Biological evaluation and molecular docking of some chromenyl-derivatives as potential antimicrobial agents

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Abstract: Various thiosemicarbazones (TSCs) and their heterocyclic thiadiazolines (TDZ) possess important biological effects. In addition, chromenyl derivatives exhibit a wide range of pharmacological activities. Based on these findings and as a continuation of our research on nitrogen and sulfur containing compounds, we investigated a series of previously reported chromenyl-TSCs (**1a-j**) and chromenyl-TDZs (**2a-j**) for their *in vitro* antimicrobial activities against two bacterial and four fungal strains. MIC and MBC/MFC (µg/mL) values of these compounds were evaluated and compared to those of Spectinomycin, Moxifloxacin and Fluconazole, used as reference drugs. For a better understanding of the drug-receptor interactions, all the compounds were further subjected to molecular docking against four targets that were chosen based on the specific mechanism of action of the reference drugs used in the antimicrobial screening. All compounds tested showed equal or higher antibacterial/antifungal activities relative to the used reference drugs. *In silico* studies (molecular docking) revealed that all the investigated compounds showed good binding energies towards four receptor protein targets and supported their antimicrobial properties.

Keywords: chromone; thiosemicarbazone; thiadiazoline; antimicrobial; molecular docking.

INTRODUCTION

Infectious microbial diseases remain a life threatening problem due to the resistance of microorganisms to prophylaxis or antimicrobial therapy, reaching an alarming level in many countries all over the world. In recent decades, the problem of multidrug-resistant microorganisms has reached an alarming level in many countries around the world (Guo *et al*., 2015). Resistance to antimicrobial agents (glycopeptides, β-lactamines, quinolones, macrolides), among a variety of clinically significant species of bacteria, is becoming an increasingly important global health problem.

In view of these facts, it is important to develop more effective antimicrobial agents (Ionuț *et al*., 2013a; Keri *et al*., 2014; Ŝarkanj *et al*., 2013).

In the area of medicinal chemistry, a considerable attention is being given to sulfur containing substances such as thiosemicarbazones (TSCs), because of their pharmacological properties and promising biological implications. Various TSCs and their heterocyclic thiadiazoline (TDZ) derivatives possess important biological effects, such as antimicrobial (Ionuț *et al*., 2013a), antioxidant (Parmar *et al*., 2011), anti-HIV-1 (Yusuf and Jain, 2014) and anticancer properties, and the literature concerning these subjects is steadily increasing. The presence of fragment -N=CH-R in Schiff bases is known for its biological activity. Many reports exist on structure-activity relationship of these classes of compounds (Ebrahimi *et al*., 2015; Parmar *et al*., 2011; Yusuf *et al*., 2014). In addition, chromone and its analogs are important pharmacophores in medicinal chemistry, chromenyl derivatives exhibiting a wide range of pharmacological activities like antibacterial, antifungal, anticancer, antioxidant, anti-HIV, anti-ulcer, immunostimulatory, biocidal and anti-inflammatory functions. Some of the chromone derivatives known for their pharmacological properties have been or are used as therapeutic agents today (cromolyn or cromoglicate, nedocromil, apigenin, diosmin, flavoxate) (Keri *et al*., 2014). Therefore, chromenyl scaffold is being considered as a privileged structure (Emami and Ghanbarimasir, 2015; Ionuț *et al*., 2013b; Keri *et al*., 2014; Walenzyk *et al*., 2005).

Molecular docking represents a primary component in many drug discovery programs, being able to predict the conformation of a ligand within the active site of a

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receptor and finding the low-energy binding modes between them (Kang *et al*., 2012).

Based on the above mentioned and in continuation of our research on Schiff bases (Ionuț *et al*., 2013a) and nitrogen and sulfur containing heterocycles (Tiperciuc *et al*., 2013; Nastasă *et al*., 2013), we hereby report the antimicrobial activity and molecular docking of some previously reported chromenyl-TSCs as well as of their corresponding 1,3,4-TDZ derivatives (Ionuț *et al*., 2013b), obtained by cyclization under acetylating conditions. The molecular docking study of all the compounds has been done for a better understanding of the drug-receptor interactions and confirmed the antimicrobial properties of the investigated chromenyl-derivatives.

MATERIALS AND METHODS

Test compounds

Twenty compounds 10 chromenyl-TSCs (**1a**-**j**) and their corresponding 1,3,4-TDZs (**2a**-**j**) were assessed for their *in vitro* antimicrobial activities and subjected to *in silico* evaluation, respectively molecular docking. The synthesis and physico-chemical characterization of these compounds, along with their *in vitro* anticancer effects were previously reported (Ionuț *et al.,* 2013b). The chemical structures of the tested compounds are compiled in table 1.

Antimicrobial activity evaluation

The MIC and MBC/MFC values of these compounds were determined *in vitro* against bacterial and fungal strains. Spectinomycin, Moxifloxacin and Fluconazole were used as reference drugs.

Test microorganisms

The microorganisms used for the antimicrobial activity evaluation were prepared according to Dezsi *et al* (Dezsi *et al.,* 2015) and Winnicka *et al* (Winnicka *et al*., 2012).

Antimicrobial agents

Stock solutions (1mg/mL) were prepared by dissolving the tested chromenyl-TSCs **1a**-**j** and chromenyl-TDZs **2a**-**j** in sterile DMSO. These solutions were stored at 4°C. Series of double diluting solutions of above compounds were prepared in RPMI 1640 medium obtaining final concentrations in the range of 500 µg/mL to 0.015µg/mL.

Broth micro dilution method

The micro dilution method was employed for minimum inhibitory concentration test. Media was placed into each 96 wells of the micro plates. Sample solutions at high concentration (100 µg/mL) were added into first rows of micro plates and two-fold dilutions of the compounds were made by dispensing the solutions into the remaining wells. 10µl culture suspensions were inoculated into all the wells. The sealed micro plates are incubated at 37°C for 18h. Antifungal activity was tested by using broth micro dilution method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (Mocan *et al.,* 2015; Winnicka *et al.,* 2012).

In silico assay: Virtual screening docking

All the investigated compounds were virtually docked against four targets 30S ribosomal protein S12, DNA gyrase subunit A, DNA topoisomerase IV subunit A and lanosterol-14α-demethylase in order to investigate the potential binding mode of these inhibitors. Prior to docking, an academic license of Marvin Sketch was used for drawing, displaying (in tables) and 3D optimisation of all ligands, Marvin Sketch 15.1.5.0, 2015, Chem Axon (http://www.chemaxon.com).

Both an experimentally high-resolution crystal structure (as best recommended for docking) (Varnek*,* 2008) and three corresponding homology models (instead of missing experimentally determined structures and low-resolution structures) from RCSB Protein Data Bank (www.rcsb.org) were used. For lanosterol-14α-demethylase, a validated high-resolution crystal structure (1.90 Å, PDB ID: 4LXJ) (Monk *et al*., 2014) was used. For 30S ribosomal protein S12, the L-chain (position 2-124) of an all-atom homology model (PDB ID: 1M5G) (Tung *et al*., 2002), while for DNA gyrase subunit A and DNA topoisomerase IV subunit A two homology models generated via SWISS-MODEL (Arnold *et al*., 2006; Biasini *et al.,* 2014; Guex *et al.,* 2009; Kiefer *et al*., 2009) based on Uni Prot ID P43702, respectively Uni Prot ID P43702 were used.

The virtual screening of all ligands against the four targets was carried out with PyRx Python Prescription 0.9.2 (Dallakyan and Olson, 2015) using Auto Dock Vina (Trott and Olson, 2010) as docking software. In order to improve the accuracy of output, the number of runs was set at 60 for each target using the exhaustiveness function of the software. The rendering of docking images was performed with an embedded module of PyRx 0.9.2 The Visualization ToolKit (VTK) by Kitware, Inc.

RESULTS

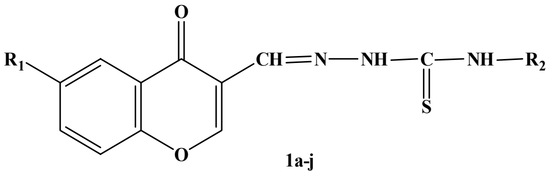
In vitro antimicrobial activity

Twenty compounds (table 1), 10 chromenyl-TSCs (**1a**-**j**) and their corresponding 1,3,4-TDZs (**2a**-**j**), were screened for their *in vitro* growth inhibitory activities against Gram-positive (*Listeria monocytogenes* ATCC 19115) and Gram-negative (*Escherichia coli* ATCC 25922) bacterial strains and also against species of Candida (*C. albicans* ATCC 10231, *C. albicans* ATCC 18804, *C. krusei* ATCC 6258, *C. parapsilopsis* ATCC 22019) by evaluating their MIC and MBC/MFC (µg/mL) values.

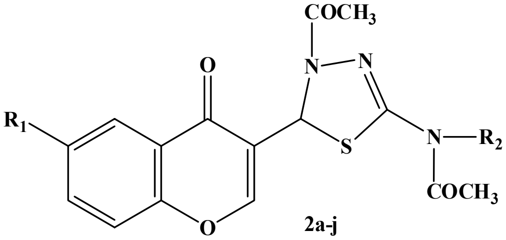
The results obtained are shown in tables 2 and 3 and were compared to those of Spectinomycin, Moxifloxacin and Fluconazole, used as reference drugs.

Subsequently preliminary SAR studies were performed to deduce how the structure variation or modification could affect the antimicrobial activity.

Table 1: The chemical structures of the investigated compounds.



1a-j



2a-j

COMPOUND		R_1	R_2
1a	**2a**	CH_3	CH_3
1b	**2b**	Cl	CH_3
1c	**2c**	CH_3	CH_2-CH=CH_2
1d	**2d**	Cl	CH_2-CH=CH_2
1e	**2e**	CH_3	C_6H_5
1f	**2f**	Cl	C_6H_5
1g	**2g**	CH_3	4-C_6H_4-OCH_3
1h	**2h**	Cl	4-C_6H_4-OCH_3
1i	**2i**	CH_3	3- C_6H_4-CF_3
1j	**2j**	Cl	3- C_6H_4-CF_3

Table 2: Minimum Inhibitory Concentrations (MIC, µg/mL) of compounds **1a-j** and **2a-j**.

Compound	***Escherichia coli*** **ATCC 25922**	***Listeria monocytogenes*** **ATCC 19115**	***Candida albicans*** **ATCC 10231**	***Candida albicans*** **ATCC 18804**	***Candida krusei*** **ATCC 6258**	***Candida parapsilosis*** **ATCC 22019**
1a	31.25	15.62	1.95	1.95	0.97	0.48
1b	31.25	15.62	1.95	0.97	1.95	0.24
1c	3.9	0.97	0.48	0.48	0.48	0.48
1d	62.5	15.62	7.81	7.81	3.9	0.48
1e	62.5	7.81	3.9	7.81	3.9	1.95
1f	62.5	1.95	0.97	0.97	7.81	0.48
1g	62.5	15.62	3.9	1.95	3.9	0.12
1h	62.5	15.62	3.9	7.81	1.95	0.48
1i	15.62	7.81	0.48	0.48	0.24	0.24
1j	15.62	7.81	7.81	3.9	7.81	0.24
2a	62.5	3.9	7.81	1.95	0.97	0.48
2b	62.5	3.9	0.48	0.97	3.9	0.24
2c	31.25	31.25	7.81	15.62	7.81	7.81
2d	62.5	62.5	3.9	7.81	1.95	3.9
2e	62.5	1.95	1.95	7.81	3.9	0.48
2f	62.5	31.25	1.95	0.97	0.97	1.95
2g	62.5	15.62	0.48	0.48	0.48	0.48
2h	7.81	3.9	0.48	0.24	0.24	0.12
2i	62.5	62.5	0.97	1.95	7.81	0.97
2j	62.5	31.25	1.95	1.95	0.97	1.95
Spectinomycin	62.5	-	-	-	-	-
Moxifloxacin	-	31.25	-	-	-	-
Fluconazole	-	-	15.62	15.62	15.62	7.81
Inoculum Control	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
Broth control	No growth	No growth	No growth	No growth	No growth	No growth

Table 3: Minimum Bactericidal/ Fungicidal Concentrations (MBC/MFC, µg/mL) of compounds **1a-j** and **2a-j**.

Compound	*Escherichia coli* ATCC25922	*Listeria monocytogenes* ATCC 19115	*Candida albicans* ATCC 10231	*Candida albicans* ATCC 18804	*Candida krusei* ATCC 6258	*Candida parapsilosis* ATCC 22019
1a	62.5	31.25	7.81	7.81	3.9	1.95
1b	62.5	62.5	7.81	3.9	3.9	0.97
1c	15.62	3.9	3.9	7.81	3.9	3.9
1d	125	31.25	15.62	15.62	7.81	0.97
1e	62.5	31.25	15.62	31.25	15.62	7.81
1f	62.5	7.81	1.95	1.95	7.81	1.95
1g	125	62.5	3.9	3.9	7.81	0.48
1h	125	125	7.81	15.62	3.9	0.97
1i	31.25	15.62	1.95	1.95	0.97	0.97
1j	31.25	15.62	15.62	7.81	15.62	0.97
2a	125	15.62	31.25	7.81	3.9	1.95
2b	125	15.62	1.95	7.81	7.81	1.95
2c	62.5	31.25	15.62	31.25	15.62	15.62
2d	125	62.5	7.81	15.62	3.9	7.81
2e	125	7.81	3.9	15.62	15.62	1.95
2f	125	31.25	3.9	3.9	1.95	3.9
2g	125	31.25	1.95	0.97	0.97	0.97
2h	31.25	7.81	1.95	0.97	0.48	0.24
2i	125	62.5	3.9	7.81	15.62	1.95
2j	125	31.25	15.62	3.9	7.81	3.9
Spectinomycin	125	-	-	-	-	-
Moxifloxacin	-	62.5	-	-	-	-
Fluconazole	-	-	31.25	31.25	31.25	15.62

All experiments were performed in triplicate and results are expressed as means, standard deviations were <5%

Molecular docking study

All the compounds investigated for their *in vitro* antimicrobial properties were further subjected to *in silico* molecular docking evaluation, against four targets (three bacterial enzymes and one fungal key-enzyme), that were chosen based on the specific mechanism of action of the reference drugs used in the *in vitro* antimicrobial screening. The results obtained are depicted in tables 4,5,6,7 and figures 1,2,3, and 4.

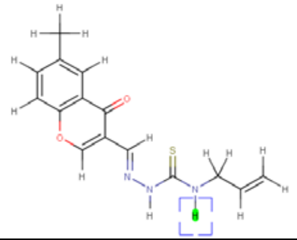
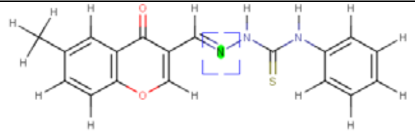
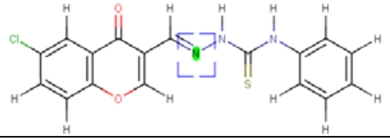
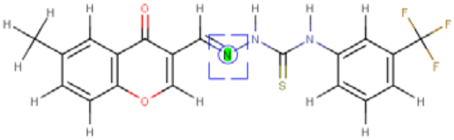
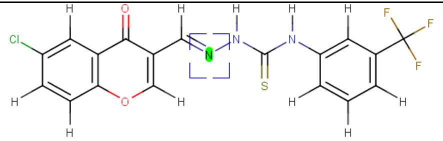
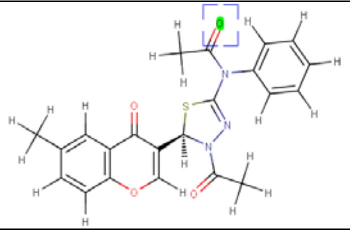
The molecular docking was performed in order to investigate the potential binding mode and binding affinity of these inhibitors.

Docked ligand conformations were analysed in terms of binding affinity and hydrogen bonding between ligands (compounds) and the four-receptor proteins. Detailed analyses of the ligand-receptor interactions were carried out, and final possible orientations of the ligands and receptors were saved.

Table 4: Binding affinity of the tested compounds towards 30S ribosomal protein S12.

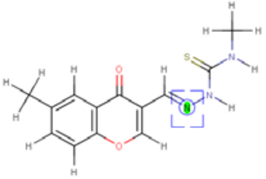
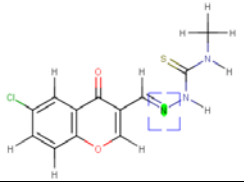
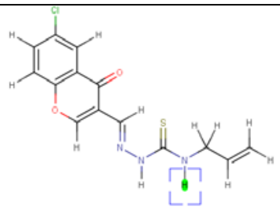
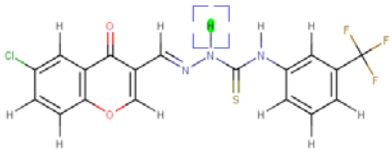
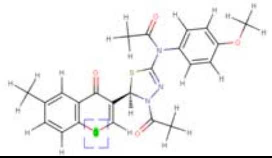
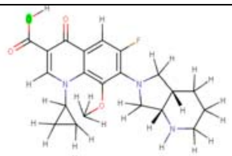
Compound	Binding energy (kcal/mol)	Compound	Binding energy (kcal/mol)
1a	-6.3	**2a**	-7.1
1b	-7.1	**2b**	-7.1
1c	-6.2	**2c**	-7.1
1d	-6.6	**2d**	-7
1e	-5.6	**2e**	-7.5
1f	-5.7	**2f**	-6.9
1g	-5.6	**2g**	-7.1
1h	-5.7	**2h**	-7.4
1i	-5.8	**2i**	-7.8
1j	-5.9	**2j**	-7.6
Spectinomycin		- 6.1	

Table 5: Binding affinity of the tested compounds towards DNA gyrase subunit A with depiction of interacting atoms (H-bonds) of the ligands.

Compound	**DNA gyrase subunit A**		
	Binding Affinity (kcal/mol)	**H-bonds**	
		AA residue(s) of target	**Interacting atom(s) of the ligand**
1a	-7.6	N/A	N/A
1b	-7.3	N/A	N/A
1c	-7.6	Pro219	
1d	-7.1	N/A	N/A
1e	-8.7	Ser98	
1f	-8.5	Ser98	
1g	-8.7	N/A	N/A
1h	-8.6	N/A	N/A
1i	-9.9	Ser98	
1j	-9.8	Ser98	
2a	-9.1	N/A	N/A
2b	-8.6	N/A	N/A
2c	-8.6	N/A	N/A
2d	-8.4	N/A	N/A
2e	-9.2	Tyr266	
2f	-9.7	N/A	N/A
2g	-9.2	N/A	N/A
2h	-9.2	N/A	N/A
2i	-10.4	N/A	N/A
2j	-10.4	N/A	N/A
Moxifloxacin	-7.9	N/A	N/A

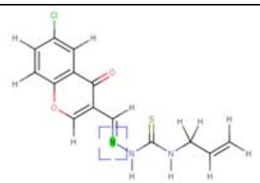
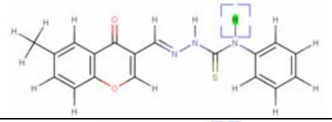
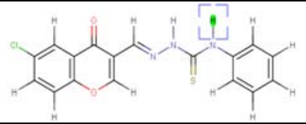
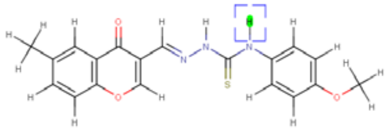
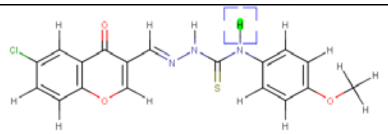
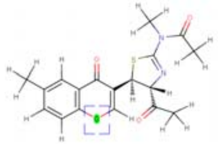
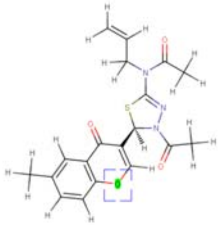
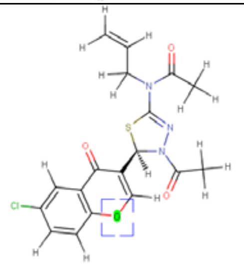
N/A - not available/assigned

Table 6: Binding affinity of the tested compounds towards DNA topoisomerase IV subunit A with depiction of interacting atoms (H-bonds) of the ligands.

Compound	**DNA topoisomerase IV subunit A**		
	Binding Affinity (kcal/mol)	**H-bonds**	
		AA residue(s) of target	**Interacting atom(s) of the ligand**
1a	-7.4	Leu338	
1b	-7.1	Leu338	
1c	-7.5	N/A	N/A
1d	-7.4	Gln159	
1e	-8	N/A	N/A
1f	-8.5	N/A	N/A
1g	-8.4	N/A	N/A
1h	-8.3	N/A	N/A
1i	-9.4	N/A	N/A
1j	-9.6	Gln159	
2a	-7.9	N/A	N/A
2b	-8.4	N/A	N/A
2c	-8.3	N/A	N/A
2d	-8.1	N/A	N/A
2e	-9.1	N/A	N/A
2f	-8.5	N/A	N/A
2g	-9.1	Gln159	
2h	-8.5	N/A	N/A
2i	-9.2	N/A	N/A
2j	-9.3	N/A	N/A
Moxifloxacin	-7.4	Phe97pp	

N/A - not available/assigned

Table 7. Binding affinity of the tested compounds towards lanosterol-14α-demethylase with depiction of interacting atoms (H-bonds) of the ligands.

Compound	**Lanosterol-14α-demethylase**		
	Binding Affinity (kcal/mol)	**H-bonds**	
		AA residue (s) of target	**Interacting atom (s) of the ligand**
1a	-8.2	N/A	N/A
1b	-8.1	N/A	N/A
1c	-8.3	N/A	N/A
1d	-8.2	Tyr140	
1e	-9	Tyr140	
1f	-8.9	Tyr140	
1g	-8.6	Tyr140	
1h	-8.5	Tyr140	
1i	-10.1	N/A	N/A
1j	-10.8	N/A	N/A
2a	-10.3	N/A	N/A
2b	-9.3	Tyr140	
2c	-9.2	Tyr140	
2d	-9	Tyr140	

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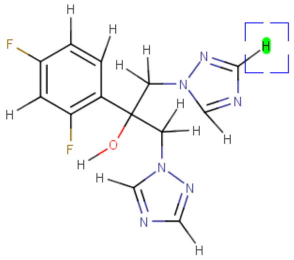
N/A - not available/assigned

Table 7. Binding affinity of the tested compounds towards lanosterol-14α-demethylase with depiction of interacting atoms (H-bonds) of the ligands.

Compound	**Lanosterol-14α-demethylase**		
	Binding Affinity (kcal/mol)	**H-bonds**	
		AA residue (s) of target	**Interacting atom (s) of the ligand**
2e	-9	N/A	N/A
2f	-9.4	N/A	N/A
2g	-9.1	N/A	N/A
2h	-8.1	N/A	N/A
2i	-8.9	N/A	N/A
2j	-9.1	N/A	N/A
Fluconazole	-8.3	Gly314	

N/A - not available/assigned

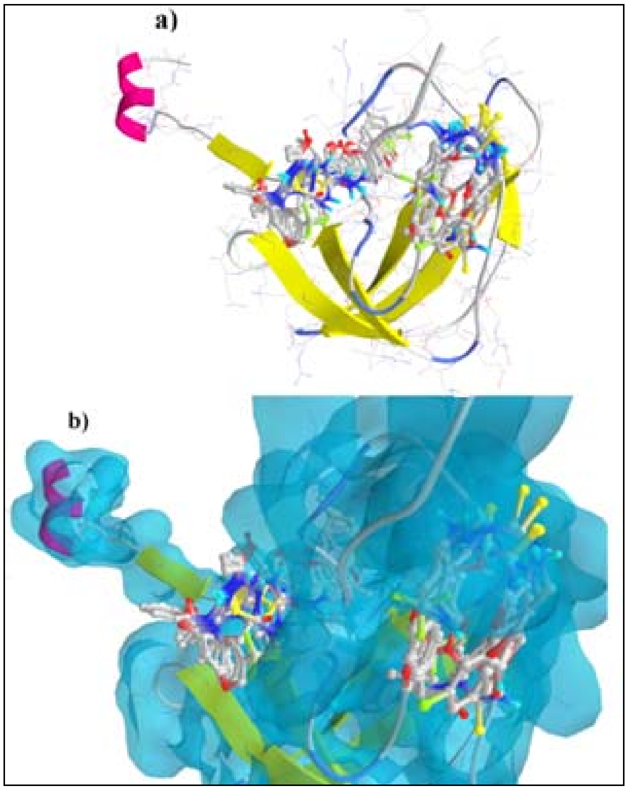


Fig. 1: Docking poses of compounds **1a-j** and **2a-j** in the active site of 30 ribosomal protein S12 (the receptor is depicted as thin sticks with secondary structure drawn as cartoon backbone, meanwhile ligands are figured as sticks): (a) without simulation of the molecular surface; (b) with simulation of the molecular surface (light blue) for a better understanding of tridimensional positioning in the active site of enzyme.

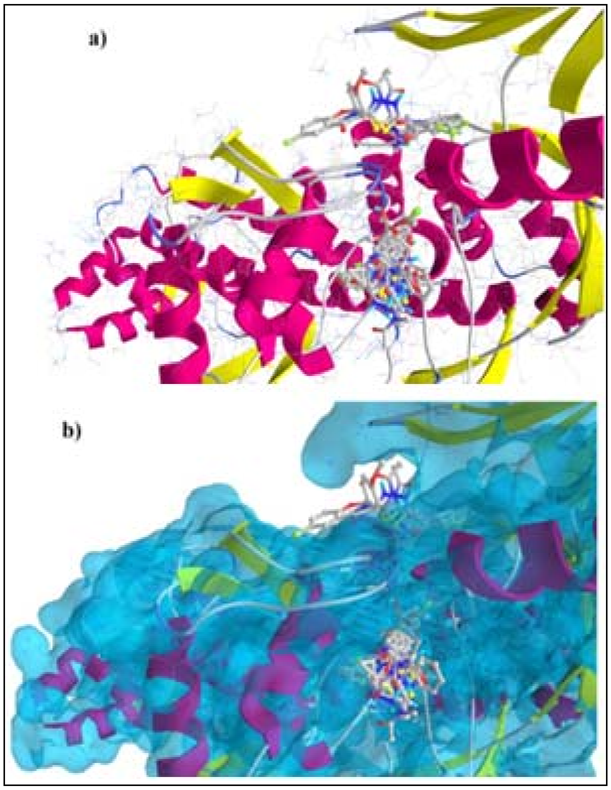


Fig. 2: Docking poses of compounds **1a-j** and **2a-j** in the active site of DNA gyrase subunit A (the receptor is depicted as thin sticks with secondary structure drawn as cartoon backbone, meanwhile ligands are figured as sticks): (a) without simulation of the molecular surface; (b) with simulation of the molecular surface (light blue) for a better understanding of tridimensional positioning in the active site of enzyme.

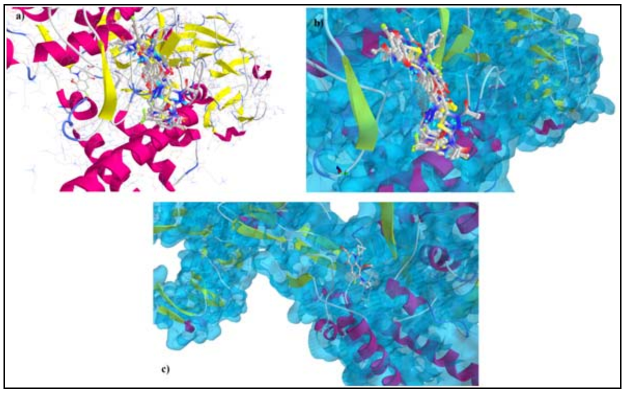


Fig. 3. Docking poses of the investigated compounds in the active site of DNA topoisomerase IV subunit A (the receptor is depicted as thin sticks with secondary structure drawn as cartoon backbone, meanwhile ligands are figured as sticks): (a) without simulation of the molecular surface; (b) with simulation of the molecular surface (light blue) for a better understanding of tridimensional positioning in the active site of enzyme; (c) the isolated positioning of Moxifloxacin blocking the access of the substrate to the binding site.

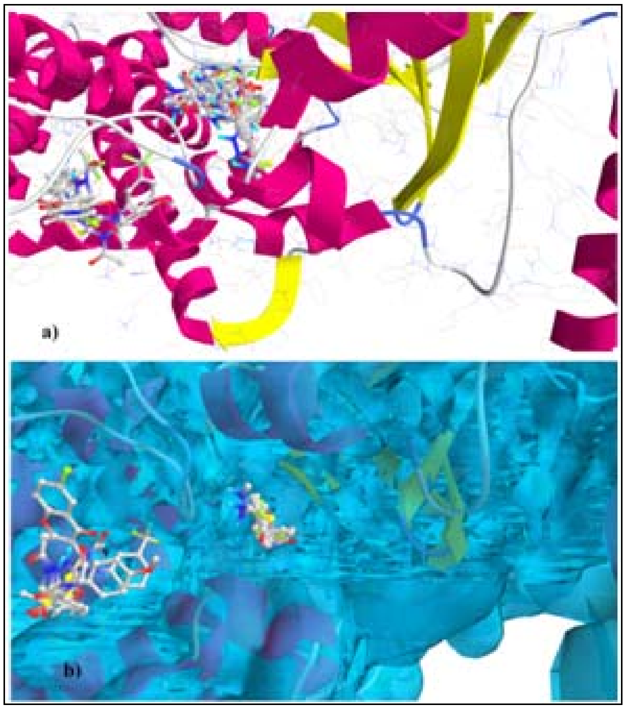


Fig. 4. Docking poses of the investigated compounds in the active site of lanosterol-14α-demethylase (the receptor is depicted as thin sticks with secondary structure drawn as cartoon backbone, meanwhile ligands are figured as sticks): (a) without simulation of the molecular surface; (b) with simulation of the molecular surface (light blue) for a better understanding of tridimensional positioning in the active site of enzyme.

DISCUSSION

Antibacterial activity

All the compounds showed good to very good antibacterial effects, some of them proved to be more potent than the used reference drugs.

Towards *E. coli* ATCC 25922 five chromenyl-TSCs (**1a**, **1b**, **1c**, **1i**, **1j**) and two chromenyl-TDZs (**2c**, **2h**) showed lower MIC and MBC values than reference Spectinomycin, while the other five TSCs and eight TDZs exhibited the same antibacterial effects as the used reference drug. The MIC values ranged between 3.9 and 62.5 µg/mL for the chromenyl-TSCs **1a**-**1j** and between 7.81 and 62.5 µg/mL for their corresponding TDZs **2a**-**j**. The most active of the Schiff bases series **1a**-**1j** was compound **1c** (MIC=3.9µg/mL, MBC=15.62µg/mL), **2h** (MIC=7.81µg/mL, MBC=31.25µg/mL) being the most potent of the tested 1,3,4-TDZs series **2a**-**j**.

Analysis of the tested compounds showed that the presence of aliphatic chemical groups (methyl, allyl) in the general structure of the tested TSCs and TDZs increased the growth inhibitory activity towards tested *E. coli* strain, while the incorporation of aromatic groups (phenyl, 4-OCH_3-phenyl) lead to higher MIC/MBC values. The presence of electron-withdrawing CF_3 group in position 3 of the benzene ring (compounds **1i**, **1j**, **2i** and **2j**) enhanced the antibacterial effects.

Against *L. monocytogenes* ATCC 19115 all the tested TSCs (**1a**-**1j**) and also five TDZs (**2a**, **2b**, **2e**, **2g**, **2h**) were found to be more potent, while other three tested TDZ derivatives (**2c**, **2f**, **2j**) had the same potency as the reference drug. The MIC values ranged between 0.97 and 15.62 µg/mL for the chromenyl-TSCs **1a**-**1j**, compound **1c** exhibiting the best activity. We could notice again the positive influence of the presence of halogen atoms on the antibacterial potential of some compounds (**1f**, **1i**, **1j**). As for the tested chromenyl-TDZ derivatives **2a**-**j**, no significant structure-activity-relationship could be established. MIC values ranged between 1.95 and 62.5 µg/mL, **2e** being the most active (MIC=1.95µg/mL).

Antifungal activity

All of the tested chromenyl-derivatives exhibited excellent antifungal effects against tested *Candida species,* all of them being more active than reference Fluconazole. For the tested chromenyl-TSCs, MIC values ranged between 0.48 and 7.81µg/mL against *C. albicans* ATCC 10231 and ATCC 18804, 0.24 and 7.81µg/mL against *C. krusei* ATCC 6258 and between 0.12 and 1.95 µg/mL against *C. parapsilopsis* ATCC 22019, compounds **1c** and **1i** being the most active. As for the TDZ derivatives 2a-j, MIC values ranged between 0.48 and 7.81 µg/mL against *C. albicans* ATCC 10231, 0.24 and 15.62 µg/mL against *C. albicans* ATCC 18804, 0.24 and

7.81 µg/mL against *C. krusei* ATCC 6258 and between 0.12 and 7.81 µg/mL against *C. parapsilopsis* ATCC 22019, compound **2h** exhibiting the best inhibitory activity.

In general, cyclization of compounds **1a**-**j** to TDZs **2a**-**j** did not have a major influence on the antimicrobial potential. As it can be observed, in some cases MIC and MBC/MFC values decreased after cyclization of TSCs to TDZs (**1h** → **2h**); in others cyclization lead to the increase of these values (**1c** → **2c**) or had no significant influence (**1g** → **2g**).

30S ribosomal protein S12

The ribosome assembly consists of about 54 proteins in eubacteria and at least 80 of them in eukaryotic cells, they being responsible for the assemblage and maintenance of the ribosome. Moreover, many ribosomal proteins are also involved in a variety of extra ribosomal functions (Sudhamalla *et al*., 2012). Ribosomal protein S12, for example, plays key roles in the ribosome's response to some error-promoting antibiotics and in modulating the accuracy of translation (Agarwal and O'Connor, 2014).

All the compounds were docked comparatively with Spectinomycin against the 30S ribosomal protein S12 and the results obtained are shown in table 4. Molecular docking revealed that all the tested compounds possessed the required energy to dock itself with the target receptor, values ranging from -5.6 to -7.8kcal/mol, comparable with that of Spectinomycin (-6.1 kcal/mol). A set of fourteen compounds (**1a**-**d**, **2a**-**j**) exhibited better binding affinity (lower free binding energy) to the target than the standard drug Spectinomycin. The data obtained also showed that all chromenyl-TDZs had better binding affinity than their corresponding TSCs, so we could say that cyclization lead to a decrease in binding energy of these compounds.

Binding poses of the docked molecules are presented in fig. 1. It can be noticed that chromenyl-TSCs (**1a**-**j**, left side of the images) are completely separated from their corresponding TDZs (**2a**-**j**) and reference Spectinomycin (right side of the images). Chromenyl-TDZs **2i** and **2j** had the best binding activities, which may suggest that the insertion of fluorine atoms in the general structure may increase the interaction with this target.

DNA gyrase subunit A and DNA topoisomerase IV subunit A

DNA topoisomerases II (also called DNA gyrase) and IV (Topo IV) are essential for maintenance of proper DNA topology during transcription and replication of bacteria (Shahavar *et al*., 2015). These enzymes are type IIA heterodimer (A2B2) topoisomerases. Both DNA gyrase and Topo IV can relax super coiled DNA, whereas only DNA gyrase can introduce negative supercoils. Topo IV is more effective than DNA gyrase at decatenation of double stranded circular DNA, hence the necessity for both enzymes (Gubaev and Klostermeier, 2011; Gubaev and Klostermeier, 2014; Roca, 1999; Sissi and Palumbo, 2010).

All the investigated compounds were docked comparatively with Moxifloxacin against DNA gyrase subunit A and DNA topoisomerase IV subunit A. The results obtained are presented in tables 5 and 6. Molecular docking revealed that all the tested compounds showed good binding energies towards the target receptors, ranging from -7.1 to -10.4 kcal/mol in the case of DNA gyrase subunit A and from -7.1 to -9.6 in the case of DNA Topo IV subunit A, comparable in both cases with that of Moxifloxacin (-7.9 and respectively -7.4 kcal/mol).

Sixteen compounds (**1e**-**j**, **2a**-**j**) exhibited better binding affinity (lower binding energy) to the target DNA gyrase subunit A than Moxifloxacin. Again, the data obtained showed that all chromenyl-TDZs had lower binding energy than their corresponding TSCs, proving that cyclization of the intermediate compounds might have an important role in enhancing the binding affinity. Compounds **1c**, **1e**, **1f**, **1i**, **1j** and **2e** exhibited well-established H-bonds with some amino acids (Pro219, Ser198, Tyr266) in the receptor active pocket. In both series, compounds bearing fluorine atoms in their structures (**1i**, **1j**, **2i**, **2j**) had the best binding energies.

Thus, similarly to fluoroquinolones, the presence of fluorine atoms enhances the affinity towards the enzyme. It can be observed (fig. 2) that compounds **1c**, **2h**, **2i** and **2j** bond separately to the A subunit of DNA gyrase than the other compounds investigated and reference Moxifloxacin.

Concerning the interactions with Topo IV Subunit A, only one compound (**1b**) had a higher, but still similar, binding energy (-7.1 kcal/mol) than standard Moxifloxacin (-7.4 kcal/mol). Two other chromenyl-TSCs (**1a** and **1d**) showed the same binding energy (-7.4 kcal/mol) with the used reference antibacterial. The other tested chromenyl-derivatives (**1c**, **1e**-**j** and **2a**-**j**) exhibited better binding affinity (lower binding energy) to this target than Moxifloxacin. In general, chromenyl-TDZs had better binding affinity than their corresponding TSCs, leading us to the previous conclusion.

Compounds **1a**, **1b**, **1d**, **1j**, **2g** and also Moxifloxacin exhibited well-established H-bonds with some amino acids (Leu338, Gln159, Phe97pp) in the receptor's active pocket. Like in the case of the other topoisomerase used, we noticed again in both series of compounds that the presence of fluorine atoms in their structures (**1i**, **1j**, **2i**, **2j**) lead to a higher affinity. Fig. 3 shows all the docked chromenyl-derivatives bind similarly to the A subunit of Topo IV, while Moxifloxacin is isolated.

Lanosterol-14α-demethylase

Cytochrome P450 14α-lanosterol-demethylase is an essential enzyme in sterol biosynthesis in eukaryotes, being thus a great target for antifungal drug design (Sheehan *et al*., 1999). The active site of this target includes a triazole ring perpendicularly positioned to the porphyrin plane with a ring nitrogen atom coordinated to the Hem iron (Bano *et al*, 2015).

All twenty molecules were docked comparatively with standard drug Fluconazole against lanosterol-14α-demethylase. The results obtained are presented in table 7. All the compounds docked showed good to very good binding energies, fifteen of them (**1e**-**j**, **2a**-**g** and **2i**-**j**) having a higher affinity than reference Fluconazole. Free binding energy values ranged from -8.1 to -10.8 kcal/mol. Docking of the chromenyl-TSC **1j** in the active site showed that this compound interacts the most effectively (-10.8 kcal/mol). It was observed that some of these compounds (**1d**-**h** and **2b**-**d**) exhibit H-bond interactions with the active site of lanosterol-14α-demethylase through the Tyr140 amino acid. As shown in fig. 4, chromenyl-TDZs **2h**, **2i** and **2j** bind separately (set on the left) from the other investigated compounds and Fluconazole (set on the right).

CONCLUSIONS

In the present study twenty previously reported chromenyl derivatives were investigated for their *in vitro* antibacterial and antifungal properties. All of the tested compounds exhibited excellent antimicrobial activities, most of them being more active than used reference drugs, which indicated their potential for further lead development. A molecular docking study of these compounds was carried out for a better understanding of the drug-receptor interactions. Most of the docked compounds showed better binding energies to the targets than the used reference antimicrobials, compounds bearing fluorine atoms in their structures having the best affinity.

We believe that research data reported in the present paper could contribute to a better general understanding of the cellular mechanisms involved in the antimicrobial effects of the new compounds. However, further studies are needed in order to elucidate the biochemical mechanism of these compounds.

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