Synthesis of 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones) of prospective anti-COVID-19 drugs

Ashraf A. Aly1 · Alaa A. Hassan1 · Asmaa H. Mohamed1 · Esraa M. Osman1 · Stefan Bräse2,3 · Martin Nieger4 · Mahmoud A. A. Ibrahim1 · Sara M. Mostafa1

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Abstract
During formylation of 2-quinolones by DMF/Et3N mixture, the unexpected 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones) were formed. The discussed mechanism was proved as due to the formation of 4-formyl-2-quinolone as intermediate. Reaction of the latter compound with the parent quinolone under the same reaction condition gave also the same product. The structure of the obtained products was elucidated via NMR, IR and mass spectra. X-ray structure analysis proved the anti-form of the obtained compounds, which were stabilized by the formation hydrogen bond. Molecular docking calculations showed that most of the synthesized compounds possessed good binding affinity to the SARS-CoV-2 main protease (Mpro) in comparable to Darunavir.

Graphic abstract

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Keywords Formylation · 3,3'-methylenebis(4-hydroxyquinolin-2(1H)-ones) · X-ray · Anti-form · Molecular docking · COVID-19

Introduction

Dimethylformamide (DMF) can react as either an electrophilic and/or a nucleophilic agent. Therefore, DMF can be considered as the source of various key intermediates mediating a plethora of important reactions [1]. More significantly, DMF can participate in many reactions by serving as a multipurpose building block for various units, such as CH3, N(CH3)2, HCO2, CHO, O, H+, H., (CH3)2CO, etc. (Fig. 1).

Alkyl-quinolones AQ analogs (Fig. 2) act synergistically to inhibit bacterial growth [2, 3] (i.e., two examples assigned as HHQ and HQNP).

Quinolones show a significant similarity to some anticancer [4], anticonvulsant [5–7], anti-dermatities [8], antibacterial [9], antimicrobial [10], anti-Alzheimer [11] and pain relief [12] in addition to their medical, agricultural and industrial uses [13–15]. In previous work with quinolones, Aly et al., synthesized various classes of 2-quinolones such as 2′-amino-2,5′-dioxo-5′,6′-dihydro-spiro(indoline-3,4′-pyrano[3,2-c]quinoline)-3′-carbonitriles [16], 3-(methyl-thio)-4-oxo-4,5-dihydrofuro[3,2-c]quinoline-2-carbonitriles [17], 3-(methylthio)-4-oxo-4,5-dihydro-furo[3,2-c]quinolone-2-carboxamides [17], naphtho[2′,3′:4,5]furo[3,2-c]quinoline-6,7,12(5H)-trione derivatives (as ERK inhibitors with efficacy in BRAF-mutant Melanoma) [18], 2,3-bis-(4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)succinates, arylmethylene-bis-3,3′-quinoline-2-ones [19], N-2,3-bis(6-substituted-4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl) naphthalene-1,4-diones and substituted N-(methyl/ethyl) bisquinolinone triethylammonium salts [20].

Han and Zhou [21] reported that the reaction of two equivalents of quinolone derivatives with one equivalent of aromatic aldehydes and potassium phtalamide under reflux at water–ethanol solution, gave the corresponding 3,3′-arylmethylene-bis(4-hydroxyquinolin-2(1H)-ones)

Aly et al. [19] also reported another method of preparing arylmethylene-bis-3,3′-quinoline-2-ones via the reaction of equal equivalents of aromatic amines and diethyl malonate together with half equivalent of the corresponding aromatic aldehydes. 3,3′-Arylmethylene-bis(4-hydroxyquinolin-2(1H)-ones) have a great biological activity especially in the composition of vitamin K [22, 23] and anticoagulation [24]. Choudhary et al. [25] synthesized some 3,3′-methylenebis(substituted-4-hydroxyquinolin-2(1H)-ones from the condensation between two molecules of quinolones and one molecule formaldehyde but also neither mechanism nor NMR spectra were discussed for the products. Previously, irradiation of only N-ethyl(methyl)-4-hydroxyquinol-2-ones, was tested in ethanol and afforded their corresponding 3,3′-methylenebis(substituted-4-hydroxyquinolin-2(1H)-ones, virtually eliminating the solvent as a source of formaldehyde [26]. The method suffered from low yields of the obtained products besides to its hazard condition. Moreover the stereochemistry of the obtained products was not discussed.

Utilizing by the expected formylation process during the reaction of 2-quinolones with dimethylformamide/triethylamine (DMF/Et3N) mixture [27, 28], we explain the abnormal formation of 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones). Also, the previous two aforementioned methods could not afford a general preparation method and suffered from low yields, hazardous conditions and no spectroscopic detailed data compared to our announced method of preparation.

Coronavirus disease (COVID-19) is a respiratory infectious disease caused by a novel virus strain, severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) [29–32]. Molecular docking is utilized as a substantial tool in the drug discovery process to predict the binding mode and affinity of a drug candidate with a target. To combat COVID-19, the main protease of SARS-CoV-2 (Mpro) would be targeted due to its significant role in the viral replication

Fig. 1 DMF as a precursor of various functional groups

Fig. 2 The structures of 2-heptylquinolin-4(1H)-one (HHQ) and 2-heptyl-4-hydroxyquinoline 1-oxide (HQNO) as alkyl-quinolone (AQ) analogues
process. Therefore, the binding modes and affinities of 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones) as prospective SARS-CoV-2 inhibitors were predicted against Mpro using Darunavir as a drug reference. Darunavir (DrugBank code: DB01264) is a human immunodeficiency virus (HIV) protease inhibitor and has been recently clinically investigated as anti-COVID-19 drug [33, 34]. The aforementioned encouraged us to synthesize various derivatives of 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones) and established a general method of preparing the former compounds. In addition, we investigate the molecular docking of 3,3′-methylenebis(4-hydroxyquinolin-2(1H)-ones) as anti-COVID-19 using Darunavir as a prospective drug reference.

Results and discussion

Upon addition of equimolar amounts of 4-hydroxy-2(1H)-quinolones 1a–g and Et3N and gently heating in an oil bath at 70–80 °C using DMF for 10–12 h, the resulting yellow-orange coloration of the solution was converted gradually to brown color and the 3,3′-methylenebis(substituted-4-hydroxyquinolin-2(1H)-ones 3a–g were precipitated in 70–87% yields (Scheme 1).

The structural assignment of all the obtained products 3a–g were based on IR, NMR (1H NMR and 13C NMR,) and mass spectra were performed; these and elemental analyses were in good agreement with the assigned structures. As an example, 3,3′-methylene-bis(1-ethyl-4-hydroxyquinolin-2(1H)-one (3g). The elemental analysis and mass spectrometry of compound 3g have the gross formula C23H22N2O4. The IR spectrum of 3g indicated the presence of OH at ν = 3500 (OH), 3030 (Ar–CH), 2867 (Aliphatic –CH) and 1643 cm⁻¹ (C=O), whereas CH2 group at ν = 1458 cm⁻¹. The 1H NMR spectrum of 3g exhibited a triplet at δH = 1.24 and a quartet at 4.38 ppm with the coupling constant J = 7.50 Hz arising from ethyl group. The 1H NMR spectrum of 3g also showed the methylene protons at δH = 3.89. Eight aromatic protons give rise to characteristic signals in the aromatic region of the spectrum, whereas the hydroxyl protons resonated at δH = 12.65. The presence of methylene (CH2) group is evident from 13C-DEPT-NMR spectra; exhibiting positive signal at δC = 131.50, 122.67, 123.30 and 116.74 ppm due to Ar–CH (C-7), (C-6), (C-5) and (C-8), respectively (Fig. 3). The 13C NMR spectrum of 3g supported the 13C NMR spectroscopic data by the distinctive appearance of carbon signals representing quinolone C-4a and C-8a (Fig. 3) and resonated at δC = 115.15 and 136.70 ppm, respectively. Also, the observed δC values for carbon atoms in C-2 at δC = 164.84, C-4 at 159.63 and C-3 at 108.53 ppm.

The structure of 3g was unambiguously determined by a single crystal structure determination showing the bisthmethylene system (Fig. 4 and see CIF file, note that the crystallographic numbering does not correspond to the systematic IUPAC numbering rules). The bond lengths C(3)–C(21) and C(13)–C(21) are 1.5085 (15) Å and 1.5104 (14) Å, respectively, and have single bond character, while C=O of 1.2536 (13) Å and 1.2605 (13) Å, has double bond character. Whereas, bond lengths C(3)–C(4) 1.3637 (15) Å, C2–C3 1.4384 (15) Å and N1–C2 1.3796 (14) Å indicate the presence of hydrogen bond between O2–H14–O14 and O12-H4-O4.

The anti-form of the formed compound is established and stabilized by the formed hydrogen bonding. On the basis of the previous reports [1, 27], the formation of 3,3′-methylenebis(substituted-4-hydroxyquinolin-2(1H)-ones 3a–g can be rationalized as depicted in Scheme 2. It would be proposed that Et3N would abstract a hydrogen proton from the active methylene in C-3 of 1a–g and therefore increasing the nucleophilicity of CH-3 of the quinolone
moiety. Thereafter, a nucleophilic addition of the anion CH-3 of 1a–g to the carbonyl carbon of DMF would give the intermediate 4 accompanied by elimination of a molecule of dimethylamine, (CH₃)₂NH to give 4-formyl-2-quinolones (5). Reaction of 5 with 2 via the nucleophilic attack of the oxygen lone pair to the carbonyl in 2 would form intermediate 6 (Scheme 2). Subsequently, elimination of another molecule of dimethylamine (CH₃)₂NH would give the intermediate 7. Further nucleophilic attack of a molecule of 1 to vinylic-carbon in 7 would form the intermediate 8. Finally, decarboxylation of 8 would form compound 3 (Scheme 3). The reaction pathway was also supported via isolation of (CH₃)₂NH, which was identified by TLC analysis.

Having established reaction conditions in hand, we investigated the formation 3a–g from the reaction of 3-formyl-4-hydroxy-2-quinolone derivatives 5a–g with 1a–g under the condition illustrated in Scheme 3. We reacted 5a–g with their resemble derivatives in 1a–g to obtain symmetric compounds like those in Scheme 1. Fortunately, the target symmetric products of 3,3′-methylenebis(substituted-4-hydroxyquinolin-2(1H)-ones) 3a–g were formed in 60–77% yields (Scheme 3).

**Molecular docking calculations**

Utilizing molecular docking technique, the binding modes and affinities of compounds 3a–g as prospective SARS-CoV-2 inhibitors were predicted against the main protease (MPro). The geometrical structures of 3a–g were prepared and docked into the active site of SARS-CoV-2 MPro using AutoDock 4.2.6 software with docking parameters of GA= 250 and eval= 25,000,000. The predicted binding scores and features are summarized in Table 1. The 2D representations of binding modes of the investigated compounds inside the active site of SARS-CoV-2 MPro are depicted in Fig. 5.

What is interesting about the data in Table 1 is that compounds 3a–g demonstrated good binding affinities toward SARS-CoV-2 MPro with docking scores ranged from −8.63 to −7.05 kcal/mol. Besides, compounds 3a–g exhibited the same binding modes inside the active site of MPro, forming an essential hydrogen bond with key amino acid GLU166 residue (Fig. 5). Further interactions including van der Waals, hydrophobic and pi-based interactions were also observed between the compound and the key amino acids inside the SARS-CoV-2 MPro active site (Fig. 5).

Among the examined compounds, 3e showed the highest binding affinity with docking score of −8.6 kcal/mol against SARS-CoV-2 MPro. The high potentiality of 3e as SARS-CoV-2 MPro inhibitor would be returned to its capability to form four hydrogen bonds with THR190, GLN192, ARG188 and GLU166 amino acid with bond lengths of 2.10, 2.38, 1.79 and 2.08 Å, respectively (Figs. 5, 6).

The binding affinity and features of Darunavir were investigated and compared to compound 3e as SARS-CoV-2 MPro inhibitors. According to molecular docking calculations, Darunavir showed a good binding affinity of −8.19 kcal/mol, forming three hydrogen bonds with GLU166, and LEU167 with bond lengths of 1.94, 2.88 and 1.96 Å, respectively (Figs. 5, 6). A comparison of the molecular docking results revealed the competing binding affinity of 3e with regard to Darunavir as prospective SARS-CoV-2 MPro inhibitor.

**Conclusion**

Formylation of 2-quinolones by DMF/Et₃N mixture caused an insertion of a methylene group between two symmetrically quinolones. DMF/Et₃N mixture was proved as a formylating agent of the parent 2-quinolones. Reaction of 4-formyl-2-quinolone with the parent 2-quinolone under the
Scheme 2  The proposed mechanism describes the formation of compounds 3a–g

Scheme 3  Formation of compounds 3a–g from the reaction of 3-formyl-4-hydroxy-2-quinolones 5a–g with 1, 2 and Et₃N

a; R¹ = R² = H, R³ = H  
b; R¹ = CH₃, R² = H, R³ = H  
c; R¹ = OCH₃, R² = H, R³ = H  
d; R¹ = H, R² = Cl, R³ = H  
e; R¹ = H, R² = Br, R³ = H  
f; R¹ = R² = H, R³ = CH₃  
g; R¹ = R² = H, R³ = CH₂CH₃

3a (60%), 3b (64%), 3c (66%), 3d (68%), 3e (73%), 3f (75%), 3g (77%)
same reaction condition gave the same product. The aforementioned 3-formyl-2-quinolones would prospectively be used to prepare various symmetrical or asymmetrical substituents of the desired compounds. Molecular docking calculations demonstrated the competing binding affinity of 3e with regard to Darunavir as a prospective SARS-CoV-2 Mpro inhibitor.

### Experimental

The IR spectra were recorded by ATR technique (ATR = Attenuated Total Reflection) with a FT device (FT-IR Bruker IFS 88), Institute of Organic Chemistry, Karlsruhe University, Karlsruhe, Germany. The NMR spectra were measured in DMSO-$d_6$ with a Bruker AV-400 spectrometer, 400 MHz for $^1$H and 100 MHz for $^{13}$C; and the chemical shifts are expressed in $\delta$ (ppm), versus internal tetramethylsilane (TMS) = 0 for $^1$H and $^{13}$C, and external liquid ammonia = 0. The description of signals includes: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet and $m$ = multiplet. Mass spectra were recorded on a FAB (fast atom bombardment) Thermo Finnigan Mat 95 (70 eV). Mass spectra were measured in DMSO-$d_6$ on a Bruker AV-400 spectrometer, 400 MHz for $^1$H and 100 MHz for $^{13}$C; and the chemical shifts are expressed in $\delta$ (ppm), versus internal tetramethylsilane (TMS) = 0 for $^1$H and $^{13}$C, and external liquid ammonia = 0. The description of signals includes: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet and $m$ = multiplet. Mass spectra were recorded on a FAB (fast atom bombardment) Thermo Finnigan Mat 95 (70 eV). Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. TLC was performed on analytical Merck 9385 silica aluminum sheets (Kieselgel 60) with Pf$_{254}$ indicator; TLC’s were viewed at $\lambda_{max}=254$ nm.

### Starting materials

1,6-Disubstituted-quinoline-2,4-(1H,3H)-diones $1a$–$g$ were prepared according to the literature [35, 36] whereas carbaldehydes $5a$, $5b$, $5c$–$f$ and $5g$ were synthesized according to the literature [37–40].

### General procedure

#### Method a:
A mixture of $1a$–$g$ (1 mmol), 15 ml of DMF (2), 0.100 g (1 mmol) Et$_3$N was gently heated with stirring in an oil bath at 70–80 °C for 10–12 h. The time period until the reactants had disappeared, as mentioned in Scheme 1, was monitored by TLC. The formed precipitate was then washed with ethanol (50 mL) and recrystallized from the stated solvents to give pure crystals of $3a$–$g$. The filtrate was concentrated on vacuum and (CH$_3$)$_2$NH was obtained and was identified by TLC analysis.

#### Method b:
A mixture of $1a$–$g$ (1 mmol), $5a$–$g$ (1 mmol) and 0.100 g (1 mmol) of Et$_3$N in 2 15 ml of 2 was gently heated with stirring for 8–10 h in an oil bath at 70–80 °C. Compounds $3a$–$g$ were obtained (i.e., Scheme 3) in pure state as above mentioned. The IR spectra were recorded by ATR technique (ATR = Attenuated Total Reflection) with a FT device (FT-IR Bruker IFS 88), Institute of Organic Chemistry, Karlsruhe University, Karlsruhe, Germany. The NMR spectra were measured in DMSO-$d_6$ with a Bruker AV-400 spectrometer, 400 MHz for $^1$H and 100 MHz for $^{13}$C; and the chemical shifts are expressed in $\delta$ (ppm), versus internal tetramethylsilane (TMS) = 0 for $^1$H and $^{13}$C, and external liquid ammonia = 0. The description of signals includes: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet and $m$ = multiplet. Mass spectra were recorded on a FAB (fast atom bombardment) Thermo Finnigan Mat 95 (70 eV). Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. TLC was performed on analytical Merck 9385 silica aluminum sheets (Kieselgel 60) with Pf$_{254}$ indicator; TLC’s were viewed at $\lambda_{max}=254$ nm.

### Table 1

| No. | Compound | Docking score (kcal/mol) | Binding features (hydrogen bond length in Å) |
|-----|----------|--------------------------|--------------------------------------------|
| 1   | $3a$     | −8.28                    | ARG188 (2.18 Å), MET165 (2.63 Å), HIS164 (2.14 Å), GLU166 (2.17 Å, 2.79 Å) |
| 2   | $3b$     | −8.14                    | ARG188 (2.81 Å), GLN192 (2.37 Å), THR190 (2.09 Å), GLU166 (2.03 Å) |
| 3   | $3c$     | −7.05                    | ARG188 (1.82 Å), THR190 (2.60 Å), GLN192 (1.93 Å), GLU166 (1.82 Å, 1.96 Å) |
| 4   | $3d$     | −8.30                    | GLU166 (2.05 Å), ARG188 (1.80 Å), THR190 (2.08 Å), GLN192 (2.38 Å) |
| 5   | $3e$     | −8.63                    | THR190 (2.1 Å), GLN192 (2.38 Å), ARG188 (1.79 Å), GLU166 (2.08 Å) |
| 6   | $3f$     | −7.72                    | GLN189 (1.94 Å), GLU166 (2.01 Å, 2.33 Å) |
| 7   | $3g$     | −7.38                    | GLU166 (2.82 Å) |
| 8   | Darunavir| −8.19                    | GLU166 (1.94 Å, 2.88 Å), LEU167 (1.96 Å) |

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3,3′-Methylenebis(4-hydroxy-6-methoxyquinolin-2(1H)-one) (3c). Orange crystals (DMF/CH3OH), yield (method a): 0.230 g (76%) or yield (method b): 0.260 g (66%); mp = 330–332 °C; IR (KBr): ν = 3450 (OH), 2910 (NH), 3008 (Ar–CH), 1660 (CO), 1453 cm⁻¹ (CH2); 1H NMR (400 MHz, DMSO-d6): δ = 3.81 (s, 6H, OCH3), 3.78 (s, 2H, CH2), 7.24–7.30 (m, 2H, Ar–H), 7.32–7.38 (m, 2H, Ar–H), 7.60–7.72 (m, 2H, Ar–H), 12.13 (s, 2H, NH), 12.96 ppm (s, 2H, OH); 13C NMR (100 MHz, DMSO-d6): δ = 19.23 (CH2), 55.37 (OCH3), 109.11 (C-3), 115.76 (C-4a), 115.87 (C-8), 122.13 (C-5), 131.62 (C-7), 132.13 (C-6), 134.82 (C-8a), 160.61 (C-4), 165.69 ppm (C-2); MS (Fab, 70 eV, %): m/z = 394 (M+, 20), 136 (63), 120 (9), 107 (18), 89 (13).

Anal. Calcd. for C21H18N2O6 (394.38): C, 63.96; H, 4.60; N, 7.10. Found: C, 63.84; H, 4.72; N, 7.19.

Fig. 5 2D representation of predicted binding mode of 3a–g inside the active site of COVID-19 main protease (MPro)

3,3′-Methylenebis(7-chloro-4-hydroxyquinolin-2(1H)-one) (3d) [25]. Orange crystals (DMF/CH3OH), yield (method a): 0.322 g (80%) or yield (method b): 0.274 g (68%); 1H NMR (400 MHz, DMSO-d6): δ = 3.78 (s, 2H, CH2); 7.22–7.28 (m, 2H, Ar–H), 7.30–7.39 (m, 2H, Ar–H),

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7.62–7.70 (m, 2H, Ar–H), 12.15 (s, 2H, NH), 12.86 ppm (s, 2H, OH); 13C NMR (100 MHz, DMSO-
\textit{d}_6): \(\delta = 20.01\) (CH-2), 109.00 (C-3), 115.70 (C-4a), 115.02 (C-8), 122.13 (C-6), 130.00 (C-5), 132.13 (C-7), 136.82 (C-8a), 160.62 (C-4), 164.69 ppm (C-2); MS (Fab, 70 eV, %): \(m/z = 403/402\) (20/18), 136 (63), 120 (9), 107 (18), 89 (13). \textit{Anal. Calcd.} for C\textsubscript{19}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{4} (402.02): C, 56.60; H, 3.00; N, 6.95. \textit{Found}: C, 56.49; H, 3.12; N, 7.14.

3,3′-Methylenebis(7-bromo-4-hydroxyquinolin-2(1H)-one) (3e) [25]. Orange crystals (DMF/EtOH), yield (\textit{method a}): 0.406 g (83%) or yield (\textit{method b}): 0.357 g (73%); \(^1\)H NMR (400 MHz, DMSO-
\textit{d}_6): \(\delta = 3.77\) (s, 2H, CH\textsubscript{2}), 7.22–7.25 (m, 2H, Ar–H), 7.26–7.30 (m, 2H, Ar–H), 7.70–7.82 (m, 2H, Ar–H), 12.14 (s, 2H, NH), 12.90 ppm (s, 2H, OH); 13C NMR (100 MHz, DMSO-
\textit{d}_6): \(\delta = 19.80\) (CH\textsubscript{2}), 109.10 (C-3), 115.76 (C-4a), 115.10 (C-8), 122.10 (C-6), 128.90 (C-5), 132.98 (C-7), 136.82 (C-8a), 160.58 (C-4), 165.12 ppm (C-2); MS (Fab, 70 eV, %): \(m/z = 490/489\) (20/18), 136 (63), 120 (10), 107 (20), 89 (10). \textit{Anal. Calcd.} for C\textsubscript{19}H\textsubscript{12}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{4} (489.12): C, 46.37; H, 2.46; N, 5.69. \textit{Found}: C, 46.49; H, 2.36; N, 7.73.

3,3′-Methylenebis(7-bromo-4-hydroxyquinolin-2(1H)-one) (3f) [26]. Orange crystals (DMF/EtOH), yield (\textit{method a}): 0.340 g (87%) or yield (\textit{method b}): 0.300 g (77%); IR (KBr): \(\nu = 3500\) (OH), 3030 (Ar–CH), 2867 (CH-Aliphatic), 1643 (CO), 1458 cm\(^{-1}\) (CH\textsubscript{2}); \(^1\)H NMR (400 MHz, DMSO-
\textit{d}_6): \(\delta = 1.24\) (t, 6H, CH\textsubscript{3}-Et), 3.89 (s, 2H, CH\textsubscript{2}), 4.38 (q, 4H, CH\textsubscript{2}), 7.00–7.05 (m, 2H, Ar–H), 7.29–7.35 (m, 2H, Ar–H), 7.59–7.70 (m, 4H, Ar–H), 7.90–8.07 (m, 2H, Ar–H), 12.65 ppm (s, 2H, OH); 13C NMR (100 MHz, DMSO-
\textit{d}_6): \(\delta = 12.95\) (CH\textsubscript{2}-Et), 21.11 (CH\textsubscript{2}), 37.59 (CH\textsubscript{3}-Et), 108.52 (C-3), 115.15 (C-4a), 116.74 (C-8), 122.67 (C-6), 123.30 (C-5), 131.50 (C-7), 136.70 (C-8a), 159.63 (C-4), 164.83 ppm (C-2); MS (Fab, 70 eV, %): \(m/z = 390\) (M\textsuperscript{+}, 18), 202 (12), 136 (62), 120 (12), 107 (20), 89 (20). \textit{Anal. Calcd.}
for C$_2$H$_2$N$_2$O$_4$ (390.42): C, 70.75; H, 5.68; N, 7.17. Found: C, 70.82; H, 5.77; N, 7.29.

**Crystal structure determination**

The single-crystal X-ray diffraction study of 3g was carried out on a Bruker D8 Venture diffractometer with Photon II detector at 123(2) K using Cu-Kα radiation ($\lambda = 1.54178$ Å). Dual space/intrinsic methods (SHELXT) [41] were used for structure solution and refinement was carried out using SHELXL-2014 (full-matrix least-squares on $F^2$) [42]. Hydrogen atoms were localized by difference electron density determination and refined using a riding model (H2O free). A semi-empirical absorption correction was applied.

3g: Orange crystals, C$_2$H$_2$N$_2$O$_4$, $M_r = 390.42$, crystal size 0.36×0.24×0.12 mm, monoclinic, space group $P2_1/c$ (No. 14), $a = 13.2293$ (3) Å, $b = 17.0327$ (4) Å, $c = 8.5503$ (2) Å, $\beta = 101.919$ (1)$^\circ$, $V = 1885.11$ (8) Å$^3$, $Z = 4$, $\rho = 1.376$ Mg/m$^3$, $\mu$(Cu-Kα) = 0.77 mm$^{-1}$, $F(000) = 824$, $2\theta_{max} = 144.4^\circ$, 16886 reflections, of which 3689 were independent ($R_{int} = 0.024$), 268 parameters, 2 restraints, $R_1 = 0.034$ (for 3587 $I > 2\sigma(I)$), w$R_2 = 0.089$ (all data), $S = 1.06$, largest diff. peak/hole = 0.27/-0.19 e Å$^{-3}$.

**Molecular docking calculations**

All molecular docking calculations were carried out using Autodock 4.2.6 software [43]. The crystal structure of SARS-CoV-2 main protease (Mpro; PDB code: 6LU7 [44]) was taken as a template for all molecular docking calculations. Water molecules, ions and the ligand were deleted. The protonation state of Mpro was evaluated using H$^{+}$. All molecular docking calculations were carried out using Molecular docking calculations

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**Supporting Information**

CCDC 2011538 (3g) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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Affiliations

Ashraf A. Aly1 ⊙ · Alaa A. Hassan1 · Asmaa H. Mohamed1 · Esraa M. Osman1 · Stefan Bräse2,3 · Martin Nieger4 · Mahmoud A. A. Ibrahim1 · Sara M. Mostafa1

1 Department of Chemistry, Faculty of Science, Minia University, Minia 61519, Egypt
2 Institute of Organic Chemistry, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany
3 Institute of Biological and Chemical Systems (IBCS-FMS), Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany
4 Department of Chemistry, University of Helsinki, A. I. Virtasenakio I, P.O. Box 55, 00014 Helsinki, Finland