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Green synthesis of bis-(β-dicarbonyl)-methane derivatives and biological evaluation as putative anticandidial agents

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ABSTRACT

In this work the effectiveness of two different reaction media, an Ionic Liquid (IL) and a Deep Eutectic Solvent (DES), as greener, alternative solvents for the synthesis of bioactive bis-(β-dicarbonyl)-methane derivatives is examined. A domino Knoevenagel-Michael reaction between selected aromatic aldehydes and heterocyclic 1,3-dicarbonyl compounds was successfully accomplished, producing the desired compounds in satisfactory yields. The solvents were recycled and reused three times without noticeable decrease in reaction yields. A putative conformation of compound **4g** was determined using NMR spectroscopy and an "anti" orientation of the fused aromatic rings was proposed. Moreover, some of the bis-(β-dicarbonyl)-methane derivatives were tested for their antifungal activity against four *Candida albicans* strains. Biscoumarin **6** and bisquinolinone **4d** exhibited promising anticandidial activity. In parallel, in silico ligand-based similarity calculations provided a putative mechanism of action of the examined compounds through CYP51 inhibition.

1. Introduction

Multicomponent reactions (MCRs) are one-pot reactions in which three or more molecules react in the same reaction vessel affording a single product without isolation of any intermediate. This methodology is characterized by high atom economy and exploratory power, whereas it is operationally simple and easily automatable offering a target and diversity-oriented synthesis [1–3].

Numerous methods and catalysts have been reported for the synthesis of heterocycles via MCRs however, some possess serious drawbacks such as long reaction time, refluxing in volatile organic solvents in high temperature, copious work-up procedures, use of expensive catalysts which cannot be recycled [4–7]. Nowadays, the increasing environmental awareness leads researchers to emphasize on the development of new methods according to the spirit of sustainable development.

In this context, the synergistic use of MCRs and Ionic Liquids (ILs) is a perfect combination for eco-compatible heterocyclic increasingly useful tools for the synthesis of pharmaceuticall synthesis. MCRs performed in green and eco friendly media, such as ILs and Deep Eutectic Solvents (DESs), have become increasingly useful tools for the synthesis of pharmaceutically active heterocyclic compounds since the development of a rapid and cleaner technology is of great importance [8–13].

In our recent work, synthesized and characterized a series of biodegradable protic based ILs [14,15]. Some of the synthesized ethanolamine-based ILs, have been used as solvents and catalysts in organic synthesis [8], as media for biooxidations [16], as media for hydrolytic and synthetic reactions catalyzed by lipase-inorganic hybrid nanoflowers [17], as entrainers for the separation of azeotropic mixtures [18] and as reducing agents in silver nanoparticles synthesis [19] and silver mesoparticles [20].

In recent years, DESs are reported as low-cost eutectic mixtures, with similar physicochemical properties and phase behavior to ILs and gaining increasing interest in many scientific fields [13,21]. From the view point of green chemistry, DESs such as choline chloride/urea (CC/U), are in some cases more attractive than ILs, as their synthetic process is relatively simple, cheap and 100% atom

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economic. DESs are of great interest as an alternative to room temperature ILs and demonstrate great importance and advantages in several organic transformations [13]. Their preparation usually involves mixing one or more hydrogen bond donors and one or more hydrogen bond acceptors from natural and readily available starting materials in the proper ratio under heating until a homogenous liquid is formed [22].

As a continuation of our previous study on biscoumarin synthesis [8], we herein present the synthesis of some new bis-(β-dicarbonyl)-methane compounds based on the quinolinone framework, under mild conditions, using the IL N-hydroxyethylammonium formate as solvent.

Moreover, as the recyclability and biodegradability of DESs in combination with the high atom economy of MCRs is bound to provide a greener approach to a wide variety of bioactive molecules, we herein report a comparative study of the use of CC/U as solvent for the domino Knoevenagel/Michael reactions resulting to the synthesis of geminal bis-(β-dicarbonyl)-methane compounds in lower reaction time and satisfactory yields under also mild conditions. The recyclability and reusability of both IL and DES were also investigated. The obtained compounds were structurally elucidated and conformationally examined.

Coumarins and quinolinones, consist an important class of heterocyclic compounds that found numerous natural products, and constitute targets in organic synthesis since they are serving as a class of substances with diverse biological and pharmacological activities [23–26]. Many of their derivatives, like biscoumarins [5,27,28] and bisquinolinones [4,6] are bis-(β-dicarbonyl)-methane compounds. According to the literature, biscoumarin analogues are endowed with significant activities such as antimicrobial [29], antimalarial [30,31], antileishmanial [32], antibacterial [33,34], antitumor [35], anticoagulant [36] etc.

To a step further, due to the well-known biological and pharmacological activity of coumarins, quinolinones and some of their derivatives, the synthesized compounds were tested for their antifungal activity against four strains of *Candida albicans*, a fungal pathogen responsible for candidiasis in human hosts [37]. An abnormal overgrowth of *Candida* species had been observed in the gastrointestinal, urinary and respiratory tracts, not only in immunocompromised patients (AIDS, diabetes) but also related to nosocomial infections and in healthy individuals [38,39].

2. Results and discussion

2.1. Chemistry

The bis-(β-dicarbonyl)-methane derivatives **4a**-**4j** were prepared by one step reaction, using as solvents an IL or DES, as presented in Scheme 1.

A plausible mechanism can be described as follows: the aldehyde carbonyl is activated by hydrogen bond formation (with either the H_3N^+ group of the IL or the NH_2 group of urea of the DES) and a nucleophilic attack by the β-dicarbonyl compound occurs. The resulting Knoevenagel adduct **3**, is an α,β-unsaturated dicarbonyl compound which can act as a good Michael acceptor with enhanced electrophilic character by H-bond formation with the IL or DES. A second molecule of the β-dicarbonyl compound acts as the Michael donor to produce the final bis(β-dicarbonyl) methane compounds **4a**-**4j** [13,40,41].

The aromatic aldehydes were chosen with respect to the electronic nature of the substituents (Cl, OMe, OH) as well as the position of substitution. We have previously developed an environmentally friendly methodology for the synthesis of biscoumarins via a domino Knoevenagel Michael reaction using N-hydroxyethylammonium formate as an alternative solvent. After optimization studies it was ascertained that the reaction between 4-hydroxy-coumarin and various benzaldehydes in the synthesized ILs proceeded smoothly at 40 °C in a period of time up to 3 h [8].

In an effort to expand the scope of our previous study, we investigated the 2:1 reaction between quinolinone derivatives such as 2,4-quinolinediol (**1a**), 4-hydroxy-1-methyl-2(1*H*)-quinolone (**1b**) and different substituted benzaldehydes (**2a**-**2f**) using as solvent N-hydroxyethylammonium formate using the same conditions (3 h at 40 °C) (Table 1, Method A).

The desired compounds were obtained in satisfactory yields, after simple trituration with methanol at 60–65 °C. The substitution on the heterocyclic nitrogen does not seem to influence the reactivity of the starting quinolinones as the yields of compounds **4a**-**4c**, produced from NH-quinolinone **1a**, are comparable to those of compounds **4d**-**4f**, produced from NCH_3-quinolinone **1b**. Higher yields were obtained by reactions with the dimethoxy-substituted benzaldehydes (70–79%) whereas the products from p-chlorobenzaldehyde were obtained in lower yields (46 and 50%).

In order to boost the eco friendly character of the reported method we set out to examine the reaction using a DES as solvent so as to make the reaction procedure more environmentally and economically viable. In this context, we chose to use CC/U. ChCl is the most common quaternary ammonium salt used in DES synthesis since it is a low cost and nontoxic compound. In addition, it is classified as a provitamin in Europe and produced on large scale as an animal feed supplement [42]. When mixing ChCl with a hydrogen bond-donor such as urea, the hydrogen bonding between ChCl and urea is responsible for reaching the eutectic point.

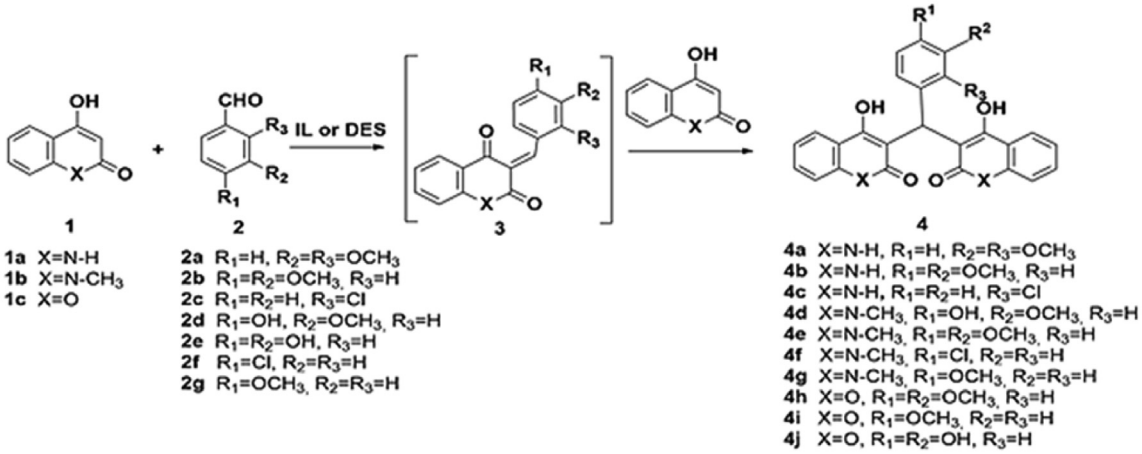
At the outset, we investigated the bisquinolinone derivatives **4a**-**4g** synthesis using CC/U as the solvent. Due to the poor solubility of 2,4-quinolinediol (**1a**) and 4-hydroxy-1-methyl-2(1*H*)-quinolone (**1b**) in CC/U, the reaction was inevitably carried out in higher temperature.

In order to optimize the reaction temperature, different temperatures were tested (Table 2). It is clear that by performing the reaction at 80 °C, the reaction proceeded smoothly in 4 h, providing the desired products in higher yields comparing to the other tested conditions.

After optimization studies, a range of aromatic aldehydes were subjected to reaction with 2,4-quinolinediol (**1a**) and 4-hydroxy-1-methyl-2(1*H*)-quinolone (**1b**) in CC/U, to afford the desired products 4b and **4d**-**4g** (Table 1, Method B).

According to results above, we can conclude that the use of CC/U as solvent, does not favour the reaction between 2,3-dimethoxybenzaldehyde (**2a**) and 2,4-quinolinediol (**1a**) (product **4a**). However, the reaction of **1a** with 3,4-dimethoxy-benzaldehyde produces compound **4b** in 58% yield. This difference in reactivity was not observed when the IL was used as a solvent. In that case, compound **4a** was obtained in 79% yield (Table 1). A plausible explanation for this observation can be the increased steric hindrance caused by the presence of a 2-methoxy-group on the aromatic ring which probably hinders the hydrogen bond formation between the carbonyl group and CC/U and does not facilitate the nucleophilic attack of **1a.**

Based on the positive results of the bisquinolinone synthesis using CC/U, we decided to test the reactivity of 4-hydroxycoumarin (**1c**). The 2:1 reaction between 4-hydroxycoumarin (**1c**) and 3,4-dimethoxy-benzaldehyde (**2b**) was investigated as a model reaction at 40 °C using CC/U as solvent. The reactants underwent a domino Knoevenagel – Michael reaction to yield the product **4h** in high purity and 41% yield within 1 h. Thus, the reaction can be accomplished in a shorter period of time, but the yield is less satisfactory comparing to our previous method where N-hydroxyethylammonium formate was used as solvent (75% at 40 °C in 3 h). Hence, in order to evaluate how the temperature affects the



Scheme 1. Bis-(β-dicarbonyl)-methane compounds (**4a**-**4j**) synthesis via a Knoevenagel−Michael condensation between aromatic aldehydes (**2a**-**2g**) and appropriate heterocyclic 1,3-dicarbonyl compound (**1a**-**1c**) in the presence of IL N-Hydroxyethylammonium formate or DES CC/U.

Table 1
Bisquinolinone synthesis via a domino Knoevenagel Michael reaction using N-hydroxyethylammonium formate (Method A) or CC/U (Method B) as solvent.

Product	Method A [a]	Method B [b]
	% Yield (after trituration procedure)	
4a	79	No reaction
4b	60	58
4c	50	_[c]
4d	78	64
4e	69	70
4f	46	25
4g	_[c]	52

[a] Reaction conditions: 3 h at 40 °C.
[b] Reaction conditions: 4 h at 80 °C.
[c] Not tested in these reaction conditions.

Table 2
Optimization of the reaction conditions of bisquinolinone synthesis via a domino Knoevenagel Michael reaction using as solvent CC/U.

Product	Temperature (°C)	Time	% Yield
4e	60	overnight	51
	80	4 h	70
4g	60	overnight	30
	80	4 h	52

reaction yields, three different temperatures were tested (40 °C, 60 °C and 80 °C) (Table 3). Through this investigation we can conclude that 3 h and 60 °C are the best conditions for the bis-coumarin synthesis using CC/U as solvent.

By comparing IL and DES as solvents in the domino Knoevenagel-Michael reactions studied here we can conclude that the reaction yields provided by Method A (using IL) are slightly higher comparing to those of Method B (using CC/U). This could be attributed to the "bulkier" structure of CC/U that may lead to steric hindrance when hydrogen bonds are formed between the CC/U and the carbonyl groups in both steps of the domino reaction. Additionally, the reaction conditions of Method A are milder and the reaction time lower, therefore, we could assume that Method A is more energy-efficient than Method B.

Table 3
Knoevenagel Michael MCR between 4-hydroxycoumarin (**1c**) and various benzaldehydes (**2b**, **2e** & **2g**) in different reaction conditions via CC/U.

Product	Temperature (°C)	Time	% Yield
4h	40	1 h	41
	60	1 h	51
	60	3 h	70
4i	40	3 h	44
	60	3 h	70
	80	3 h	68
4j	60	3 h	64

2.2. Recyclability and reusability

Recyclability and reusability of the solvent was performed in order to satisfy the green chemistry criteria. A model reaction was chosen and the reactants were mixed in 2:1 M ratio the appropriate amount of solvent, according to the appropriate method each time. After completion of the reaction (monitoring with TLC), the reaction mixture was shaken with water and was filtered to separate the solvent. The isolated solvent was evaporated and dried in high vacuum and heating until constant weight. Recyclability and reusability of the solvent was performed in order to satisfy the green chemistry criteria.

The solvent, reused efficiently up to successive 3 times providing the bis-(β-dicarbonyl)-methane derivative in good yields and high purity. The solvent´s purity was as tested through ^{1}H NMR spectroscopy before reuse (Fig. 1).

2.3. NMR based structural and conformational elucidation of selected compounds

In order to evaluate the main conformational features of the title compounds, 2D NMR experiments were implemented on bisquinolinone **4g,** which was selected as a representative compound. More specifically, 2D COSY was used to assign the protons of the aromatic region of **4g**. Specifically, cross peaks between 8.24 and 7.33 ppm, 7.33−7.62 ppm 7.62−7.42 ppm and 6.81−7.08 ppm present correlations between protons H-5,H-5′ − H-6,H-6′, H-6,H-6′ − H-7,H-7′and H-7,H-7′ − H-8, H-8′ and H-4″,H-6″ with H-3″,H-7″, respectively (see Supplementary material, Fig. S1).

The 2D NOESY spectrum is one of the most powerful methods for conformational analyses, since it allows tocorrelate nuclei through space at a distance smaller than 5 Å (NOE phenomenon). The presence of OH-4 − NCH_3-1′ and OH-4′ − NCH_3-1 off diagonal peaks (see Supplementary material, Fig. S2) clearly indicates an "anti" orientation of the fused aromatic rings providing the

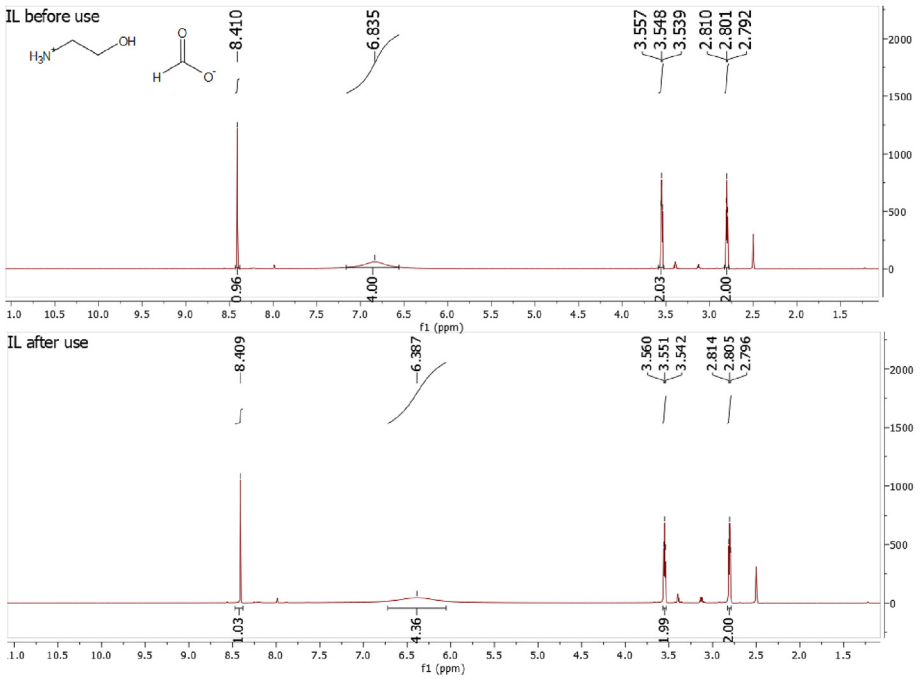


Fig. 1. ^{1}H NMR (DMSO-d_6) of N-Hydroxyethylammonium formate before (a) and after use (b) as solvent.

possibility for H bonds formation between the hydroxyl groups at positions 4 and 4′ with the corresponding carbonyl groups (Fig. 2 left).

In an effort to explore the conformational features, compound **4g** was minimized and subjected to random sampling conformational search from which 4 representative low energy conformations were generated (see Supplementary material, Fig. S3). All four conformers satisfied the above crucial NOE distance constrains and presented the formation of H bonds between the hydroxyl groups at positions 4 and 4′ with the corresponding carbonyl groups, reinforcing the initial hypothesis. The lowest energy conformation of compound **4g** that satisfying the NOE distance constraints is illustrated in Fig. 2 (right).

Furthermore, the existence of peaks between OH-4 and NCH_3-1′ with aromatic protons H-3″, H-7″ further describes the specific orientation of the fused rings with the para substituted aromatic ring.

A detailed assignment of the ^{13}C NMR spectrum was performed for the novel compound **4d** using ^{13}C, 2D heteronuclear HSQC and HMBC spectra. This analysis clearly shows that carbons 1N—CH_3 & 1′N—CH_3, C2 & C-2′, C4 & C-4′, C4a & C-4a′ and C8a & C-8a' (see Supplementary material, Table S1), give different signals in the spectrum. This observation can be attributed to the "anti" orientation of the fused aromatic rings which results to the chemical non-equivalence of these carbon atoms.

2.4. Evaluation of anticandidial activity

The antimicrobial activity of coumarins and quinolinones is known in the literature but bibliographic data of the antimicrobial activity and particularly the antifungal activity of bis-β-dicarbonyl methane products is still scarce. Some biscoumarin analogues have

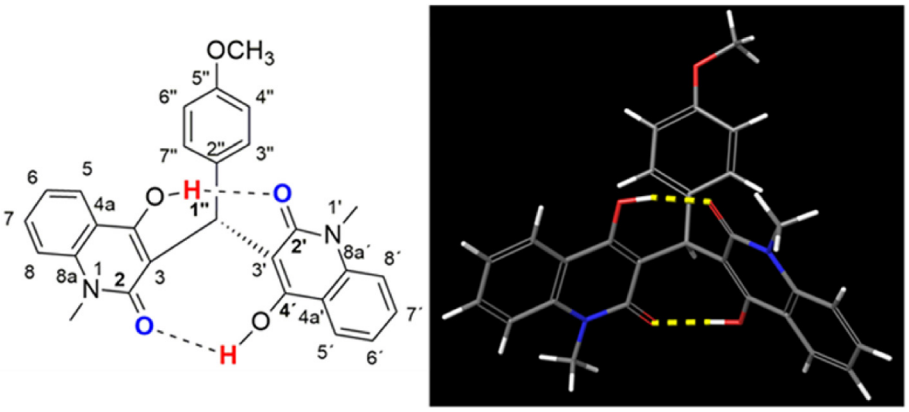


Fig. 2. (left) 3,3'-((4-methoxyphenyl)methylene)bis(4-hydroxy-1-methylquinolin-2(1*H*)-one) (**4g**) (right) The lowest energy conformation (E = −3.95 kcal mol^{-1}) of compound **4g** derived from random sampling conformational search, in accordance with crucial NOE distance constraints. Hydrogen bonds formation between 4-OH and 4′-OH with the corresponding 2-C═O and 2′-C═O are depicted with yellow lines. The calculated distances between OH-4 – NCH_3-1′ and OH-4′ – NCH_3-1 were 3.81 and 3.86 Å, respectively (pink lines) satisfying the NOE signals. The corresponding angles, including the following atoms OH-4/2′-C═O (163.7°) and OH-4′/2-C═O (172.3°) are presented with green color.

been tested for their antifungal activity against Staphylococcus aureus ATCC 6538 [29] and against *C. albicans* [43] with very promising results, whereas for the bisquinolinone analogues, to our knowledge, there is no corresponding bibliographic data. Thus, we set out to test some of the bis-coumarin and bis-quinolinone products for their antifungal activity and in particular against four *Candida albicans* isolates. Amphotericin B and Ketoconazole, both important drugs widely used in humans as antifungal agents [44,45] were used as controls.

In this context, five biscoumarins (**5**–**9**) synthesized in our previous work (Scheme 2) [8], biscoumarin **4h** and bisquinolinones (**4a** & **4e-f**) synthesized in the present work, were tested against four isolates of *C. albicans.*

The antifungal susceptibility of the tested compounds was evaluated as Minimum Inhibitory Concentrations (MIC) and as Minimum Fungicidal Concentration (MFC). MIC and MFC assay results of the tested compounds are shown in Table 4.

According to these results, it seems that biscoumarin **6** (MIC $0.794 \cdot 10^{-2}$ and MFC $1.587 \cdot 10^{-2}$ μmol·mL^{-1} for all the strains) and the novel bisquinolinone **4d** (MIC $0.774 \cdot 10^{-2}$ μmol·mL^{-1} και MFC $1.549 \cdot 10^{-2}$ μmol·mL^{-1} for all the strains) are the most effective compounds of the series, against all tested *Candida* strains comparing to Ketoconazolele that was used as the control antifungal drug (MIC $0.583 \cdot 10^{-2}$ μmol·mL^{-1} for strains 7/15, 8/15 & 527/14 and 0.301 for the strain 1/6/15 and MFC $9.408 \cdot 10^{-2}$ μmol·mL^{-1} for the strain 7/15, $1.167 \cdot 10^{-2}$ μmol·mL^{-1} for the strains 7/15 & 8/15 and $0.583 \cdot 10^{-2}$ μmol·mL^{-1} for the strain 1/6/15).

A more detailed analysis of the results shows that the position and electronic character of the substituents on the phenyl ring are important for activity. The most active biscoumarin **6** possesses two electron-donating methoxy groups at positions 2 and 3 of the aromatic ring. Changing the position of the 2-OCH_3 group to position 4 (compound **4h**) results to significant loss of activity against strains 7/15 and 8/15 (MIC $3.175 \cdot 10^{-2}$ μmol·mL^{-1} και MFC $6.349 \cdot 10^{-2}$ μmol·mL^{-1} for both strains) and a moderate loss of activity against strains 527/14 and 1/2/15 (MIC $1.587 \cdot 10^{-2}$ μmol·mL^{-1} και MFC $3.175 \cdot 10^{-2}$ μmol·mL^{-1} for both strains). However, the presence of a 4-OH and a 3-OCH_3 groups (compound **5**) as well as only a 4-OH (compound **9**) leads to loss of activity against all strains, thus we can postulate that a polar, hydrogen bond forming OH group does not favour anticandidial activity in the biscoumarin series tested. On the other hand, the presence of electron withdrawing substituents at position 4, like Cl (compound **7,** MIC $0.839 \cdot 10^{-2}$ μmol·mL^{-1} και MFC $1.678 \cdot 10^{-2}$ μmol·mL^{-1} for all strains) gives rise to a molecule with activity comparable to compound **6** whereas the presence of a 4-NO_2 group (compound **8**) decreases the activity.

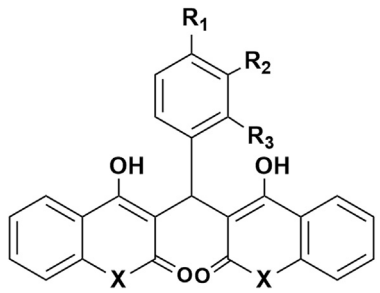
As far as bisquinolinones are concerned, compound **4d** possessing a N–CH_3 structural feature as well as 4-OH and 3-OCH_3 substituents is the most active compound among all those tested in this work. Replacement of the 4-OH with 4-OCH_3 (compound **4e**, MIC $1.504 \cdot 10^{-2}$ μmol·mL^{-1} και MFC $3.001 \cdot 10^{-2}$ μmol·mL^{-1} for strains 7/15 and 8/15) leads to loss of activity whereas the 4-Cl substituted compound **4f** is inactive.

Comparing biscoumarin **6** with bisquinolinone **4a,** it is obvious that the bioisosteric replacement of the heterocyclic O with the N–H group does not promote activity thus, it can be postulated that the presence of a hydrogen bond donor such as the N–H moiety is not a desirable structural feature for this kind of activity. It is evident though that a much larger number of analogues should be tested in order to obtain a better insight on the structure -activity relationship of bis-(β-dicarbonyl)-methane derivatives.

All compounds showed lower antifungal effect than Amphotericin B, which exhibited MIC at 0.068 10^{-2} μmol·mL^{-1} and MFC at $0.135 \cdot 10^{-2}$ μmol·mL^{-1}.

Table 4
Anticandidal activity of bis-β-dicarbonyl methane compounds (10^{-2} μmol·mL^{-1}) against four isolates of *Candida albicans.*

Compound		*C. albicans*			
		7/15	***8/15***	***527/14***	***1/6/15***
5	MIC	3.272			
	MFC	6.544			
6	MIC	0.794			
	MFC	1.587			
7	MIC	0.839			
	MFC	1.678			
8	MIC	1.639			
	MFC	3.279			
9	MIC	3.501			
	MFC	7.002			
4h	MIC	3.175		1.587	
	MFC	6.349		3.175	
4a	MIC	1.594			
	MFC	3.188			
4d	MIC	0.774			
	MFC	1.548			
4e	MIC	1.504			
	MFC	3.001		1.504	
4f	MIC	3.172		3.172	
	MFC	6.343			
Ketoconazole	MIC	0.583			0.301
	MFC	9.408	1.167		0.583
Amphotericin B	MIC	0.068			
	MFC	0.135			



5 X=O, R_1=OH, R_2=OCH_3, R_3=H
6 X=O, R_1=H, R_2=R_3=OCH_3
7 X=O, R_1=Cl, R_2=R_3=H
8 X=O, R_1=NO_3, R_2=R_3=H
9 X=O, R_1=OH, R_2=R_3=H

Scheme 2. Biscoumarins **5**–**9** [8].

2.5. In silico calculations

In order to propose a putative mechanism of antifungal activity for bis-β-dicarbonyl methane compounds, ligand-based similarity calculations were performed. The similarity property principle (SPP) states that similar compounds should have similar properties [46]. Based on this principle a combination of similarity metrics was performed between the most widely used antifungal agents, the azoles (see Supplementary material, Fig. S4) and the bis-β-dicarbonyl methane synthetic compounds (Table 4). Particularly, the methodology includes molecular and chemical similarity

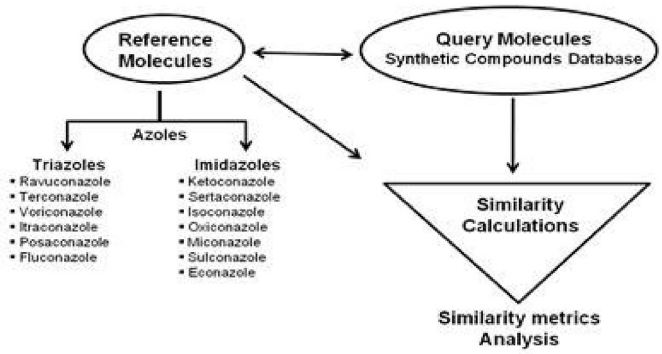


Fig. 3. Flowchart of the methodology for similarity measurements.

concepts [47] and Fig. 3 presents the flowchart of the methodology.

The similarity metrics analysis indicates that the synthetic compounds (query molecules) present high similarity with both triazoles and imidazoles subsets of azoles drug class (see Supplementary material, Table S2) with a Tanimoto good coefficient values, in many cases higher than the literature threshold of 0.85 [48]. Thus, all synthetic compounds present acceptable similarity correlation compared to both triazole and imidazole subsets ($0.5<Tc<0.9$).

Especially compounds **6** and **4d**, which present the highest anticandidial activity, show a high scoring similarity in comparison with Ravuconazole, 0.92 and 0.905, respectively (see Supplementary material, Table S2). Obviously, since the synthetic compounds follow the same molecular and chemical pattern as azoles a proposed mechanism for their anticandidial activity could be the inhibition of the lanosterol 14α-demethylase (CYP51) blocking ergosterol synthesis, a unique component in fungal cell membrane.

3. Conclusions

In conclusion, we have successfully developed a simple and efficient method for the synthesis of bis-(β-dicarbonyl)-methane derivatives, some of which are reported for the first time in the literature (**4a**, **4c** & **4d**). The efficiency of two different solvents in the domino Knoevenagel-Michael reaction was examined: the IL N-hydroxyethylammonium formate (Method A) and the DES CC/U (Method B). Both solvents yielded the final products in high purity and satisfactory yields after simple work-up procedure. Method A seems to be more energy efficient comparing to Method B due to the milder reaction conditions and shorter reaction time. Both N-hydroxyethylammonium formate and CC/U can be easily recycled and successfully reused up to 3 times, highlighting the proposed method's advantageous and environmentally friendly character. Finally, some of the bis-(β-dicarbonyl)-methane derivatives were tested for their antifungal activity against four strains of *C. albicans,* responsible for candidiasis, one of the most common nosocomial infections. Comparing to Ketoconazole (reference drug) the best results were given from biscoumarin **6** (X = O, $R_2 = R_3 = OCH_3$), with MIC $0.794 \cdot 10^{-2}$ and MFC $1.587 \cdot 10^{-2}$ $\mu mol \cdot mL^{-1}$ as well as the novel bisquinolinone **4d** (X = CH_3, $R_2 = OCH_3$, $R_3 = OH$), with MIC $0.774 \cdot 10^{-2}$ $\mu mol \cdot mL^{-1}$ and MFC $1.549 \cdot 10^{-2}$ $\mu mol \cdot mL^{-1}$ for all the fungal strains. Finally, similarity calculations results indicate that the bis-β-dicarbonyl methane compounds could possibly exert their antifungal activity through inhibition of the *Candida Albicans* CYP51 enzyme.

4. Experimental section

4.1. Materials

Ethanolamine analytical grade (Fischer Scientific), formic acid 98−100% (Sigma Aldrich), choline chloride 98+% (Alfa Aesar), urea purum (Fluka), 4-hydroxy-coumarin 98+% (Alfa Aesar), 2,4-quinolinediol 97% (Sigma Aldrich), 4-hydroxy-1-methyl-2(1H)-quinolone 98% (Sigma Aldrich), 3,4-dimethoxy-benzaldehyde (Sigma Aldrish), 2,3-dimethoxy-benzaldehyde 98% (Sigma Aldrich), 3,4-dihydroxy-benzaldehyde 97% (Fluka), anisaldehyde 98% (Fluka), 4-chloro-benzaldehyde 96% (Acros Organics), 2-chloro-benzaldehyde,vanillin >98% (Fluka). All reagents were used without additional purification.

4.2. Methods

NMR spectroscopy. Synthesized compounds were structurally elucidated using 300 & 600 MHz Varian NMR spectrometers using DMSO-d_6 and $CDCl_3$ 99,9 atom % D as solvents. ^1H NMR spectra were recorded at 298 K under the following parameters. Spectral width (SW) = -2−14 ppm, number of scans (ns) = 32. For the 2D homonuclear NMR experiments a Varian 600 MHz spectrometer equipped with a triple resonance probe{HCN} was used at 298 K. 2D $^1H-^1H$ COSY and 2D $^1H-^1H$ NOESY spectra were recorded using SW = 9689.9 Hz with 90° pulse = 9.38 us. For the 2D $^1H-^1H$ COSY, number of increments were (ni) = 16 and number of scans were (ns) = 32. For the 2D $^1H-^1H$ NOESY, mixing time was set tm = 250 ms, ns = 64 and ni = 256. The assignment of the carbon chemical shifts (ppm) for the novel compound **4d** was performed through ^{13}C, 2D heteronuclear HSQC and HMBC spectra. The $^1H-^{13}C$ HSQC and $^1H-^{13}C$ HMBC spectrum were recorded with 256 t_1 (ni) increments, 128 (ns) scans per increment and a relaxation delay of 1s. The ^{13}C spectral width was 36199.1 Hz for both heteronuclear experiments. Spectra processing was performed using MestReNovav.11.0.0 software.

Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected. HR-MS spectra were obtained using a UHPLC-MSn Orbitrap Velos-Thermo mass spectrometer.

Molecular Modeling. Macromodel module of Schrödinger 2017-2 package (Maestro, version 11.2.013, Schrödinger, LLC, New York, NY, 2017) was utilized for conformational analysis. Compound **4g** was designed and minimized with OPLS3 force field in $CHCl_3$ solvent. Minimization was performed with Powell-Reeves Conjugate Gradient (PRCG) method, using 5000 iterations and convergence threshold of 0.001 kcal/mol Å, to discover a local minimum.

For the generation of random conformers, the minimized structure of **4g** was subjected to Conformational Search, using the Mixed torsional/Low-mode sampling method. The main advantage of this method is that combined the random changes in torsion angles and/or molecular position from the torsional sampling (MCMM) method with the low mode steps from the LMOD method, which is highly efficient and has the advantage that ring structures and variable torsion angles do not need to be specified. The maximum number of steps was defined equal to 1000, using 100 steps per rotatable bonds, the energy window was set equal to 100 $kj \cdot mol^{-1}$ and the RMSD cut-off was equal to 0.5 Å.

4.3. Synthesis of IL N-Hydroxyethylammonium formate

In a round-bottom flask mounted in an ice bath and equipped with a magnetic stirrer, were added 1 eq of ethanolamine and 1eq of formic acid was added dropwise. The reaction mixture was stirred for 24 h at room temperature. The resulting light yellow

liquid was dried under high vacuum at 50 °C for 3 h prior to use. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm 8.39 (1H, s, HCOO), 7.72 (4H, br s, $-NH_3^+$ and $-OH$), 3.55 (2H, t, J = 5.1Hz, $-OCH_2-$), 2.82 (2H, t, J = 5.1 Hz, $CH_2NH_3^+$)

4.4. Synthesis of DES CC/U

In a round-bottom flask equipped with a magnetic stirrer were added 1 eq of choline chloride (CC) and 2 eq of dry urea (U). The reaction mixture was stirred for 30 min at 70 °C under inert conditions (nitrogen atmosphere). The resulting colorless liquid was dried under high vacuum at 50 °C for 3 h prior to use. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm 5.54 (8H, s, $4NH_2$), 3.83–3.79 (2H, m, CH_2), 3.44–4.40 (3H, m, CH_2, OH), 3.41 (2H, d, J = 4.9Hz), 3.11 (9H, s, $3CH_3$).

4.5. General procedure of bis-(β-dicarbonyl)-methane compounds (4a-4j)

Method A. In a round-bottom flask equipped with a magnetic stirrer were added 2 mmol of the appropriate heterocyclic 1,3-dicarbonyl compound, 2,4-quinolinediol (**1a**) or 4-hydroxy-1-methyl-2(1*H*)-quinolone (**1b**), 1 mmol of a substituted benzaldehyde **2** and 4 mL of N-hydroxyethylammonium formate. The IL was dried prior to use in high vacuum at 50 °C for at least 3 h. The reaction mixture was heated at 40 °C and stirred under inert atmosphere for 3–4 h. The completion of the reaction was monitored by TLC. The reaction mixture was progressively turned to a viscous white solid mass. At the end of the reaction, the mixture was cooled to room temperature, and an appropriate quantity of water was added. The precipitate formed was filtered off by vacuum filtration. For further purification, the product was triturated with methanol at 60–65 °C and the pure product, collected after cooling, by filtration and dried under vacuum.

Method B. In a round-bottom flask equipped with a magnetic stirrer were added 2 mmol of the appropriate heterocyclic 1,3-dicarbonyl compound, 2,4-quinolinediol (**1a**), 4-hydroxy-1-methyl-2(1*H*)-quinolone (**1b**) or of 4-hydroxy-coumarin (**1c**), 1 mmol of a substituted benzaldehyde and 4–5 mL of the DES CC/U as solvent. The DES was dried prior to use in high vacuum at 50 °C for at least 3 h. The reaction mixture was heated and stirred under inert atmosphere. The completion of the reaction was monitored by TLC. Depending on the starting materials, temperature of the reaction mixture varies between 60 °C and 80 °C for 3–4 h. The reaction mixture progressively turned to a viscous white solid mass. At the end of the reaction, the mixture was cooled to room temperature, and an appropriate quantity of water was added. The precipitate formed was filtered off by vacuum filtration. For further purification, the product was triturated with methanol at 60–65 °C and the pure product, collected after cooling, by filtration and dried under vacuum.

All the synthesized compounds **4a-4j** were characterized by ^{1}H & ^{13}C NMR and HR-MS (see Supplementary material for full experimental and spectral data).

Both IL and DES can be reused after simple recovery procedure involving the evaporation of the aqueous filtrate in vacuo and high vacuum drying at 50 °C until constant weight.

4.6. Antifungal activity of bis-(β-dicarbonyl)-methane compounds against four strains of Candida albicans

Four strains of *C. albicans* were isolated from oral cavities of patients at ENT Clinic, Clinical Hospital Centre Zvezdara, Belgrade, Serbia. Strains were determined on CHROMagar plates (Biomerieux, France) and maintained on Sabourand Dextrose Agar (Merck, Germany) at 4 °C and subcultured once a month.

Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were tested by the use of microdilution method (EUCAST(2002)) [49]. Fresh overnight yeast cultures were adjusted with sterile saline to a concentration 1.0×10^5 CFU/per well. Tested compounds were added in dilution order in microplate wells. The microplates were incubated at 37 °C for 24 h after which MIC and MFC were determined.

The MIC values were considered as the lowest concentrations without microscopically observed growth. Following the serial subcultivations of 10 μL into microtiter plates containing 100 μL of broth/well, as well as subsequent 24 h incubation at 37 °C, the lowest concentrations with no visible growth were defined as the MFC values, indicating 99.5% killing of the original inoculum. Ketoconazole and Amphotericin B were used as a positive control (Sigma Aldrich, Germany).

4.7. Similarity calculations

The similarity calculations were performed, using MAESTRO (MAESTRO, v. 11.2.013, Schrödinger, LLC, New York, NY, 2017) software. Two data sets were created and similarity calculations were implemented. Data set 1 contains the commercial available antifungal drug class of azoles (see Supplementary material, Fig. S4), which was selected as reference molecules, and the set 2 includes the bis-β-dicarbonyl methane compounds database.

Subsequently, an atom pair fingerprint was generated for all data sets compounds and the similarity coefficient (Tanimoto coefficient) was calculated. Then, the similarity was calculated focused not only on the fingerprint similarity measurement but also on physicochemical properties (Descriptors: molecular weight, number of heavy atoms, number of rotatable bonds, lipophilicity, number of atoms in a ring) of all the examined datasets (see Supplementary material, Table S3).

The data sets compounds (Sets 1–2) were sketched in 2D format and were energy minimized in MAESTRO (MAESTRO, v. 11.2.013, Schrödinger, LLC, New York, NY, 2017) software. The fingerprint similarity was generated using the Atom Pairs type of Canvas fingerprint similarity. This type of fingerprint focuses on the concept of an atom pair, which is differentiated by the type and the topological distance separating them [50] Canvas module (Canvas, Schrödinger, LLC, New York, NY, 2017) of MAESTRO (MAESTRO, v. 11.2.013, Schrödinger, LLC, New York, NY, 2017) was applied to calculate similarities based on molecular properties of all examined compounds. Specifically, the descriptors for each compound includes in the data sets, were estimated by Qikprop module (Qikprop, Schrödinger, LLC, New York, NY, 2017) of MAESTRO (MAESTRO, v. 11.2.013, Schrödinger, LLC, New York, NY, 2017).

The Tanimoto coefficient was utilized as the similarity metric and is defined by the following equation:

$$Tc\ (A, B) = c/(a+b-c)$$

a and b are the number of features present in compared structures A and B, respectively and c is the number of shared features by A and B [45].

Declaration of competing interest

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

CRediT authorship contribution statement

Andromachi Tzani: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Christos Vaitsis:**

Investigation. **Eftichia Kritsi:** Investigation, Formal analysis. **Marija Smiljkovic:** Investigation, Formal analysis. **Marina Sokovic:** Investigation, Formal analysis, Writing - original draft. **Panagiotis Zoumpoulakis:** Conceptualization, Formal analysis, Writing - review & editing. **Anastasia Detsi:** Conceptualization, Supervision, Validation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2020.128276.

References

[1] R.C. Cioc, E. Ruijter, R.V.A. Orru, Multicomponent reactions: advanced tools for sustainable organic synthesis, Green Chem. 16 (6) (2014) 2958–2975.
[2] M. Haji, Multicomponent reactions: a simple and efficient route to heterocyclic phosphonates, Beilstein J. Org. Chem. 12 (1) (2016) 1269–1301.
[3] C.M. Pasko, A.A. Dissanayake, B.S. Billow, A.L. Odom, One-pot synthesis of pyrroles using a titanium-catalyzed multicomponent coupling procedure, Tetrahedron 72 (9) (2016) 1168–1176.
[4] R. Grigg, S. Whitney, V. Sridharan, A. Keep, A. Derrick, Iridium catalysed alkylation of 4-hydroxy coumarin, 4-hydroxy-2-quinolones and quinolin-4 (1H)-one with alcohols under solvent free thermal conditions, Tetrahedron 65 (36) (2009) 7468–7473.
[5] K. Kandasamy, M.P. Pachamuthu, M. Muthusamy, S. Ganesabaskaran, A. Ramanathan, Synthesis of novel pyrazolylbiscoumarin derivatives using FeTUD-1 as a mesoporous solid acid catalyst, RSC Adv. 3 (47) (2013) 25367–25373.
[6] P. Gunasekaran, P. Prasanna, S. Perumal, A.I. Almansour, ZnCl2-catalyzed three-component domino reactions for the synthesis of pyrano [3, 2-c] quinolin-5 (6H)-ones, Tetrahedron Lett. 54 (25) (2013) 3248–3252.
[7] V.N. Murthy, S.P. Nikumbh, S.P. Kumar, L.V. Rao, A. Raghunadh, An efficient one pot synthesis of 2-amino quinazolin-4 (3H)-one derivative via MCR strategy, Tetrahedron Lett. 56 (42) (2015) 5767–5770.
[8] A. Tzani, A. Douka, A. Papadopoulos, E.A. Pavlatou, E. Voutsas, A. Detsi, Synthesis of biscoumarins using recyclable and biodegradable task-specific ionic liquids, ACS Sustain. Chem. Eng. 1 (9) (2013) 1180–1185.
[9] A. Taheri, B. Lai, J. Yang, J. Zhang, Y. Gu, Facile synthesis of densely substituted chroman derivatives through Brønsted acid ionic liquid catalyzed three-component reactions of aromatic aldehydes, 1, 1-diarylethylenes and nucleophiles, Tetrahedron 72 (4) (2016) 479–488.
[10] E. Rushell, Y.K. Tailor, S. Khandewal, K. Verma, M. Agarwal, M. Kumar, Deep eutectic solvent promoted synthesis of structurally diverse hybrid molecules with privileged heterocyclic substructures, New J. Chem. 43 (31) (2019) 12462–12467.
[11] S. Sobhani, M. Honarmand, 2-Hydroxyethylammonium acetate: a reusable task-specific ionic liquid promoting one-pot, three-component synthesis of 2-amino-3,5-dicarbonitrile-6-thio-pyridines, Compt. Rendus Chem. 16 (3) (2013) 279–286.
[12] N. Isambert, M.d.M.S. Duque, J.-C. Plaquevent, Y. Genisson, J. Rodriguez, T. Constantieux, Multicomponent reactions and ionic liquids: a perfect synergy for eco-compatible heterocyclic synthesis, Chem. Soc. Rev. 40 (3) (2011) 1347–1357.
[13] P. Liu, J.-W. Hao, L.-P. Mo, Z.-H. Zhang, Recent advances in the application of deep eutectic solvents as sustainable media as well as catalysts in organic reactions, RSC Adv. 5 (60) (2015) 48675–48704.
[14] A. Tzani, M. Elmaloglou, C. Kyriazis, D. Aravopoulou, I. Kleidas, A. Papadopoulos, E. Ioannou, A. Kyritsis, E. Voutsas, A. Detsi, Synthesis and structure-properties relationship studies of biodegradable hydroxylammonium-based protic ionic liquids, J. Mol. Liq. 224 (2016) 366–376.
[15] A. Tzani, D. Skarpalezos, A. Papadopoulos, D. Aravopoulou, I. Kleidas, E. Ioannou, E. Voutsas, A. Kyritsis, A. Detsi, Synthesis of novel non-toxic naphthenic and benzoic acid ionic liquids. Structure-properties relationship and evaluation of their biodegradability potential, J. Mol. Liq. 296 (2019) 111927.
[16] A.A. Papadopoulou, A. Tzani, D. Alivertis, M.H. Katsoura, A.C. Polydera, A. Detsi, H. Stamatis, Hydroxyl ammonium ionic liquids as media for biocatalytic oxidations, Green Chem. 18 (4) (2016) 1147–1158.
[17] A.A. Papadopoulou, A. Tzani, A.C. Polydera, P. Katapodis, E. Voutsas, A. Detsi, H. Stamatis, Green biotransformations catalysed by enzyme-inorganic hybrid nanoflowers in environmentally friendly ionic solvents, Environ. Sci. Pollut. Control Ser. 25 (27) (2018) 26707–26714.
[18] N. Gjineci, E. Boli, A. Tzani, A. Detsi, E. Voutsas, Separation of the ethanol/water azeotropic mixture using ionic liquids and deep eutectic solvents, Fluid Phase Equil. 424 (2016) 1–7.
[19] A. Tzani, S. Koutsoukos, D. Koukouzelis, A. Detsi, Synthesis and characterization of silver nanoparticles using biodegradable protic ionic liquids, J. Mol. Liq. 243 (2017) 212–218.
[20] D. Koukouzelis, A.R.N. Pontillo, S. Koutsoukos, E. Pavlatou, A. Detsi, Ionic liquid – assisted synthesis of silver mesoparticles as efficient surface enhanced Raman scattering substrates, J. Mol. Liq. (2020) 112929.
[21] D. Skarpalezos, A. Detsi, Deep eutectic solvents as extraction media for valuable flavonoids from natural sources, Appl. Sci. 9 (19) (2019) 4169.
[22] Q. Zhang, K.D.O. Vigier, S. Royer, F. Jérôme, Deep eutectic solvents: syntheses, properties and applications, Chem. Soc. Rev. 41 (21) (2012) 7108–7146.
[23] M. Roussaki, K. Zelianaios, E. Kavetsou, S. Hamilakis, D. Hadjipavlou-Litina, C. Kontogiorgis, T. Liargkova, A. Detsi, Structural modifications of coumarin derivatives: determination of antioxidant and lipoxygenase (LOX) inhibitory activity, Bioorg. Med. Chem. 22 (23) (2014) 6586–6594.
[24] M. Roussaki, C.A. Kontogiorgis, D. Hadjipavlou-Litina, S. Hamilakis, A. Detsi, A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and lipoxygenase inhibitory activity, Bioorg. Med. Chem. Lett 20 (13) (2010) 3889–3892.
[25] E. Kavetsou, L. Gkionis, G. Galani, C. Gkolfinopoulou, L. Argyri, E. Pontiki, A. Chroni, D. Hadjipavlou-Litina, A. Detsi, Synthesis of prenyloxy coumarin analogues and evaluation of their antioxidant, lipoxygenase (LOX) inhibitory and cytotoxic activity, Med. Chem. Res. 26 (4) (2017) 856–866.
[26] X.-M. Chu, C. Wang, W. Liu, L.-L. Liang, K.-K. Gong, C.-Y. Zhao, K.-L. Sun, Quinoline and quinolone dimers and their biological activities: an overview, Eur. J. Med. Chem. 161 (2019) 101–117.
[27] G.-X. Gong, J.-F. Zhou, L.-T. An, X.-L. Duan, S.-J. Ji, Catalyst-free synthesis of α, α-bis (4-hydroxycoumarin-3-yl) toluene in aqueous media under microwave irradiation, Synth. Commun. 39 (3) (2009) 497–505.
[28] N. Tavakoli-Hoseini, M.M. Heravi, F.F. Bamoharram, A. Davoodnia, M. Ghassemzadeh, An unexpected tetracyclic product isolated during the synthesis of biscoumarins catalyzed by [MIM (CH2) 4SO3H][HSO4]: characterization and X-ray crystal structure of 7-(2-hydroxy-4-oxo-4H-chromen-3-yl)-6H, 7H-chromeno [4, 3-b] chromen-6-one, J. Mol. Liq. 163 (3) (2011) 122–127.
[29] N. Hamdi, M.C. Puerta, P. Valerga, Synthesis, structure, antimicrobial and antioxidant investigations of dicoumarol and related compounds, Eur. J. Med. Chem. 43 (11) (2008) 2541–2548.
[30] J.L. Vennerstrom, W.Y. Ellis, A.L. Ager Jr., S.L. Andersen, L. Gerena, W.K. Milhous, Bisquinolines. 1. N, N-bis (7-chloroquinolin-4-yl) alkanediamines with potential against chloroquine-resistant malaria, J. Med. Chem. 35 (11) (1992) 2129–2134.
[31] K. Raynes, Invited review Bisquinoline antimalarials: their role in malaria chemotherapy, Int. J. Parasitol. 29 (3) (1999) 367–379.
[32] Z. Zaheer, F.A.K. Khan, J.N. Sangshetti, R.H. Patil, Efficient one-pot synthesis, molecular docking and in silico ADME prediction of bis-(4-hydroxycoumarin-3-yl) methane derivatives as antileishmanial agents, EXCLI journal 14 (2015) 935.
[33] J. Li, C.-W. Lv, X.-J. Li, D. Qu, Z. Hou, M. Jia, X.-X. Luo, X. Li, M.-K. Li, Synthesis of biscoumarin and dihydropyran derivatives and evaluation of their antibacterial activity, Molecules 20 (9) (2015) 17469–17482.
[34] D. Završnik, S. Muratović, D. Makuc, J. Plavec, M. Cetina, A. Nagl, E.D. Clercq, J. Balzarini, M. Mintas, Benzylidene-bis-(4-hydroxycoumarin) and benzopyrano-coumarin derivatives: synthesis, 1H/13C-NMR conformational and X-ray crystal structure studies and in vitro antiviral activity evaluations, Molecules 16 (7) (2011) 6023–6040.
[35] Y.-P. Sui, H.-R. Huo, J.-J. Xin, J. Li, X.-J. Li, X.-L. Du, H. Ma, H.-Y. Zhou, H.-D. Zhan, Z.-J. Wang, Antibacterial and antitumor activities of biscoumarin and dihydropyran derivatives, Molecules 20 (9) (2015) 17614–17626.
[36] I. Manolov, C. Maichle-Moessmer, I. Nicolova, N. Danchev, Synthesis and anticoagulant activities of substituted 2, 4-diketochromans, biscoumarins, and chromanocoumarins, Arch. Pharmazie: Int. J. Pharmaceut. Med. Chem. 339 (6) (2006) 319–326.
[37] M. Pfaller, D. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin. Microbiol. Rev. 20 (1) (2007) 133–163.
[38] D. Kuhn, J. Chandra, P. Mukherjee, M. Ghannoum, Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces, Infect. Immun. 70 (2) (2002) 878–888.
[39] N. Martins, I.C. Ferreira, L. Barros, S. Silva, M. Henriques, Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment, Mycopathologia 177 (5–6) (2014) 223–240.
[40] C. Yue, D. Fang, L. Liu, T.-F. Yi, Synthesis and application of task-specific ionic liquids used as catalysts and/or solvents in organic unit reactions, J. Mol. Liq. 163 (3) (2011) 99–121.
[41] H. Hu, F. Qiu, A. Ying, J. Yang, H. Meng, An environmentally benign protocol for aqueous synthesis of tetrahydrobenzo [b] pyrans catalyzed by cost-effective ionic liquid, Int. J. Mol. Sci. 15 (4) (2014) 6897–6909.
[42] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep eutectic solvents (DESs) and their

applications, Chem. Rev. 114 (21) (2014) 11060–11082.
[43] S. Rehman, M. Ikram, R.J. Baker, M. Zubair, E. Azad, S. Min, K. Riaz, K. Mok, S.-U. Rehman, Synthesis, characterization, in vitro antimicrobial, and U2OS tumoricidal activities of different coumarin derivatives, Chem. Cent. J. 7 (1) (2013), 68-68.
[44] R.J. Hamill, Amphotericin B formulations: a comparative review of efficacy and toxicity, Drugs 73 (9) (2013) 919–934.
[45] J. Campion, J. Martinez, Ketoconazole, an antifungal agent, protects against adiposity induced by a cafeteria diet, Horm. Metab. Res. 36 (7) (2004) 485–491.
[46] M.A. Johnson, G.M. Maggiora, Concepts and Applications of Molecular Similarity, Wiley, 1990.
[47] G. Maggiora, M. Vogt, D. Stumpfe, J. Bajorath, Molecular similarity in medicinal chemistry: miniperspective, J. Med. Chem. 57 (8) (2014) 3186–3204.
[48] D.E. Patterson, R.D. Cramer, A.M. Ferguson, R.D. Clark, L.E. Weinberger, Neighborhood behavior: a useful concept for validation of “molecular diversity” descriptors, J. Med. Chem. 39 (16) (1996) 3049–3059.
[49] EUCAST, Method for determination of minimal inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Discussion Document E. Dis. 7.1, European Society of Clinical Microbiology and Infectious Diseases Taufkirchen, 2002.
[50] R.E. Carhart, D.H. Smith, R. Venkataraghavan, Atom pairs as molecular features in structure-activity studies: definition and applications, J. Chem. Inf. Comput. Sci. 25 (2) (1985) 64–73.