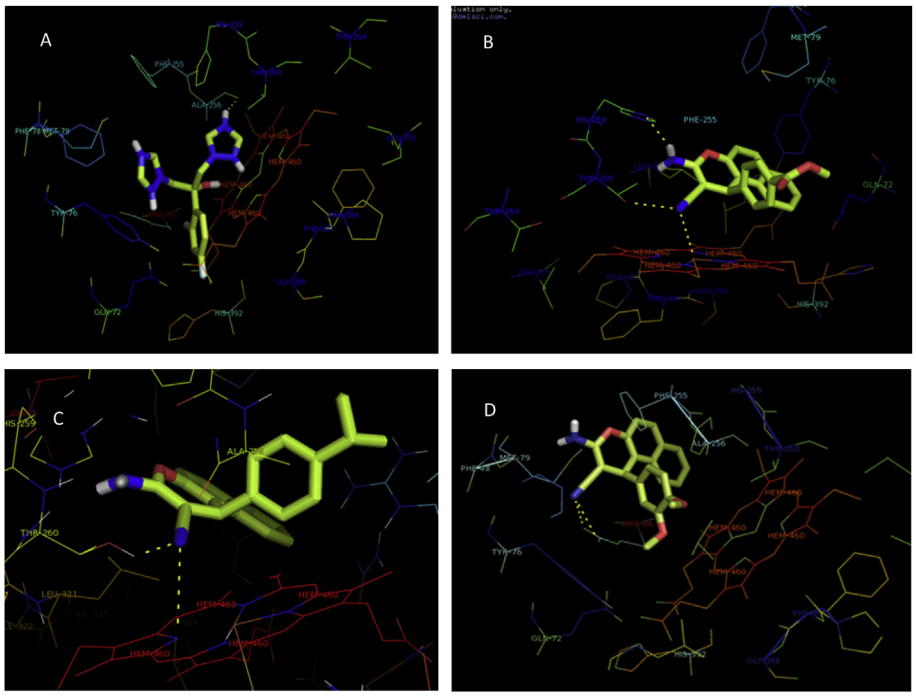


Fig. 2. (***a***). Molecular docking modeling perpendicular orientation of fluconazole to the heme iron of CYP51, (***b***) Orientation compound ***C3***, (***c***) Orientation compound ***C4*** and (***d***) Orientation compound ***C9*** in active site of CYP51. Note: For clarity, only interacting residues in 12 Å were displayed.

Acknowledgments

The Research Council of Yazd University is gratefully acknowledged for the financial support of design and synthesis of compounds. The anti fungal activities of the synthetic compounds were evaluated in Shiraz University of Medical Sciences by grant number 93-01-05-7688. The authors would like to thank the Research Consulting Center (RCC) at Shiraz University of Medical Sciences for their assistant in editing this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2016.03.002.

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