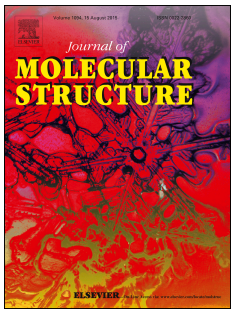


Novel 1,3,4-oxadiazole linked benzopyrimidinones conjugates: Synthesis, DFT study and antimicrobial evaluation

Sarra Chortani, Hayet Edziri, Marwa Manachou, Youssef O. Al-Ghamdi, Sami G. Almalki, Yaser E. Alqurashi, Hichem Ben Jannet, Anis Romdhane

PII: S0022-2860(20)30682-7

DOI: https://doi.org/10.1016/j.molstruc.2020.128357

Reference: MOLSTR 128357

To appear in: *Journal of Molecular Structure*

Received Date: 12 March 2020

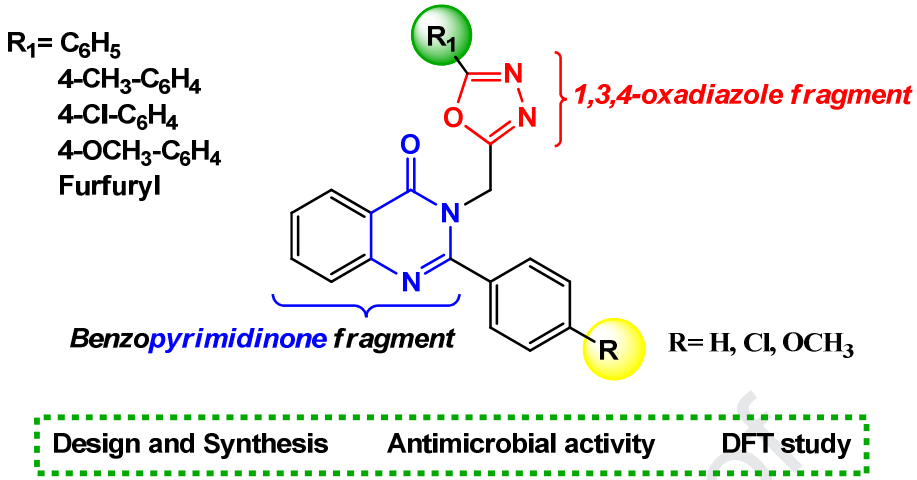
Revised Date: 12 April 2020

Accepted Date: 27 April 2020

Please cite this article as: S. Chortani, H. Edziri, M. Manachou, Y.O. Al-Ghamdi, S.G. Almalki, Y.E. Alqurashi, H. Ben Jannet, A. Romdhane, Novel 1,3,4-oxadiazole linked benzopyrimidinones conjugates: Synthesis, DFT study and antimicrobial evaluation, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.128357.

Sarra Chortani did the synthesis work. Hayet Edziri tested the antimicrobial activity. Marwa Manachouperformed the DFT study. Youssef O. Al-Ghamdi helped in the structure analysis. Sami G. Almalki helped in the interpretation of biological results. Yaser E. Alqurashi guided some biological assays. Hichem Ben Jannet contributed to the discussion of the results and completed the redaction of the manuscript. Anis Romdhane was the supervisor of the present work, he also checked the structure determination of the synthesized compounds and the biological study, and he completed the redaction of the article.

Graphical abstract



Novel 1,3,4-oxadiazole linked benzopyrimidinones conjugates: Synthesis, DFT study and antimicrobial evaluation

Sarra Chortani[a], Hayet Edziri[b], Marwa Manachou[c], Youssef O. Al-Ghamdi[d,*], Sami G. Almalki[e], Yaser E. Alqurashi[e], Hichem Ben Jannet[a], Anis Romdhane[a,*]

[a]Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Team: Medicinal Chemistry and Natural Products (LR11ES39), Department of Chemistry, Faculty of Science of Monastir, University of Monastir, Avenue of Environment, 5019 Monastir, Tunisia.

[b]Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, 5000 Monastir, Tunisia

[c]Laboratory of Material Characterization, Applications and Modeling, Faculty of Science of Tunis, University of Tunis El Manar, 2092, Tunis, Tunisia

[d]Department of Chemistry, College of Science Al-zulfi, Majmaah University, Al-Majmaah, 11952, Saudi Arabia

[e]Department of Biology, College of Science Al-zulfi, Majmaah University, Al-Majmaah, 11952, Saudi Arabia

*Corresponding authors. A. Romdhane (T Tel.: +21673500279, Fax: +21673500278; E-mail: anis_romdhane@yahoo.fr). Y O. Al-Ghamdi (Tel.: +966164044094, Fax: +966164044094; E-mail: yo.alghamdi@mu.edu.sa).

Abstract:

This work describes the synthesis, characterization and antimicrobial evaluation of a series of new 1,3,4-oxadiazole linked benzopyrimidinones. Their structures were confirmed on the basis of ^{1}H, ^{13}C NMR and ES-HRMS analysis. Their molecular geometry are also studied theoretically by the Density Functional Theory (DFT) method with B3LYP/6-31++G(d,p). All the calculations were done in DMSO, using conductor like polarizable continuum (CPCM). The target compounds were screened for their antimicrobial activity against four types of

pathogenic bacteria and three fungal strains. Biological results revealed that some of these compounds demonstrated excellent to moderate antimicrobial activities with minimum inhibitory concentration (MIC) values ranging from 10.8 to 140.7 µM. Notably, compounds **5g** and **5h** showed the highest inhibitory activity against all bacterial strains with MIC values ranging from 111.3 and 10.8 µM against *S. aureus*, *E. coli* and *P. aeruginosa*, and displayed a good antifungal activity against *C. albicans* with MIC values of 10.8 and 27,8 µM, respectively. The structure-activity relationship (SAR) was discussed.

Keywords: benzopyrimidinone, 1,3,4-oxadiazole, antibacterial, antifungal, DFT

1. Introduction

Heterocyclic compounds furnish a basic platform for the building of newer entities and have captured wide spread interest because of their abundance in natural products and their diverse biological properties [1]. Amongst all, heterocyclic molecules containing nitrogen and oxygen are known to display interesting biological activities [2]. In this framwork, pyrimidinones cores have demonstrated a broad spectrum of biological properties including anticancer [3], anti-acetylcholinesterase [4], antityrosinase [5], antibacterial [6] and antifungal [7]. Furthermore, pyrimidine molecules have become an important building blocks for the development of new commercial drugs such as Trimethoprim (antimicrobial) (Fig. 1), Metioprim (antibacterial) (Fig. 1) and Flucytosine (antifungal) (Fig. 1)[8].

In the other hand, oxadiazoles and their derivatives are very attractive targets on account of their diverse biological properties such as cytotoxic [9], antibacterial [10], antioxidant [11] and anti-inflammatory activities [12]. Particularly, 1,3,4-oxadiazoles core has aroused considerable attentions on account of its derivatives are characterized with a wide range of biological activities, including anti-diabetic [13], anti-malarial [14], antimicrobial [15], anticancer [16], analgesic [17] and anti-inflammatory [18]. Moreover, this fragment is present in skeleton of several important commercialized medicinal derivatives such as Raltegravir

(antiretroviral agent) [19] (Fig. 1), Zibotentan (anticancer drug) [20](Fig.1), Fenadiazole (hypnotic drug) [21] (Fig. 1), and Nesapidil (antihypertensive agent) [22](Fig. 1).

The diverse biological properties of these heterocyclic scaffolds prompted us to continue to the development of highly antimicrobial bioactive molecules. In this context, a new series of 1,3,4-oxadiazole linked benzopyrimidinones were synthesized and evaluated for their *in vitro* antimicrobial activity. To support the experimental results, the synthesis of these new hybrid molecules is described through DFT calculations at the B3LYP/6–31 ++G(d,p) level of theory. The reactivity of these series of compounds was studied using hardness, softness and global electrophilicity index as well as the FMO theory.

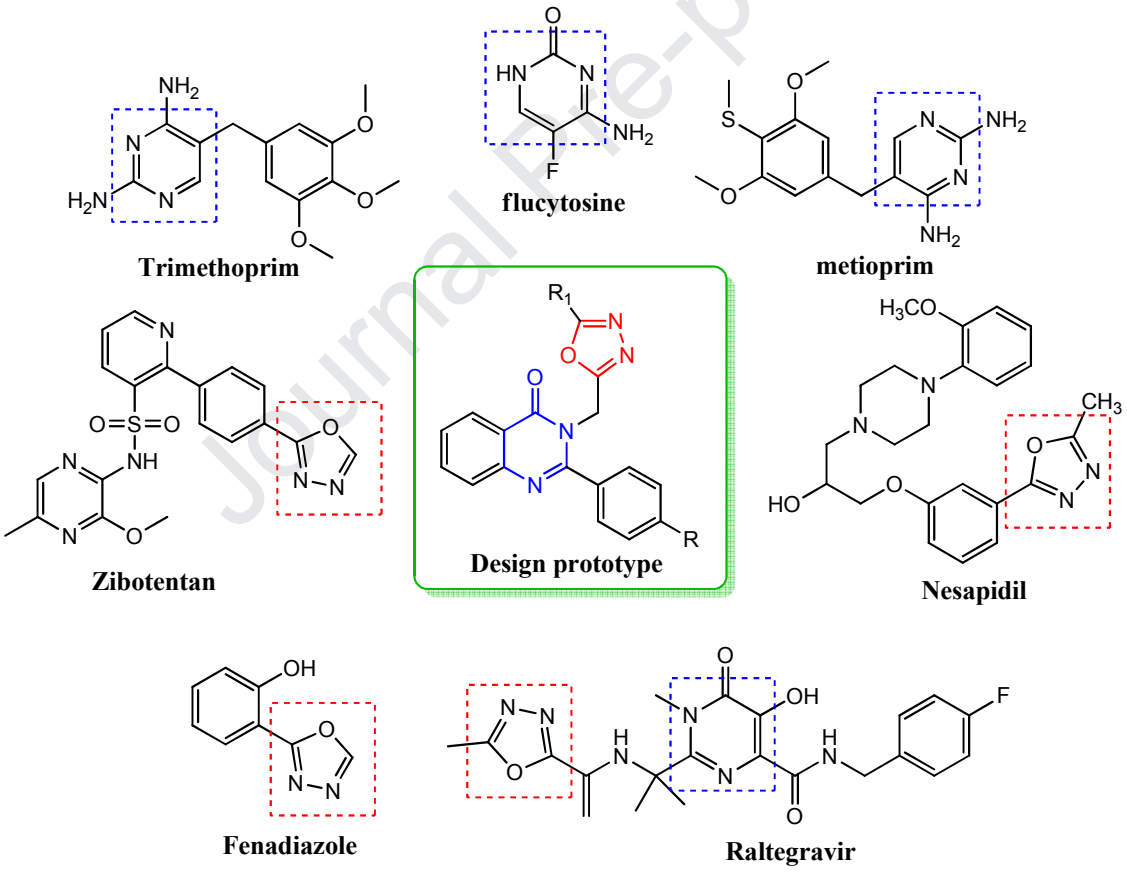


Fig. 1. Representative examples of biologically active drugs containing pyrimidine and 1,3,4-oxadiazole derivatives.

2. Experimental section

2.1. General information

All reactions were monitored by TLC using aluminium sheets of Merck silica gel 60 F254, 0.2 mm. Melting temperatures were determined on an electrothermal 9002 apparatus and were reported uncorrected. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). All chemical shifts were reported as δ values (ppm) relative to residual non deuterated solvent. High Resolution Mass Spectra (ES-HRMS) were obtained with Micromass LCT (ESI technique, positive mode) spectrometers.

*2.1.1. General procedure for the synthesis of 2-aryl-benzopyrimidinone **2***

A mixture of 2-aminobenzamide **1** (5 mmol) and substituted aromatic aldehydes (5 mmol) was heated under reflux for 24 h in dry acetonitrile (50 mL) in the presence of equimolar molecular of iodine. After the reaction was completed, the mixture was cooled to room temperature. A solution of sodium thiosulphate (5%) was added and the resulted solid was filtered off and washed with water. The crude product was recrystallized from ethanol.

*2.1.2. General procedure for preparation of hydrazides **3***

Equimolar solution 1mmol of 2-arylbenzopyrimidinones **2**, anhydrous potassium carbonate and ethyl chloroacetate was refluxed in dry DMF (40 mL) with continuous stirring for 6 h. The reaction mixture was allowed to cool and then it was poured in water and extracted using EtOAc. The organic layer was dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residual was treated with an excess of hydrazine hydrate in ethanol at room temperature for 1 hour. The precipitated solid formed was filtered, washed with ethanol, and dried to obtain compounds **3a-c**.

*2.1.3. General procedure for the synthesis of compounds **5***

Equimolar solution (1mmol) of hydrazide **3** and the arylaldehyde was refluxed in dry ethanol (15 mL) for 2 h under argon atmosphere. The white precipitate formed thus obtained was

filtered to afford compounds **4** which were refluxed by potassium carbonate (1mmol) in dimethyl sulphoxide at 100°C in the presence of catalytic amount of iodine for 8 h. After the reaction was completed, the solvent was evaporated and the residue thus obtained was purified by column chromatography (silica gel, PE /EtOAc 8:2) to give compounds **5a-o**.

2.2. In vitro Antimicrobial Activity

2.2.1. Microorganisms

The microorganisms tested in this work were *S. aureus (ATCC 25923), E. coli (ATCC25922), P. aeruginosa (ATCC 27950),* and *E. faecalis* (ATCC 29212). The antifungal activity was tested against three pathogenic reference yeasts including *Candida albicans ATCC 90028, C. glabrata ATCC 90030* and *C. parapsilosis ATCC 22019.*

2.2.2. Micro-well dilution assay

Minimum inhibitory concentration (MIC) values were determined by a micro dilution method [23]. All product stock solutions were dissolved in 10% dimethyl sulfoxide (DMSO). The inocula of the bacteria and yeasts were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Media were placed into each 96 wells of the microplates. Test solutions at 500 µg/mL were added in the first rows of the microplates and two-fold dilution of the compounds (250–0.125 µg/mL) were made by dispensing the solutions to the remaining wells. Ten microliter culture suspensions were inoculated into all the wells. The plate was covered with a sterile plate sealer and then incubated for 18 h at 37 °C. Ampicillin and Griseofulvin (for bacteria and fungi), and Amoxicillin (for bacteria) were used as standard antibiotics. All tests were performed in triplicate. The MIC was defined as the lowest concentration of the compounds that inhibits the growth of microorganisms after incubation.

2.3. Computational details

The optimization of all geometries was performed by density functional theory using the Gaussian 09 set of programs [24]. Geometry optimization of all products was carried out using DFT methods at the B3LYP/6-31++G(d,p) level of theory [25]. For these systems, we carried out full geometry optimization in the C_1 point group, followed by harmonic frequencies simulations to confirm that they correspond to true minima (all positive frequencies). All the calculations were done in DMSO, using conductor like polarizable continuum (CPCM). The conceptual DFT descriptors [26-28] have been computed using the so-called frozen core approximation and the Koopmans' theorem [29]. Thus, global indexes have been calculated using frontier densities, frontier energies[30-32] such as the chemical potential $\mu \cong (E_{HOMO} + E_{LUMO})/2$ [33], hardness $\eta \cong (E_{LUMO} - E_{HOMO})$ and the electrophilicity index $\omega = \mu^2/2\eta$[34-36]. Molecular structure drawings and spin densities plots were generated using the ADF-GUI auxiliary program [37a,b,c,d].

3. Results and discussion

3.1. Synthesis

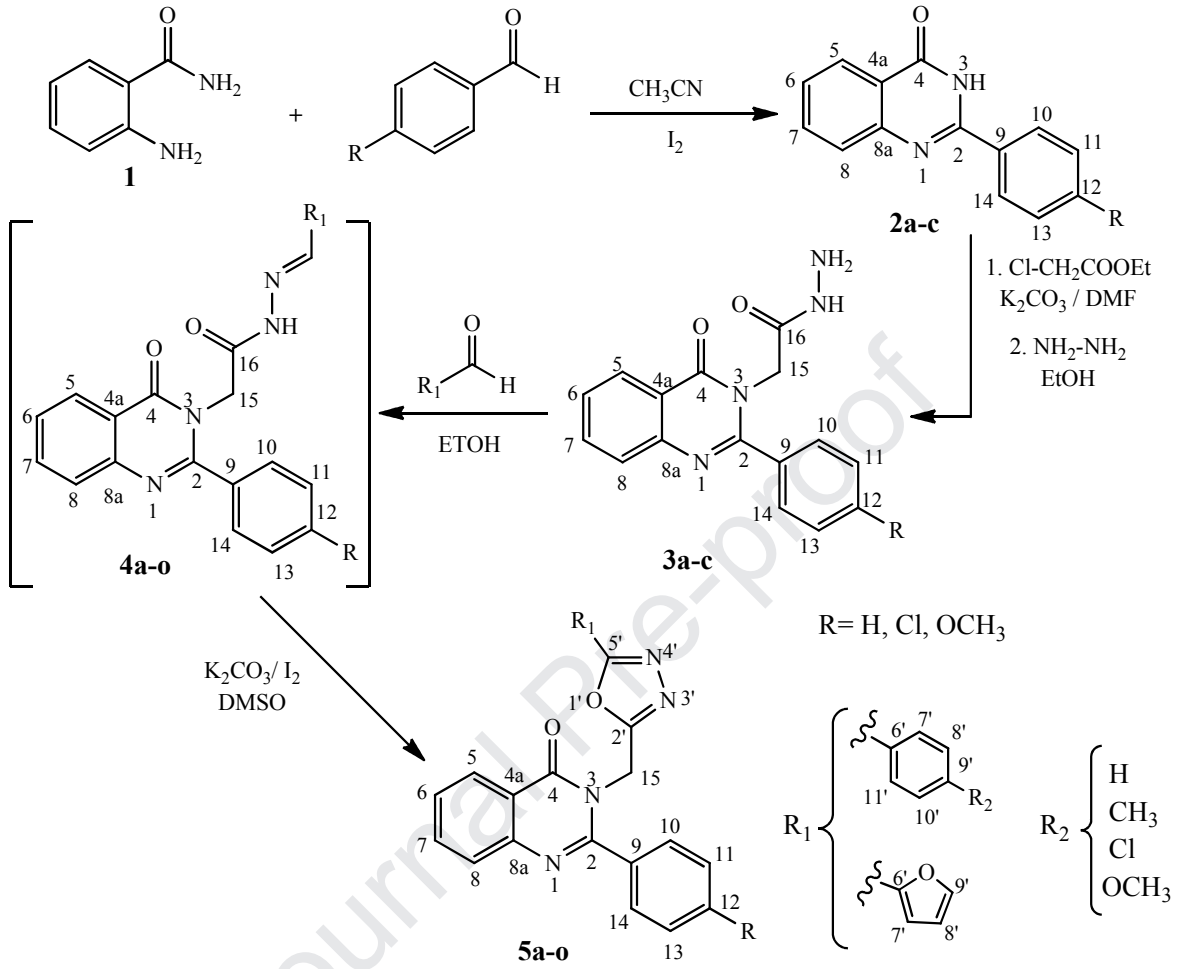
The synthetic route, used for the synthesis of a new 1,3,4-oxadiazoles linked benzopyrimidinones is depicted in Scheme 1. Initially, the first step involved the formation of benzopyrimidinones derivatives, which were efficiently generated by the condensation reaction of 2-aminobenzamide **1** with various substituted aromatic aldehydes in the presence of equimolar molecular iodinein acetonitrile [3]. The structures of the formed benzopyrimidinones **2a-c** were characterized by their ^{1}H NMR and ^{13}C NMR spectra. In fact, the ^{1}H NMR spectra showed, in addition of the signals corresponding to the protons introduced by the arylaldehyde and the 2-aminobenzamide moiety, the presence of the singlet at 12.26 - 12.53 ppm due to the NH proton. Unambiguous proofs for the obtained compounds aroused from their ^{13}C NMR data. Particularly the shift-values attributed to the quaternary

carbons C-2 (151.4 - 151.9 ppm) and C-4 (162.2 - 162.3 ppm) were consistent with the strong deshielding effects caused by heteroatom's proximity.

Esterification of benzopyrimidinones **2a-c** with ethyl chloroacetate in DMF in the presence of potassium carbonate gave the synthesized esters, which were converted to the corresponding hydrazides **3a-3c** by stiring with hydrazine hydrate in ethanol at room temperature, in good yields [38-39]. The structures of **3a-3c** were ascertained by their ^{1}H NMR spectra showing a new singlet at 5.11-5.14 ppm relative to the methylene group (N-CH_2-) and the presence of a new two singlets due to mobile protons NH and NH_2 at 9.48-950 and 3.65-4.32 ppm, respectively. The ^{13}C NMR spectra of compounds **3a-3c** showed the appearance of new signals at 64.1-64.3 and 166.1-166.3 ppm attributed to the methylene carbon (CH_2) and C=O, respectively.

In addition, the synthetic hydrazides **3a-3c** were reacted with substituted aromatic aldehydes in dioxane affording the hydrazones **4a-4o** which were cyclized *in situ*, in presence of iodine and potassium carbonate in dimethyl sulphoxide, to give the desired 1,3,4-oxadiazole derivatives **5a-5o** [40]. The physical properties of compounds **5a-5o** are shown in Table 1. The structures of the target compounds **5a-5o** were confirmed by their spectral data (^{1}H and ^{13}C NMR and ES-HRMS). In fact, the ^{1}H NMR spectra of these compounds exhibited the absence of two singlets related to the mobile protons (NH and NH_2), the presence of a singlet corresponding to the methylene group (CH_2) at 5.91-6.06 ppm and the appearance of a new characteristic signals at 6.61-8.56 ppm attributable to the aromatic protons introduced by aromatic aldehyde. In the ^{13}C-NMR spectra of these products, we observed essentially the appearance of two new signals attributable to C-2 and C-5 carbons of the oxadiazole ring at 158.5-165.9 ppm and the disappearance of a signal at 166.1-166.3 ppm corresponding to the carbonyl of the hydrazid group (C-16). Moreover, high resolution mass spectrometry (ES-

HRMS) data of all the examined compounds **5a-5o** were also in agreement with the proposed structures.



Scheme 1. Synthetic route of benzopyrimidinones linked 1,3,4-oxadiazoles **5a-o**.

Table 1. Structures and yields of compounds **5a-o**.

	Substituent		
Compounds	**R**	**R_1**	**Yields %**
5a	H	C_6H_5	66
5b	H	4-CH_3-C_6H_4	74
5c	H	4-Cl-C_6H_4	60
5d	H	4-OCH_3-C_6H_4	77
5e	H	furyl	63
5f	Cl	C_6H_5	65
5g	Cl	4-CH_3-C_6H_4	74

5h	Cl	4-Cl-C_6H_4	61
5i	Cl	4-OCH_3-C_6H_4	78
5j	Cl	furyl	62
5k	OCH_3	C_6H_5	69
5l	OCH_3	4-CH_3-C_6H_4	79
5m	OCH_3	4-Cl-C_6H_4	64
5n	OCH_3	4-OCH_3-C_6H_4	80
5o	OCH_3	furyl	68

3.2. Biological activity

The synthesized compounds **2a-c**, **3a-c** and **5a-o** were screened for their *in vitro* antibacterial and antifungal activity.

3.2.1 *In vitro Antibacterial activity*

The antibacterial activity of the target compounds was assayed *in vitro* against Gram-positive bacteria, namely, *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), and Gram-negative bacteria, namely, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Escherichia coli* (*E. coli*). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the product inhibiting the visible growth of each microorganism which was ascertained by the broth microdilution method.

The results presented in Table **2** showed that most compounds from the series **2a-c** and **3a-c** were found to have slight to moderate antibacterial against the four tested bacteria compared to the reference antibiotics (Ampicillin and Amoxicillin).On the other hand, compounds **5a-o** showed in most cases interesting antibacterial potential. The compound **5c** displayed significant antibacterial activity against *E. coli* and *P. aeruginosa* with MIC values of 45 and 22.5 µM, respectively compared to the reference antibiotics. The results showed that compound **5g** exhibited a good activity against all the strains used with MIC values ranging from 10.8 to 21.7µM. This finding shows that the presence of a chlorine atom in *para* position in the phenyl group introduced by the arylaldehyde, used in the first step of the synthesis,

seems at the origin of the high activity. This conclusion was reinforced by the weak activity of the derivative **5b** where only the chlorine atom has been changed by a hydrogen atom. The compound **5h** displayed an interesting activity against *S. aureus* and *E. faecalis* with MIC value of 27.8µM. The existence of two chlorine atoms in this molecule may be at the origin of the noted antibacterial activity. The activity of this derivative compared to that of **5c** (MIC = 90.1µM against *S. aureus* and *E. faecalis*) where the chlorine atom introduced by the arylaldehyde is replaced by a hydrogen atom can explain the effect of this halogen on the antibacterial activity. Compounds **5j** and **5o** were found to be active towards *S. aureus* and *E. faecalis* with MIC values of 71.3 and 87.4, respectively. The activity of these two derivatives (**5j** and **5o**), compared to certain analogs, could be explained by the presence in each one of a furyl ring.

Table 2. *In vitro* Antibacterial activity of synthesized compounds 2a-c, 3a-c and 5a-o

	MIC(µM)			
	Gram-positive bacteria		**Gram-negative bacteria**	
Compounds	*Staphylococcus aureus* ATCC 25923	*Enterococus faecalis* ATCC 29212	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853
2a	337.7	337.7	337.7	337.7
2b	390.5	390.5	390.5	195.2
2c	247.9	247.9	123.9	123.9
3a	255.0	255.0	255.0	255.0
3b	106.6	106.6	106.6	106.6
3c	192.8	192.8	192.8	96.4
5a	131.5	131.5	263.0	131.5
5b	253.7	253.7	253.7	126.8
5c	90.1	90.1	45.0	22.5
5d	121.8	121.8	121.8	121.8
5e	135	135.0	135.0	67.5
5f	181.1	181.1	181.1	181.1
5g	10.8	10.8	21.7	10.8
5h	27.8	27.8	111.3	27.8
5i	140.7	140.7	140.7	70.3
5j	77.3	77.3	154.6	154.6
5k	152.3	152.3	152.3	152.3
5l	117.8	117.8	117.8	117.8
5m	140.7	140.7	140.7	140.7
5n	113.5	113.5	113.5	113.5

5o	87.4	87.4	174.9	87.4
Ampicillin[a]	71.5	-	28.62	28.62
Amoxicillin[a]	-	0.27	70.0	-

[a] positive control

3.2.2 *In vitro Antifungal activity*

The *in vivo* fungicidal results of the synthesized compounds **2a-c**, **3a-c** and **5a-o** against *Candida parapsilosis* (*C. parapsilosis*), *Candida albicans* (*C. albicans*) and *Candida glabrata* (*C. glabrata*) were presented in Table 3. The most synthesized compounds displayed variable degrees of inhibition against the three yeasts used. Griseofulvin and Ampicillin were used as standard antifungal agents.

However, Compounds **2a-c**, and **3a-c** did not show any interesting activity towards the used fungal strains by comparison with the positive controls employed.

On the other hand, certain derivatives from the series **5a-o** exhibited a promising antifungal activity against *C. albicans* with MIC values ranging from of 140.7 (**5n**) and 10.8 (**5g**) µM compared to Griseofulvin (MIC = 141.7µM).

Compound **5g**, the only derivative among those having R = chlorine, has a fairly high activity (MIC = 10.8µM) against the three fungi used. The coexistence of this chlorine atom and the 4-CH_3-C_6H_4- group in the same molecule can explain this strong activity compared to other analogs bearing in each case a chlorine with a C_6H_5-(**5f**), 4-Cl-C_6H_4-(**5h**), 4-OCH_3-C_6H_4- (**5i**) and furyl (**5j**). The substitution of the chlorine atom in **5g** by a hydrogen atom in compound **5b** significantly attenuated this activity which goes from 10.8 µM to 45.0 µM against *C. parapsilosis*, *C. albicans* and to 90.1µM towards *C. glabrata*. The activity of compound **5h** against *C. albicans* with an MIC value of 27.8 µM can be also interpreted by the coexistence of the two chlorine atoms in both aromatic rings.

With respect to the structure-activity relationship (SAR), The results indicated that the presence of a chlorine atom in the aromatic ring and the introduction of methyl or chlorine

group in the phenyl ring of the 1,3,4-oxadiazole system were in favor of the high activity of compounds **5g** and **5h**.

Table 3. ***In vitro*** **Antifungal activity of synthesized compounds 2a-c, 3a-c and 5a-o**

	MIC(µM)		
Compounds	*C. parapsilosis ATCC 22019*	*C. albicans ATCC 90028*	*C. glabrata* ATCC 90030
2a	337.7	337.7	337.7
2b	195.2	195.2	195.2
2c	123.9	123.9	247.9
3a	255.0	255.0	255.0
3b	106.6	106.6	106.6
3c	192.8	96.4	192.8
5a	131.5	131.5	131.5
5b	45.0	45.0	90.1
5c	63.4	126.8	126.8
5d	121.8	121.8	5121.8
5e	67.5	67.5	67.5
5f	181.1	181.1	181.1
5g	10.8	10.8	10.8
5h	55.6	27.8	55.6
5i	140.7	70.3	140.7
5j	77.3	154.6	77.3
5k	152.3	152.3	76.1
5l	117.8	117.8	117.8
5n	140.7	140.7	140.7
5m	113.5	113.5	113.5
5o	87.4	87.4	174.9
Griseofulvin[a]	-	141.7	-
Ampicillin[a]	0.143	-	0.143

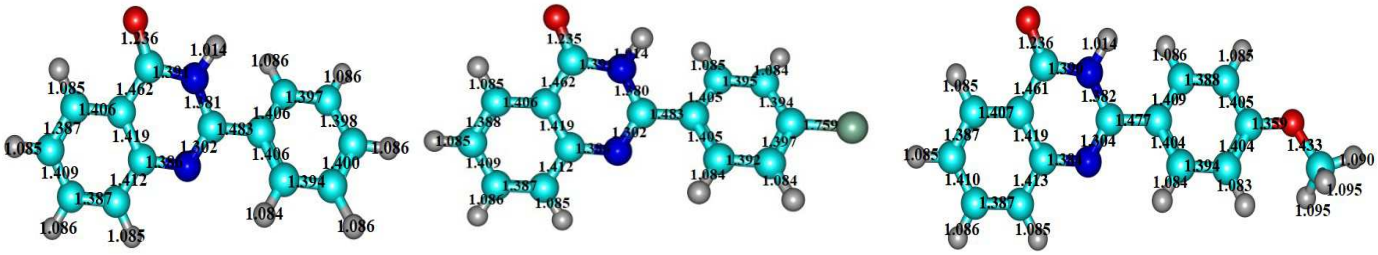
[a]positive control

3.3. DFT study

In order to understand the behavior of molecules in reactions, it is advisable to use reliable theoretical reactivity indices based on computational chemistry methods to interpret experimental data. The density functional theory DFT [41] is the most often used quantum chemical methods because of its low cost and satisfactory performance.

Equilibrium structure and Vibrational analysis of benzopyrimidinone:

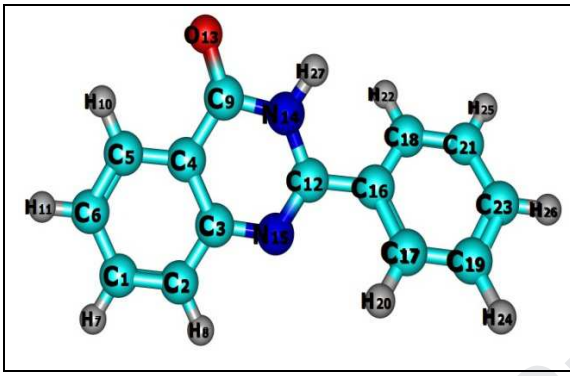
Figure 2 and Table 4 show the main geometrical parameters of the optimized equilibrium geometry of benzopyrimidinone considered as the core of the prepared molecules. These data are obtained using B3LYP method in conjunction with the 6-31G++ (d,p) basis sets. Close examination of these structures shows that the benzopyrimidinone moiety within these compounds has very close structure. Indeed, C=O, C-C, C-N and N-H distances of 1.23, 1.46, 1.39, 1.01 (all values are in Å), respectively, have been computed. The deviations between the distances and the angles calculated using B3LYP are less than 0.01 Å and less than 1°. Thus, the functionalization of this heterocycle (benzopyrimidinone) slightly disturbs its structure. This is confirmed by the vibrational analysis performed for this series. Anharmonic frequencies can be obtained after scaling the corresponding harmonic frequencies by the appropriate scaling factor (by 0.9662 for $\omega > 1000$ cm^{-1} and by 0.9833 for $\omega < 1000$ cm^{-1}) as discussed in Ref. [42]. Table 5 presents the respective data for C=N, C=O and NH elongations. This table shows that similar C=N (of~1557 cm^{-1}), C=O (of ~1641 cm^{-1}), NH (of ~3476 cm^{-1}) elongations values are computed for all the compounds. Indeed, these differ by less than 5 cm^{-1} within the series. This little difference cannot be considered as significant for reactivity.



2a **2b** **2c**

Figure 2. 3D structures and bond lengths (in Å) of benzopyrimidinone obtained using B3LYP/6-31G++ (d,p)

Table 4. Valence angles (in degree) of benzopyrimidinone derivatives computed using B3LYP/6-31G++(d,p)



Angles	2a	2b	2c
C3-N15-C12	118.1	118.1	118.2
N15-C12-N14	122.2	122.3	121.9
C12-N14-C9	124.8	124.7	124.9
N14-C9-C4	113.4	113.4	113.5
C9-C4-C3	118.5	118.5	118.4
O13-C9-N14	120.3	120.3	120.3

Table 5. Scaled anharmonic vibrational frequencies (in cm^{-1}) (scaling factor 0.9662 [42]) of some elongation modes of benzopyrimidinone derivatives as computed using B3LYP/6-31++G(d,p).

Compounds	2a	2b	2c	Assignement
C=O	1641.3	1643.6	1640.1	C=O stretching
NH	3476.5	3477.6	3475.8	N–H stretching
C_{12}=N	1557.8	1542.4	1541.0	C=N stretching

Chemical reactivity of synthesized compounds 2a-c, 3a-c and 5a-o

To investigate the relation between electronic parameters and experimental results, the electronic parameters of these compounds have been calculated. The energies of HOMO and LUMO are related to the reactivity of the molecule. For instance, molecules with electrons at accessible (near-zero) HOMO levels tend to be good nucleophiles because donation of these electrons toward making a new bond is easy. Similarly, molecules with low LUMO energies

tend to be good electrophiles because placing an electron into such an orbital is facilitated. Both properties can be connected *via* the energy gap (ΔE) between the HOMO and the LUMO.ΔE = E_{LUMO} − E_{HOMO} showkinetically stability of the molecules. Figures 3-5 shows the dispersion of the HOMO, LUMO orbitals calculated with DFT methods at the B3LYP/6-31++G(d,p) level of theory.

The HOMO and LUMO distributions of phenylquinazolinone and their derivatives of group I II and III are illustrated in Figures 3-5. As shown in this figures, the LUMO distributions are almost the same, while the HOMO distributions are different. The HOMO distributions are localized around quinazolinone, which is most important part among involving constituents in the antibacterial and antifungal activity. These characteristics may be helpful for a qualitative understanding of the electrostatic interactions that may take place between reagents or enzyme active sites and the phenylbenzopyrimidinone derivatives under study.

On the other hand, electron-donating like (Cl, CH_3…) groups delocalize the HOMO all over the molecules, which support, in most cases, the activity of the corresponding compounds.

Concerning the value of the energy of the gap ΔE, the larger values of the energy difference will provide the low reactivity to a chemical species. The chemical hardness, electronic chemical potential and electronegativity are also important tools to study stability or reactivity of species. Regarding to the Pearson's maximum hardness principle [43] which shows the relation between energy and chemical hardness. All structures, which have the lowest value of chemical hardness and energy gap, are the highest reactivity species.

In this context, Table 6 shows that 2-phenylbenzopyrimidin-4(*3H*)-one **2a** has a large energy gap (4.530 eV) compared to **2c** (4.381 eV). According to these data we can conclude that the 2-(4-methoxyphenyl) benzopyrimidin-4(*3H*)-one **2c** is softer and more reactive and less stable in comparison with the other analogsfrom the series **2**. The result is the same for the other series. Indeed, as shown in Tables 7-9, the compounds **3c**, **5m** (R=OCH_3, R_1=4-Cl-C_6H_4) and

5o (R=OCH_3, R_1=furyl)) have the lowest energy gap (4.653, 4.360 and 4.521eV, respectively) and the lowest hardness (2.326, 2.180 and 2.260 eV, respectively) compared to the other derivatives in their respective series. These findings were found to being agreement with the results of biological activity. Therefore, the lowest chemical hardness, the energy gap, and the highest chemical potential for these molecules, make these them a most active species in their respective groups. All other descriptors converge to the same result.

A distinct feature of the systems containing electron-donating group is the small HOMO-LUMO gap which can be correlated with a stronger reactivity [44] as was observed in the antibacterial and antifungal activity. This small interval is always related to the substitution of a donor group whatever the series studied in our case we find it at the level of **2c**, **3c** and **5o** (Figures 3-5). Indeed, energy gap descreases when a electro-donor group is substituted at the radicals, in a case of **3a-c** (ΔE= 4.87, 4.82, 4.65 eV, respectively). As can be seen, compound **3c** has the smallest energy gap (ΔE= 4.65 eV). Thus, this compound is the most reactive one in this series.

Therefore, electron-donating like (Cl, OCH_3…) groups substituted in *para* position of the phenylquinazolinone core, according to their softness improve the antimicrobial activity and their reactivity [45]. This order is proportion to the reaction yield and to the biological activity noted. In fact, compounds **5g** shows a good activity against all the strains used with MIC values ranging from 10.8 to 21.7 µM. In overall, this result supports the experimental trend. So to increase the value of the activity, we can decrease the E_{LUMO} value, for which we suggest the substitution of the phenylquinazolinone core with a stronger donor electron ability group.

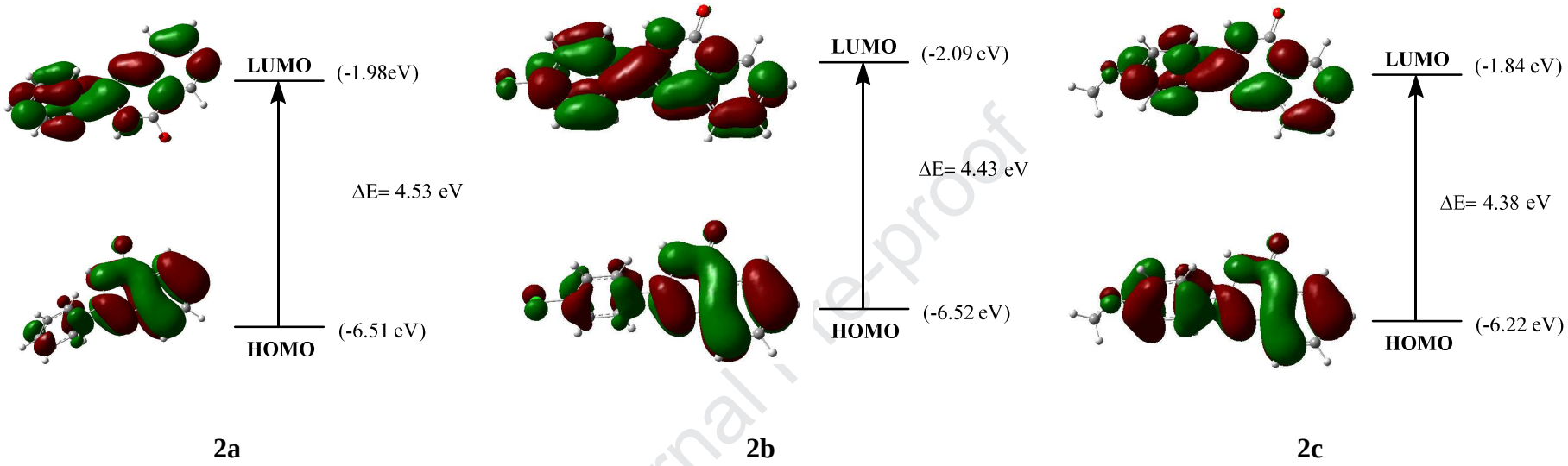


Figure 3. The frontier molecular orbitals of group (I)

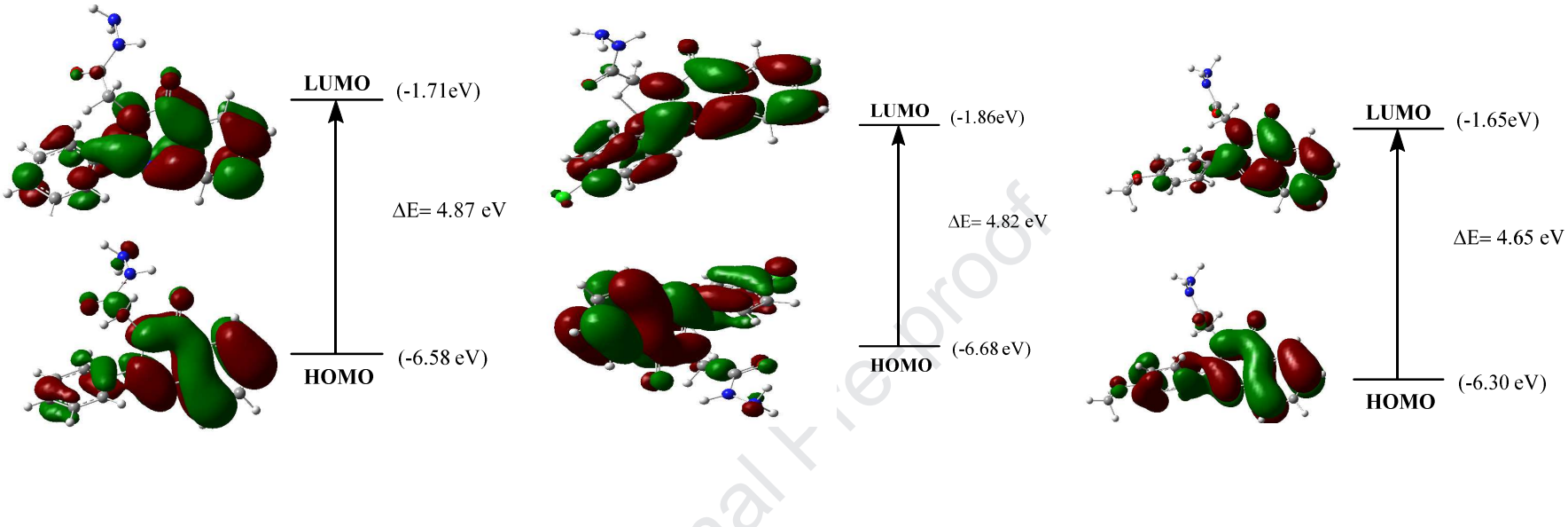
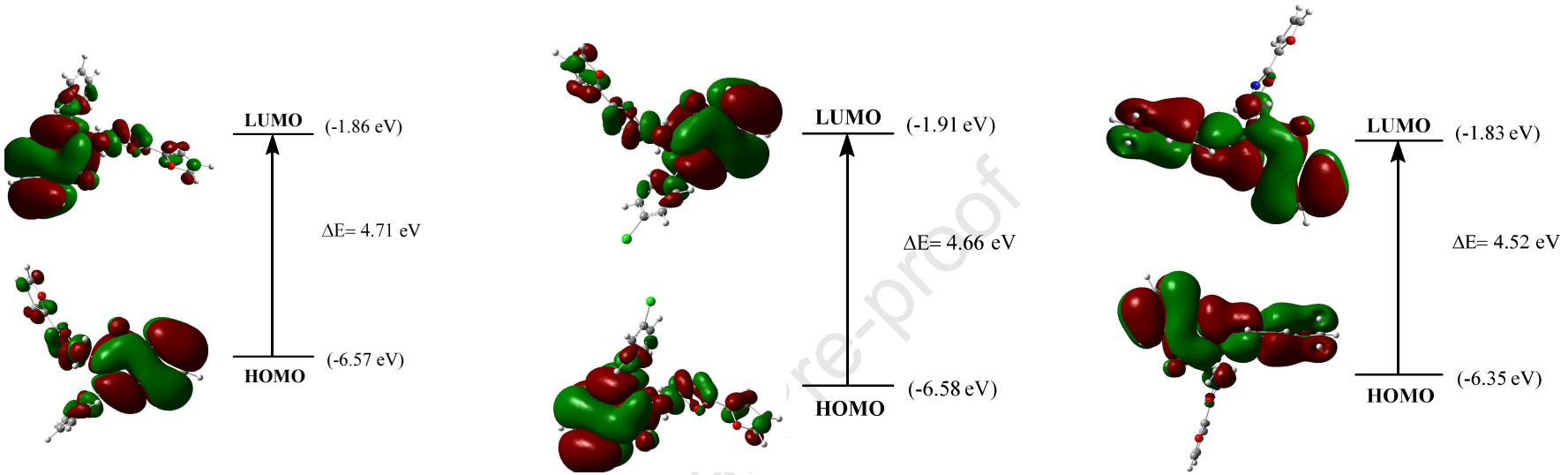


Figure 4. The frontier molecular orbitals of group (II)



5 e **5 j** **5 o**

Figure 5. The frontier molecular orbitals of group (III)

Table 6. Frontier orbital energy HOMO, LUMO, energy gap (ΔE), the global reactivity descriptors (μ, η, S, w) in eV, and yields of reaction for group (I)

entry	yield %	R	E_{HOMO}	E_{LUMO}	χ	μ	η	S(eV^{-1})	ω
2a	**55**	**H**	-6.515	-1.984	4.250	-4.250	2.265	0.220	3.987
2b	**60**	**Cl**	-6.529	-2.092	4.310	-4.310	2.218	0.225	4.187
2c	**68**	**OMe**	-6.226	-1.845	4.036	-4.036	2.190	0.228	3.718

Table 7. Frontier orbital energy HOMO, LUMO, energy gap (ΔE), the global reactivity descriptors (μ, η, S, w) in eV, and yields of reaction for group (II)

entry	yield %	R	E_{HOMO}	E_{LUMO}	χ	μ	η	S(eV^{-1})	ω
3a	**58**	**H**	-6.588	-1.717	4.152	-4.152	2.435	0.205	3.540
3b	**64**	**Cl**	-6.688	-1.865	4.276	-4.277	2.411	0.207	3.793
3c	**72**	**OMe**	-6.303	-1.650	3.976	-3.977	2.326	0.214	3.398

Table 8. Frontier orbital energy HOMO, LUMO, energy gap (ΔE), the global reactivity descriptors (μ, η, S, w) in eV, and yields of reaction for group (III)

entry	yield %	R	E_{HOMO}	E_{LUMO}	χ	μ	η	S(eV^{-1})	ω
5e	**63**	**H**	-6.572	-1.860	4.216	-4.216	2.355	0.212	3.773
5j	**62**	**Cl**	-6.580	-1.911	4.245	-4.246	2.334	0.214	3.861
5o	**68**	**OMe**	-6.353	-1.832	4.093	-4.093	2.260	0.221	3.705

Table 9. Frontier orbital energy HOMO, LUMO, energy gap (ΔE), the global reactivity descriptors (μ, η, S, w) in eV, and yields of reaction

entry	yield %	R	R1	E_{HOMO}	E_{LUMO}	ΔE	χ	μ	η	$S(eV^{-1})$	ω
5a	66	**H**	**C_6H_5**	-6.727	-1.862	4.864	4.295	-4.295	2.432	0.205	3.792
5b	74		**4-CH_3-C_6H_4**	-6.681	-1.789	4.892	4.235	-4.235	2.446	0.204	3.666
5c	60		**4-Cl-C_6H_4**	-6.701	-1.993	4.708	4.347	-4.347	2.354	0.212	4.014
5d	77		**4-OMe-C_6H_4**	-6.360	-1.760	4.600	4.060	-4.060	2.300	0.217	3.584
5f	65	**Cl**	**C_6H_5**	-6.661	-1.922	4.738	4.291	-4.291	2.369	0.211	3.887
5g	74		**4-CH_3-C_6H_4**	-6.705	-1.820	4.885	4.262	-4.262	2.442	0.204	3.719
5h	61		**4-Cl-C_6H_4**	-6.725	-2.007	4.718	4.366	-4.366	2.359	0.211	4.041
5i	78		**4-OMe-C_6H_4**	-6.368	-1.783	4.585	4.075	-4.075	2.292	0.218	3.622
5k	69	**OMe**	**C_6H_5**	-6.354	-1.865	4.488	4.109	-4.109	2.244	0.222	3.763
5l	79		**4-CH_3-C_6H_4**	-6.351	-1.792	4.559	4.071	-4.071	2.279	0.219	3.636
5m	64		**4-Cl-C_6H_4**	-6.356	-1.995	4.360	4.176	-4.176	2.180	0.229	3.999
5n	80		**4-OMe-C_6H_4**	-6.333	-1.737	4.596	4.035	-4.035	2.298	0.217	3.543

4. Conclusion

In conclusion, a new series of benzopyrimidinones linked 1,3,4-oxadiazoles were designed and synthesized by reacting the hydrazide **3a-c**, previously prepared from 2-aminobenzamide **1** in two steps, with various substituted aromatic aldehydes provided the *N*-substituted hydrazides **4a-c**, which were cyclized in presence of iodine and potassium carbonate. The newly prepared compounds **2-5** have been tested for *in vitro* antimicrobial activity. Amongst all the tested compounds **5g** and **5h** exhibited excellent antibacterial activity particularly against *S. aureus, E. coli* and *P. aeruginosa* and showed the highest antifungal activity against *C. albicans*. The study of the SAR permitted to conclude in most cases the presence of chlorine atom attached to aromatic ring and the importance of the nature of the substituents attached the aryl group on the 1,3,4-oxadiazoles moiety improved the activity against fungi and some bacterial organisms tested. Theoretical DFT/B3LYP study has been carried out in order to explain the reactivityof the different product and to understand the experimental findings. The energies of HOMO and LUMO are related to the reactivity and the biological activity of the molecule and this properties can be connected via the energy gap (ΔE) between the HOMO and the LUMO. As we saw previously, **5m** and **5o** have the lowest energy gap in comparison with the other molecules their respective groups. Further, the lowest chemical hardness, and the highest chemical potential for these molecules, make these compounds a most active species in their respective groups.

Conflict of interest

None declared.

Acknowledgments

The authors are grateful to the Ministry of Higher Education and Scientific Research of Tunisia for financial support. The authors also would like to thank Deanship of Scientific Research at Majmaah University, Saudi Arabia, for supporting this work.

References:

[1] M. Jha, S. Guy, Y. C. *Tetrahedron Lett*. 52 (2011) 4337-4341.

[2] H. Doshi, S. Thakkar, P. Khirsariya, M. Thakur, A. Ray, *Appl. Biochem. Biotechnol*. 175 (2015) 1700-1709.

[3] A. Rahmouni, S. Souiei, M. A. Belkacem, A. Romdhane, J. Bouajila, H. B. Jannet, *Bioorg.Chem*, 66 (2016) 160-168.

[4] A. Romdhane, A. B. Said, M. Cherif, H. B. Jannet, *Med. Chem. Res*. 25 (2016) 1358-1368.

[5] M. Debbabi, V. D. Nimbarte, S. Chekir, S. Chortani, A. Romdhane, H. B. Jannet, *Bioorg. Chem*. 82 (2019) 129-138.

[6] L. Suresh, P. S. VijayKumar, Y. Poornachandra, C. G. Kumar, G. V. P. Chandramouli, *Bioorg. Chem*. 68 (2016) 159-165.

[7] J. Zhang, J. F. Peng, T. Wang, P. Wang, Z. T. Zhang, *J. Mol. Struct*. 1120 (2016) 228-233.

[8] M. M. Sekhar, U. Nagarjuna, V. Padmavathi, A. Padmaja, N. V. Reddy, T. Vijaya, *Eur. J. Med. Chem*. 145 (2018) 1-10.

[9] A. B. Saïd, A. Romdhane, N. Elie, D. Touboul, H. B. Jannet, J. Bouajila, *J. Enzyme Inhib. Med. Chem*. 31 (2015) 1277-1285.

[10] N. P. Rai, V. K. Narayanaswamy, T. Govender, B. K. Manuprasad, S. Shashikanth, P. N. Arunachalam, *Eur. J. Med. Chem*. 45 (2010) 2677-2682.

[11] S. Ningaiah, U. K. Bhadraiah, S. Keshavamurthy, *Bioorg. Med. Chem. Lett*. 23 (2013) 4532-4539.

[12] M. Farooqui, R. Bora, C. R. Patil, *Eur. J. Med. Chem*. 44 (2009) 794-799.

[13] R. Bhutani, D. P. Pathak, G. Kapoor, A. Husain, M. A. Iqbal, *Bioorg. Chem.* 83 (2019) 6-19.

[14] L. H. Al-Wahaibi, N. S. Kumar, A. A. El-Emam, N. S. Venkataramanan, H. A. Ghabbour, A. M. S. Al-Tamimi, J. Percino, S. Thamotharan, *J. Mol. Struct*. 1175 (2019) 230-240.

[15] H. Khalilullah, S. Khan, M. S. Nomani, B. Ahmed, *Arab. J. Chem*. 9 (2016) S1029-S1035.

[16] J. Sławinski, K. Szafranski , A. Pogorzelska, B. Zołnowska, A. Kawiak, K. Macur, M. Belka , T. Baczek, *Eur. J. Med. Chem*.132 (2017) 236 -248.

[17] A. Husain, A. Ahmad, M. M. Alam, M. Ajmal, P. Ahuja, *Eur. J. Med. Chem*. 44 (2009) 3798-3804.

[18] M. Amir, S. Kumar, *Acta. Pharm*. 57 (2007) 31-45.

[19] A. Savarino, *Expert Opin. Inv. Drug.* 15 (2006**)** 1507-1522.

[20] N. D. James, J. W. Growcott, *Drug. Future*, 34 (2009) 624-633.

[21] H. Varshney, A. Ahmad, A. Rauf, A. Sherwani, M. Owais, *Med. Chem. Res*. 24 (2015) 944-953.

[22] N. C. Desai, N. Bhatt, H. Somani, A. Trivedi, *Eur. J. Med. Chem*, 67 (2013) 54-59.

[23] R. Sakly, H. Edziri, M. Askri, M. Knorr, C. Strohmann, M. Mastouri, *C. R. Chim*. 21 (2017) 41-53.

[24] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, HB. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, HP. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Jr. Montgomery, J. E. Peralta,

F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, (2009)

[25] C. Lee, W. Yang, R.G. Parr, *Phys. Rev*. B 37 (1988) 785-789.

[26] M. Chebbi, H. Chebbi, H. M'rabet, Y. Arfaoui, *Chem. Res. Chin. Univ*. 3 (2017) 765-772.

[27] S. Daoudi, M. Chebbia, N. Hamdi, Y. Arfaoui, *MOJ Drug Des DevelopTher*. 1 (2017) 78-87.

[28] L. R. Domingo, M. Ríos-Gutiérrez, P. Pérez, *J. Org. Chem*. 83, 4, (2018), 2182-2197.

[29] T. A. Koopmans, *Physica*. 1 (1933) 104-113.

[30] L. R. Domingo, M. Ríos-Gutiérrez, P. Pérez, *Molecules*, 21 (2016) 748-769, https://doi.org/10.3390/molecules21060748.

[31] G. Klopman, *J. Am. Chem. Soc*. 90 (1986) 223-234.

[32] R. G. Parr, L. Von Szentpaly, S. Liu, J. *Am. Chem. Soc*. 121, 9, (1999) 1922-1924.

[33] K. Harrath, S. Boughdiri, R. Linguerri, M. Hochlaf, *Theor. Chem. Acc*. 2 (2016) 135-142.

[34] R. G. Parr, R. G. Pearson, *J. Am. Chem. Soc*. 105, 26, (1983) 7512-7516.

[35] J. Frau, D. Glossman-Mitnik, *Front Chem*. 6 (2018) 136, doi: 10.3389/fchem.2018.00136.

[36] P. Geerlings, F. De Proft, W. Langenaeker, *Chem. Rev*. 103 (2003) 1793-1873

[37] (a) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford, CT, (2010). (b) G. teVelde, F. M. Bickelhaupt, E. J. Baerends, C. Fonseca Guerra, S. J. A. Van Gisbergen, J. G. Snijders, T. Ziegler, *Jour. Comput. Chem.* 22 (2001) 931-967. (c) C. F. Guerra, J. G. Snijders, G. teVelde, E. J. Baerends, Theor. *Chem. Acc*. 99 (1998) 391-403. (d) ADF2016.04, Theoretical Chemistry; Vrije University: Amsterdam, The Netherlands; http://www.scm.com/.

[38] M. Zayane, A. Romdhane, M. Daami-Remadi, H. B. Jannet, *J. Chem. Sc*. 127 (2015)1619-1626.

[39] S. Medimagh-Saidana, A. Romdhane, M. Daami-Remadi, H. Jabnoun-Khiareddine, D. Touboul, H. B. Jannet, M. A. Hamza, *Med. Chem. Res*. 24 (2015**)** 3247-3257.

[40] R. N. Kumar, Y. Poornachandra, P. Nagender, G. S. Kumar, D. K. Swaroop, C. G. Kumar, B. Narsaiah, *Bioorg. Med. Chem.Lett*. 26 (2016) 4829-4831.

[41] K. Harrath., K. Essalah, C. Morell, H. Chermette, S. Boughdiri, *Theor. Chem. Acc*. 134 (2015) 98-103.

[42] M. L. Laury, M. J.Carlson, A. K. Wilson, *J. Comput. Chem.* 33 (2012) 2380-2387.

[43] Pearson, R.G., *Coord. Chem. Rev.* 100 (1990) 403-425.

[44] J. Aihara, *Phys. Chem. Chem. Phys.* 2 (2000) 3121-3125.

[45] V. Mkpenie, I. Mkpenie, E. Essien, *Der Pharma Chem*. 7 (2015) 330-334.

Highlights

- A series of new 1,3,4-oxadiazole linked benzopyrimidinones **5a-o** was synthesized
- The structures of these hybrid compounds were established by spectroscopic methods.
- All compounds were evaluated *in vitro* for their antibacterial and antifungal activities.
- Biological results revealed that all synthesized compounds **3a-3i** possess significant antibacterial activity against selected strains.
- Some of compounds **5a-o** demonstrated excellent to moderate antimicrobial activities.
- The DFT analysis results showed the importance of the 1,3,4-oxadiazolobenzopyrimidinone system to get a potent antimicrobial activity.

Declaration of interests

X ☐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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: