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

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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-TiCl₄.SiO₂ 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-3carbonitrile (*C9*) had the most antifungal activity against *Aspergillus clavatus, Candida glabarata, Candida dubliniensis, Candida albicans* and *Candida tropicalis* at concentrations ranging from 8 to ≤ 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.

Keywords: 2-Amino-4*H*-benzochromenes; Nano-TiCl₄.SiO₂; Docking study; Synthesis; Antifungal; Antibacterial.

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 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 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[/]chromenes which are generally prepared by reaction of malononitrile, aldehydes and naphthols in one-pot procedure. This multicomponent reaction has been catalyzed with K₂CO₃ [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], Na₂CO₃ [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-TiCl₄.SiO₂. 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), ¹H-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 ¹H 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 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 (1mmol), malononitrile (1mmole), naphthol (1mmol) and nano-TiCl₄.SiO₂ (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 CHCl₃ 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.

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

		$ \begin{array}{c} x \\ y \\ x \\ y \\ y$
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
	1	(C1, C2, C3, C4, C6, C7, C8, C9, C10) (C5, C11, C12)
Compound	Mp (°C)	Spectral data
	Found Reported	
CI	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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.8 (m, 2H _{7,10}), 7.7 (d, <i>J</i> = 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, <i>J</i> = 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 , ¹ H 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).
СЗ	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 , ¹ H 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 ; <i>s</i> : <i>s</i> : <i>c</i>), 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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.8 (d, <i>J</i> = 8.4, 2H _{7,10}), 7.7 (m, 1H ₆), 7.4 (m, 2H _{8,9}), 7.25 (d, <i>J</i> = 9.6 Hz,1H ₅), 6.7 (d, <i>J</i> = 6.8, 2H _{5.6}), 6.66 (d, <i>J</i> = 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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.9 (d, <i>J</i> =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, <i>J</i> =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 , ¹ H 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 ⁻¹), 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).
С7	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 , ¹ H 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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.9 (m, 3H _{7,10,6}), 7.4 (m, 2H ₈), 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).
С9	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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.9 (d, <i>J</i> =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, <i>J</i> =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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 7.3 (m, 9H _{6,7,8,9,10,3',4',5',6'}), 7.1 (d, <i>J</i> = 6.8 Hz, 1H ₂), 7.0 (d, <i>J</i> = 8 Hz 1H ₅), 5.0 (s, 1H ₁), 3.8 (s, 2H, NH ₂), 3.05 (t, <i>J</i> = 7.6 Hz, 2H _{methylene}), 2.7 (t, <i>J</i> = 8 Hz, 2H _{methylene}), MS: (m/z, %), 326 (M ⁺ , 15), 221 (100).
CII	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 , ¹ H NMR (400 MHz, CDCl ₃), δ (ppm) = 8.18 (d, <i>J</i> =8 Hz, 1H ₇), 7.79 (d, <i>J</i> =7.6 Hz, 1H ₁₀), 7.5(m, 3H _{6.8.9}), 7.15(s br, 4H _{2',3',5',6'}), 7.06 (d, <i>J</i> =8.4, 1H ₅), 4.85 (s, 1H ₄), 4.72 (s, 2H, NH ₂), 2.87(sept. <i>J</i> =6.8 Hz, 1H _{isopropyl}), 1.2 (d, <i>J</i> =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 , ¹ H 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).

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), Candida albicans (ATCC 1905), Candida albicans (SUCC 2303), Candida albicans (SUCC 625), Candida glabarata (ATCC 2192), Candida glabarata (ATCC 863), Candida glabarata (ATCC 2175), Candida glabarata (ATCC 2175), Candida dubliniensis (ATCC 7988), Candida tropicalis (SUCC 194), Candida tropicalis (SUCC 611), Candida 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].

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 μ g/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 \times 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 $0.4-5 \times 106$ 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 the presence of nano-TiCl₄.SiO₂ (0.1 g) under solvent free conditions (Table 2, Entry 8).



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

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 Figure 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.

Table 2

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

	(1)	CN CN (2) $OH H$ (3)	Catalyst		(E) (F) (F) (F) (F) (F) (F) (F) (F) (F) (F	
Entry	Catalyst	Mol% (g)	ArOH	Condition	Solvent	Product
						[Yield(%)][ref]
1	Na ₂ CO 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 ₂ O	-	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 ₂ O	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	<i>n</i> -Hexane	5 [80%]-
18	Nano-TiCl ₄ .SiO ₂	0.1	β -naphthol	Sonication	EtOH: H ₂ O	5 [75%]-

^aThe molar ratio of benzaldehyde : naphthol : and malonitrile is 1 : 1 : 1.

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 minutes at 90 °C (Scheme 1).



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

Table 3

Preparation of 2-amino-4H-benzochromene-3-carbonitrile in the presence of nano-TiCl₄.SiO₂^a



^aA mixture of aldehyde (1mmol), malononitrile (1mmol), naphthol (1mmol), and nano-TiCl₄.SiO₂ (0.1 g) was stirred at 90 °C under solvent– free conditions

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



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

In reaction of 3-phenyl propionaldehyde as an aliphatic aldehyde with malononitrile and 2naphthol, corresponding chromene product was obtained in 80% yield after 100 minutes 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 minutes 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 minutes 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* 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 *E. 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 μ g/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- 8 μ g/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 8-16 μ g/mL. Also this compound showed good fungistatic and fungicidal activity against some species of Candida at concentrations ranging from 16-128 μ g/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 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 *Candida 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 Figure 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. Figure 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-TiCl₄.SiO₂ 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.



Figure 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.

Table 4

Result of Minimum inhibitory concentrations of the 2-amino-4*H*-benzochromenes drivatives (µg/mL) against fungi

	Compounds	Compounds C1 C2 C3		C	4	C5		С6		C7		<i>C8</i>		<i>C9</i>		Fluconazolel					
	Fungi(number of strains)	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Fungi	A. flavus (ATCC 64025))	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
	A. fumigatus(ATCC 14110))	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
	A. clavatus (ATCC)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
	C. glabarata (ATCC 863)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	1>	1>
	C. dubliniensis (ATCC 8501)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	1>	1>
\$	C. dubliniensis (ATCC 7988)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	1>	1>
ast	C. glabarata (ATCC 863)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	1>	1>
Ye	C. albicans (ATCC 1912)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	1>	1>
	C. glabarata (ATCC 2192)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	16	>128	1>	1>
	C. glabarata (ATCC 2175)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	1>	1>
	C. albicans (ATCC 1905)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	16	>128	1>	1>
	C. albicans (SUCC 2303)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
	C. albicans (SUCC 625)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
s	C. tropicalis (SUCC 194)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
yte	C. tropicalis (SUCC 611)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	1>
Ido	C. tropicalis (ATCC 750)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	1>	1>
ıat	M. canis	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	128
ern	T. rubrum	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1>	128
Q	E. floccosum	>128	>128	>128	>128	2	8	1	32	>128	>128	>128	>128	>128	>128	>128	>128	8	16	1>	1>
MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration																					

4. Conclusion

ACCEPTED MANUSCRIPT

We have demonstrated a simple method for the synthesis of 2-amino-4*H*-benzochromene-3-carbonitrile by nano-TiCl₄.SiO₂ 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.

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Highlights

► A series of novel 2-amino-4*H*-benzochromene-3-carbonitrile derivatives in the presence of nano-TiCl₄.SiO₂ were synthesized. ► Some of the compounds showed good fungi static and fungicidal activity against some species of Candida ► Docking simulation was performed to insert compounds in to CYP51 active site to determine the probable binding model.