Moxifloxacin-Ester derivatives: Synthesis, characterization and pharmacological evaluation

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Abstract: It is known that resistance of bacteria is one of the major issues in drug treatment. To cope this issue, it is required to synthesize new analogues which contest against mutated bacteria. This research study included synthesis of several derivatives of moxifloxacin by adding different phenol and alkyl halide at third position of carboxylic group with esterification reaction and the structures of synthesized derivatives were characterized by spectroscopic techniques i.e. ^{1}H NMR, FT-IR and mass-spectrometry. In continuation, antimicrobial activities of the analogues were also evaluated against number of Gram-positive, Gram-negative bacteria and fungi. The experimental results of novel derivatives exhibit significant antibacterial and antifungal profile in which so many synthesized derivatives influenced a similar and enhanced activity against selected microbes that were *S. typhi*, *P. mirabilis*, *P. aeruginosa*, *S. flexneri*, *B. subtilis* as compared to the moxifloxacin. Moreover, few innovative derivatives were also produced better anti-fungal activity against *F. solani* and *T. rubrum*. Furthermore, the enzymatic activity of all analogues has been analyzed against urease and carbonic anhydrase and concluded that C2 was selected inhibitor of urease enzyme.

Keywords: Moxifloxacin, Ester derivatives, ANOVA, antifungal activity, antibacterial activity.

INTRODUCTION

The fluoroquinolone is an important class of nitrogen containing heterocycles moiety. It is broad spectrum antibiotic agent which is extensively used as drug of interest for derivative synthesis to overcome the problem of bacterial resistance (Casal *et al*., 2017; Huang et. al., 2016; Graza *et al*., 2017). SAR of fluoroquinolones clarify that 3rd position and 7th position are essential for antibacterial spectrum (Hu *et al*., 2018; Xu *et al*., 2018; Towle *et al*., 2018). The carboxylic acid at 3rd position provides binding side for DNA gyrase so that any alteration on this position of fluoroquinolone could be abolish its antimicrobial activity (Foroumadi *et al*., 2005). But here many exceptions to this rule; of which, one is ester pro-drug analogues that are converted in-vivo back to the acid. In literature, some derivatives of quinolones are available in which C-3 position was modified and antibacterial activities were increased. In 1990, Chu *et al*., (Chu et. al., 1990) modified C-3 position of ciprofloxacin without damaging its antibacterial activities. Jefferson *et al*., (Jefferson *et al*., 2003) introduced macromolecule at C-3 in fluoroquinolones with effect against S. aureus and E. coli and suggested that same modification was useful for the expansion of novel fluoroquinolones derivatives.

In the present work, esterification of 3-carboxylic group of moxifloxacin (MFX, fig. 1) was performed by reacting with alkyl halide and phenol. The reaction solvent was methanol and H_2SO_4 was used as catalyst.

MATERIALS AND METHODS

The moxifloxacin was gifted by Getz pharmaceuticals, Karachi and other analytical grade reagents and solvents were procured from Merck, Germany. Similarly, all glass-wares were obtained from Pyrex. As an experimental design, the Gallen Kamp apparatus was used to check melting points of derivatives. IR was noted by Shimadzu prestige-21 200 VCE Spectrophotometer in region of 400-4000cm^{-1} (KBr pellets). 1H-NMR spectra were obtained by Bruker/XWIN-NMR spectrophotometer ($CDCl_3$ and methanol used as solvent) and internal standard for NMR was TMS. Mass-spectra were recorded by JEOL MS Route. The aluminum TLC cards were used for thin layer chromatography (TLC) and iodine vapors was used as detector.

Synthesis of derivative C1 and C2

Fischer method was used for the formation of 3-carboylate derivatives from MFX with phenol (resorcinol and vanillin) in acidic medium. To achieve this goal, equal mole of MFX and selected phenol were carefully measured and methonolic solutions were prepared. The prepared solutions were mixed in reaction flask and reflux at 80 C (scheme 1). The concentrated sulphoric acid used

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as catalyst. TLC (methanol: butanol: Ammonia 30%, 1:4:3) was taken twice an hour. At the end of reaction, the product was acceptable to evaporate and convert into crystal and the experimental results were carefully estimated for the Percent yield, melting point, and solubility.

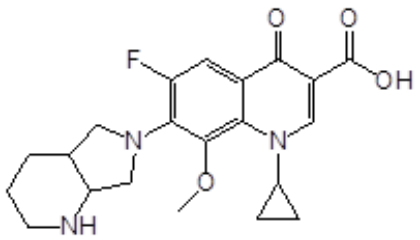


Fig. 1: Moxifloxacin

Synthesis of derivative C3

In this reaction, we demonstrated the esterification of 3-carboxylicgroup of MFX with benzyl chloride. The potassium fluoride (KF) was used as catalyst. Similarly, methanol and water used as reaction solvent (scheme 1). It is a single step inexpensive and less time saving process (Brinchi *et al*., 2003)

Antimicrobial bioassay

The synthesized derivatives were assessed against seven Gram -ve, four Gram +ve bacteria and three fungi by disk susceptibility technique. Four different concentrations (5, 10, 20 and 40 μgmL^{-1}) of standard and derivatives were used to prepare discs. Nutrient agar was prepared, autoclave, cool, and set in Petri dishes. Bacterial culture was uniformly applied on the agar surface by using sterile cotton swab. The prepared discs of MFX and derivatives were placed then incubated at 37ºC±1ºC, for 24h. Positive control was also run to check solvent effect. The experiment was repeated at three times. The mean values, standard deviation and one-way ANOVA with 95% level of significance were evaluated. Dunnett's test was used to analyze significant differences between individuals. Similarly, antifungal activities were performed using SDS medium plates with fungal culture. The fungal cultures were incubated at room temperature, for 48 hours. Inhibition zones were calculated by Vernier caliper.

Enzymatic analysis

Urease assay

Reaction mixture solution 25µL of jack bean urease (enzyme), a mixture of buffers (55µL), urea (100mM) and derivatives (concentration was 0.5mM; 5µL) was incubated in 96-well plates at 30°C for 15min. The indophenol technique was adopted to evaluated urease activity (Wetherburn, 1967), in which ammonia production was measured. Into the each well; phenol reagent was 45µL (1% w/v phenol, 0.005% w/v sodium nitro-prussside) and alkali reagent was 70µL (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added. After 50 mins, the absorbance was calculated at 630nm by microplater reader (Molecular Device, USA). 6.8 was the pH of experiment and the ultimate volume of test solution was 200µL (n=3). Rates of change in absorbance were calculated by softMax Pro software (molecular Device, USA). Percent inhibitions were measured by formula 100-($OD_{testwell}/OD_{control}$) ×100. The internal standard was thiourea. (Khan *et al*., 2004)

Carbonic anhydrase assay

The yellow compound of 4-nitrophenol was synthesized by the hydrolysis of 4-NPA (colorless compound) and acetate which was evaluated in HEPES buffer, 7.2-7.9 was the pH range at 25-28ºC. 140µL of HEPES-Tris solution was present in reaction tube for each sample, 20µL of aqueous solution of purified bovine erythrocyte CA-II (0.1-0.2mg/2000µL of deionized water for 96-well) was freshly prepared, Fluka biochemical. Synthesized derivatives dissolved in DMSO to made 20µL of formerly ready solution (1%), 20µL (0.6-0.8mM) of substrate 4-PNA was prepared in ethanol. The process was start after 15min incubation of target derivative by addition of 4-PNA, synthesized derivatives were screened minimum 3 times at all selected concentrations. The process was started by placing the plate in a SPECTRA max 340 spectrophotometer and the result was monitored at 1 min interval for 30mins at 400nm (Ho *et al*., 2003; Bayram *et al*.,2008).

Spectral data

C1

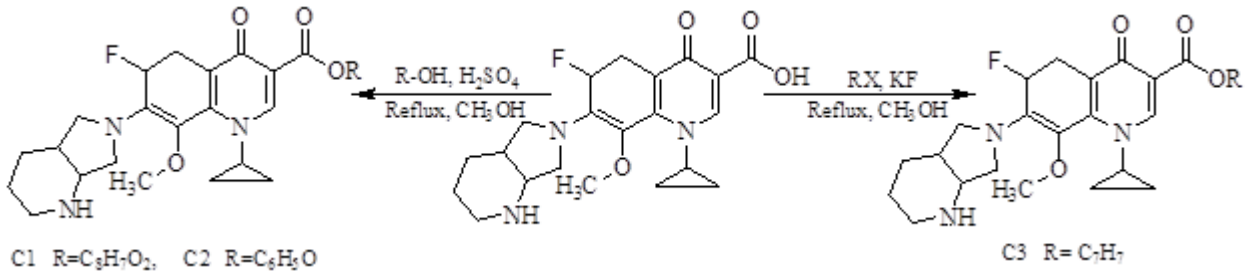
4-formyl-2-methoxyphenyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydropyrrolo-[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4-formyl-2-methoxyphenyl) ($C_{29}H_{30}FN_3O_6$)

Percent yield: 72%, molecular weight: 535.56, melting point: 108°C; IR (KBr) ν_{max}: 3535, 3473, 1737, 1687, 1558, 1512, 1161; 1H-NMR (400MHz- $CDCl_3$): δ:0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine) 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H, methoxy), 3.91 (s, 3H, OCH_3), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48 (d, 1H, J=0.046 of ring), 7.28-7.59 (m, benzyl ring), 8.65 (s, 1H, H2 position), 9.67 (s, 1H of CHO); EIMS: m/z (rel. abundance %): 535(M^{+1} 0.97), 401 (base peak 100), 357 (17.4), 231 (4.3), 205 (6.1), 163 (3.9), 106 (2.5), 96 (38.6), 78 (2.3), 68 (4.5).

C2

2-hydroxyphenyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydropyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (2-hydroxyphenyl) ($C_{27}H_{28}FN_3O_5$)

Percent yield: 55%, molecular weight: 493.53, melting point: 94°C; IR (KBr) ν_{max}: 3524, 3385, 1732, 1689, 1557, 1522, 1165; 1H-NMR (400MHz- $CDCl_3$): δ:0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine) 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H,



Scheme 1 Synthesis of ester linkage at position 3 by using phenol and alkyl halide

Table 1: Physical characteristic of MFX analogues

Compounds	Molecular Formula	Molecular Weight	Melting Point	Percent Yield	State/Colour	Solubility
C1	$C_{29}H_{30}FN_3O_6$	535.56	108 °C	52%	Solid/Orange	Water, Methanol, Ethanol, DMSO
C2	$C_{27}H_{28}FN_3O_5$	493.53	94 °C	55%	Solid/Brown	Water, Methanol, Ethanol, DMSO
C3	$C_{28}H_{30}FN_3O_4$	491.55	222°C	61%	Solid/Light Brown	Water, Methanol, Ethanol, DMSO

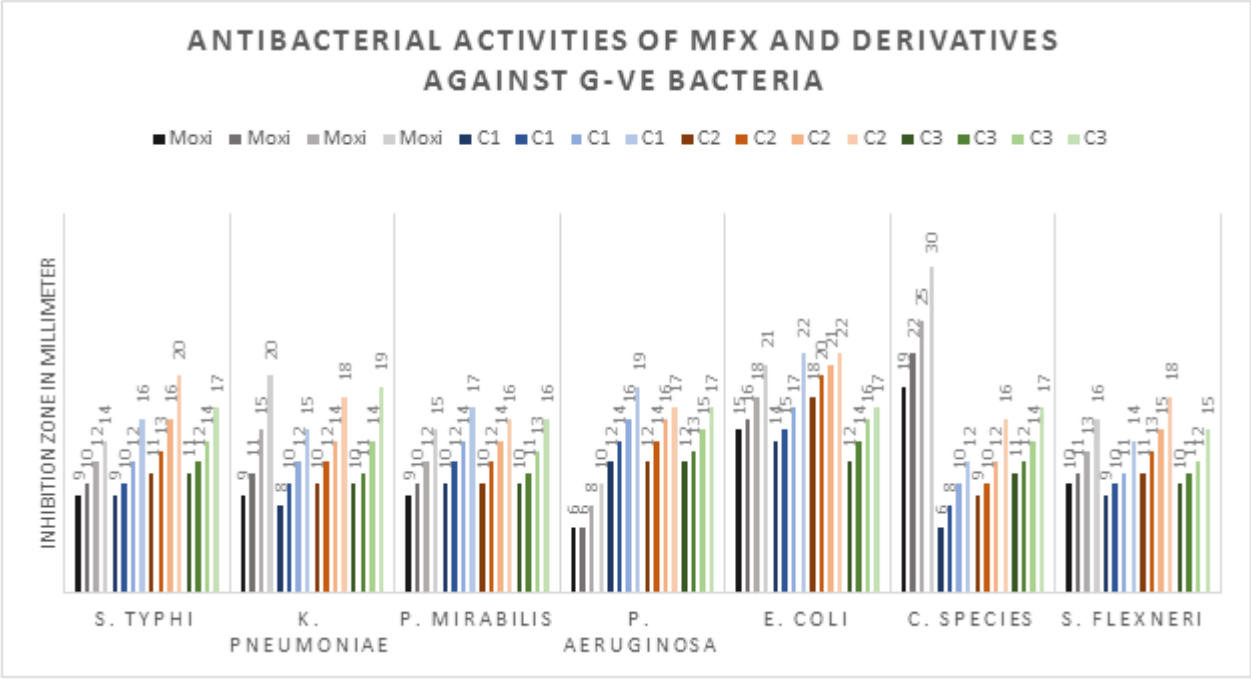


Fig. 2: Antibacterial activities against Gram negative bacteria

methoxy), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48 (d, 1H, J=0.046 of ring), 7.24-7.7.65 (m, benzyl ring), 8.71 (s, 1H, H2 position); EIMS: m/z (rel. abundance %); 493(M^{+1} 0.85), 401 (base peak 100), 357 (27.3), 231 (5.2), 205 (7.2), 163 (4.4), 96 (41.1), 94 (2.3), 78 (2.2), 68 (3.2).

C3

Benzyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(octahydropyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate benzyl ($C_{28}H_{30}FN_3O_4$)

Percent yield: 61%, molecular weight: 491.55, melting point: 222°C; IR (KBr) ν_{max}: 3535, 3473, 1734, 1687, 1562, 1543, 1317; 1H-NMR (400MHz- $CDCl_3$): δ:0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine) 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H, methoxy), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48 (d, 1H, J = 0.046 of ring), 7.44-7.72 (m, benzyl ring), 8.73 (s, 1H, H2 position); EIMS: m/z (rel. abundance %): 491(M^{+1} 1.02), 401 (28.8), 357 (7.6), 231 (3.9), 205 (3.31), 163 (7.4), 96 (base peak 100), 78 (3.09), 68 (10.72).

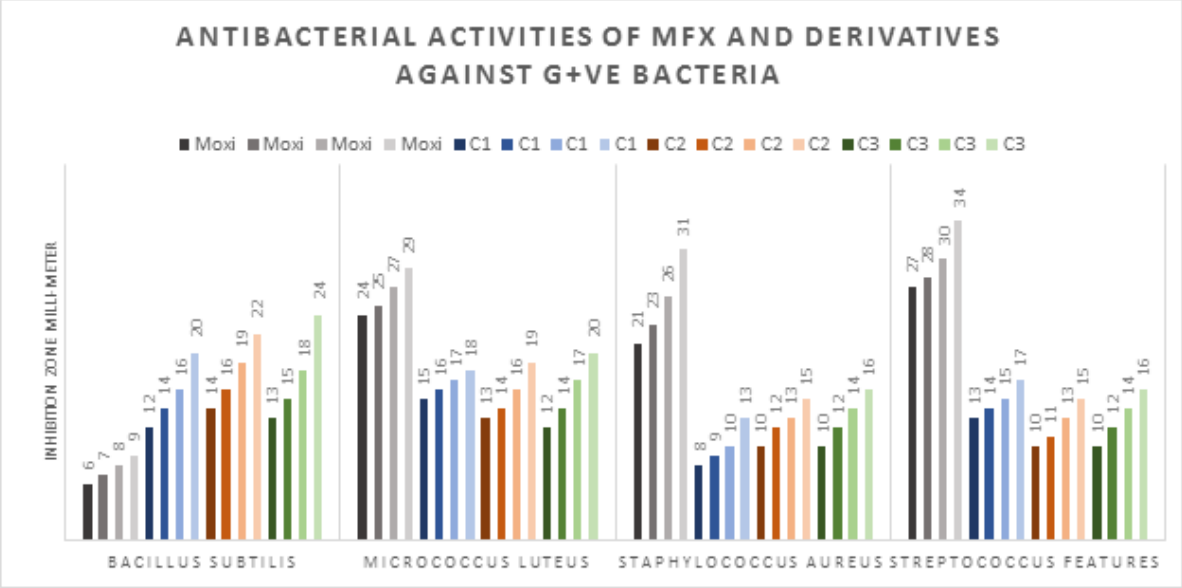


Fig. 3: Antibacterial activities against Gram positive bacteria

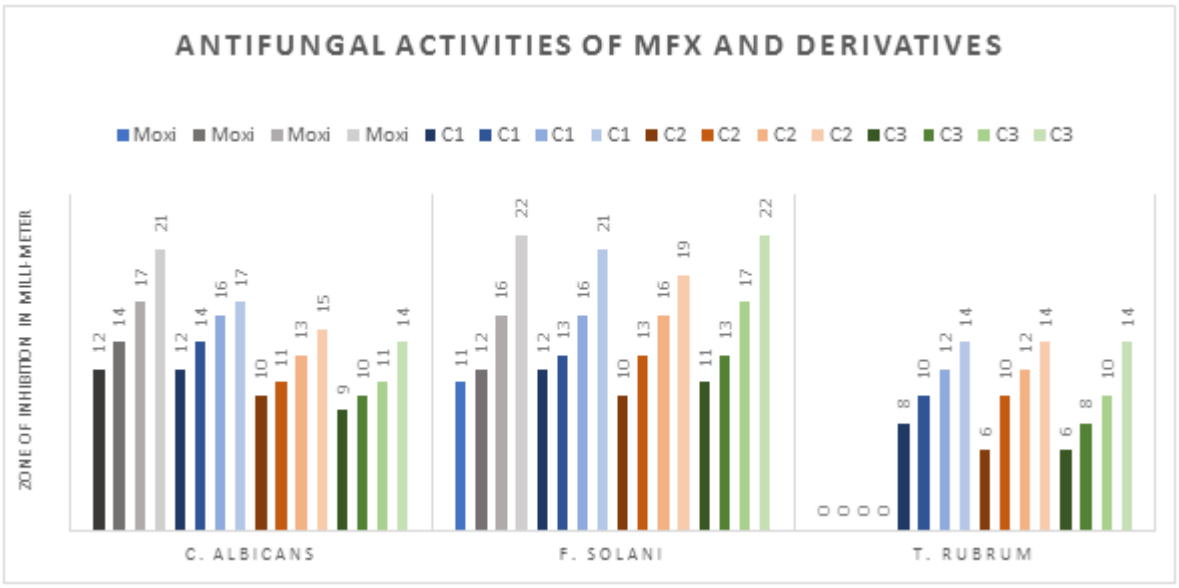


Fig. 4: Antifungal activities of MFX & derivatives

RESULTS

All physical characteristic of MFX analogues was presented in table 1. Spectroscopic studies were present in experimental and discussion section. The antibacterial and antifungal activities were presented in the form of graphs (fig. 2-3). The enzymatic activities were also comprised by graphical representation in fig. 4.

DISCUSSIONS

Spectroscopic studies

The novel 3-carboxamide derivatives of MFX have been characterized by physical, spectroscopic, analytical, and spectral data. FTIR spectra of novel analogues of MFX produced strong band of ester (C=O) at 1732-1737 cm^{-1} in all derivatives (Czock *et al*., 2006; Culley *et al*., 2001). Carboxylic acid (C-O, C=O) produced bands at 1705 and 1317 cm^{-1} which missing in all analogues (Czock *et al*., 2006). It was established that the carboxylic group MFX was involved in ester formation. This fact was also established by vanishing of OH band at 3524 (Culley *et al*., 2001). ^{1}HNMR spectrum of all analogues have not any band at 11 ppm, H-2 hydrogen move which was also established that the 3-carboxylic acid of MFX was participated in esterification with phenols and new peaks of aromatic ring of phenol produced in at 7.2 to 7.59 ppm. The signal of aldehyde group was appeared at 9.67 ppm

in C1 and hydrogen peak of methoxy group was showed at 3.71 ppm. New peaks appeared at 7.0 to 7.72 ppm of aromatic ring of C3 and furthermore, the alkyl halide form ester by removing chloride group with 3-carboxylic acid of MFX which produced peak at 3.86 instead of 4.41 ppm.

Antimicrobial bioassay

The antibacterial data of analogues were recorded in the form of zone of inhibition (diameter, mm) the synthesized analogues and standard was compared by the help of one-way analysis of variance (ANOVA). Selected post-hoc test was Dunnett's test which used to check significance differences between analogues and MFX at $p \leq 0.05$ (figs. 2, 3).

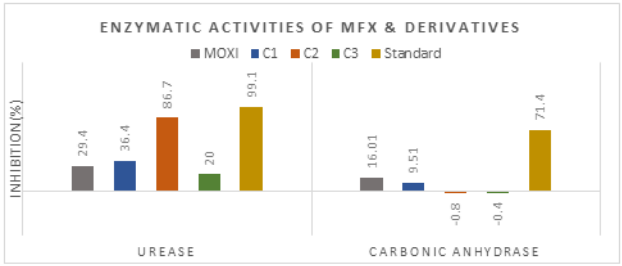


Fig. 5: Graphical representation of enzymatic inhibition C1 to C3

One-way analysis of variance provided significance differences against all Gram-negative organism between derivatives and MFX (used as standard). Dunnett's test evaluated that almost entire analogues were significantly increased ($p<0.001$) against *P. aeruginosa*, K. pneumonia, *S. typhi*, *P. mirabilis* and *S. flexneri*. However, all analogues were significantly decrease against citro-bacter species. The derivative C1 and C2 were insignificant against E. coli and C3 was significantly decrease. Similarly, the Dunnett's test examined that almost whole derivatives were decrease significantly against all selected Gram-positive organism.

ANOVA produced notable difference against *C. albicans* among whole synthesized derivatives and MFX. Dunnett's test examined activity derivative C1 was insignificant where as C2 and C3 were significantly decreased. Similarly, Dunnett's test evaluated that activities of entire derivatives against *F. solani* in which C2 and C3 were significantly decreased. However, derivatives C1 did not show any significant difference. Significant difference produced, as a result of ANOVA, between all synthesized derivatives against T. rubrum. Dunnett's test studied that entire derivatives produced significant difference in increased manner (fig. 4).

Enzymatic activity

The inhibitor activities of analogues C1 to C3 have been checked against carbonic anhydrase and urease (fig. 5). The excellent inhibitory activity (IC_{50}=9.43) of C2 analogues was observed against urease. However, rest of the compounds was inactive. Prepared compounds were also assayed against carbonic anhydrase, it was seen that all compounds were inactive.

CONCLUSION

We have designed and synthesized new C-3 modified ester derivatives of MFX. Structure elucidation was performed by mass spectroscopy and FT-IR, H^1NMR techniques and also screened against their antimicrobial activities. Synthesized analogues of MFX were produces higher activities against Gram-negative and Gram-positive organism including *S typhi*, *P mirabilis*, *P aeruginosa*, *S flexneri*, *B subtilis* as compared with standard. We also observed very high antifungal activities of all synthesized analogues against *F. solani* and *T. rubrum*. It was concluded that C2 was selected inhibitor of urease enzyme.

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