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Synthesis and Biological Evaluation of Quinolinone Compounds as SARS CoV 3CL^{pro} Inhibitors

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SARS CoV 3CL^{pro} is known to be a promising target for development of therapeutic agents against the severe acute respiratory syndrome (SARS). A quinolinone compound **1** was selected via virtual screening, and it was synthetized and tested for enzymatic inhibition *in vitro*. Compound **1** showed potent inhibitory activity (IC_{50}=0.44 μmol/L) toward SARS CoV 3CL^{pro}. Further work on a series of quinolinone derivatives resulted in the discovery of the most potent compound **23**, inhibiting SARS CoV 3CL^{pro} with an IC_{50} of 36.86 nmol/L. The structure-activity relationships were also discussed.

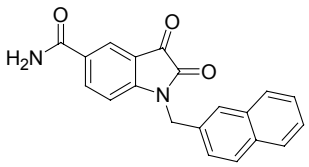
Keywords SARS, SARS CoV 3CL^{pro}, inhibitors, quinolinone

Introduction

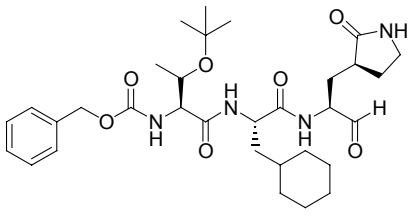
Severe acute respiratory syndrome (SARS), caused by a novel coronavirus (SARS CoV), is a highly infective upper respiratory tract disease.[1-4] Due to the high mortality rate (up to 10%) SARS quickly became a global threat in 2003.[5,6] Although about a year later from its first discovery the initial outbreak of the virus was stymied, the reemergence of SARS is quite possible and the development of new anti-SARS drugs is particularly important.[7,8]

The SARS CoV is a positive-sense single-stranded RNA virus. Its genome is comprised of about 29700 nucleotides, most of them (over 21000 nucleotides) belongs to the replicase gene, which encodes two overlapping polyproteins, pp1a (486 kDa) and pp1ab (790 kDa). The SARS CoV 3CL^{pro}, a ~33 kDa cysteine protease, releases functional polypeptides from pp1a and pp1ab. Without these polypeptides the viral replication and transcription will be disabled. As the integral role of SARS CoV 3CL^{pro} in SARS CoV lifecycle, it is an ideal target for drug discovery.[9-12]

Previously, a large number of SARS CoV 3CL^{pro} inhibitors have been reported and their scaffolds are diverse. Yang *et al*.[6] developed a potent SARS CoV 3CL protease inhibitor (TG-0205221, Ki=50 nmol/L, Figure 1). Sydnes *et al*.[9] synthetized a series of glutamic acid and glutamine peptides possessing a trifluoromethyl ketone group with effective inhibitory activity. Zhou *et al*.[11] have reported 1-alkylisatin-5-carboxamide (IC_{50} =0.37 μmol/L, Figure 1) and its analogues to be highly potent SARS CoV 3CL^{pro} inhibitors.



1-Alkylisatin-5-carboxamide



TG-0205221

Figure 1 1-Alkylisatin-5-carboxamide and TG-0205221.

We identified novel SARS CoV 3CL^{pro} inhibitors among a database containing more than 600000 compounds by virtual screening. Compound **1** (Figure 2) was selected, synthetized and tested against SARS CoV 3CL^{pro} *in vitro*. **1** showed potent inhibitory activity (IC_{50}

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=0.44 μmol/L) toward SARS CoV 3CL^{pro}.

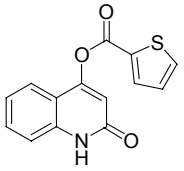


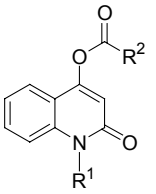
Figure 2 Structure of compound **1**.

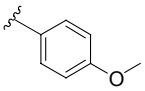
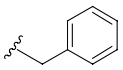
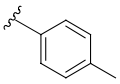
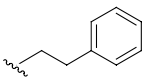
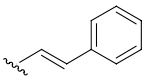
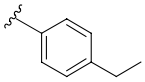
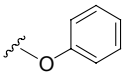
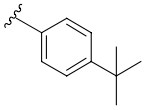
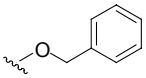
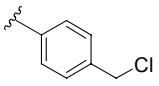
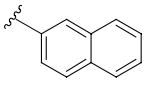
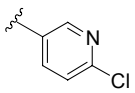
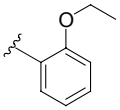
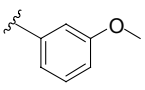
Results and Discussion

23 compounds were synthesized and tested against SARS CoV 3CL^{pro} *in vitro*. Biological evaluation results of the title compounds are listed in Table 1.

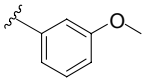
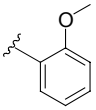
As shown in Table 1, replacement of the thiophene moiety (compound **1**) with benzene ring (compound **2**) leads to the decrease of the inhibitory activity, especially when the benzene ring was substituted. It is worth

Table 1 Inhibition activities of quinolinone compounds against SARS CoV 3CL^{pro}



No.	R^1	R^2	Inhibition or IC_{50}	No.	R^1	R^2	Inhibition or IC_{50}
1	H		0.437±0.03 μmol/L	**13**	H		68.06%
2	H		0.66±0.10 μmol/L	**14**	H		52.20%
3	H		11.19%	**15**	H		39.83%
4	H		62.63%	**16**	H		43.62%
5	H		38.15%	**17**	H		39.37%
6	H		31.03%	**18**	H		50.97%
7	H		44.47%	**19**	H	CH_2CH_3	32.22±4.65 μM
8	H		83.70%	**20**	H	$CH_2CH_2COOCH_3$	41.70±10.35 μmol/L
9	H		3.29±0.49 μmol/L	**21**	H	$OCH_2CH(CH_3)_2$	58.49±11.60 μmol/L
10	H		54.20%	**22**	CH_3		158.7±4.95 nmol/L

Continued

No.	R^1	R^2	Inhibition or IC_{50}	No.	R^1	R^2	Inhibition or IC_{50}
11	H		1.41±0.12 μmol/L	**23**	CH_3		36.86±1.50 nmol/L
12	H		4.45±0.76 μmol/L				

noting that the introduction of substituent at *meta*-position or *ortho*-position of benzene ester carbonyl shows more inhibition potency than *para*-position. For instance, the IC_{50} of compounds **11** and **12** are 1.41 and 4.45 μmol/L respectively, significantly more powerful than compound **13** (68.06% inhibition at 1 mmol/L). Meanwhile, to extend the distance between the benzene ring and the ester carbonyl carbon, compounds **14**—**18** were prepared. Biological evaluation results showed these compounds were mostly inactive against SARS CoV 3CL^{pro} compared with compound **2**. Compounds **19**—**21** with a carbon chain instead of benzene ring were partly kept the inhibitory potency, got an IC_{50} from 32.22 to 58.49 μmol/L. Dramatically, compound **23** (IC_{50}=36.86 nmol/L), methylation derivatives of the N—H group of the quinolinone of compound **1**, exhibited approximately 12-fold higher potency relative to compound **1** (IC_{50}=0.44 μmol/L). As well as compound **22**, it was about 9-fold higher potency than compound **11**.

The nitrogen, oxygen, and sulfur atoms are colored blue, red, and orange, respectively. Hydrogen bonds are displayed as blue dashed lines. Figure 3 showed docking model of compounds **1** and **23**, from which we can conclude that compound **1** binds competitively and strongly to the active site, and the quinolinone portion was recognized by S1 pocket. Moreover, the thiophene moiety extends into S1' site with hydrophobic interactions. The S1' pocket is not quite large, this may explain why compounds **14** and **15** , with long carbon chain between the aromatic ring and ester carbonyl, show lower potency than compound **2**. Unlike compound **1**, methylation of the N-H of the quinolinone brings a different binding interaction, the thiophene moiety of compound **23** occupies S2 pocket, and the methyl group fills into the crack of S1 pocket closely. As S1' pocket is much nearer the surface of the protein than S2 pocket, the new binding model makes compound **23** bind more tightly than compound **1** with the protein. This may partly explain why compound **23** showed more potent inhibitory effect compared with compound **1**. Molecular insight into the two different binding modes of compound **1** and **23** was shown in Figure 4, the oxygen at C-2 of quinolinone moiety of compound **1** makes a H-bond with the NH of His 163 (1.78 Å), and the NH of quinolinone forms a H-bond with Glu 166 (2.26 Å). The ester carbonyl oxygen forms a hydrogen bond with the NH of Gly 143 (2.26 Å). To compound **23**, the hydro-

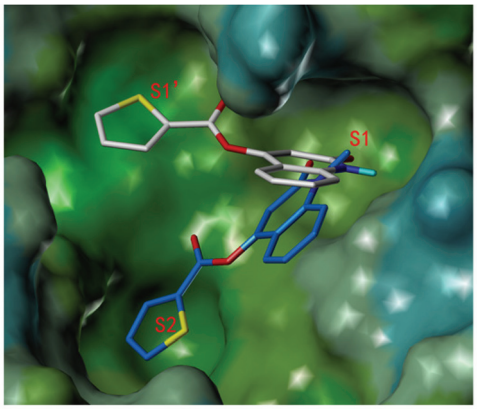


Figure 3 The docking model of compound **1** (grey) and **23** (blue) in the active site of SARS CoV 3CL^{pro}.

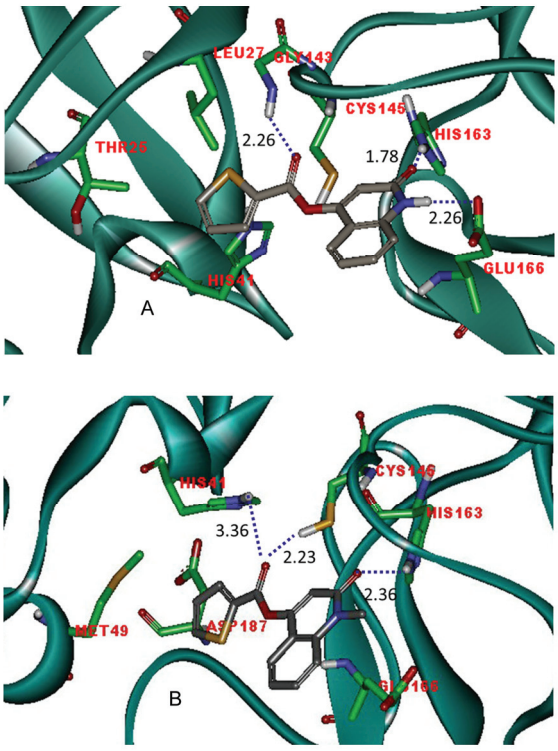


Figure 4 Simulated binding models of compound **1** (A) and **23** (B) in SARS CoV 3CL^{pro}.

gen bond of the amide carbonyl oxygen with the NH of His 163 (2.36 Å) was also conserved as well. But there is no hydrogen bond with Glu 166, because of the methylation. The ester carbonyl oxygen of compound **23** makes hydrogen bonds with the active site residues, His 41 (3.36 Å) and Cys 145 (2.23 Å) . This is the most difference between the two binding modes. The active site of SARS CoV $3CL^{pro}$ contains a catalytic dyad constituted by Cys 145 and His 41, which functions as the common nucleophile in the proteolytic process.[11] That means that although the methylation disturbed the hydrogen bond of N—H of quinolinone, the distance between ester carbonyl oxygen and the S—H group of Cys 145 and N—H group of His 41 might be changed favorably resulting in dramatic inhibitory potency. In addition, the carbonyl of compound **23** may potentially react with the Cys 145 residue. This suppose remains to be further studied.

Conclusions

In conclusion, twenty-three quinolinone compounds were synthesized and tested *in vitro* as inhibitors against SARS CoV $3CL^{pro}$. Compound **1**, selected via virtual screening, was proved to have a potent inhibitory activity (IC_{50} = 0.44 μmol/L). Following optimization of 4-quinolinone ester derivatives resulted in the discovery of compound **23** with the most powerful potency (IC_{50} =36.86 nmol/L). The present work demonstrated that 4-quinolinone ester analogs could be used as new leads for future Anti-SARS drugs discovery. Further structural optimization and *in vivo* activities about quinolinone compounds are well under way.

Experimental

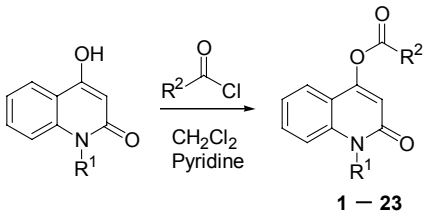
Materials and instruments

Unless otherwise noted, all chemicals and solvents were commercially available and treated with standard methods before use. Melting points were determined using an SGW-X4B digital melting point apparatus and uncorrected. SHANGHAI SANPONT GF254 plates were used as analytical TLC. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance DPX spectrometers and using DMSO as solvent. All ^{13}C NMR spectra were recorded proton-decoupled. Chemical shifts for ^{1}H and ^{13}C spectra are quoted in ppm downfield from TMS. Coupling constants are referred to as *J* values in hertz. ESI mass spectra were acquired using a Bruker ESQUIRELCTM ESI ion trap spectrometer. FT-IR spectra were recorded at room temperature in the region of 4000—400 cm^{-1} with a Perkin-Elmer spectrum 65 FT-IR spectrometer using KBr pellets. Elemental analyses of carbon, hydrgen and nitrogen were obtained with an Elementar Vario MICRO cube Elemental Analyser.

General procedure

To a suspension of 4-hydroxy-2-quinolinone or 4-hydroxy-1-methyl-2-quinolinone (1 mmol) in 25 mL of anhydrous CH_2Cl_2, pyridine (1.2 mmol) was added, then the mixture was stirred at room temperature. Corresponding acyl chloride (1.2 mmol) dissolved in 10 mL of anhydrous CH_2Cl_2 was added dropwise over 25 min. The reaction mixture was stirred for a further 5—8 h, then washed several times with diluted hydrochloric acid in a separatory funnel. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed by evaporation. Product was purified by recrystallization with CH_2Cl_2 and ethyl acetate.

Scheme 1 General procedure for the synthesis of target compounds **1**—**23**



1 — **23**

4-(Thiophene-2-carbonyl)oxy-quinol-2-one (1) Compound **1** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 2-thiophenecarbonyl chloride, obtained a white solid in 93% yield. m.p. 234 — 235 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.62 (s, 1H), 7.23 (t, J=8.0 Hz, 1H), 7.41—7.36 (m, 2H), 7.64—7.58 (m, 2H), 8.20—8.16 (m, 2H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.0, 115.1, 116.1, 122.5, 122.7, 129.5, 131.1, 132.1, 136.7, 136.9, 139.4, 155.9, 159.1, 162.6; IR (KBr) v: 3436, 1737, 1670, 1256, 1103, 733 cm^{-1}; ESI-MS m/z: 272.02 ([M+H$^+$]). Anal. calcd for $C_{14}H_9NO_3S$: C 61.98, H 3.34, N 5.16; found C 62.10, H 3.35, N 5.43.

4-Benzoyloxy-quinol-2-one (2) Compound **2** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and benzoyl chloride, obtained a white solid in 94% yield. m.p. 214—215 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.61 (s, 1H), 7.22 (t, J=7.6 Hz, 1H), 7.41 (d, J=8.4 Hz, 1H), 7.69—7.58 (m, 4H), 7.82 (t, J=7.6 Hz, 1H), 8.23 (d, J=8.4 Hz, 2H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.1, 115.3, 116.1, 122.7, 128.4, 129.7, 130.6, 132.0, 135.1, 139.4, 156.5, 162.7, 163.8; IR (KBr) v: 3462, 1740, 1671, 1251, 1093, 749 cm^{-1}; ESI-MS m/z: 266.09 ([M+H$^+$]). Anal. calcd for $C_{16}H_{11}NO_3$: C 72.45, H 4.18, N 5.28; found C 72.74, H 4.21, N 5.31.

4-(4-Methylbenzoyl)oxy-quinol-2-one (3) Compound **3** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 4-methylbenzoyl chloride, obtained a white solid in 90% yield. m.p. 239—241 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.46 (s, 3H), 6.59 (s, 1H), 7.21 (t, J=7.6 Hz, 1H), 7.40 (d, J=8.0 Hz, 1H), 7.47 (d, J=8.0 Hz, 2H), 7.59 (t, J=7.6 Hz, 2H), 8.12 (d, J=8.0 Hz, 2H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.8, 113.0, 115.3, 116.1, 122.6, 122.7, 125.6, 130.2, 130.7, 132.0, 139.4, 139.8,

145.8, 156.6, 162.7, 163.7; IR (KBr) *v*: 3436, 1740, 1672, 1257, 1092, 752 cm^{-1}; ESI-MS *m*/*z*: 280.01 ([M+H$^+$]). Anal. calcd for $C_{17}H_{13}NO_3$: C 73.11, H 4.69, N 5.02; found C 73.02, H 4.53, N 4.98.

4-(2-Methylbenzoyl)oxy-quinol-2-one (4) Compound **4** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 2-methylbenzoyl chloride, obtained a white solid in 91% yield. m.p. 220−221 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.61 (s, 3H), 6.60 (s, 1H), 7.22 (t, *J*=7.6 Hz, 1H), 7.41 (d, *J*=8.0 Hz, 1H), 7.49−7.45 (m, 2H), 7.67−7.58 (m, 3H), 8.24 (d, *J*=7.6 Hz, 1H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.5, 112.9, 113.4, 115.4, 116.1, 118.0, 120.9, 122.7, 122.8, 132.0, 132.4, 135.8, 139.5, 156.6, 159.7, 162.7, 163.1; IR (KBr) *v*: 3430, 1727, 1675, 1492, 1300, 1231, 745 cm^{-1}; ESI-MS *m*/*z*: 280.33 ([M+H$^+$]). Anal. calcd for $C_{17}H_{13}NO_3$: C 73.11, H 4.69, N 5.02; found C 73.38, H 4.71, N 5.47.

4-(4-Ethylbenzoyl)oxy-quinol-2-one (5) Compound **5** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 4-ethylbenzoyl chloride, obtained a white solid in 96% yield. m.p. 233−234 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.24 (t, *J*=7.6 Hz, 3H), 2.75 (q, *J*=7.6 Hz, 2H), 6.59 (s, 1H), 7.21 (t, *J*=8.0 Hz, 1H), 7.41 (d, *J*=8.0 Hz, 1H), 7.50 (d, *J*=8.0 Hz, 2H), 7.59 (t, *J*=8.0 Hz, 2H), 8.14 (d, *J*=8.0 Hz, 2H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.7, 28.8, 113.0, 115.3, 116.1, 122.7, 125.9, 129.1, 130.8, 132.0, 139.4, 151.8, 156.6, 162.7, 163.7; IR (KBr) *v*: 3435, 1756, 1655, 1243, 1087, 750 cm^{-1}; ESI-MS *m*/*z*: 293.99 ([M+H$^+$]). Anal. calcd for $C_{18}H_{15}NO_3$: C 73.71, H 5.15, N 4.78; found C 73.98, H 5.28, N 4.89.

4-(4-*tert*-Butylbenzoyl)oxy-quinol-2-one (6) Compound **6** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 4-*tert*-butylbenzoyl chloride, obtained a white solid in 95% yield. m.p. 263−264 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.35 (s, 9H), 6.58 (s, 1H), 7.21 (t, *J*=7.6 Hz, 1H), 7.41 (d, *J*=7.6 Hz, 1H), 7.60 (t, *J*=7.6 Hz, 2H), 7.68 (d, *J*=8.4 Hz, 2H), 8.15 (d, *J*=8.4 Hz, 2H), 11.96 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.2, 35.5, 113.0, 115.3, 116.1, 122.7, 125.7, 126.5, 130.6, 132.0, 139.4, 156.6, 158.4, 162.7, 163.7; IR (KBr) *v*: 3467, 1758, 1661, 1254, 1108, 766 cm^{-1}; ESI-MS *m*/*z*: 322.12 ([M+H$^+$]). Anal. calcd for $C_{18}H_{15}NO_3$: C 74.75, H 5.96, N 4.36; found C 74.52, H 5.88, N 4.41.

4-(4-Chloromethylbenzoyl)oxy-quinol-2-one (7) Compound **7** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 4-(chloromethyl)benzoyl chloride, obtained a white solid in 97% yield. m.p. 234−236 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.92 (s, 2H), 6.62 (s, 1H), 7.21 (t, *J*=8.4 Hz, 1H), 7.41 (d, *J*=8.4 Hz, 1H), 7.65−7.58 (m, 1H), 7.72 (d, *J*=8.4 Hz, 2H), 8.23 (d, *J*=8.4 Hz, 2H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 45.6, 113.1, 115.2, 116.1, 122.69, 122.72, 128.2, 129.9, 131.0, 132.1, 139.4, 144.7, 156.5, 162.7, 163.4; IR (KBr) *v*: 3436, 1741, 1675, 1264, 1097, 762, 711 cm^{-1}; ESI-MS *m*/*z*: 314.02 ([M+H$^+$]). Anal. calcd for $C_{17}H_{12}ClNO_3$: C 65.08, H 3.86, N 4.46; found C 65.34, H 3.78, N 4.31.

4-(2-Naphthoyl)oxy-quinol-2-one (8) Compound **8** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 2-naphthoyl chloride, obtained a white solid in 95% yield. m.p. 259−260 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.66 (s, 1H), 7.22 (t, *J*=8.0 Hz, 1H), 7.42 (d, *J*=8.0 Hz, 1H), 7.61 (t, *J*=8 Hz, 1H), 7.71−7.67 (m, 2H), 7.76 (t, *J*=8.0 Hz, 1H), 8.10 (d, *J*=8.0 Hz, 1H), 8.20−8.15 (m, 2H), 8.26 (d, *J*=8.0 Hz, 1H), 8.97 (s, 1H), 11.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.2, 115.3, 116.1, 122.7, 122.8, 125.6, 125.7, 127.8, 128.3, 129.3, 129.8, 130.2, 132.1, 132.6, 132.6, 136.1, 139.5, 156.7, 162.7, 164.0; IR (KBr) *v*: 3436, 1744, 1675, 1276, 1191, 750 cm^{-1}; ESI-MS *m*/*z*: 315.99 ([M+H$^+$]). Anal. calcd for $C_{20}H_{13}NO_3$: C 76.18, H 4.16, N 4.44; found C 76.49, H 4.34, N 4.23.

4-(6-Chloronicotinoyl)oxy-quinol-2-one (9) Compound **9** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 6-chloronicotinoyl chlorid, obtained a white solid in 97% yield. m.p. 250−251 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.65 (s, 1H), 7.21 (t, *J*=8.0 Hz, 1H), 7.41 (d, *J*=8.0 Hz, 1H), 7.60 (t, *J*=8.0 Hz, 1H), 7.74 (d, *J*=8.0 Hz, 1H), 7.82 (d, *J*=8.4 Hz, 1H), 8.58 (dd, *J*=8.4, 2.4 Hz, 1H), 9.18 (d, *J*=2.4 Hz, 1H), 11.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ: 113.1, 115.0, 116.0, 122.7, 123.0, 124.6, 125.3, 132.1, 139.4, 141.6, 152.0, 155.8, 156.2, 162.1, 162.6; IR (KBr) *v*: 3436, 1753, 1683, 1276, 1112, 752 cm^{-1}; ESI-MS *m*/*z*: 301.14 ([M+H$^+$]). Anal. calcd for $C_{15}H_9ClN_2O_3$: C 59.91, H 3.02, N 9.32; found C 60.12, H 3.11, N 9.41.

4-(2-Ethoxybenzoyl)oxy-quinol-2-one (10) Compound **10** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 2-ethoxybenzoyl chloride, obtained a white solid in 90% yield. m.p. 178−179 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.37 (t, *J*=6.8 Hz, 3H), 4.21 (q, *J*=6.8 Hz, 2H), 6.53 (s, 1H), 7.13 (t, *J*=7.6 Hz, 1H), 7.28−7.22 (m, 2H), 7.41 (d, *J*=8.0 Hz, 1H), 7.60 (td, *J*=8.0, 1.2 Hz, 1H), 7.67 (td, *J*=8.0, 1.6 Hz, 1H), 7.75 (d, *J*=8.0 Hz, 1H), 7.95 (dd, *J*=7.6, 1.6 Hz, 1H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.0, 64.8, 112.9, 114.2, 115.3, 116.1, 118.5, 120.8, 122.5, 123.0, 132.0, 132.2, 135.5, 139.5, 156.8, 158.8, 162.7, 163.6; IR (KBr) *v*: 3422, 1717, 1672, 1308, 1233, 1109, 744 cm^{-1}; ESI-MS *m*/*z*: 210.09 ([M+H$^+$]). Anal. calcd for $C_{18}H_{15}NO_4$: C 69.89, H 4.89, N 4.53; found C 69.52, H 4.88, N 4.61.

4-(3-Methoxybenzoyl)oxy-quinol-2-one (11) Compound **11** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 3-methoxy-benzoyl chloride, obtained a white solid in 94% yield. m.p. 199−201 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.88 (s, 3H), 6.60 (s, 1H), 7.22 (t, *J*=8.0 Hz, 1H), 7.42−7.37 (m, 2H), 7.62−7.56 (m, 3H), 7.67 (t, *J*=2.0 Hz, 1H), 7.82 (d, *J*=8.0 Hz, 1H), 11.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.0, 113.1,

115.0, 115.2, 116.1, 121.2, 122.7, 122.7, 122.9, 129.7, 130.9, 132.1, 139.4, 156.5, 160.0, 162.7, 163.6; IR (KBr) *v*: 3435, 1752, 1668, 1277, 1196, 1102, 742 cm^{-1}; ESI-MS *m/z*: 296.11 ([M + H^+]). Anal. calcd for $C_{17}H_{13}NO_4$: C 69.15, H 4.44, N 4.74; found C 68.75, H 4.28, N 4.81.

4-(2-Methoxybenzoyl)oxy-quinol-2-one (12) Compound **12** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and oanisoyl chloride, obtained a white solid in 91% yield. m.p. 208 −209 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.93 (s, 3H), 6.55 (s, 1H), 7.15 (t, J=7.6 Hz, 1H), 7.25 (t, J=7.6 Hz, 1H), 7.30 (d, J=8.4 Hz, 1H), 7.40 (d, J=8.4 Hz, 1H), 7.60 (t, J=7.6 Hz, 1H), 7.71 (dd, J=13.2, 7.6 Hz, 2H), 8.01 (d, J=7.2 Hz, 1H), 11.94 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.8, 113.2, 115.4, 116.1, 122.69, 122.72, 126.9, 127.6, 131.6, 131.8, 132.0, 132.6, 134.1, 139.5, 141.4, 156.7, 162.7, 164.2; IR (KBr) *v*: 3468, 1750, 1679, 1236, 1195, 1136, 728 cm^{-1}; ESI-MS *m/z*: 296.06 ([M+H^+]). Anal. calcd for $C_{17}H_{13}NO_4$: C 69.15, H 4.44, N 4.74; found C 69.32, H 4.72, N 5.06.

4-(4-Methoxybenzoyl)oxy-quinol-2-one (13) Compound **13** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and 4-methoxybenzol chloride, obtained a white solid in 95% yield. m.p. 232−233 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (s, 3H), 6.57 (s, 1H), 7.18 (d, J=8.8 Hz, 2H), 7.22 (d, J=7.6 Hz, 1H), 7.40 (d, J=8.8 Hz, 1H), 7.59 (t, J=7.6 Hz, 2H), 8.18 (d, J=8.8 Hz, 2H), 11.94 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.2, 113.0, 115.0, 115.4, 116.1, 120.4, 122.7, 132.0, 132.9, 139.4, 156.6, 162.7, 163.4, 164.7; IR (KBr) *v*: 3436, 1743, 1655, 1605, 1252, 1093, 761 cm^{-1}; ESI-MS: *m/z* 295.78 ([M+H^+]). Anal. calcd for $C_{17}H_{13}NO_4$: C 69.15, H 4.44, N 4.74; found C 69.01, H 4.52, N 4.33.

4-(2-Phenylacetyl)oxy-quinol-2-one (14) Compound **14** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and phenylacetyl chloride, obtained a white solid in 92% yield. m.p. 174 −175 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) *v*: 4.16 (s, 2H), 6.41 (s, 1H), 7.16 (t, J=8.0 Hz, 1H), 7.46−7.31 (m, 6H), 7.49 (d, J=8 Hz, 1H), 7.56 (t, J=8.0 Hz, 1H), 11.92 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 71.1, 112.0, 114.7, 116.1, 122.4, 122.7, 129.0, 129.1, 129.2, 132.1, 135.1, 139.4, 151.7, 156.1, 162.6; IR (KBr) *v*: 3436, 1756, 1668, 1123, 755 cm^{-1}; ESI-MS *m/z*: 280.35 ([M+H^+]). Anal. calcd for $C_{17}H_{13}NO_3$: C 73.11, H 4.69, N 5.02; found C 72.98, H 4.46, N 4.91.

4-(3-Phenylpropanoyl)oxy-quinol-2-one (15) Compound **15** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and hydrocinnamoyl chloride, obtained a white solid in 93% yield. m.p. 167−168 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.02 (t, J=7.2 Hz, 2H), 3.12 (t, J=7.2 Hz, 2H), 6.30 (d, J=1.2 Hz, 1H), 7.12 (t, J=8.0 Hz, 1H), 7.29−7.23 (m, 1H), 7.35−7.33 (m, 6H), 7.55 (t, J=8.0 Hz, 1H), 11.88 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.5, 35.4, 112.7, 115.2, 115.9, 122.4, 122.8, 126.8, 128.8, 128.9, 131.9, 139.4, 140.5, 156.5, 162.6, 170.5; IR (KBr) *v*: 3436, 1760, 1671, 1125, 750 cm^{-1}; ESI-MS *m/z*: 294.10 ([M+H^+]). Anal. calcd for $C_{18}H_{15}NO_3$: C 73.71, H 5.15, N 4.78; found C 73.24, H 5.03, N 4.96.

4-(Cinnamoyl)oxy-quinol-2-one (16) Compound **16** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and cinnamoyl chloride, obtained a white solid in 95% yield. m.p. 207−208 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.53 (s, 1H), 7.03 (d, J=16.0 Hz, 1H), 7.23 (t, J=8.0 Hz, 1H), 7.39 (d, J=8.0 Hz, 1H), 7.51−7.48 (m, 3H), 7.59 (t, J=8.0 Hz, 1H), 7.66 (d, J=8.0 Hz, 1H), 7.88−7.86 (m, 2H), 7.99 (d, J=16.0 Hz, 1H), 11.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.7, 115.3, 116.0, 116.7, 122.6, 122.8, 129.4, 129.5, 131.7, 132.0, 134.2, 139.4, 148.3, 156.5, 162.7, 164.1; IR (KBr) *v*: 3436, 1755, 1682, 1631, 1118, 750 cm^{-1}; ESI-MS *m/z*: 291.93 ([M+H^+]). Anal. calcd for $C_{18}H_{13}NO_3$: C 74.22, H 4.50, N 4.81; found C 74.02, H 4.86, N 4.98.

2-Oxo-1,2-dihydroquinolin-4-yl phenyl carbonate (17) Compound **17** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and phenyl chloroformate, obtained a white solid in 91% yield. m.p. 191 − 192 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.74 (s, 1H), 7.28 (t, J=7.6 Hz, 1H), 7.41 −7.34 (m, 1H), 7.53−7.45 (m, 4H), 7.62 (t, J=7.6 Hz, 1H), 7.79 (d, J=7.6 Hz, 1H), 12.00 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.3, 114.6, 116.1, 121.7, 122.5, 122.8, 127.3, 130.3, 132.3, 139.4, 150.4, 151.0, 156.0, 162.6; IR (KBr) *v*: 3444, 1784, 1672, 1243, 765 cm^{-1}; ESI-MS *m/z*: 282.00 ([M+H^+]). Anal. calcd for $C_{16}H_{11}NO_4$: C 68.32, H 3.94, N 4.98; found C 68.56, H 4.02, N 5.11.

Benzyl 2-oxo-1,2-dihydroquinolin-4-yl carbonate (18) Compound **18** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and benzyl chloroformate, obtained a white solid in 92% yield. m.p. 169−170 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.35 (s, 2H), 6.58 (s, 1H), 7.22 (t, J=8.0 Hz, 1H), 7.38 (d, J=8.0 Hz, 1H), 7.46−7.41 (m, 3H), 7.50 (d, J=6.8 Hz, 2H), 7.59 (t, J=6.8 Hz, 2H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.7, 115.2, 116.0, 122.5, 122.7, 127.7, 129.0, 130.2, 132.0, 134.0, 139.4, 156.4, 162.6, 169.4; IR (KBr) *v*: 3435, 1762, 1654, 1226, 1195, 770 cm^{-1}; ESI-MS *m/z*: 295.89 ([M+H^+]). Anal. calcd for $C_{17}H_{13}NO_4$: C 69.15, H 4.44, N 4.74; found C 69.54, H 4.71, N 4.58.

4-(Propionyl)oxy-quinol-2-one (19) Compound **19** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and propionyl chloride, obtained a white solid in 92% yield. m.p. 179−180 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.18 (t, J=7.6 Hz, 3H), 2.79 (q, J=7.6 Hz, 2H), 6.40 (s, 1H), 7.21 (t, J=8.0 Hz, 1H), 7.36 (d, J=8.0 Hz, 1H), 7.57 (t, J=8.0 Hz, 1H), 7.63 (d, J=8.0 Hz, 1H), 11.88 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 9.2, 27.3, 112.7, 115.3, 116.0, 122.5, 122.9, 131.9, 139.4, 156.5, 162.7, 171.9; IR (KBr) *v*: 3436, 1761, 1674, 1134, 752 cm^{-1}; ESI-MS *m/z*:

218.02 ([M+H$^+$]). Anal. calcd for $C_{12}H_{11}NO_3$: C 66.35, H 5.10, N 6.45; found C 66.54, H 5.32, N 6.74.

Methyl 2-oxo-1,2-dihydroquinolin-4-yl succinate (20) Compound **20** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and methyl 4-chloro-4-oxobutanoate, obtained a white solid in 90% yield. m.p. 170−171 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.75 (t, J=6.8 Hz, 2H), 3.02 (t, J=6.8 Hz, 2H), 3.65 (s, 3H), 6.35 (s, 1H), 7.22 (t, J=8.0 Hz, 1H), 7.37 (d, J=8.0 Hz, 1H), 7.58 (t, J=8.0 Hz, 1H), 7.66 (d, J=8.0 Hz, 1H), 11.91 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 28.8, 29.3, 52.1, 112.6, 115.2, 116.0, 122.5, 122.9, 132.0, 139.4, 156.5, 162.6, 170.3, 172.8; IR (KBr) v: 3439, 1772, 1733, 1651, 1329, 1117, 773 cm^{-1}; ESI-MS m/z: 276.04 ([M+H$^+$]). Anal. calcd for $C_{14}H_{13}NO_5$: C 61.09, H 4.76, N 5.09; found C 61.21, H 4.34, N 4.96.

Isobutyl 2-oxo-1,2-dihydroquinolin-4-yl carbonate (21) Compound **21** was prepared according to general procedure by using 4-hydroxy-2-quinolinone and isobutyl chloroformate, obtained a white solid in 94% yield. m.p. 126−127 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.95 (d, J=6.8 Hz, 6H), 2.07−1.97 (m, 1H), 4.08 (d, J=6.8 Hz, 2H), 6.55 (s, 1H), 7.24 (t, J=7.6 Hz, 1H), 7.38 (d, J=8.0 Hz, 1H), 7.59 (t, J=7.6 Hz, 2H), 11.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.0, 27.7, 75.4, 112.1, 114.8, 116.1, 122.3, 122.7, 132.1, 139.4, 151.9, 156.2, 162.7; IR (KBr) v: 3436, 1774, 1655, 1231, 1196, 759 cm^{-1}; ESI-MS m/z: 262.33 ([M+H$^+$]). Anal. calcd for $C_{14}H_{15}NO_4$: C 64.36, H 5.79, N 5.36; found C 64.12, H 5.89, N 5.08.

1-Methyl-4-(3-methoxybenzoyl)oxy-quinol-2-one (22) Compound **22** was prepared according to general procedure by using4-hydroxy-1-methyl-2-quinolinone and 3-methoxybenzol chloride, obtained a white solid in 96% yield. m.p. 160−162 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.67 (s, 3H), 3.88 (s, 3H), 6.74 (s, 1H), 7.32 (t, J=8.0 Hz, 1H), 7.39 (dd, J=8.0, 2.0 Hz, 1H), 7.59 (t, J=8.0 Hz, 1H), 7.75−7.65 (m, 4H), 7.84 (d, J=8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.7, 56.0, 112.5, 115.1, 115.7, 116.2, 121.3, 122.88, 122.91, 123.2, 129.7, 130.9, 132.5, 140.3, 155.4, 160.0, 161.9, 163.6; IR (KBr) v: 1742, 1664, 1275, 1102, 1023, 740 cm^{-1}; ESI-MS m/z: 310.19 ([M+H$^+$]). Anal. calcd for $C_{18}H_{15}NO_4$: C 69.89, H 4.89, N 4.53; found C 70.36, H 5.02, N 4.86.

1-Methyl-4-(thiophene-2-carbonyl)oxy-quinol-2-one (23) Compound **23** was prepared according to general procedure by using 4-hydroxy-1-methyl-2-quinolinone and 2-thiophenecarbonyl chloride, obtained a white solid in 91% yield. m.p. 148−149 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.65 (s, 3H), 6.75 (s, 1H), 7.32 (t, J=8.0 Hz, 1H), 7.37 (t, J=4.4 Hz, 1H), 7.63 (d, J=8.0 Hz, 1H), 7.73−7.70 (m, 2H), 8.20−8.16 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.7, 112.3, 115.7, 116.1, 122.9, 123.0, 129.5, 131.1, 132.5, 136.7, 136.9, 140.2, 154.7, 159.1, 161.8; IR (KBr) v: 3092, 1733, 1663, 1413, 1354, 1249, 1105, 739 cm^{-1}; ESI-MS m/z: 285.95 ([M+H$^+$]). Anal. calcd for $C_{15}H_{11}NO_3S$: C 63.14, H 3.89, N 4.91; found C 63.53, H 3.57, N 4.65.

Biology enzyme assays

The sequence of SARS-CoV 3CL^{pro} cloned into the pGEX-6p-1 vector was transformed into *E. coli* BL21 (DE3) cells. The recombinant protein with GST-tag was purified by GST-glutathione affinity chromatography and the ion exchange column. Eventually purified protein was of high purity (>95%) as judged by SDS-PAGE analysis and the concentration is 0.5 μmol/L, and the buffer is 50 mmol/L Tris-HCl, pH 7.3, 1 mmol/L EDTA. The substrate synthesized in Shanghai Biological Engineering Company is dissolved in DMSO, with 0.8 mmol/L liquid storage for used.

The SARS-CoV 3CL^{pro} inhibition assays were conducted via fluorescence resonance energy transfer (FRET). The natural substrate amino acid sequence (AVLQ. SGFRKK) of SARS-CoV 3CL^{pro} ends with MCA fluorescent group and Dnp fluorescence quenching group. The system of screening is as follows (Table 2): The settled concentrations of proteins, compounds and substrate were preheated at 37 ℃ and oscillated. Excitation/emission light is 320/405 nm, test was carried out every 3 s for 60 times. Drawing curves, the maximum value of the negative control curve slope is V_0, and the largest compound curve slope is V_1. The inhibition ratio can be defined ($1-V_1/V_0$). And the IC_{50} value was calculated by the following equation:

$$V_0/V=1+[I]/IC_{50}$$

V_0 shows the initial rate of the reaction without inhibitor, V means the initial rate of the reaction with the inhibitor at various concentrations, [I] indicates the concentration of the inhibitor.

Table 2 SARS CoV 3CL^{pro} compound screening system

System	Volume	Final concentration
SARS-Cov 3CL^{pro}	97 μL	0.5 μmol/L
Substrate	2 μL	16 μmol/L
Compound	1 μL	1 mmol/L
Total	100 μL	

Docking study

The crystal structure of SARS-CoV 3CL^{pro} (code: 3SN8), obtained from The Protein Data Bank as a complex bound with inhibitors, was used in this docking study. And the docking studies were carried out in the Discovery Studio 3.0. To prepare the protein for docking, water molecules and inhibitors were removed and hydrogen atoms were added by using Prepare Protein Structure module, from the protein.

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