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Original article

Conventional and microwave irradiated synthesis, biological activity evaluation and molecular docking studies of highly substituted piperazine-azole hybrids

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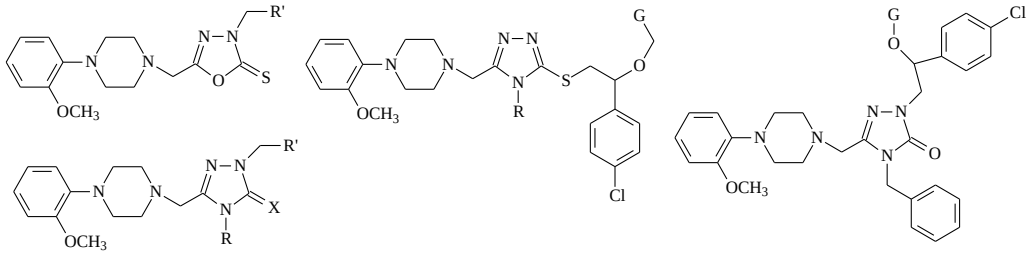
Graphical Abstract



New hybrid molecules consisting of fluoroquinolone, methoxyphenylpiperazine and azole moieties were synthesized by microwave irradiated and conventional methods. The newly synthesized compounds were screened for their antimicrobial, antiurease, antiglucosidase and antioxidant activities. Also, molecular docking studies were performed.

ABSTRACT

Azole derivatives (**3**, **6**) obtained starting from 1-(2-methoxyphenyl)piperazine were converted to the corresponding Mannich bases containing *β*-lactame or flouroquinolone core *via* a one pot three component reaction. The synthesis of conazole analogues was carried out starting from triazoles by three steps. Reactions were carried out under conventional and microwave mediated conditions. All the newly synthesized compounds were screened for their antimicrobial, enzyme inhibition and antioxidant activity, and most of them displayed good-moderate activity. Binding affinities and non-covalent interactions between enzyme-ligand complexes were predicted with molecular docking method at molecular level. Docking results complemented well the experimental results on α-glucosidase and urease inhibitory effects of the compounds. Higher binding affinities and much more interaction networks were observed for active compounds in contrary to inactive ones. It was predicted with the docking studies that triazole and anisole moieties in the structure of the synthesized compounds contributed to the stabilization of corresponding enzymes through noncovalent interactions.

Keywords:
Fluoroquinolone
1,2,4-Triazole
Microwave
Mannich reaction
Biological activity
Molecular docking

1. Introduction

The goal of the anti-infective therapy is to rid the host of the pathogens, whereas the therapy of other diseases takes aim at direct the host [1]. Although significant progress has been made for the treatment and control of microbial infections by introducing new strategies and combinatorial therapy, antimicrobial resistance continues to be one of major concerns to the public health and scientific communities worldwide. Because, infections caused by resistant pathogens fail to response to treatment resulting in prolonged illness and greater risk of death. The history of antimicrobial chemotherapy has clearly demonstrated that the drugs used for the treatment of microbial infections are also responsible for making these diseases more difficult to treat in future due to the increasing antimicrobial resistance [1, 2]. In order to overcome the antimicrobial resistance, several strategies have been developed; one of which contains the development of hybrid molecules synthesized by combination of several pharmacophores that are selected on the basis of their known bio profiles [3-6].

Nitrogen-containing heterocycles constitute an extraordinary class of synthetic organic chemistry, natural products, drugs and food industry [7]. Among them, piperazine nucleus locates in the structures of a number of important drugs such as ciprofloxacin, norfloxacin, pefloxacin, ofloxacin, lomefloxacin, which are fluoroquinolone class antibacterials used for the treatment of various infections caused by Gram negative bacteria (Fig. S1 in Supporting information) [8-10].

The science of organic synthesis is constantly enriched by the improvement of synthetic methodologies. Microwave assisted techniques were reported to be more effective in perspective of environment, reaction time, high yields, ease of work-up and isolation of products. Moreover, solvents which are often expensive, toxic, difficult to remove in the case of aprotic dipolar solvents with high boiling point, and are environmentally polluting agents, are not necessary most of the microwave assisted synthesis [11]. Recently, multicomponent reactions (MCRs) have received considerable attention by synthetic organic and medicinal chemists for the construction of complex molecules having biological activity. When compared with conventional organic reactions, MCRs have some superior properties including high conversion rate, minimal reaction time and structural complexity. Thus, MCRs are also considered as green chemical processes [12]. Among these, Mannich reaction, a one pot three-component condensation reaction, provide synthetically and biologically important β-aminoalkylated compounds, which are important intermediates for the construction of various nitrogen-containing natural products and pharmaceuticals [13].

Most of free radicals are unstable species and capable of abstracting electrons from other molecules. Hydrogen peroxide (H_2O_2), the hydroxyl radical (HO), the superoxide anion radical (HOA), which are the predominant reactive oxygen species generated by cell metabolism or by exogenous factors have essential roles in cell signaling, apoptosis and gene expression. The damages of DNA, proteins and lipids caused by excessive free radical attacks can resulted in some diseases such as cancer, neurological degeneration and arthritis, as well as the process of aging. The discovery of new agents with antioxidant properties has become another extraordinary active area of preventive medicinal chemistry [14-16].

In light of these considerations, as the continuation of our ongoing efforts endowed with the discovery of hybrid compounds, we reported here the synthesis and biological activities of new hybrid molecules containing several heterocyclic nuclei with biological activity by ecofriendly and traditional methods (Fig. S2 in Supporting information). Docking some of the synthesized compounds into the active sites of the α-glucosidase and urease enzymes was performed as well to predict the binding affinities and non-covalent interactions between enzyme-ligand at molecular level.

2. Results and discussion

2.1. Chemistry

In the present study, the ecofriendly synthesis, antimicrobial, enzyme inhibition and antioxidant activity screening, and molecular docking studies of new hybrid molecules was intended. The structures of newly synthesized compounds were established on the basis of physicochemical, elemental analysis and spectral data (FT IR, ^{1}H NMR, ^{13}C NMR and EI-MS).

Compounds **2, 5a** and **6a** were synthesized following the procedure reported by us earlier [2]. The cyclocondensation of the hydrazide (**2**) with CS_2 in ethanol-water in basic media generated the compound, {[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2-thiol (**3**) under reflux, and also microwave irradiated conditions (Scheme 1). This idea originated from the aim to obtain an active hydrogen compound with biological activity that can be used as an intermediate for further condensation reactions leading to the formation of new bioactive products. In order to optimize the method, microwave (MW) irradiation was applied at different power values of 80, 100, 150 and 200 W and the progress of reaction was monitored by TLC. The complete conversion of the starting hydrazide (**2**) was observed after microwave irradiation at 200 W for 4 min. It is noteworthy to underline that shorter reaction time or lower microwave energy power caused to lower conversion rate, while increasing

reaction time or MW power resulted in decomposition of the target product as revealed by TLC analysis. Assignment of the structure of **3** was based on correct elemental analysis results. FT IR spectrum of compound **3** revealed the presence of -SH group at 2832 cm^{-1}. A singlet characteristic for the -SH group was recorded at 14.35 ppm in the ^{1}H NMR spectra of compound **3**. In the mass spectrum, the molecular ion peak (*m/z*= 307.36, M+1) is present corresponding to the molecular weight of the assigned for compound **3**.

The nucleophilic addition of compound **2** to alkyliso(thio)cyanates afforded the corresponding carbo(thio)amides (**5a-d**) (Scheme 1). The reaction conditions were examined in DCM under reflux conditions as well as under solvent free microwave irradiation conditions with a view to maximizing the yield of the product. To optimize reaction conditions, the synthesis of compound **5a** was selected as model reaction and microwave (MW) irradiation was applied at different power values of 100, 150, 200, 250 and 300 W (the progress of reaction was monitored by TLC). The complete conversion of the starting hydrazide (**2**) was observed after microwave irradiation at 100 W for 10 min. Compared with conventional thermal heating, microwave irradiation decreased the reaction time from 20 h to 10 min. It is noteworthy to underline that shorter reaction time or lower microwave energy power caused to lower conversion rate, while increasing reaction time or MW power resulted in decomposition of the target product as revealed by TLC analysis. FT IR spectra of compounds **5a, 5b** and **5d** revealed the presence of -C=S group 1235-1244 cm^{-1}. On the other hand, the FT-IR spectrum of compound **5c** exhibited -C=O stretching band at 1658 cm^{-1}. In the ^{13}C NMR spectra of these compounds (**5a-d**), -C=S or -C=O function resonated between 162.44-181.25 ppm. Another evidence for the formation of carbo(thio)amides was the presence of three -NH- signals at 7.16-9.22 ppm in the ^{1}H NMR spectra as D_2O exchangeable singlet. The elemental analysis data of carbon, hydrogen and nitrogen were formed to be satisfactory and within the permissible limit error.

The synthesis of triazoles (**6a-d**) was carried out by the treatment of NaOH in ethanol-water under reflux condition in order to introduce the 1,2,4-triazole nucleus to piperazine skeleton, because it is well known that more efficacious bioactive compounds can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework [2, 14-16] (Scheme 1). In addition, type of compounds **6a-d** can be considered as useful tolls having active NH group for further one pot three component aminoalkylation reactions leading to the formation new bioactive compounds. Then, the scope of these reactions was investigated under microwave irradiation. Compared with conventional thermal heating, microwave irradiation decreased the reaction time from 4-13 h to 5-13 min and increased the yields from 80%–88% to 92%–100% (Table 1).

It is well known that 1,2,4-triazol-3-(thi)ol derivatives can exist as mercapto-thioxo (or enol-keto) tautomeric forms. The –SH proton due to mercapto form resonates at about 13-14 ppm, while NH signal originated from thioxo tautomer appears at 9-12 ppm as D_2O exchangeable signals [2, 17-20]. In the FT-IR spectra of compounds **6a** and **6d**, the presence of stretching bands at 2829 (for **6a**) and 2822 cm^{-1} (for **6d**) originated from –SH function supported the mercapto form for compounds **6a** and **6d**. The –SH proton resonated at 13.85 and 13.84 ppm as D_2O exchangeable peaks, respectively, in the ^{1}H NMR spectra of these compounds. On the other hand, the signal due to thioxo (for **6b**) or keto (for **6c**) group was recorded at 1238 or 1707 cm^{-1} as expected, while no signal due to –SH (for **6b**) or –OH (for **6c**) function was observed. Moreover, the D_2O exchangeable signal belonging to NH group pointing the thioxo or keto form for compounds **6b** and **6d** appeared at 11.62 and 11.72 ppm, respectively. The additional support for the formation of the targeted compounds, **6a-d** was obtained by the appearance of [M+1], [M+2], [M+Na] and/or [M+K] ion peaks at corresponding *m/z* values confirming their molecular masses; and these compounds have given elemental analysis results consistent with the proposed structures.

The one-pot, three-component Mannich type reaction of compounds **3** and **6a-d** with several amines namely 6-aminopencillanic acid, 7-aminocephalosporanic acid, norfloxacin and ciprofloxacin in the presence of formaldehyde generated the aminoalkylated products (**4a, 4b**; **7a-h**) (Scheme 1). This reaction proceeds *via* the formation of immonium salt which subsequently attacks the *N*-1 of triazole or oxadiazole *N*-3 giving rise to the corresponding Mannich bases. Two methods were applied for this treatment including conventional and microwave assisted techniques. Time and yield data for the synthesis of compounds **4** and **7a-h** by microwave and conventional methods were given in Table 1.

In comparison with the long refluxing time, microwave irradiation provided more efficient and green way for one pot Mannich type condensation with relatively higher product yield. The number of signals and their chemical shifts are in accordance with the assigned structures for the Mannich bases. In the ^{1}H NMR and ^{13}C NMR spectra, additional signals corresponding to the 6-aminopenicillanic acid, norfloxacin or ciprofloxacin, were recorded at the related chemical shift values, while the spectra of these compounds showed the disappearance of the characteristic bands of triazole (or oxadiazole) -NH or -SH. Moreover, the preparation of Mannich bases was verified by registration of their mass spectrums which were in accordance with their molecular masses and the elemental analysis data (carbon, hydrogen and nitrogen) were ±0.4% of the theoretical values.

The synthesis of new analogues (**10a-i** and **13a-c)** of triazole class antifungals was carried out starting from the corresponding 3*H*-1,2,4-triazole compounds (**6a-d)** *via* three steps, because in recent years, serious fungal infections have emerged as an important complication and a major cause of disease and mortality [21, 22]. The structures of

these compounds were confirmed on the basis of spectroscopic methods and elemental analysis (Scheme 2 and Scheme 3).

2.2. Biological Activity

2.2.1. Antimicrobial Activity

All the newly synthesized compounds were screened for their antimicrobial activity and the results obtained were presented in Table 2. Among these, compound **3**, that contains a 1,3,4-oxadiazole nucleus linked to piperazine skeleton *via* a methylene linkage, was found to be active on *Escherichia coli* (Ec), a Gram-negative, facultatively anaerobic bacterium, *Yersinia pseudotuberculosis* (Yp), a Gram-negative bacterium, *Staphylococcus aureus* (Sa) and *Enterococcus faecalis* (Ef), which are Gram positive cocci, and *Mycobacterium smegmatis* (Ms), a nonpigmented rapidly growing mycobacterium with the mic values varying 31.25-250 µg/mL. The conversion of compound **3** to the corresponding Mannich bases (**4a-d**) containing a *β*-lactame or fluoroquinolone unit resulted in an increase in the antimicrobial activity. Among these, compounds **4a** and **4b,** which contain a fluoroquinolone nucleus attached to piperazinyl-oxadiazole skeleton displayed excellent antimicrobial activities. In fact, the activities of these compounds (**4a** and **4b)** is better than standard drug ampicillin with the MIC values <0.24 µg/mL. Other mannich bases derived from compound **3** (**4c** and **4d**), having a *β*-lactam nucleus instead of fluoroquinolone, exhibited good-moderate activity on some of the test microorganisms. Among these, the MIC values of compound **4c** on Ec and Ms varying 7.81 and 3.91 µg/mL are better than standard drugs, ampicillin and streptomycin.

Among the carbon(thio)amides, compounds **5b-d** were found to have good-moderate activity against the test microorganisms with the MIC values between 7.8-125 µg/mL except Pa and Ef. Carbothioamide **5a** containing a *N*-phenyl nucleus linked to thioamide carbon exhibited activity towards none of the test microorganisms. Among the 1,2,4-triazole derivatives (**6a-d**), the best activity was observed for **6d**, that includes a benzyl group at the position 4 of 4*H*-1,2,4-triazole-3-thiol skeleton, on Ec and Ms with the MIC values 7.81 µg/mL and 3.91 µg/mL, respectively. On the other hand, excellent activities with the MIC values varing <0.12-1.95 µg/mL were observed for compounds **7a-h**, the synthesis of which was carried out by the aminoalkylation of the corresponding compounds **6a-d** with norfloxacine or ciprofloxacine, concequently, these compounds can be regarded as fluorquinolone-triazole-phenylpiperazine hydrids. On the other hand, these compounds are inactive against *Candida albicans* (Ca) and *Saccharomyces cerevisiae* (Sc), yeast like fungy. Only slight activity was observed for **7d** and **7h** on Ca and Sc with the MIC values 500 µg/mL.

Table 2 revealed that compounds **10a**, **10i** and **13a-c,** which can be considered as new analogues of miconazole, econazole or sulconazole (Figure 1) containing triazole nucleus instead of imidazole exhibited good antifungal activity on *Saccharomyces cerevisiae* (Sc) and *Candida albicans* (Ca) with the MIC values 62.5 or 78 µg/mL as expected.

2.2.2. Principal component analysis (PCA)

The analyzed results for comparing the microwave irradiation method (MIM) and conventional method (CM) by PCA are shown in Fig. 1a. PC of Mannich bases listed in Table 1 (**4a-d** and **7a-h**) explained 78.43 % of total variation, where PC1 accounts for 61.59% of the variance and PC2 for 16.85%. PCA indicated that compounds **4b, 4a** and **7e** at the right lower plan and compounds **7f-h** at the right upper plan on PC1 were mostly closely associated and strong correlated with the CM-yield, and MIM-yield (r = 0.958, 0.878, 0.897, P< 0.05), while MIM-time and CM-time did not correlate with any of them. In contrast, compounds **4c**, **7d** and **4d** at the right lower plan on PC2 were closely associated with CM-time-Acat and negatively strong correlated with MIM-yield (r = -0.948), CM-yield (r = -0.972).

Table 3 shows antioxidant capacity (µmol TE/g) values of 35 synthesized novel compounds measured by DPPH, FRAP and CUPRAC assays. The AC values given in the table significantly (P< 0.05) differed within and among assayed tests. The highest AC values were obtained from compound **4b** (832.91), following **4a** (556.05) as fluoroquinolone for DPPH, **5d** (4744.08 mmol/TE g) following **1** (3094.08) for FRAP and **4b** (1192.38) following **7g** (894.22 µmol) as fluoroquinolone for CUPRAC. The table includes compound **1**, hydrazide (**2**), carbothioamide (**5d**) and carboxamide (**5c**) each, five compounds in triazole (**6a-d**), three compounds in ethanone (**8a, b, d**), four compounds in ethanol (**9a-d**), ten compounds in conazole (**10a-i** and **13c**) and nine compounds in fluoroquinolone (**4a**, **4b**, **7a**, **b**, **d**, **e**, **f**, **h**, **g**) groups.

The Fig. 1b represents the antioxidant capacity (AC) data of the synthesized 35 novel compounds assayed using the three assays applied to PCA. The PCA of the compounds' AC values explained 91.03% of total variation, where PC1 accounts for 58.56% of the variance and PC2 for 32.47%. PC1 separated DPPH and CUPRAC from the other AC assay, FRAP. First, DPPH, following CUPRAC having a positive loading along the axis on PC1, was associated and positively high correlated (r = 0.729, P< 0.05) with higher AC of compounds, **4b**, **7d**, **7h**, **5c** and **6c,** which are three fluoroquinolones, one carboxamide and one triazole derivatives, respectively. FRAP was associated more with

AC of compounds **4a**, **7a**, **7g** and **7b** as fluoroquinolone, **6a**, **6b** and **6d** as triazole and **2** as hydrazide derivatives and did not correlate with other two AC tests (r = 0.062). In contrast, the remaining 22 compounds did not lead to a complete separation for AC values depending on the AC assay types. They were situated on the negative and positive axes on PC2, contributing more or less equal numbers of compounds at the left lower and upper plans of the principal component.

The average AC values of hydrazide (**2**), carbo(thio)amide (**5a-d**) triazole (**6a-d**), ethanone (**8a, b, d**), ethanol (**9a-d**), conazole (**10a-i** and **13c**), fluoroquinolone (**4a**, **4b**, **7a**, **b**, **d**, **e**, **f**, **h**, **g**) derivatives were also applied to PCA (Fig. 1c). PC of AC values of the compounds at nine derivative levels explained 90.80 % of total variation, where PC1 accounts for 51.60 % of the variance and PC2 for 39.20 %. PCA indicated that carboxamide derivative with CUPRAC and fluoroquinolone, triazole and hydrazide with DPPH at the right lower and upper plan on PC1 and carbothioamide, benzyl-ethanone and phenylyethanone with FRAP at the left upper plan were most closely significantly associated and correlated with 18 compounds in these groups. However, the remaining five derivative groups with 17 compounds situated at the left lower plan on PC2 were associated and correlated within alone, but not the three AC assay tests (Fig. 1c).

2.2.3. Urease inhibitory activity

The newly prepared compounds were assayed for their *in vitro* urease inhibitory activity against Jack bean urease with the inhibition rate 59% and 57%, respectively. Two of those compounds (**10d** and **13a**), which are new analogues of triazole class antifungals, showed promising antiurease activity. The remaining compounds displayed no inhibitory effect (Table 4).

2.2.4. α-Glucosidase inhibitory activity

All compounds were evaluated with regard to α-glucosidase inhibition. Compounds **10a, 13a** and **13b**, which may be regarded as new analoques of triazole class antifungals, exhibited good inhibitory effect at various concentrations with inhibitory rates of 80%, 91% and 99% of 100 µmol/L, respectively (Table 4). Acarbose, an α-glucosidase inhibitor used as an anti-diabetic drug, exhibited an inhibitory effect of 83% at the same concentration. No significant inhibitory effect was detected for other compounds.

2.3. Molecular docking results

In order to predict the binding mode and affinities of compounds to target enzymes *saccharomyces cerevisiae* α-glucosidase and *H. pylori* urease, molecular docking was carried out. For comparison, an active and an inactive compound were selected for the corresponding enzymes according to the experimental results on their inhibitory effects.

The most energetically profitable poses of the active compound **13b** (99±5% inhibition) and inactive compound **9b** (5±1% inhibition) in the active site of *saccharomyces cerevisiae* α-glucosidase enzyme were presented in Fig. 2 and Fig. S3 (in supporting information), respectively in both three–dimensional (3D) and two-dimensional (2D) spaces.

For the docking of the compounds **13b** and **9b** to α-glucosidase, binding affinity values of -8.2 and -7.9 kcal mol^{-1} were predicted, correspondingly. Weak hydrogen bonds of the C–H···O, C–H···π and O–H···π were the characteristic interactions for the active compound **13b** (Fig. 2b). Three C–H···O interactions were obtained between the different methylene groups of **13b** and both Pro312 and Asp242 amino acids in the distance range of 3.16–3.30 Å. Aromatic dichlorobenzene ring of **13b** was constituted two C–H···π interactions with Pro243 residue at 3.98 and 4.38 Å distance. Another C–H···π interaction was formed between Tyr158 and **13b** at a distance of 3.54 Å. In addition, the presence of water molecules in the binding site of α-glucosidase caused an O–H···π interaction with anisole moiety of **13b** (3.96 Å). In case of inactive compound **9b**, it was formed a π ···π interaction of His280 and triazole ring at 3.91 Å and a C–H···π interaction between the endmost methyl group of compound and Tyr158 amino acid at 4.11 Å distance (Fig. S3b in Supporting information). Smaller molecular structure of **9b** with less functional group resulted with fewer non-covalent interactions and lower binding affinity comparison to **13b**.

Active compound **10d** (59±3% inhibition) and inactive compound **10g** (4±1% inhibition) were given as an example of interaction with *H. pylori* urease in Fig. S4 and Fig. S5, respectively.

Cys321 and piperazine ring of **10d** formed S–H···N hydrogen bond interaction at 3.45 Å distance. **10d** was interacted with Met317 amino acid *via* C–H···S (at 3.70 Å) and arene-H (3.81 Å) interactions. The other arene-H contacts were obtained for the compound and His322, His323 and Ala169 residues (Fig. S4b in Supporting information). In contrary to large non-covalent interactions network between **10d** and urease, only a C–H···π interaction was formed among inactive compound **10g** and His322 residue of the enzyme. A noticeable difference in the energy of binding with urease was also predicted for two compounds: -9.6 kcal mol^{-1}for the active compound **10d** and 7.3 kcal/mol for the inactive **10g**. Lower binding affinity value of **10g** can be attributed to the weak interaction with the enzyme urease.

3. Conclusions

This study reports the successful synthesis of some new hybrid compounds or conazole derivatives starting from methoxyphenyl piperazine. The effect of MW irradiation on the progress of the reactions was investigated. The antimicrobial, antioxidant and enzyme inhibitory activity studies were also performed in the study. The antimicrobial screening suggested that the compounds containing norfloxacin or ciprofloxacin nucleus displayed excellent antimicrobial activity. Some of them displayed inhibition properties on *S. cerevisiae* better or equivalent to fluconazole, while, most of the synthesized compounds exhibited good antioxidant activity. Moreover, two compounds showed promising antiurease activity and three of them displayed good anti α-glucosidase activity.

Docking some of the synthesized compounds into the active sites of the α-glucosidase and urease was carried out and the obtained results complemented well the experimental results on α-glucosidase and urease inhibitory effects of the compounds. Higher binding affinities and much more interaction networks were observed for active compounds in contrary to inactive ones. It was predicted with the docking studies that triazole and anisole moieties in the structure of the synthesized compounds contributed to the stabilization of corresponding enzymes through noncovalent interactions.

4. Experimental

4.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz NMR spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, *J* values are given in Hz. The elemental analysis was performed on *a Costech Elemental Combustion System* CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4 % of the theoretical values. The mass spectra were obtained on a Quattro LC-MS (70 eV) Instrument. Microwave irradiated reactions were performed in a CEM Discovery monomode synthesis reactor. Compounds **2, 5a** and **6a** were reported by us earlier [10].

*4.1.1. {[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (**3**)*

Method 1: Compound **2** (10 mmol) and CS_2 (0.60 mL, 10 mmol) were added to a solution of KOH (0.56 g, 10 mol) in 50 mL H_2O and 50 mL ethanol and the reaction mixture was refluxed for 7 h. Then, the reaction content was acidified with conc. HCl to pH 6. The precipitate formed was filtered off, washed with H_2O and recrystallized from ethanol to afford the desired compound. Yield: 89 %.

Method 2: The solution of compound **2** (1 mmol) and CS_2 (0.60 mL, 1 mmol) in H_2O+ethanol (5 mL+ 5 mL) containing KOH (1 mmol) was irradiated in monomode microwave reactor in closed vessel with the pressure control at 100 ºC, 200 W maximum power for 4 min (hold time). After the completion of the reaction, (monitored by TLC), the mixture was acidified with conc. HCl to pH 6. The precipitate formed was filtered off, washed with H_2O and recrystallized from ethanol to afford the desired compound. Yield: 94%.

*4.1.2. General method for the synthesis of compounds **5a-d***

Method 1: A mixture of compound **2** (10 mmol) and the corresponding iso(thio)cyanate (10 mmol) in dichloromethane was stirred at room temperature for 20 h (monitored by TLC). After evaporating the solvent under reduced pressure, a solid obtained. The crude product was purified by crystallisation from ethanol (for **5a** and **5c**) or ethyl acetate (for **5b**).

Method 2: A mixture of compound **2** (1 mmol) and the corresponding iso(thio)cyanate (1 mmol) was irradiated in closed vessels with the pressure control at 100 ºC, 100 W (the progress of reaction was monitored by TLC) for 10 min. Then, the mixture was poured into ice water and a solid formed. The crude product was collected by filtration and purified by crystallization from dimethyl sulfoxide.

*4.1.3. General method for the synthesis of compounds **6a-d***

Method 1: A solution of corresponding carbo(thio)amide **5a-d** (10 mmol) in ethanol/water (1:1) was refluxed in the presence of 2 mol/L NaOH (20 mmol) for 4 h (for **6a**), 13 h (for **6b**) or 9 h (for **6c**) and 8 h (for **6d**) (the progress of the reaction was monitored by TLC). Then, the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethanol to give the target compound.

Method 2: The mixture of compound **5a-d** (1 mmol) and 2 mol/L NaOH (2 mmol) in ethanol (10 mL) was irradiated in monomode microwave reactor in open vessel (physical parameters were given in Table 1). Then the resulting solution was cooled to room temperature and acidified to pH 5 with 37 % HCl. The precipitate formed was filtered off, wash with water, and recrystallized from ethyl acetate (for **6a** and **6c**), ethanol (for **6d**) and methanol (for **6b**) to give the target compounds.

*4.1.4. General method for the synthesis of compounds **4a-d** and **7a-h***

Method 1: The solution of 7-aminocephalosporanic acid (for **4d**), 6-aminopenicillanic acid (for **4c**), ciprofloxacin (for **4b**, **7b**, **7d**, **7f**, **7h**) or norfloxacin (for **4a**, **7a**, **7e**, **7g**), (10 mmol) in dimethyl formamide was stirred at room temperature in the presence of formaldehyde (37%, 30 mmol) for 15 min. Then, the corresponding compound **3** or **6a-d** (10 mmol) was added into it and stirred for additional 20-24 h. The reaction mixture was poured to ice-water and a solid obtained. This crude product was recrystallized from an appropriate solvent to give the desired compound.

Method 2: The mixture of 7-aminocephalosporanic acid (for **4d**), 6-aminopenicillanic acid (for **4c**), ciprofloxacin (for **4b**, **7b**, **7d**, **7f**, **7h**), norfloxacin (for **4a**, **7a**, **7e**, **7g**), (1 mmol), formaldehyde (37%, 3 mmol) and the corresponding compound **3** or **6** (1 mmol) in dimethyl formamide (10 mL) was irradiated in monomode microwave reactor in closed vessel at 100 ^{o}C (physical parameters were given Table 1). The solid formed after the mixture was poured to ice-water was filtered off and purified by crystallisation from an appropriate solvent to give the desired compound.

*4.1.5. General method for the synthesis of compounds **8a**, **8b**, **8d** and **11***

Method 1: The solution of compounds **6a, 6b** and **6d** (10 mmol) in ethanol was stirred in the presence of sodium ethoxide (10 mmol) at room temperature for 15h (the progress of the reaction was monitored by TLC). Then, 2-bromo-1-(4-chlorophenyl)etanone (30 mmol) was added into it and stirred for additional 12h. After evaporating the solvent under reduced pressure, a solid obtained. The crude product was recrystallized from an appropriate solvent to afford the desired product.

Method 2: The solution of compounds **6a, 6b** and **6d** (1 mmol) and sodium ethoxide (1 mmol) in ethanol (10 mL) was irradiated in monomode microwave reactor in closed vessels at 90 ^{o}C, 100 W, for 1.5 h (for **8a, 8b** and **8d**) or 2 h (for **11**) (the progress of the reaction was monitored by TLC). Then, 2-bromo-1-(4-chlorophenyl)etanone (3 mmol) was added into it and irradiated for additional 1h. The mixture was poured into ice-water and a solid obtained. This crude product was collected by filtration and recrystallized from an appropriate solvent to afford the desired product.

*4.1.6. General method for the synthesis of compounds **9a**, **9b**, **9d** and **12***

Method 1: $NaBH_4$ (50 mmol) was added the solution of the corresponding compound **8a, 8b, 8d** and **11** (10 mmol) in ethanol and the mixture was stirred at room temperature for 24 h (the progress of the reaction was monitored by TLC). After evaporating the solvent under reduced pressure, a solid obtained. The crude product was recrystallized from an appropriate solvent to afford the desired product.

Method 2: The solution of compound **8a, 8b, 8d** and **11** (1 mmol) in ethanol (10 mL) was irradiated in monomode microwave reactor in closed vessel in the presence of $NaBH_4$ (3 mmol) with pressure control (physical parameters were given in Table 1). Then, the mixture was poured into ice-water and a solid obtained. This crude product was collected by filtration and recrystallized from an appropriate solvent to afford the desired product.

*4.1.7. General method for the synthesis of compounds **10a-i** and **13a-c***

Method 1: NaH (10 mmol) was added the solution of the corresponding compound **9** or **12** (10 mmol) in THF and the mixture was refluxed for 5 h. Then, the corresponding substituted benzylchloride was added into it and the refluxed for additional 9 h (for **10a-i**) or 12 h (for **13a-c**). After the completion of the reaction, solvent was evaporated under reduced pressure, and the obtained oily product was extracted with 15 mL of ethyl acetate three times in the presence of K_2CO_3. The organic layer was dried on Na_2SO_4. After the removal of solvents at a reduced pressure, an oily product formed which was purified by column chromatography (*n*-hexane/ ethyl acetate) on silica gel.

Method 2: NaH (1 mmol) was added the solution of the corresponding compound **9** or **12** (1 mmol) in THF (10 mL) and the mixture was irradiated in monomode microwave reactor in closed vessel with pressure control at 100 °C and 100 W, for 10 min. Then, the corresponding substituted benzylchloride (3 mmol) was added into it and irradiation was continued for 45 minute (for **10a-i**) or 50 min (for **13a-c**) at 125 °C, 150 Watt. Solvent was evaporated under reduced pressure, and the obtained oily product was extracted with 15 mL of ethyl acetate three times in the presence of K_2CO_3. The organic layer was dried on Na_2SO_4. After the removal of solvents at a reduced pressure, an oily product formed which was purified by column chromatography (n-hexane/ ethyl acetate) on silica gel.

4.2. Antimicrobial activity

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/milliliter (µg/mL).

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35 °C [23]. Ampicillin (10 µg) and fluconazole (5 µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with dilution of 1:10 was used as solvent control. Only positive results were presented in Table 5.\

4.3. Antioxidant activity

4.3.1. Antioxidant activity studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity: The scavenging activity of different chemicals was determined using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by Blois [24]. A 100-µL: chemical solution was mixed with 1 mL of freshly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. The activity was expressed as µmol Trolox equivalent.

FRAP (the ferric reducing ability of plasma): FRAP was measured using the method described by Benzie & Strain [25] with some modification. To 100 µL of each sample was added 2900 µL freshly prepared FRAP reagent containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyle-s-triazine) and 20 mmol/L $FeCl.6H_2O$ in proportions of 10:1:1 (v/v/v). The mixture was incubated for 30 min at 37 ºC and measured at 593 nm. The values were expressed as µmol of Trolox/g.

CUPRAC (cupric ion reducing antioxidant capacity): CUPRAC was measured following the procedure described by Apak *et al.* [26] with some modification. Briefly, 100 µL of each chemical solution was mixed with 900 µL bi-distilled water, 1 mL acetate buffer solution (1 mmol/L, pH: 7.0), 1 mL $CuCl_2$ (10 mmol/L) and 1 mL 7.5 mmol/L neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 450 nm against a water blank. Trolox was used as the standard calibration curves, and the results were expressed as µmol Trolox equivalent per g.

4.4. α-Glucosidase inhibition assay [27]

α-Glucosidase inhibition assay was performed spectrophotometrically. *α*-Glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich) was dissolved in phosphate buffer (pH 6.8, 50 mmol/L). Test compounds were dissolved in DMSO. To 96-well microtiter plates was added 20 µL of test sample, 20 µL of enzyme (20 mU/mL) and 135 µL of buffer This was then incubated for 15 minutes at 37º C. After incubation, 25 µL of *p*-nitrophenyl-*α*-D-glucopyranoside (2 mmol/L, Sigma Aldrich) was added and change in absorbance was monitored for 20 minutes at 400 nm. The test compound was replaced by DMSO (10% final) as the control. Acarbose (Sigma-Aldrich) was used as a standard inhibitor. The assays were performed in triplicate. The IC_{50} value was determined as the concentration of compound giving 50% inhibition of maximal activity.

4. 5. Urease inhibition assay [28]

Reaction mixtures comprising 25 µL of Jack Bean urease, 55 µL of buffer (0.01 mol/L K_2HPO_4, 1 mmol/L EDTA and 0.01 mol/L LiCl, pH 8.2) and 10 mmol/L urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured following the indophenol method and was used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well. Increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (SpectraMax M5, Molecular Device, USA). The percentage inhibition was calculated from the formula $100–(OD_{testwell}/OD_{control})\times100$. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed under the same reaction conditions.

4.6. Molecular docking

Before the docking, initial structures of the compounds were built and optimized by the GAUSSIAN 09 program [29]. Geometry optimizations were performed using Density Functional Theory (DFT) at the B3LYP (Becke–3 parameter–Lee-Yang-Parr) /6-31G (d, p) level [30], [31] with dual Xeon workstation (2.3 GHz, RAM 64 GB).

The crystal structures of the *saccharomyces cerevisiae* α-glucosidase and *H. pylori*urease enzymes were obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb/), under the accession codes, 3A4A [32] and 1E9Y [33], respectively. Molecular Operating Environment (MOE) software [34] was used for molecular docking studies. Enzyme-ligand complexes were energy minimized to a gradient of 0.01 kcal/(mol Å), and protonated by means of the force field AMBER99. Charges on the enzyme and ligands were assigned using the force field AMBER99 and force field MMF94X, correspondingly. The active sites of enzymes were identified by the site finder application in MOE. Triangle Matcher Algorithm and two rescoring functions, London dG and GBVI/WSA dG were used to produce 20 poses of each ligand. All poses generated with docking were analyzed and the best-scored pose for each compound was selected for further investigation of interactions with the corresponding enzyme.

4.7. Statistical analysis

All measurements were performed in triplicate (n=3), and the data were presented as mean ± pooled standard deviation (mean ± SD). The data were analyzed using a one-way ANOVA and Duncan's multiple range test for comparison among means at significance levels of P <0.01 or 0.05 (IBM SPSS Statistics V22.0). A statistical software package (XLSTAT version 2014.6) using ADDINSOFT (Damremont, Paris, France) was used to perform principal component analysis (PCA).

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References

[1] N. Khardory, Antibiotics-past, present and future, Med. Clin. North Am. 90 (2006) 1049-1076.

[2] M.Y. Mentese, H. Bayrak, Y. Uygun, et al., Microwave assisted synthesis of some hybrid molecules derived from norfloxacin and investigation of their biological activities, Eur. J. Med. Chem. 67 (2013) 230-242.

[3] V.R. Solomon, C.K. Hu, H. Lee, Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach, Bioorg. Med. Chem. 18 (2010) 1563-1572.

[4] E. Rajanarendar, K. Thirupathaiah, S. Ramakrishna, D. Nagaraju, A facile and convenient synthesis of novel imidazo[1, 2-*b*] isoxazoles and their Mannich bases as potential biodynamic agents, Chin. Chem. Lett. 26 (2015) 1511-1513.

[5] C.K. Hu, V.R. Solomon, P. Cano, H. Lee, A 4-aminoquinoline derivative that markedly sensitizes tumor cell killing by Akt inhibitors with a minimum cytotoxicity to non-cancer cells, Eur. J. Med. Chem. 45 (2010) 705-709.

[6] S. Demirci, A. Demirbas, S. Ulker, S. Alpay-Karaoglu, N. Demirbas, Synthesis of some hetero functionalized penicillanic acid derivatives and investigation of their biological activities, Arch. Pharm. 347 (2014) 200-220.

[7] A. Alizadeh, R. Ghanbaripour, L.G. Zhu, Piperidine-iodine a dual system catalyst for synthesis of coumarin bearing pyrrolo [1, 2-*a*] quinoxaline derivatives via a one-pot three-component reaction,Tetrahedron 70 (2014) 2048-2053.

[8] S. Emami, E. Ghafouri, M.A. Faramarzi, et al., Mannich bases of 7-piperazinylquinolones and kojic acid derivatives: synthesis, *in vitro* antibacterial activity and *in silico* study, Eur. J. Med. Chem. 68 (2013) 185-191.

[9] J.A. Wiles, B.J. Bradbury, M.J. Pucci, New quinolone antibiotics: a survey of the literature from 2005 to 2010, Expert Opin. Ther. Pat. 20 (2010) 1295-1319.

[10] J. Huang, M.H. Wang, B. Wang, et al., Synthesis, antimycobacterial and antibacterial activity of 1-(6-amino-3, 5-difluoropyridin-2-yl)fluoroquinolone derivatives containing an oxime functional moiety, Bioorg. Med. Chem. Lett. 26 (2016) 2262-2267.

[11] K.K. Sivakumar, A. Rajasekaran, P. Senthilkumar, P.P. Wattamwar, Conventional and microwave assisted synthesis of pyrazolone Mannich bases possessing anti-inflammatory, analgesic, ulcerogenic effect and antimicrobial properties, Bioorg. Med. Chem. Lett. 24 (2014) 2940-2944.

[12] R.G. Fiorot, J.F.A. Filho, T.M.C. Pereira, et al., A simple and convenient method for synthesis of new amino naphthoquinones derived from lawsone by catalytic multicomponent Mannich reaction, Tetrahedron Lett. 55 (2014) 4373-4377.

[13] S.S. Mansoor, K. Aswin, K. Logaiya, S.P.N. Sudhan, An efficient synthesis of β-amino ketone compounds through one-pot three-component Mannich-type reactions using bismuth nitrate as catalyst, J. Saudi Chem. Soc. 19 (2015) 379-386.

[14] P.S. Nayak, B. Narayana, B.K. Sarojini, et al., Design, synthesis, molecular docking and biological evaluation of imides, pyridazines, and imidazoles derived from itaconic anhydride for potential antioxidant and antimicrobial activities, J. Taibah Univ. Sci. 10 (2016) 823-838.

[15] S.A.F. Rostom, A.A. Bekhit, Microwave-assisted synthesis of certain pyrrolyl pyridines, some derived ring systems and their evaluation as anticancer and antioxidant agents, Eur. J. Med. Chem. 92 (2015) 712-722.

[16] A. Balabani, D.J. Hadjipavlou-Litina, K.E. Litinas, et al., Synthesis and biological evaluation of (2, 5-dihydro-1*H*-pyrrol-1-yl)-2*H*-chromen-2-ones as free radical scavengers, Eur. J. Med. Chem. 46 (2011) 5894-5901.

[17] H. Bayrak, A. Demirbas, N. Demirbas, S.A. Karaoglu, Synthesis of some new 1, 2, 4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities, Eur. J. Med. Chem. 44 (2009) 4362-4366.

[18] S. Basoglu, A. Demirbas, S. Ulker, S. Alpay-Karaoglu, N. Demirbas, Design, synthesis and biological activities of some 7-aminocephalosporanic acid derivatives, Eur. J. Med. Chem. 69 (2013) 622-631.

[19] M. Yolal, S. Basoglu, H. Bektas, et al., Synthesis of eperezolid-like molecules and evaluation of their antimicrobial activities, Russ. J. Bioorg. Chem. 38 (2012) 539-549.

[20] N. Demirbas, S.A. Karaoglu, A. Demirbas, E. Çelik, Synthesis and antimicrobial activities of some new [1, 2, 4]triazolo[3, 4-b][1, 3, 4]thiadiazoles and [1, 2, 4]triazolo[3, 4-b] [1, 3, 4]thiadiazines, Arkivoc 1 (2005) 75-91.

[21] N.G. Aher, V.S. Pore, N.N. Mishra, et al., Synthesis and antifungal activity of 1, 2, 3-triazole containing fluconazole analogues, Bioorg. Med. Chem. Lett. 19 (2009) 759-763.

[22] X.Y. Che, C.Q. Sheng, W.Y. Wang, et al., New azoles with potent antifungal activity: design, synthesis and molecular docking, Eur. J. Med. Chem. 44 (2009) 4218-4226.

[23] National Committee for Clinical Laboratory Standard, NCCLS Document M7-A3, 13(25), P.A. Willanova, PA, USA, 1993.

[24] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature 181 (1958) 1199-1200.

[25] I.F.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol. 299 (1999) 15-27.

[26] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir, Novel total antioxidant capacity ındex for dietary polyphenols and vitamins c and e, using their cupric ıon reducing capability in the presence of neocuproine: CUPRAC method, J. Agric. Food Chem. 52 (2004) 7970-7981.

[27] M.I. Choudhary, A. Adhikari, S. Rasheed, et al., Cyclopeptide alkaloids of *Ziziphus oxyphylla* Edgw as novel inhibitors of α-glucosidase enzyme and protein glycation, Phytochem. Lett. 4 (2011) 404-406.

[28] M.W. Weatherburn, Phenol-hypochlorite reaction for determination of ammonia, Anal. Chem. 39 (1967) 971-974.

[29] M.J. Frisch, G.W. Trucks, H.B. Schlegel, et al., Gaussian 09, Revision A.01., Gaussian, Inc., Wallingford, CT, USA, 2009.

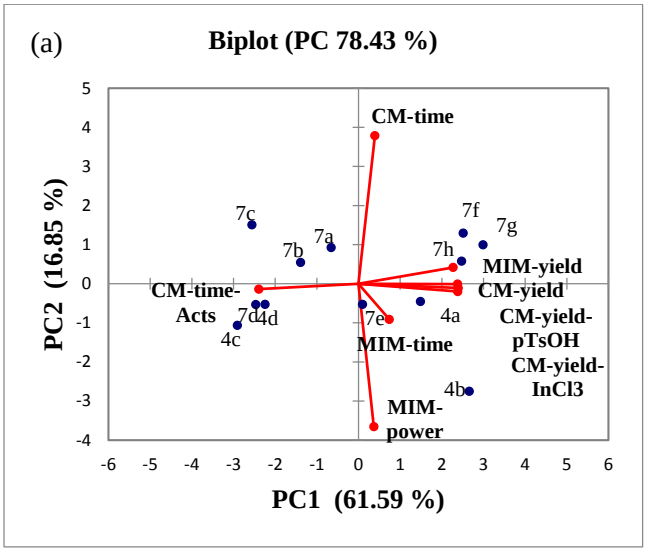
[30] A.D. Becke, Density‐functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648-5652.

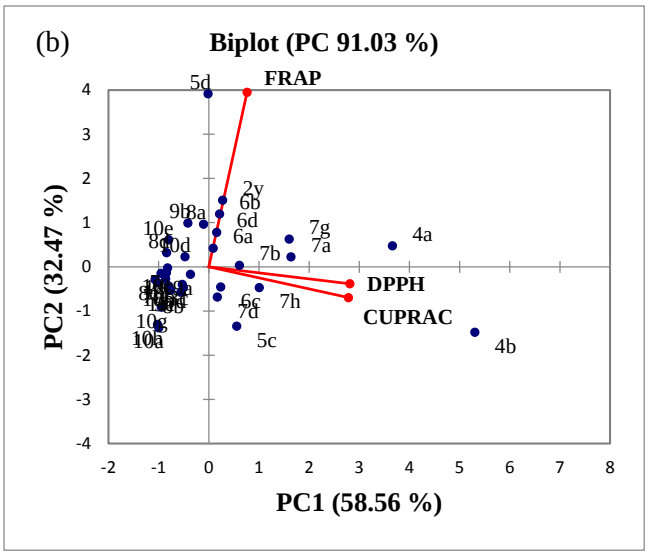
[31] C.T. Lee, W.T. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B 37 (1988) 785-789.

[32] K. Yamamoto, H. Miyake, M. Kusunoki, S. Osaki, Crystal structures of isomaltase from *Saccharomyces cerevisiae* and in complex with its competitive inhibitor maltose, FEBS J. 277 (2010) 4205-4214.

[33] N.C. Ha, S.T. Oh, J.Y. Sung, et al., Supramolecular assembly and acid resistance of Helicobacter pylori urease, Nat. Struct. Mol. Biol. 8 (2001) 505-509.

[34] Molecular Operating Environment (MOE), 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.





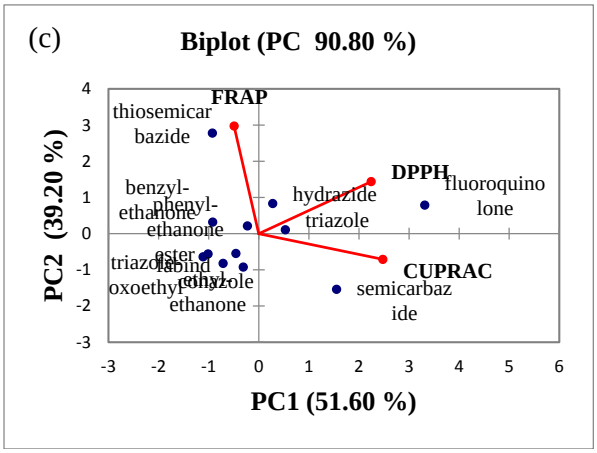


Fig. 1. Bi-plot of scores and loadings comparing microwave irradiation method (MIM), conventional method (CM) and conventional method with acid catalyst (CM) with compounds **4a-d** and **7a-h** listed in Table 1 (A), antioxidant capacity (AC) values obtained from DPPH, FRAP and CUPRAC results for 35 synthesized novel compounds listed in Table 3(B) and their compound classes based distinguishing (C).

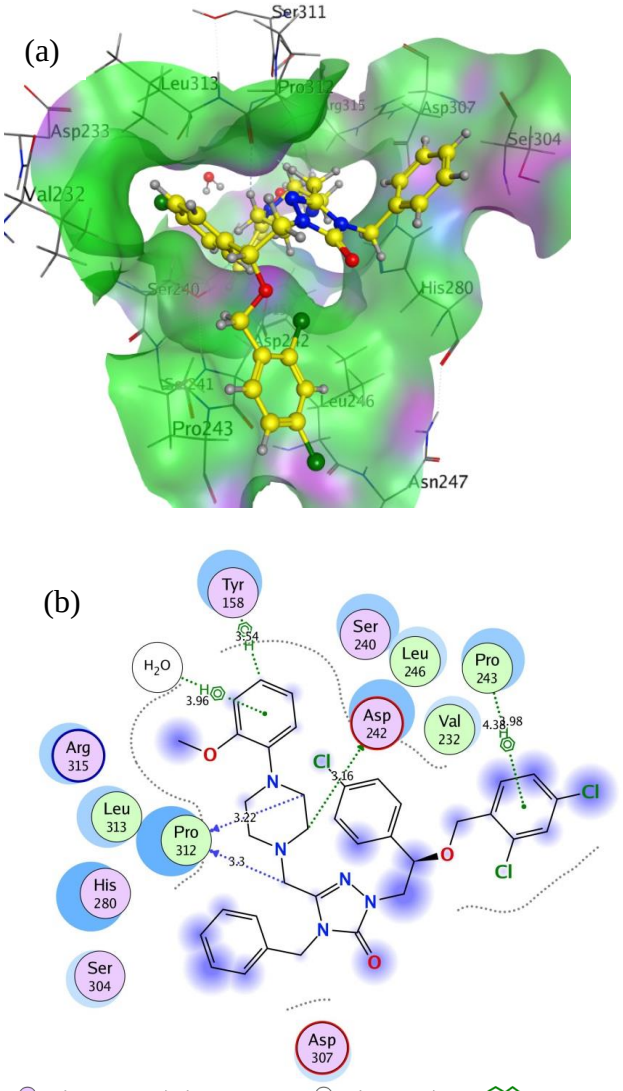
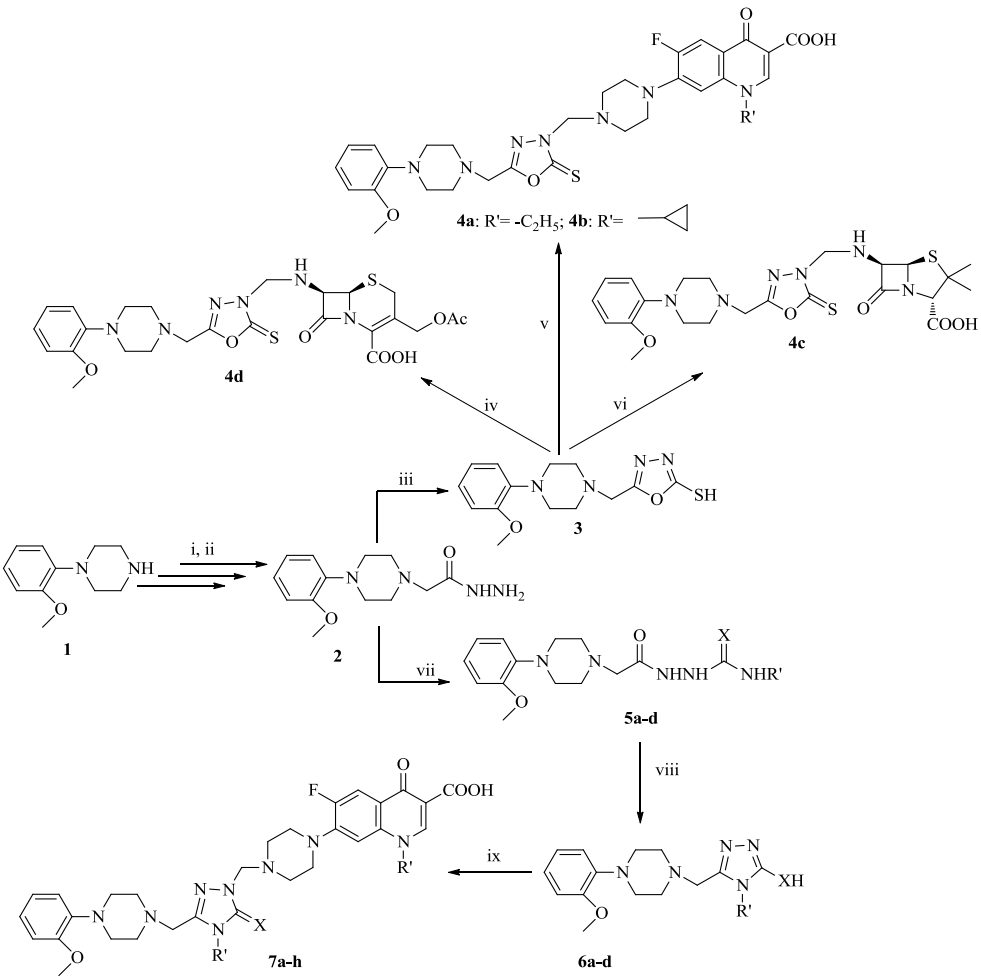
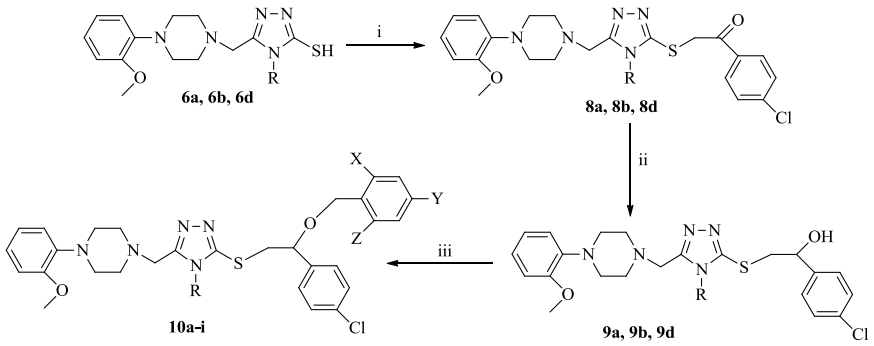


Fig. 2. 3D (a) and 2D (b) representation of docking pose for the active compound **13b** in the active site of *saccharomycescerevisiae* α-glucosidase (PDB code: 3A4A).

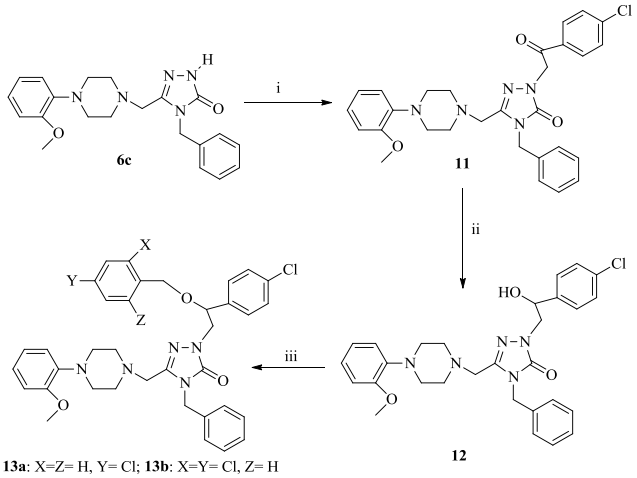


Scheme 1. Synthesis of compounds **5a-d**, **6a-d**, and **7a-h**. Reaction and conditions. *i*: $BrCH_2CO_2Et$; *ii*: H_2NNH_2; *iii*: KOH, CS_2; *iv*: HCHO, 7-aminocephalosporanic acid; *v*: HCHO, norfloxacin (for **4a**) or ciprofloxacin (for **4b**); *vi*: HCHO, 6-aminopencillanic acid; *vii*: suitable alkyliso(thio)cyanate; *viii*:NaOH, EtOH; *ix*: HCHO, norfloxacin (for **7a**, **7c**, **7e**, **7g**) or ciprofloxacin (for **7b**, **7d**, **7f**, **7h**).



6a, 8a, 9a: R= -C_6H_5; **6b, 8b, 9b**: R= -C_2H_5; **6c, 8c, 9c**: R= -$CH_2C_6H_5$;
10a: R= -C_6H_5; X=Z=H, Y= Cl; **10b**: R= -C_6H_5; X=Y=Cl, Z= H; **10c**: R= -C_6H_5; X=Z=Cl, Y= H;
10d: R= -C_2H_5; X=Z=H, Y= Cl; **10e**: R= -C_2H_5; X=Y=Cl, Z= H; **10c**: R= -C_2H_5; X=Z=Cl, Y= H;
10g: R= -$CH_2C_6H_5$; X=Z=H, Y= Cl; **10h**: R= -$CH_2C_6H_5$; X=Y=Cl, Z= H; **10i**: R= -$CH_2C_6H_5$; X=Z=Cl, Y= H

Scheme 2. Synthesis of compounds **9a, 9b, 9d**, and **10a-i**. Reaction and conditions. *i*: NaOEt, EtOH, $ClC_6H_4COCH_2Br$; *ii*: NaBH4, EtOH; *iii*: NaH, the corresponding halogenated benzyl chloride



Scheme 3. Synthesis of compounds **11**, **12**, and **13a-c**. Reaction and conditions. *i*: NaOEt, EtOH, $ClC_6H_4COCH_2Br$; *ii*: $NaBH_4$, EtOH; *iii*: NaH, the corresponding halogenated benzyl chloride.

Table 1. Time, power and yield data for compounds **4 -13**.

Compd.	Microwave irradiation method			Conventional method	
	Time (min)	Power (W)	Yield (%)	Time (h)	Yield (%)
4a	5	100	98	20	80
4b	5	150	100	20	91
4c	5	125	45	22	30
4d	6	100	57	21	35
5a	10	100	98	20	91
5b	10	100	84	20	71
5c	10	100	89	20	80
5d	10	100	94	20	86
6a	5	200	97	9	85
6b	4	150	100	9	88
6c	4	150	96	9	80
6d	3	200	97	9	88
7a	5	100	79	24	52
7b	4	100	83	22	52
7c	4	100	62	25	45
7d	5	100	65	20	41
7e	5	100	78	20	66
7f	5	100	99	25	86
7g	6	100	100	25	88
7h	5	100	97	23	85
8a	90	100	98	27	91
8b	90	100	83	27	78
8d	90	100	79	27	70
9a	10	100	89	24	83
9b	10	100	87	24	81
9d	10	100	96	24	89
10a	55	100	77	14	76
10b	55	100	88	14	83
10c	55	100	93	14	86
10d	55	100	74	14	69
10e	55	100	89	14	78
10f	55	100	88	14	82
10g	55	100	100	14	91
10h	55	100	89	14	71
10i	55	100	95	14	89
11	120	100	84	27	74
12	13	100	92	24	80
13a	60	100	83	17	74
13b	60	100	89	17	82
13c	60	100	85	17	77

Table 2. Screening for the activity of newly synthesized compounds **3-13**.

Compd.	Microorganisms and minimal inhibition concentration (μg/mL)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
3	31.25	125	-	125	250	-	125	-	-
4a	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	62.5
4b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-
4c	7.81	125	-	31.25	500	250	3.91	-	500
4d	500	125	-	31.25	-	500	-	-	-
5a	-	-	-	-	-	-	-	-	-
5b	62.5	125	-	62.5	-	125	15.65	125	62.5
5c	500	62.5	-	62.5	-	62.5	<7.8	62.5	31.3
5d	500	125	-	31.25	-	500	-	-	-
6a	-	-	-	62.5	62.5	62.5	62.5	-	-
6b	62.5	125	-	62.5	-	125	15.65	125	62.5
6c	500	31.3	-	31.3	-	31.3	-	-	-
6d	7.81	125	-	31.25	500	250	3.91	-	500
7a	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-
7b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-
7c	<0.12	0.12	0.98	0.98	0.98	0.24	<0.12	-	-
7d	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	500
7e	<0.24	<0.24	0.49	0.49	0.49	0.24	<0.24	-	-
7f	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-
7g	<0.24	<0.24	1.95	0.98	0.98	0.24	0.98	-	-
7h	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	0.49	-	500
8a	250	-	-	-	-	-	-	-	-
8b	-	-	-	156	-	-	62.5	-	-
8d	-	7.8	-	156	19	39	3.9	312.5	78
9a	-	-	-	-	500	-	125	-	62.5
9b	-	-	-	7.8	7.8	15.6	62.5	-	-
9d	-	-	-	250	-	500	-	-	-
10a	-	-	-	7.8	-	7.8	-	62.5	62.5
10b	-	-	-	3.9	3.9	7.8	-	62.5	62.5
10c	-	-	-	1.9	3.9	3.9	-	62.5	62.5
10d	-	-	3.9	3.9	3.9	-	-	62.5	-
10e	-	-	-	-	3.9	-	62.5	62.5	-
10f	-	-	1.9	1.9	3.9	-	62.5	62.5	78
10g	-	-	-	15.6	7.8	-	-	62.5	78
10h	-	-	-	7.8	3.9	-	-	62.5	78
10i	-	-	3.9	7.8	3.9	-	-	62.5	62.5
11	250	-	-	250	500	500	-	-	-
12	-	-	-	250	-	-	-	-	-
13a	-	-	-	3.9	1.9	-	-	125	62.5
13b	-	7.8	-	15.6	7.8	15.6	-	125	62.5
13c	-	-	-	3.9	1.9	3.9	-	125	62.5
Amp.	3.9	250	19	9.7	9.7	19	-	-	-
Strep.	-	-	-	-	-	-	7.8	-	-
Flu	-	-	-	-	-	-	-	39	78

Ec: *Escherichia coli* ATCC 35218, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 10145, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 709 Roma, Ms: *Mycobacterium smegmatis*ATCC607, Sc: *Saccharomyces cerevisiae* RSKK 251, Ca: *Candida albicans* ATCC 60193.
Amp.: Ampicillin, Flu.: Fluconazole, Strep.: Streptomycin.

Table 3. Antioxidant capacity (AC) values of 35 synthesized novel compounds. Values represent the mean ± SD of three determinations. An analysis of variance (SPSS version 11.5. one-way ANOVA) was used for comparisons among the means. The superscripts show significant digits. Values with the same letter within a column are not significantly different at $P<0.05$.

Compd.	DPPH*	FRAP*	CUPRAC*
1	8.98 ± 0.22^{de}	1722.63 ± 53.80^{h-j}	36.84 ± 0.82^{a}
2	54.74 ± 0.09^{k}	3096.08 ± 63.62^{s}	321.68 ± 2.93^{q}
4a	556.05 ± 2.73^{p}	2789.66 ± 87.54^{r}	870.89 ± 2.79^{x}
4b	832.91 ± 6.28^{r}	1601.87 ± 18.15^{e-g}	1192.38 ± 3.93^{z}
5c	34.00 ± 0.83^{i}	1135.52 ± 61.16^{b}	691.60 ± 6.61^{u}
5d	64.45 ± 0.01^{L}	4744.08 ± 54.27^{t}	N.D.**
6a	64.42 ± 0.02^{L}	2289.87 ± 50.03^{m}	312.75 ± 3.48^{p}
6b	62.50 ± 0.06^{L}	2861.32 ± 23.81^{r}	307.13 ± 2.42^{o}
6c	33.29 ± 0.84^{i}	1706.07 ± 83.05^{hi}	496.79 ± 4.16^{t}
6d	64.26 ± 0.07^{L}	2555.93 ± 32.32^{o}	313.98 ± 2.07^{p}
7a	207.39 ± 4.93^{o}	2377.03 ± 24.36^{n}	697.54 ± 2.48^{v}
7b	127.47 ± 0.62^{n}	2085.03 ± 58.27^{L}	440.81 ± 1.34^{r}
7d	47.27 ± 3.62^{j}	1528.50 ± 75.15^{c-e}	462.72 ± 1.79^{s}
7e	6.94 ± 0.48^{c-e}	1833.08 ± 43.70^{k}	84.62 ± 0.12^{c}
7f	2.56 ± 0.03^{a}	1562.13 ± 76.69^{c-g}	38.48 ± 2.14^{ab}
7g	70.61 ± 2.41^{m}	2701.88 ± 51.98^{p}	894.22 ± 3.28^{y}
7h	45.23 ± 0.89^{j}	1829.16 ± 22.26^{k}	785.64 ± 4.76^{w}
8a	22.56 ± 0.41^{gh}	2655.12 ± 25.92^{p}	267.88 ± 3.81^{n}
8b	13.20 ± 0.60^{f}	1489.00 ± 44.50^{cd}	222.56 ± 2.43^{k}
8c	9.32 ± 0.39^{e}	1600.57 ± 49.04^{e-g}	N.D.**
8d	35.03 ± 0.50^{i}	2064.98 ± 4.19^{L}	N.D.**
9a	23.67 ± 0.10^{h}	1806.53 ± 2.71^{jk}	248.80 ± 5.12^{m}
9b	0.58 ± 0.04^{a}	2624.77 ± 11.40^{op}	179.04 ± 3.85^{j}
9c	2.30 ± 0.01^{a}	1490.83 ± 19.42^{cd}	156.67 ± 0.2^{i}
9d	21.47 ± 0.29^{gh}	1572.75 ± 27.44^{d-g}	219.35 ± 1.67^{k}
10a	15.47 ± 0.53^{f}	841.92 ± 20.52^{a}	102.96 ± 4.48^{e}
10b	7.54 ± 0.09^{c-e}	1548.67 ± 49.46^{c-f}	122.23 ± 2.67^{g}
10c	8.65 ± 0.19^{de}	1752.49 ± 36.98^{i-k}	79.98 ± 2.96^{c}
10d	20.11 ± 0.92^{g}	2070.28 ± 45.06^{L}	180.26 ± 3.69^{j}
10e	7.26 ± 0.55^{c-e}	2288.83 ± 74.87^{m}	42.44 ± 0.79^{b}
10f	5.43 ± 0.11^{bc}	1652.03 ± 42.33^{gh}	90.28 ± 1.72^{d}
10g	6.79 ± 0.19^{c-e}	1182.78 ± 30.88^{b}	102.77 ± 1.43^{r}
10h	3.31 ± 0.15^{ab}	894.92 ± 55.44^{a}	108.53 ± 1.37^{f}
10i	6.46 ± 0.28^{c-e}	1479.40 ± 65.33^{c}	133.93 ± 3.08^{h}
13c	5.99 ± 0.19^{b-d}	1621.60 ± 52.65^{fg}	233.09 ± 1.34^{L}

*DPPH.FRAP and CUPRAC expressed as µmol TE/g.
**Not Detected

Table 4. Inhibitory activities of the synthesized compounds against Jack bean urease and α-glucosidase.

α-Glucosidaseinhibitionactivity			Ureaseinhibitonactivity	
Compd.	% inhibition	IC_{50} (µmol/L)	Compd.	% inhibition
5c	15 ± 1		**3**	7 ± 4
5d	11 ± 2		**5b**	24 ± 14
6a	9 ± 3		**5c**	45 ± 12
6b	8 ± 2		**5d**	24 ± 13
6c	9 ± 1		**6a**	35 ± 1
6d	11 ± 1		**6b**	15 ± 4
8a	19 ± 3		**6c**	39 ± 12
8b	12 ± 1		**6d**	26 ± 8
8d	24 ± 3		**8a**	19 ± 7
9a	13 ± 2		**8b**	23 ± 3
9b	5 ± 1		**8d**	13 ± 9
9d	39 ± 5		**9a**	15 ± 3
10a	80 ± 1	97.37 ± 8,86	**9b**	59 ± 4
10b	62 ± 3		**9d**	13 ± 2
10c	54 ± 2		**10a**	45 ± 9
10d	33 ± 5		**10b**	4 ± 2
10e	6 ± 4		**10c**	25 ± 8
10f	0 ± 0		**10d**	59 ± 3
10g	37 ± 3		**10e**	16 ± 1
10h	40 ± 1		**10f**	21 ± 3
10i	60 ± 2		**10g**	4 ± 1
11	50 ± 5		**10h**	23 ± 8
12	21 ± 2		**10i**	12 ± 7
13a	91 ± 12	16.71 ± 1.11	**11**	21 ± 1
13b	99 ± 5	7.8 ± 0.35	**12**	40 ± 3
13c	19 ± 2		**13a**	57 ± 7
			13b	31 ± 1
			13c	25 ± 4