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In vitro anticandidal evaluation of novel highly functionalized *bis* cyclohexenone ethyl carboxylates

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Abstract. – OBJECTIVES: Novel highly functionalized bis cyclohexenone ethyl carboxylates 7-12 were designed, synthesized and their structures were elucidated by their elemental analysis, MS, FT-IR, one-dimensional ^{1}H, and ^{13}C NMR spectroscopic data.

MATERIALS AND METHODS: All the synthesized compounds 7-12 were tested for their *in vitro* antifungal activities against *Candida sp.* namely *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida dubliniensis* and *Candida krusei*.

RESULTS: A close inspection of the *in vitro* anticandidal activity profile in differently electron withdrawing (-F, -Cl, and -Br) functional group and electron donating (CH_3 and OCH_3) substituted phenyl rings of novel highly functionalized bis cyclohexenone ethyl carboxylates 7-12 exerted strong anticandidal activity against all the tested *Candida* species. All the synthesized compounds 7-12 exhibited MIC value in the range of 6.25-200 µg/mL against all the tested *Candida* (C.) species.

CONCLUSIONS: Compound 8 against *C. albicans*, 9,11 against *C. glabrata*, 8,10 against *C. parapsilosis*, 7,9 against *C. dubliniensis*, 8,10 against *C. krusei* exhibited excellent anticandidal activity at a MIC value of 6.25 µg/mL. Likewise compound 7, 9-11 against *C. albicans,* 8, 9, 11 against *C. tropicalis*, 8 against *C. glabrata*, 9 against *C. parapsilosis*, 10 against *C. dubliniensis*, 9 against *C. krusei* revealed superior activity at a MIC value of 12.5 µg/mL.

Key Words:
Functionalized *bis* cyclohexenones, Cyclocondensation, Michael addition, Synthesis, *Candida sp.*

Introduction

Infections due to *Candida* (C.) species are the most common of the fungal infections[1]. *Candida* species produce a broad range of infections, ranging from non-life-threatening mucocutaneous illnesses to invasive process that may involve virtually any organ. Such a broad range of infections requires an equally broad range of diagnostic and therapeutic strategies. In general, both amphotericin B and the azoles have a role to play in treatment. Choice of therapy is guided by weighing the greater activity of amphotericin B for some non-*albicans* species (e.g., *Candida krusei*) against the lesser toxicity and ease of administration of the azole antifungal agents. Flucytosine has activity against many isolates of *Candida* but is not often used. Vaginal candidiasis[2] is an infection caused by *Candida albicans* (80-90%) or related fungi such as *C. glabrata* and *C. tropicalis* (10%). Fluconazole, a bis-triazole antifungal agent has the potential for reducing episodes of vaginal candidiasis[3]. In animal models, fluconazole has been shown to be more potent than Ketoconazole against *Candida* infections[4]. Clotrimazole is effective against dermatophyte and other fungal infections[5], which has been used for local treatment.

A growth of interest is growing now-a-days in exploiting more than one proximal functional pharmacophoric groups for designing novel structures capable of performing a variety of functions[6,7]. One of the essential components of the search for new leads in drug designing programme is to synthesis molecules, which are novel still resembling known biologically active molecules by virtue of the presence of some critical pharmacophoric structural features[8]. The motive for the preparation of higly functionalized cyclohexenone ethyl carboxylates is due to the fact that they are excellent carriers of different types of biological activity[9-12]. Cyclohexenoic long chain fatty alcohols are used in the treatment of neurological disorders[13]. Ambuic acid, a highly functionalized cyclohexenones exhibits antifungal activity[14]. Jesterone and hydroxyl jesterone are highly functionalized cyclohexeneyl ester derivatives with potent antifungal activity[15].

In view of the above mentioned biological properties and as part of our research program aimed at

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the synthesis of biologically active small yet novel structurally diverse compounds[6-8], herein is reported the highly functionalized cyclohexenone ethyl carboxylates has been designed and synthesized and to study their *in vitro* anticandidal activity against clinically isolated fungal strains namely *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. dubliniensis* and *C. krusei* and their structure-activity relationship results are discussed.

Materials and Methods

Chemistry

The progress of the reaction is monitored by thin layer chromatography (TLC) analysis. All the reported melting points are taken in open capillaries and are uncorrected. IR spectra are recorded in KBr (pellet forms) on a Nicolet-Avatar–330 FT-IR spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA, US) and note worthy absorption values (cm^{-1}) alone are listed. ^{1}H and ^{13}C NMR spectra are recorded at 400 MHz and 100 MHz respectively on Bruker Avance II 400 NMR spectrometer (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) using DMSO-*d* as solvent. The ESI +ve MS spectra are recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalyses are obtained on Carlo Erba 1106 CHN analyzer (Thermo Fisher Scientific Inc, Waltham, MA, US). By adopting the previous literature[16] *bis* chalcones **1-6** are prepared.

Typical Experimental Procedure for the Synthesis of Highly Functionalized *bis* Cyclohexenone Ethyl Carboxylates 7-12

To a solution of sodium ethoxide (0.001 mol) in 30 mL of absolute ethanol, freshly distilled ethyl acetoacetate (0.01 mol) and respective *bis* chalcones **1-6** (0.01 mol) in absolute ethanol (40 mL) is mixed and it is refluxed in a water bath for 3-6 h by maintaining the temperature around (70-80)ºC. The reaction mixture is allowed to cool and filtered. Then the crude product is recrystallized from absolute ethanol to afford *bis* cyclohexenone ethyl carboxylates **7-12.**

Compound 7

Reflux time: 5 h, Yield 82%, m.p. 62°C, m.f. $C_{36}H_{34}O_6$, 562 $M^{+\bullet}$, C 76.71_{found} 76.85_{cal} H 5.99_{found} 6.09_{cal}; IR (KBr) ν (cm^{-1}): 3052, 2980, 2924, 2854, 1663, 1738, 1607, 757, 694; ^{1}H NMR (δ ppm), (*J* Hz): 0.93 (6H, t, CH_2CH_3 at C-1, J=5.2), 2.99-2.95 (2H, H_{5a}, m), 3.14-3.00 (2H, H_{5a}, m), 3.68-3.61 (1H, H_6, m), 3.95-3.87 (4H, m, CH_2CH_3 at C-1), 4.11 (1H, H_1, d, J=13.6), 6.54 (1H, d, H_3, J=2.0), 7.72-7.37 (14H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.28 C-5, 43.46 C-6, 59.89 CH_2CH_3 at C-1, 58.67 C-1, 122.89 C-3, 159.29 C-4, 169.31 C=O at C-1, 194.27 C-2, 130.10-124.16 -$C_{arom.}$, 140.28, 139.86, 138.00, 137.32 *ipso*-C's.

Compound 8

Reflux time: 3 h, Yield 86%, m.p. 90°C, m.f. $C_{36}H_{32}F_2O_6$, 598 $M^{+\bullet}$, C 72.14_{found} 72.23_{cal} H 5.31_{found} 5.39_{cal}; IR (KBr) ν (cm^{-1}): 3063, 2986, 2925, 1664, 1738, 1600, 832, 756; ^{1}H NMR (δ ppm), (*J* Hz): 0.92 (6H, t, CH_2CH_3 at C-1, J=7.2), 2.99-2.94 (2H, H_{5a}, m), 3.12-3.05 (2H, H_{5a}, m), 3.67-3.59 (1H, H_6, m), 3.95-3.85 (4H, m, CH_2CH_3 at C-1), 4.09 (1H, H_1, d, J=14.3), 6.52 (1H, s, H_3), 7.81-7.18 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.27 C-5, 43.39 C-6, 59.89 CH_2CH_3 at C-1, 58.59 C-1, 122.84 C-3, 158.07 C-4, 169.15 C=O at C-1, 194.19 C-2, 128.91-115.64 -$C_{arom.}$, 162.11, 140.25, 133.80, 129.00 *ipso*-C's.

Compound 9

Reflux time: 4 h, Yield 80 %, m.p. 72°C, m.f. $C_{36}H_{32}Cl_2O_6$, 630 M^{+} , C 68.31_{found} 68.47_{cal} H 4.98_{found} 5.11_{cal}; IR (KBr) ν (cm^{-1}): 3052, 2980, 2927, 1665, 1738, 1609, 825, 679; ^{1}H NMR (δ ppm), (*J* Hz): 0.92 (6H, t, CH_2CH_3 at C-1, J=7.0), 2.97-2.93 (2H, H_{5a}, m), 3.07-3.00 (2H, H_{5a}, m), 3.66-3.63 (1H, H_6, m), 3.93-3.87 (4H, m, CH_2CH_3 at C-1), 4.11 (1H, H_1, d, J=15.6), 6.56 (1H, d, H_3, J=2.0), 7.76-7.38 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 13.79 CH_2CH_3 at C-1, 35.20 C-5, 43.35 C-6, 59.91 CH_2CH_3 at C-1, 58.60 C-1, 123.28 C-3, 157.89 C-4, 169.09 C=O at C-1, 194.21 C-2, 129.78-127.61 -$C_{arom.}$, 140.22, 136.17, 135.14 *ipso*-C's.

Compound 10

Reflux time: 3 h, Yield 78 %, m.p. 128°C, m.f. $C_{36}H_{32}Br_2O_6$, 718 $M^{+\bullet}$, C 59.87_{found} 60.02_{cal} H 4.37_{found} 4.48_{cal}; IR (KBr) ν (cm^{-1}): 3063, 2974, 2923, 2849, 1664, 1737, 1607, 825, 756; ^{1}H NMR (δ ppm), (*J* Hz): 0.85-0.97 (6H, m, CH_2CH_3 at C-1), 2.99-2.92 (2H, H_{5a}, m), 3.08-3.00 (2H, H_{5a}, m), 3.68-3.56 (1H, H_6, m), 3.94-3.86 (4H, m, CH_2CH_3 at C-1), 4.03 (1H, H_1, d, J=14.5), 6.58 (1H, d, H_3, J=3.0), 7.67-7.28 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.31 CH_2CH_3 at C-1, 35.44 C-5, 43.90 C-6, 60.51 CH_2CH_3 at C-

1, 59.22 C-1, 123.78 C-3, 158.41 C-4, 169.82 C=O at C-1, 194.59 C-2, 128.23-124.46 -$C_{arom.}$, 137.04, 132.22, 129.07 *ipso*-C's.

Compound **11**

Reflux time: 6 h, Yield 85 %, m.p. 186°C, m.f. $C_{38}H_{38}O_{8,}$ 622 $M^{+\bullet}$, C 73.11_{found} 73.29_{cal} H 6.03_{found} 6.15_{cal}; IR (KBr) ν (cm^{-1}): 3030, 2958, 2924, 2850, 1653, 1737, 1601, 831, 756; ^{1}H NMR (δ ppm), (*J* Hz): 0.94 (6H, m, CH_2CH_3 at C-1, J=7.2), 3.02-2.94 (2H, H_{5a}, m), 3.09-3.03 (2H, H_{5a}, m), 3.64-3.60 (1H, H_6, m), 3.81 (6H, s, OCH_3 at phenyl rings), 3.92-3.90 (4H, m, CH_2CH_3 at C-1), 4.06 (1H, H_1, d, J=14.5), 6.51 (1H, d, H_3, J=1.5), 7.71-6.99 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.30 CH_2CH_3 at C-1, 35.40 C-5, 43.95 C-6, 60.34 CH_2CH_3 at C-1, 59.17 C-1, 55.85 OCH_3 at phenyl rings, 121.53 C-3, 159.11 C-4, 169.78 C=O at C-1, 194.50 C-2, 128.70-114.68 -$C_{arom.}$, 161.74, 140.88, 129.74 *ipso*-C's.

Compound **12**

Reflux time: 6 h, Yield 90 %, m.p. 222°C, m.f. $C_{38}H_{38}O_{6,}$ 590 $M^{+\bullet}$, C 77.12_{found} 77.26_{cal} H 6.38_{found} 6.48_{cal}; IR (KBr) ν (cm^{-1}): 3030, 2977, 2926, 2854, 1658, 1738, 1601, 811, 756; ^{1}H NMR (δ ppm), (*J* Hz): 0.92 (6H, m, CH_2CH_3 at C-1, J=7.0), 2.33 (6H, s, CH_3 at phenyl rings), 2.99-2.95 (2H, H_{5a}, m), 3.13-3.04 (2H, H_{5a}, m), 3.68-3.57 (1H, H_6, m), 3.92-3.90 (4H, m, CH_2CH_3 at C-1), 4.09 (1H, H_1, d, J=14.3), 6.52 (1H, s, H_3), 7.63-7.24 (12H, m, $H_{arom.}$); ^{13}C NMR (δ ppm): 14.30 CH_2CH_3 at C-1, 21.32, 21.34 CH_3 at phenyl rings, 35.66,35.56 C-5, 43.96, 44.08 C-6, 60.40, 60.37 CH_2CH_3 at C-1, 59.17 C-1, 123.88, 122.55 C-3, 159.63, 158.93 C-4, 169.85, 169.73 C=O at C-1, 194.71 C-2, 129.93-126.78 -$C_{arom.}$, 143.02, 141.00, 140.83, 140.53, 135.54, 134.87 *ipso*-C's.

Microbiology

Materials

All the clinically isolated fungal strains namely *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. dubliniensis* and *C. krusei* are obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

In vitro Anticandidal Activity By Two Fold Serial Dilution Method

Minimum inhibitory concentration (MIC) in μg/mL values is carried out by two-fold serial dilution method[17]. The respective test compounds **(15-21)** are dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial fungal spores) is prepared at 37 ± 1°C from 1 to 7 days old Sabouraud's agar (Hi-media, Mumbai, India) slant cultures were suspended in seeded broth (SDB). The colony forming units (CFU) of the seeded broth are determined by plating technique and adjusted in the range of 10^4-10^5 CFU/mL. The final inoculum's size was 1.1-1.5 Ξ 10^2 CFU/mL for antifungal assay. Testing is performed at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this is diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions are obtained. A set of assay tubes containing only seeded broth is kept as control. The tubes are incubated in BOD incubators (Sigma Instruments, Chennai, India) at 28±1°C for fungi. The minimum inhibitory concentrations (MICs) are recorded by visual observations after 72-96 h (for fungi) of incubation. Fluconazole is used as standard drug for *Candida species*.

Results

The straight forward approach for the synthesis of highly functionalized cyclohexenone ethyl carboxylates **7-12** is as follows: Novel *bis* chalcones **1-6** are synthesized by the Claisen-Schmidt condensation of terephthaldehyde with substituted acetophenones in the presence of alcoholic sodium hydroxide base catalyst. Treatment of *bis* chalcones **1-6** with ethyl acetoacetate in the presence of sodium ethoxide in refluxing ethanol (Figure 1) afford highly functionalized cyclohexenone ethyl carboxylates 7-12. The reaction mechanism (Figure 2) involves the formation of Michael addition product by ethyl acetoacetate with *bis* chalcones **1-6** in the presence of base, sodium ethoxide. Afterwards the Michael addition product undergoes intramolecular aldol reaction in the presence of sodium ethoxide base, to yield the title compounds **7-12**. The structures of all the synthesized compounds **7-12** are confirmed by m.p.'s, FT-IR, MS, ^{1}H NMR, ^{13}C NMR spectra and elemental analysis.

In vitro anticandidal activity of highly functionalized cyclohexenone ethyl carboxylates **7-12** is studied against the *Candida* species *viz., C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. dubliniensis* and *C. krusei.* Fluconazole is used as a standard drug. Minimum inhibitory

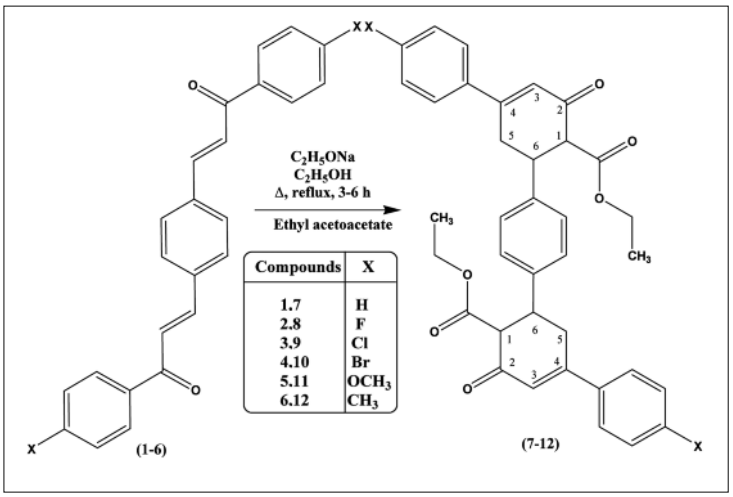

Figure 1. Synthetic route for the formation of highly functionalized cyclohexanone ethyl carboxylates.

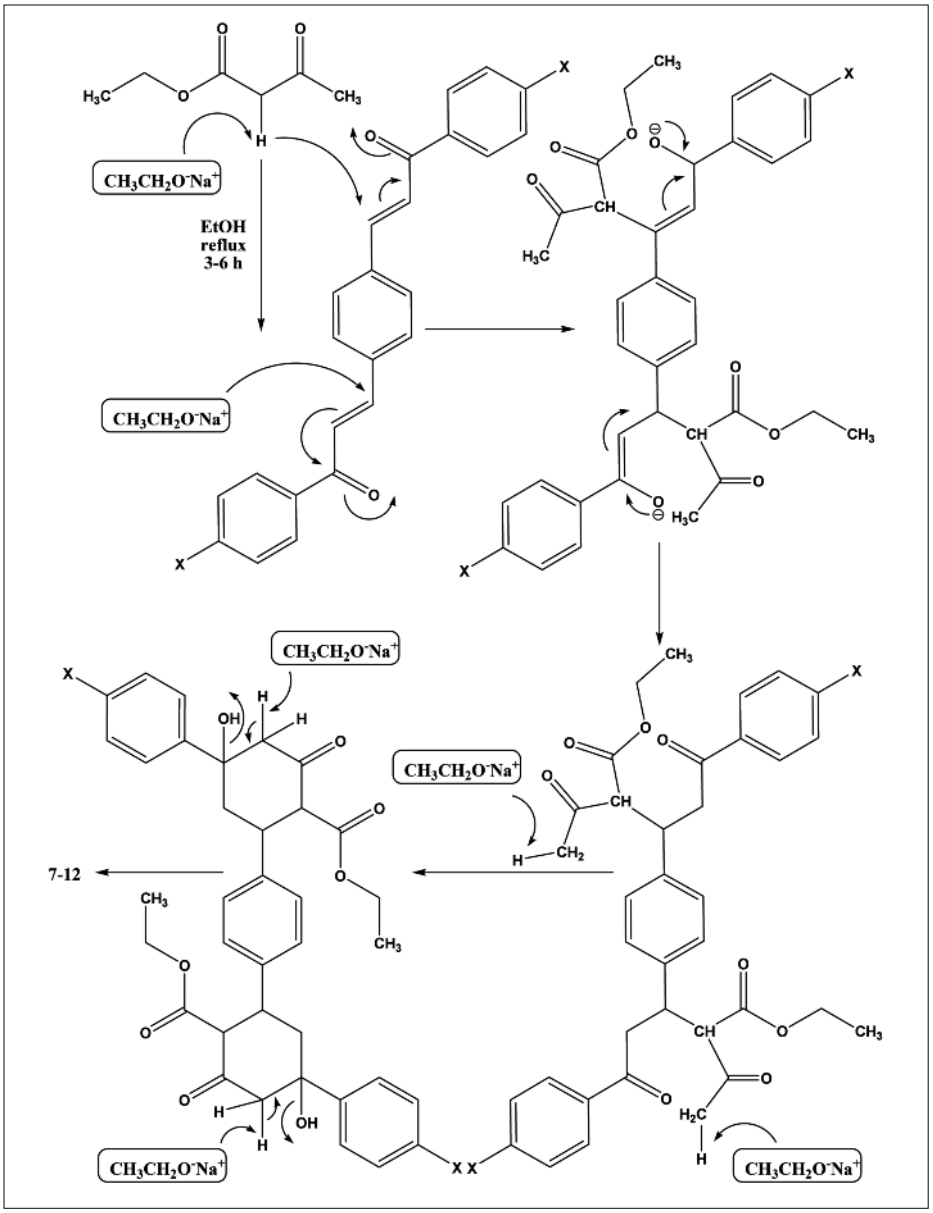

Figure 2. Mechanistic pathway for the formation of title compounds **7-12**.

Table I. *In vitro* anticandidal evaluation of synthesized **7-12**.

Compounds	X	Minimum inhibitory concentration (MIC) in µg/mL					
		C. albicans	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. dubliniensis*	*C. krusei*
7	H	12.5	25	200	100	6.25	50
8	F	6.25	12.5	12.5	6.25	25	6.25
9	Cl	12.5	12.5	6.25	12.5	6.25	12.5
10	Br	12.5	25	25	6.25	12.5	6.25
11	OCH_3	12.5	12.5	6.25	50	200	100
12	CH_3	25	25	100	200	25	25
Fluconazole		12.5	12.5	25	12.5	25	12.5

concentration (MIC) in µg/mL values is reproduced in Table I and their pictorial representation is shown in Figure 3.

Discussion

Structural Elucidation of bis Cyclohexenone Ethyl Carboxylate 7

In order to discuss the spectral data of the synthesized compounds **7-12,** compound **7** is chosen as the representative compound.

Analysis of Ft-Ir Spectrum of bis Cyclohexenone Ethyl Carboxylate 7

FT-IR spectrum of compound **7** shows two strong characteristic absorptions at 1738 and 1663 cm^{-1}due to ester carbonyl and ketone functional groups respectively. The band at 1607 cm^{-1} is due to the presence of C=C stretching frequency. The absorption frequency at 3052, 2980 cm^{-1} is assigned to aromatic C-H stretching vibration and the absorption frequencies at 2924 and 2854 cm^{-1} is assigned to aliphatic C-H stretching vibration. The observed ester carbonyl, ketone and C=C stretching vibrational bands are supporting evidence for the formation of synthesized compound **7**.

Analysis of ^{1}H NMR spectrum of bis Cyclohexenone Ethyl Carboxylate 7

In the ^{1}H NMR spectrum of **7**, a triplet observed at 0.93 ppm (J=5.2 Hz) corresponding to six protons and this signal is due to ester methyl protons at C-1. A multiplet observed at 3.95-3.87 ppm corresponding to four protons and this signal is due to ester methylene protons at C-1. Three multiplets are obtained in the range 2.99-2.95, 3.14-3.00 and 3.68-3.61 ppm and they are due to H-5a, H-5e and H-6 protons. The doublet at 4.11 ppm (J=13.6 Hz) has been assigned to H-

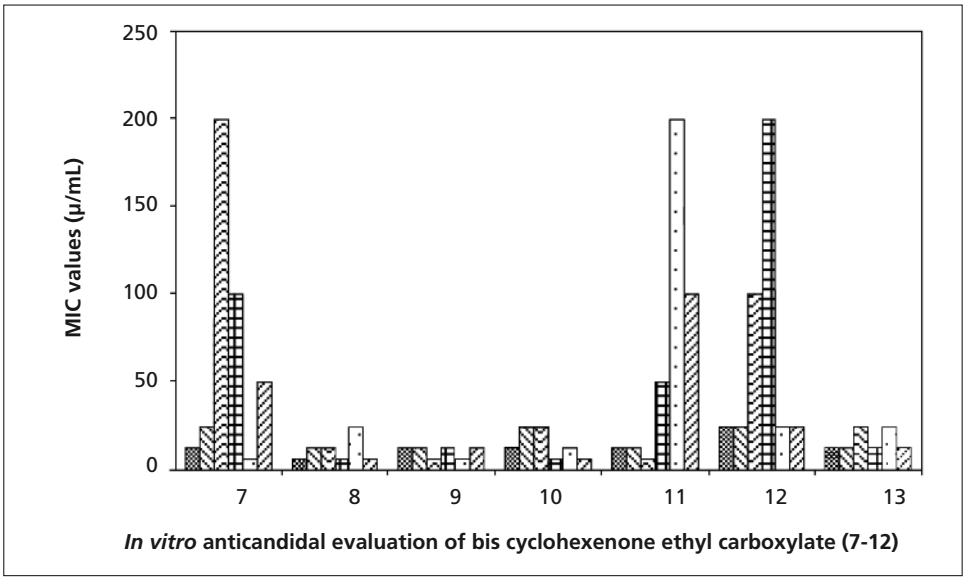

Figure 3. Pictorial representation of *in vitro* anticandidal activity MIC values for **7-12**.

1 proton. The doublet observed in downfield region at 6.54 ppm (J=2.0 Hz) is due to H-3 proton. The aromatic protons appeared as a multiplet in the range 7.72-7.37 ppm.

Analysis of ^{13}C NMR Spectrum of bis Cyclohexenone Ethyl Carboxylate 7

The ^{13}C resonances at 194.27 ppm is assigned to C-2 carbonyl carbon whereas carbon resonances observed at 169.31 ppm are assigned to ester carbonyl carbons. The ^{13}C resonances at 35.28 and 43.46 ppm are due to the C-5 and C-6 carbons respectively. The ^{13}C resonance observed at 59.89 and 13.79 ppm are assigned to ester methylene and methyl carbons at C-1 respectively. The signal observed at 58.67 ppm is assigned to C-1 carbon, whereas the signal at 122.89 ppm is assigned to C-3 carbon. The aromatic carbons are observed in the range of 130.10-124.16 ppm. C-4 carbon resonates at 159.29 ppm. The remaining ^{13}C signals at 140.28, 139.86, 138.00 and 137.32 are due to *ipso* carbons.

In vitro Anticandidal Evaluation of Highly Functionalized *bis* Cyclohexenone Ethyl Carboxylates 7-12

A close survey of the MIC values indicates that all the tested derivatives **7-12** exhibited a varied range (6.25-200 µg/mL) of anticandidal activity against all the tested *Candida* species. Compound **7**, having no substitution at the phenyl rings attached to C-4 carbon of cyclohexenone moiety exerts excellent to moderate activity against all the tested *Candida* species and show MIC value in the range of 6.25-200 µg/mL. Compound **7** shows four fold increases in activity (MIC value = 6.25 µg/mL) against *C. dubliniensis* when compare to standard drug, Fluconazole which show MIC value of 25 µg/mL. But compound **7** shows potent equal activity like that of drug Fluconazole against *C. albicans*and shows MIC value of 12.5 µg/mL.

Electron withdrawing functional groups like fluoro, chloro and bromo groups in compounds **8, 9** and **10** at the phenyl rings attached to C-4 carbon of cyclohexenone moiety all exert excellent anticandidal activity against all the tested *Candida* species. All these compounds **8, 9** and **10** exhibit MIC value in the range of 6.25-25 µg/mL against all the tested strains. Compound **8** which have fluoro functional group exhibits excellent activity against *C. albicans, C. parapsilosis* and *C. krusei* at a MIC value of 6.25 µg/mL whereas against *C. tropicalis* and *C. glabrata* it shows activity at a MIC value of 12.5 µg/mL. Two fold increase in activity is noticed by compound **8** (MIC value = 6.25 µg/mL) against *C. albicans, C. parapsilosis* and *C. krusei* than the standard drug Fluconazole (MIC value = 12.5 µg/mL). Chloro substituted compound **10** exhibits superior activities against *C. glabrata* and *C. dubliniensis* at a MIC value of 6.25 µg/mL whereas against *C. albicans, C. tropicalis, C. parapsilosis and C. krusei* it shows activity at a MIC value of 12.5 µg/mL. Four fold increase in activity is noticed by compound **10** (MIC value = 6.25 µg/mL) against *C. glabrata and C. dubliniensis* than the standard drug Fluconazole (MIC value = 25 µg/mL). Bulky bromo substituted compound reveals excellent activity against *C. parapsilosis* and *C. krusei* at a MIC value of 6.25 µg/mL whereas against *C. albicans and C. dubliniensis* it shows activity at a MIC value of 12.5 µg/mL. Two fold increase in activity is noticed by compound **10** (MIC value = 6.25 µg/mL) against *C. parapsilosis* and *C. krusei* than the standard drug Fluconazole (MIC value = 12.5 µg/mL). Replacement of electron withdrawing functional groups like fluoro, chloro and bromo groups in compounds 8, 9 and 10 by electron donating methoxy or methyl functional groups at the phenyl rings attached to C-4 carbon of cyclohexenone moiety for compounds **11** and **12** exert intermediate to good activity against all the tested *Candida* species which all show MIC in the range of 6.25-200 µg/mL. Methoxy substituted compound **11** against *C. albicans* and *C. tropicalis* show potent equal activity like that of standard drug Fluconazole and all of them show MIC value of 12.5 µg/mL. But four fold increase in anticandidal activity is noticed than drug Fluconazole by compound **11** against *C. glabrata* and shows MIC value of 6.25 µg/mL. Methyl substituted compound **12** shows moderate activity against all the tested *Candida sp.,* except against *C. parapsilosis* which shows activity only at a higher concentration of 200 µg/mL.

Conclusions

In crunch, a series of novel highly functionalized cyclohexenone ethyl carboxylates **7-12** are designed and synthesized from *bis* chalcones **1-6** and their structures are elucidated by their by their physical and analytical data. This reaction may have wide applicability in building a variety of heterocycles by choosing highly functionalized cyclohexenone ethyl carboxylates **7-12** as synthon, which has three versatile functional groups i.e., ketone, olefin and ester for the synthesis of structurally diverse organic compounds. Compound **8** against *C. albicans*, **9,11** against *C. glabrata*, **8,10** against *C. parapsilosis*, **7,9**

against *C. dubliniensis*, **8, 10** against *C. krusei* exhibited excellent anticandidal activity at a MIC value of 6.25 μg/mL. Likewise compound **7, 9-11** against *C. albicans*, **8, 9, 11** against *C. tropicalis*, **8** against *C. glabrata*, **9** against *C. parapsilosis*, **10** against *C. dubliniensis*, **9** against *C. krusei* revealed superior activity at a MIC value of 12.5 μg/mL. Results of the biological activity show that electron withdrawing substitutents like fluoro, chloro and bromo substituted derivatives exerted excellent antifungal activities, since electron withdrawing substituent increases the lipophilicity due to the strong electron withdrawing capability[18]. Moreover, electron withdrawing substitutents namely fluorine substitution was commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein ligand interactions[19]. These observations may promote a further development of our research in this field. Furthermore, the observed marked anticandidiasis activity of this group of highly functionalized cyclohexenone ethyl carboxylate derivatives may be considered as key steps for the building of novel chemical entities with comparable pharmacological profiles to that of the standard drugs.

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References

1) Rex HJ, Walsh JT, Sobel JD, Filler GS, Pappas GP, Dismukes EW, Edwards EJ. Practice guidelines for the treatment of candidiasis. Clin Infect Dis 2000; 30: 662-678.
2) Odds FC. Genital candidiasis. Clin Exp Dermatol 1982; 7: 345-354.
3) Humphrey MJ, Jevons S, Tarbit MH. Pharmacokinetic evaluation of UK-49, 858, a metamolically stable triazole antifungal drug, in animals and humans. Antimicrob Agent Chemother 1985; 28: 648-653.
4) Richardson K, Brammer KW, Marriot MS, Troke PF. Activity of UK-49,858 (fluconazole), a bistriazole derivative, against experimental infections with candida albicans ans Trichophyton mentagrophytes. Antimicrob Agent Chemother 1985; 28: 832-835.
5) Bisschop MPJM, Merkus JMWM, Scheygrond H, Van Cutsem J. Patients preference of oral treatment in vaginal candidiasis (a double-blind study). J Drug Ther Res 1985; 10: 587-589.
6) Kanagarajan V, Thanusu J, Gopalakrishnan M. Synthesis and in vitro microbiological evaluation of an array of biolabile 2-morpholino-N-(4,6-diarylpyrimidin-2-yl)acetamides. Eur J Med Chem 2010; 45: 1583-1589.
7) Thanusu J, Kanagarajan V, Gopalakrishnan M. Synthesis, spectral analysis and in vitro microbiological evaluation of 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones as a new class of antibacterial and antifungal agents. Bioorg Med Chem Lett 2010; 20: 713-717.
8) Gopalakrishnan M, Thanusu J, Kanagarajan V, Govindaraju R. Odd formation of 3-chloro-1-hydroxy-2,6-diarylpiperidin-4-ones: Synthesis, antibacterial and antifungal activities. J Enz Inhib Med Chem 2009; 24: 52-58.
9) Mori K, Kato M. Synthesis and absolute configuration of (+)-hernandulcin. A new sesquiterpene with intensely sweet taste. Tetrahedron Lett 1986; 27: 981-982.
10) Bringmann G, Lang G, Muhlbacher J, Schaumann K, Steffens S, Rytik PG, Hentschel U. Sorbicillactone A, a structurally unprecedented bioactive novel-type alkaloid from a spongederived fungus. Marine Mol Biotech 2003; 1: 231-253.
11) Kong C, Xu X, Zhou B, Hu F, Zhang C, Zhang M. Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. Phytochemistry 2004; 65: 1123-1128.
12) Nagarajan K, David J, Shah RK. Central nervous system active 5-oxo-1, 4, 5, 6, 7, 8-hexahydrocinnolines. J Med Chem 1976; 19: 508-511.
13) Luu B, Aguilar JLGD, Junges CG. Cyclohexenonic longchain fatty alcohols as neuronal growth stimulators. Molecules 2000; 5: 1439-1460.
14) Li Y, Harper JK, Grant DM, Tombe BO, Bashyal B, Hess WM, Strobel GA. Ambuic acid, a highly functionalized cyclohexenones with antifungal activity from Pestalotiopsis spp. and Monochaetia sp. Phytochemistry 2001; 57: 463-468.
15) Li JY, Strobel GA. Jesterone and hydroxy-jesterone antioomycetcyclohexenenone epoxides from the endophytic fungus. Phytochemistry 2001; 57: 261-265.
16) Guthrie W, Wang XP. The aldol condensation of acetophenone with acetone. Can J Chem 1991; 69: 339-344.
17) Dhar MH, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants biological activity. Part I. Indian J Exp Biol 1968; 6: 232- 247.
18) Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliver Rev 1997; 23: 3-25.
19) Purser S, Moore PR, Swallow S, Gouverneur V. Fluorine in medicinal chemistry. Chem Soc Rev 2008; 37: 320-330.