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Synthesis, modification and docking studies of 5-sulfonyl isatin derivatives as SARS-CoV 3C-like protease inhibitors

Wei Liu [a,*], He-Min Zhu [b], Guo-Jun Niu [d], En-Zhi Shi [b], Jie Chen [c], Bo Sun [d], Wei-Qiang Chen [d], Hong-Gang Zhou [c], Cheng Yang [c,d]

[a] College of Sciences, Tianjin University of Science and Technology, Tianjin 300457, China
[b] College of Life Sciences, NanKai University, Tianjin 300071, China
[c] College of Pharmacy, NanKai University, Tianjin 300071, China
[d] High Throughput Molecular Drug Discovery Center, Tianjin International Joint Academy of Biotechnology and Medicine, TEDA, Tianjin 300457, China

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ABSTRACT

The Severe Acute Respiratory Syndrome (SARS) is a serious life-threatening and strikingly mortal respiratory illness caused by SARS-CoV. SARS-CoV which contains a chymotrypsin-like main protease analogous to that of the main picornavirus protease, $3CL^{pro}$. $3CL^{pro}$ plays a pivotal role in the viral replication cycle and is a potential target for SARS inhibitor development. A series of isatin derivatives as possible SARS-CoV $3CL^{pro}$ inhibitors was designed, synthesized, and evaluated by in vitro protease assay using fluorogenic substrate peptide, in which several showed potent inhibition against the $3CL^{pro}$. Structure–activity relationship was analyzed, and possible binding interaction modes were proposed by molecular docking studies. Among all compounds, **$8k_1$** showed most potent inhibitory activity against $3CL^{pro}$ (IC_{50} = 1.04 μM). These results indicated that these inhibitors could be potentially developed into anti-SARS drugs.

1. Introduction

The Severe Acute Respiratory Syndrome (SARS) is a serious life-threatening upper respiratory tract disease with the most common symptoms of cough, high fever, headache, rigor, myalgia, and dizziness.[1] In 2003, SARS abruptly emerged and spread widely, becoming an epidemic that seriously affected public health and the economy of many countries.[2,3] Although SARS has been controlled and no known SARS transmission has been recorded anywhere in the world, the mutant characteristic of the coronavirus that is the causative agent of SARS indicated the possibility of a re-emergence.[4–6] The emergence of the novel human coronavirus EMC (HCoV-EMC) in the Middle East since April 2012 has so far led to 17 cases of human infection (with 11 being fatal) as of 26 March 2013.[7]

SARS virus is a novel human coronavirus featuring the largest positive-stranded RNA genomes known to date (27–31 kb) and is also termed SARS-CoV. CoV encodes a chymotrypsin-like protease ($3CL^{pro}$) that plays a pivotal role in the virus replication. The cysteine protease $3CL^{pro}$ is fuctionally analogous to the main *Picornaviridae* protease $3C^{pro}$ with a catalytic dyad (Cys-145 and His-41) in the active site.[8] $3CL^{pro}$ Cleaves the replicase polyprotein at 11 conserved sites with canonical Leu-Gln (Ser, Ala, Gly) sequences, in which the P1 position has a well-conserved Gln residue and the P2 position has a hydrophobic amino acid residue.[9,10] Given the essential role in viral processing of SARS-CoV $3CL^{pro}$, it is considered as an attractive target for anti-SARS and other coronavirus infections.

A large number of peptidomimetic and small-molecule inhibitors of $3CL^{pro}$ have been reported to date, including peptideomimetic,[5,11-20] stilbene derivatives,[21] hydroxyferroquine derivatives,[22] TG-0205221,[23] natural products,[24-27] ketones,[1,28] glycyrrhizic acid,[29] pyrimidines,[30] α,β-unsaturated esters,[31] ML188,[32] pyrazolones,[4] nucleoside analogs,[33,34] and isatin derivatives.[35,36] Although so many candidate anti-SARS CoV agents have been identified, no effective therapeutic drug and vaccine have been developed so far.

High-throughput screening revealed that 5-bromoisatin was a potent inhibitor, and then 5-bromoisatin was also soaked into a crystal of $3CL^{pro}$ recently obtained in our laboratory (Fig. 1). Although this complex has not been deposited in the protein data bank because of the poor structural quality, this fact provided us with an important clue that isatin derivatives can have inhibitory activity against $3CL^{pro}$. Previously, Chen and Zhou et al.[35,36] reported a new class of potent, small-molecule isatin-based $3CL^{pro}$ inhibitors (Fig. 2). They concluded that the C-5 position favors a carboxamide group and the N-1 position favors large hydrophobic substituents. In this Letter, we investigated a replacement of the

* Corresponding author. Tel.: +86 22 65378878.
E-mail address: liuwei2006@tust.edu.cn (W. Liu).

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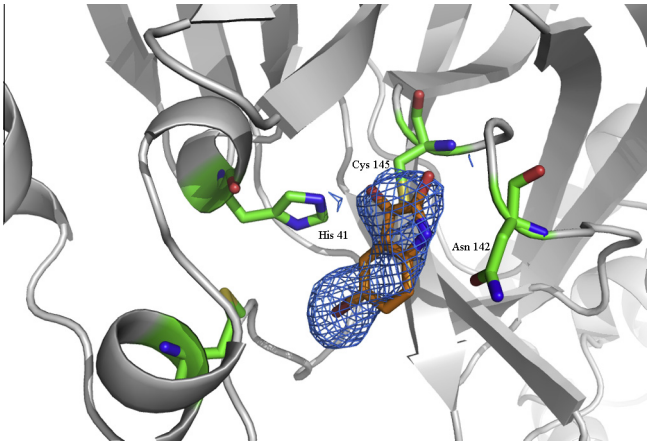


Figure 1. 5-Bromoisatin soaked into a crystal of $3CL^{pro}$ (2.8 Å).

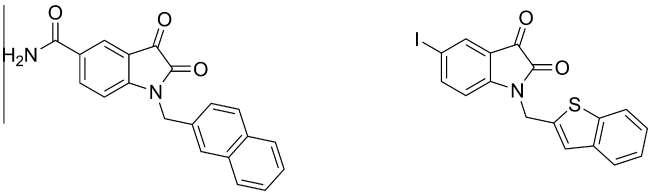


Figure 2. Structures of isatins I and II that reportedly demonstrate in vitro SARS-CoV $3CL^{pro}$ inhibition.

carboxamide group using a series of substituted sulfonamide groups in isatin. We believe that this modification can improve inhibitory activity against SARS CoV $3CL^{pro}$. We also carried out molecular docking studies to obtain the potential binding mode of these inhibitors.

2. Results and discussion

2.1. Chemistry

Compounds **3a**–**f**, **3h**, and **3j** were obtained from commercial suppliers. The synthesis of isatin derivatives **3g** and **3i** is summarized in Scheme 1. Intermediates **2g** and **2i** were prepared by the Sandmeyer reaction of substituted anilines with hydroxylamine hydrochloride and chloral hydrate. The resulting hydroxyiminoacetanilide intermediates **2g** and **2i** were cyclized by heating in concentrated sulfuric acid to give the corresponding isatin derivatives **3g** and **3i**, respectively.[37]

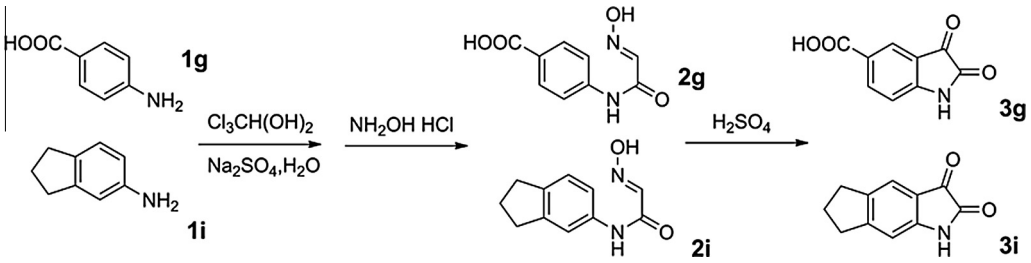
A series of compounds **8** was prepared from isatin following literature methods[38,39] (Scheme 2). In a typical procedure, isatin was sulfonated at the 5-position in the presence of chlorosulfonic acid. However, in contrast to previously published results, both 5-sulfonated gem-dichloroisatin **4** and 5-sulfonated isatin **5** were isolated, and the yield ratio was 9:1. Moreover, no desired sulfonyl chloride product was obtained. Interestingly, both **4** and **5** can react with secondary amines, respectively. Especially, **5** can directly produce compound **7** in high yield. In this paper, the mixture of compounds **4** and **5** was not isolated and was applied to synthesize target compounds **7** and **8**, and the gem-dichloro moiety of intermediate **6** was subjected to acidic hydrolysis to afford **7**. Alkylation of **7** was achieved in the presence of various bromides or iodides and NaH in DMF. Both analytical and spectral data of all target compounds **7** and **8** were accordant with their structures.

2.2. Biological activity

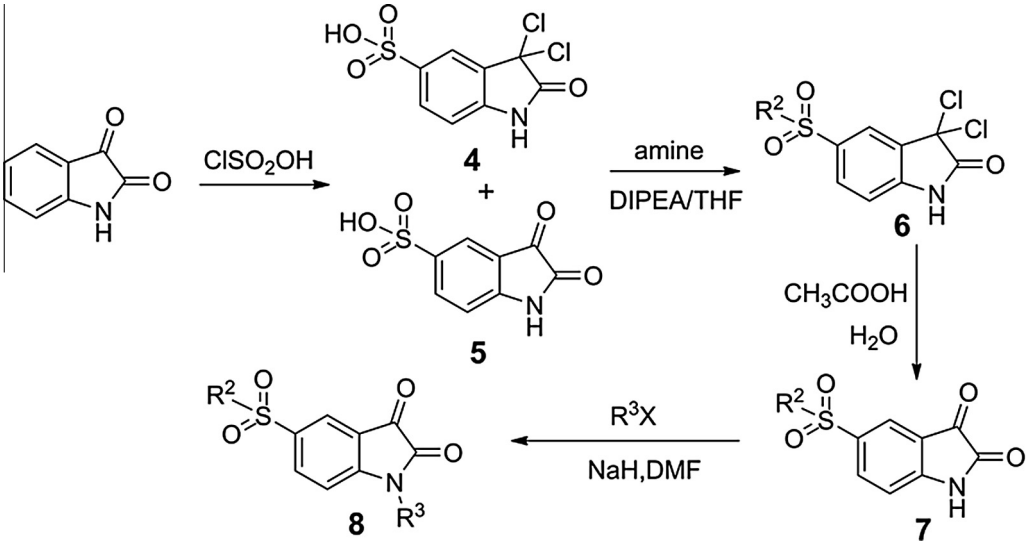
A series of compounds **3** substituted at the benzene ring of isatin was initially screened against SARS CoV $3CL^{pro}$ in vitro, and results are summarized in Table 1. SARS CoV $3CL^{pro}$ was found to be sensitive to the substituent position. Inhibitory activity results showed that compounds **3a**–**f** and **3h** substituted at the 5-position were superior to those of compounds **3i** and **3j** substituted at other positions. This result indicated that modification at the 5-position of the isatin ring was suitable and much more effective in increasing inhibitory potency. The replacement of bromine **3a** at the 5-position by iodine **3b**, chlorine **3c**, and fluorine **3d** did not increase inhibitory activity. Table 1 shows that most of compounds containing the oxy substituent **3e**–**h** displayed high inhibitory activity, except **3g**. Results revealed that the substituent containing the oxy group at the 5-position was important in improving inhibitory activity.

Considering the inhibitory tendency in Table 1 and 5-(piperazin-1-ylsulfonyl)isatin derivatives **7a**–**h** were prepared and evaluated by inhibition assays, and the results are shown in Table 2. Compared with **3**, compound **7a** exhibited a higher inhibition ratio (98.64%), with IC_{50} = 76.74 μM. Although compounds **7b** and **7c** were less active than **7a**, **7d**–**h** exhibited the increased inhibitory potency. Both one carbon (**7d**, IC_{50} = 31.71 μM; **7e**, IC_{50} = 32.08 - μM) and two carbons (**7f**, IC_{50} = 34.91 μM) between aromatic ring and piperazine showed a similar profile, which was an approximately twofold higher potency than **7a**. Compound **7h** containing more hydrophilic moiety (pyridinyl) instead of the phenyl ring slightly increased activity (IC_{50} = 51.33 μM) compared with compounds **7b** and **7c**. In particular, the substituent with flexibility increased potency (**7d**–**g**, IC_{50} <35 μM) compared with **7b**–**c**. These results suggested that the steric effect in isatin scaffold was crucial to ensuring inhibitory potency.

Replacement of the piperazinyl moiety by other simple cyclic secondary amines was also examined (Table 2) to determine the most suitable substituent. Unexpectedly, **7i**–**m** induced a more apparent improvement in inhibitory potency than bicyclic substituents **7b**–**h**, and 5-(piperidin-1-ylsulfonyl)isatin derivatives **7i** and **7k**–**m** showed better potency with IC_{50} <5 μM. Among them, 4-methylpiperidinyl (**7k**, IC_{50} = 1.18 μM) and 2-methylpiperidinyl (**7l**, IC_{50} = 2.25 μM)

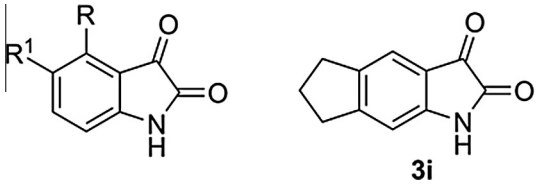


Scheme 1. Synthesis of compounds **3g** and **3i**.



Scheme 2. Synthesis of compounds 7 and 8.

Table 1
Inhibitory activities of compound **3** against SARS CoV 3CL^{pro}



Compound	R^1	R	Inhibition ratio (1 mM)
3a	Br	H	90.54%
3b	I	H	86.73%
3c	Cl	H	84.17%
3d	F	H	82.55%
3e	NO_2	H	93.96%
3f	OCH_3	H	95.32
3g	COOH	H	44.77%
3h	$COCH_3$	H	95.37%
3i	—	—	79.36%
3j	H	Br	68.44%

were the optimal substituents for enzyme inhibition, which enhanced potency by about 80- and 40-fold compared with **7a**, respectively.

After determining the influence of the substituent at the 5-position, we synthesized and examined a series of N-substituted isatin derivatives **8** to determine whether the inhibitory potency against SARS CoV 3CL^{pro} Can be enhanced by modified isatin derivatives at the N-1 position. As shown in Table 3, introduction of methyl, benzyl, and β-naphthyl methyl into the N-1 position showed significant activity variation. Most remarkably, methyl at the N-1 position resulted in a 1- to 10-fold enhancement in the inhibitory potency compared with the parent compounds **7** in Table 2, and these compounds displayed higher potency than the ones with benzyl or β-naphthyl methyl. However, **7k** (IC_{50} = 1.18 μM) was not sensitive to methyl (**8k$_1$**, IC_{50} = 1.04 μM) or benzyl (**8k$_2$**, IC_{50} = 1.69 μM), which exhibited high inhibitory potency. Explaining such similar activity tendencies was difficult, so docking studies were conducted.

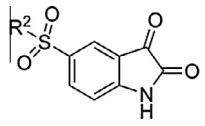
2.3. Docking studies

To predict the binding mode of isatin 5-sulfonylamide onto the active site of SARS CoV 3CL^{pro} (PDB code 1UK4) using Glide 5.5 (Glide model in Schrodinger software), compounds **7k**, **8k$_1$**, and **8k$_2$** were docked into the active site. As shown in Figure 3, compound **8k$_1$** was hydrogen bonded in Gly143 and Cys145 of protease, and **8k$_2$** generated hydrogen bonds with Gly143, Ser144, and Asn142. Notably, both carbonyl group at the 2-position and nitrogen atom at the 1-position of isatin are important in the formation of hydrogen bonds. Meanwhile, the modeling shown in Figure 4 revealed that the isatin scaffold was docked at the S1′ site, and the side chain R^2 and R^3 were located at the S2 and S1 sites of SARS CoV 3CL^{pro}, respectively. The model of compounds and SARS-CoV 3CL^{pro} presented here differed from previously reported 5-carboxamide isatin derivatives.[36] The orientation of the isatin core was flipped here in Fig. 4. The substitutions at the 5-position enabled the compounds **8k$_1$** and **8k$_2$** to fit the S2 hydrophobic site in both docking results. Furthermore, the simple six-membered ring sulfonyl group of isatin at the 5-position well fitted S2, as demonstrated in the potent inhibition of **7a** and **7i–m**. However, the 5-sulfonyl isatin derivatives **7b–h** modified by rigid bicyclic substituents at the 5-position did not fit the limited space of S2, which led to diminished inhibitory activity. Interestingly, increasing the length of substitution enabled compounds containing the flexible group **7d–g** to extend into the S3 site, which has not been mentioned in previous references. These docking experiments supported the observation in the enzymatic assay that the potency of these derivatives followed the order **7d** (31.71 μM) > **7a** (76.74 μM) > **7b** (>100 μM). Regarding the substituent at the N-1 position, the 5-carboxamide group[36] was replaced by N-1 substitution here and the methyl and benzyl substitutions more or less accommodated the hydrophobic characteristics at the S1 site. In addition, **8k$_1$** molecule (total score = 8.70) was predicted to show the analogous binding affinity relative to **8k$_2$** (total score = 8.58), which was consistent with the experimental results. Based on the findings above, we believe that the activity of the title compounds can be improved by modifying with the simple six-membered ring at the 5-position and substitution at the N-1 position with a methyl group, which can lead to a better combination of compounds with the protein pocket.

3. Conclusion

5-Bromoisatin **3a** selected by high-throughput screening and socking experiments, was proved to have potent inhibitory activity against SARS 3CL^{pro} in vitro. Optimization of 5-sulfonyl isatin

Table 2
Inhibitory activities of compounds **7a–m** against SARS CoV 3CL^{pro}



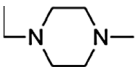
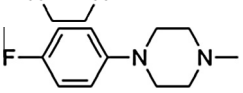
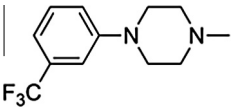
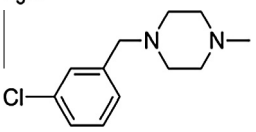
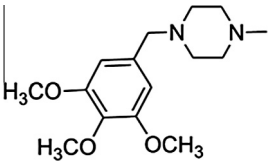
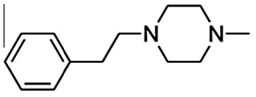
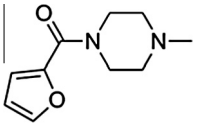
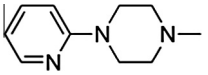
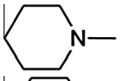
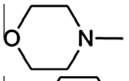
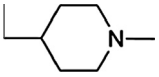
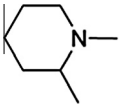
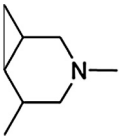
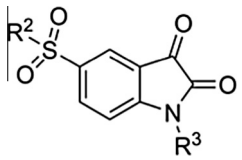
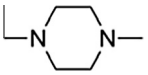
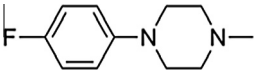
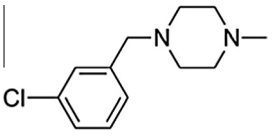
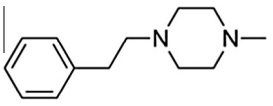
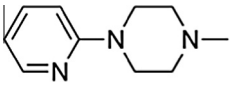
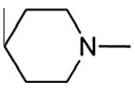
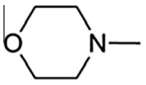
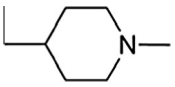
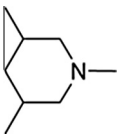
Compound	R^2	IC_{50} (μM)
7a		76.74 ± 2.99
7b		>100
7c		>100
7d		31.71 ± 1.41
7e		32.08 ± 2.83
7f		34.91 ± 9.48
7g		10.07 ± 0.59
7h		51.33 ± 2.47
7i		4.45 ± 0.13
7j		12.66 ± 0.28
7k		1.18 ± 0.11
7l		2.25 ± 0.14
7m		4.30 ± 0.07

Table 3
Inhibitory activities of compound **8** against SARS CoV 3CL^{pro}



Compound	R^2	R^3	IC_{50} (μM)
8a$_1$		CH_3	11.83 ± 1.87
8a$_2$		$PhCH_2$	67.20 ± 8.50
8a$_3$		β-$C_{10}H_7CH_2$	82.91 ± 12.91
8b$_2$		$PhCH_2$	ND
8b$_3$		β-$C_{10}H_7CH_2$	ND
8d$_2$		CH_3	ND
8d$_3$		$PhCH_2$	ND
8f$_1$		β-$C_{10}H_7CH_2$	13.86 ±
8f$_2$		CH_3	ND
8f$_3$		$PhCH_2$	ND
8h$_1$		β-$C_{10}H_7CH_2$	5.52 ± 0.33
8h$_2$		CH_3	ND
8h$_3$		$PhCH_2$	ND
8i$_2$		β-$C_{10}H_7CH_2$	14.00 ± 2.472
8i$_3$		β-$C_{10}H_7CH_2$	ND
8j$_1$		CH_3	9.91 ± 0.79
8j$_2$		$PhCH_2$	13.86 ± 2.96
8j$_3$		β-$C_{10}H_7CH_2$	39.87 ± 0.62
8k$_1$		$PhCH_2$	1.04 ± 0.01
8k$_2$		β-$C_{10}H_7CH_2$	1.69 ± 0.01
8k$_3$		CH_3	17.82 ± 0.72
8m$_1$		$PhCH_2$	2.82 ± 0.17
8m$_2$		β-$C_{10}H_7CH_2$	4.70 ± 0.12
8m$_3$		$PhCH_2$	ND

ND: not done because of quenching rate >20%.

derivatives resulted in the discovery of compound **8k$_1$**, with the most powerful potency (IC_{50} = 1.04 μM). The results of the current study suggested that 5-sulfonyl isatin derivatives had similar inhibitory activities to the reported 5-carbonyl isatins. Meanwhile, the computer model of the associated complex between the title compounds and protease rationalized their inhibitory activities. Apparently, 5-sulfonyl isatin modified by a simple six-membered ring or a bulky substituent with sufficient flexibility at the 5-position coupled with methyl at the N-1 position could dramatically promote inhibitory activity. In addition, similar orientations of the isatin core were found in both docking and socking studies. This result could serve as an important basis for more structural optimizations.

4. Experimental

4.1. Chemistry

4.1.1. General

All reactions were carried out under N_2 atmosphere unless otherwise noted. All commercial reagents were of the highest

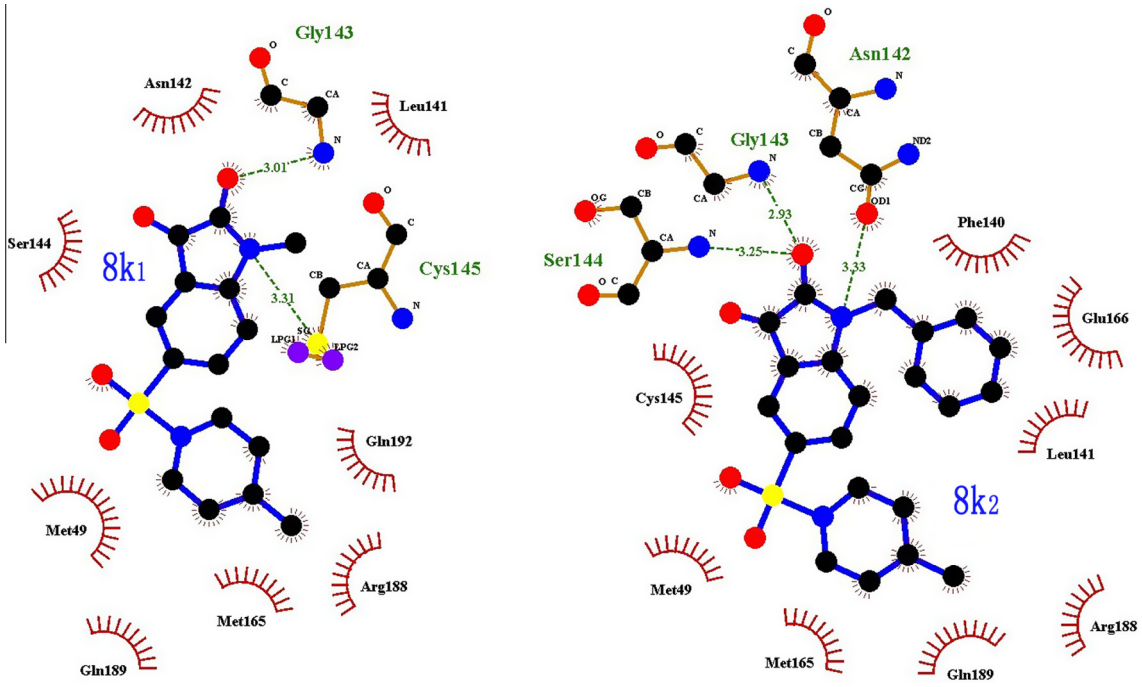


Figure 3. Binding interactions of **8k$_1$** and **8k$_2$** in the protein structure 1UK4, respectively. Hydrogen bonds are shown as green lines and hydrophobic contacts are shown as radial hemispheres.

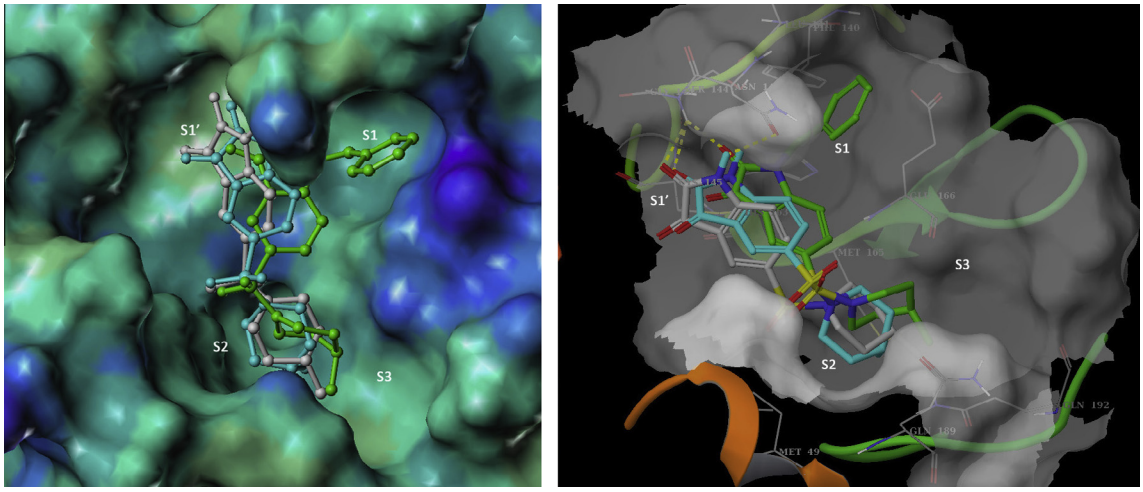


Figure 4. (left) Three molecules (**7k**, gray; **8k$_1$**, cyan; and **8k$_2$**, green) are bound in the SARS 3CL^{pro} active site. Four parts of the active site are shown. (right) Surface representation of SARS 3CL^{pro} (PDB ID 1UK4) with three inhibitors, **7k** (gray), **8k$_1$** (cyan), and **8k$_2$** (green).

purity available. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Analytical TLC was performed with SHANGHAI SANPONT GF254. Column chromatography was carried out on SHANGHAI SANPONT Gel (200–300 mesh). NMR spectra were recorded on a Bruker AM-400 or DMX-600. All NMR spectra were recorded in $CDCl_3$ or DMSO at room temperature (20 °C). Chemical shifts for ^1H and ^{13}C spectra were quoted in ppm downfield from TMS. Coupling constants are referred to as *J* values. ESI mass spectra were obtained using a Bruker ESQUIRELCTM ESI ion trap spectrometer. FT-IR spectra were determined at room temperature (20 °C) within 4000–400 cm^{-1} with a PerkinElmer spectrum 65 FT-IR spectrometer using KBr pellets. Melting points were determined using an SGW-X4B digital melting point apparatus. Elemental analysis of carbon, hydrogen and nitrogen were obtained with an Elementar Vario MICRO cube Elemental Analyser.

4.1.2. General procedure for the synthesis of compounds 3g and 3i[37]

Chloral hydrate (14.7 g, 88.8 mmol) was added to 500 ml of water with dissolved sodium sulfate (84 g, 0.59 mol). When the temperature reached 40 °C, the appropriate aniline (74.0 mmol) in 25 ml of 2 M aqueous hydrochloric acid was added dropwise. The reaction mixture was stirred for 1 h. and the suspension of hydroxylamine hydrochloride (18.5 g, 0.27 mol) was rapidly added to the reaction mixture. The mixture was then heated at 90 °C for 5 h with stirring. After cooling to room temperature, the corresponding hydroxyiminoacetanilide **2g** (or **2i**) was collected by filtration, washed with water, and dried in a vacuum. Under nitrogen atmosphere, one gram of the intermediate **2g** (or **2i**) was added in small portions with stirring to 30 ml of concentrated sulfuric acid that had been preheated and maintained at 50 °C.

After all intermediates had been added, the dark-colored solution was heated at 55 °C for an additional 30 min, cooled to room temperature, poured onto 225 ml of crushed ice, and allowed to stand for 30 min. The precipitate was collected by filtration, washed with water three times, and dried under vacuum to yield the product **3g** (or **3i**).

4.1.2.1. 5-Carboxy-1*H*-indole-2,3-dione (3g). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.01 (s, 1H), 11.34 (s, 1H), 8.12 (dd, J = 8.2, 1.7 Hz, 1H), 7.88 (d, J = 1.3 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H). ESI-MS: m/z 192.33 ([M+H$^+$]).

4.1.2.2. 1,5,6,7-Tetrahydro-cyclopent[*f*]indole-2,3-dione (3i). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.44 (s, 1H), 6.80 (s, 1H), 2.95 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 2.13 (t, J = 7.6 Hz, 2H). ESI-MS: m/z 188.12 ([M+H$^+$]).

4.1.3. Synthesis of compounds 4 and 5

Under nitrogen and ice bath, 20.0 ml (302 mmol) of chlorosulfonic acid was added dropwise to 3.6 g (24.5 mmol) isatin. Then, the mixture was heated to 70 °C for 3 h. The mixture was cooled down to room temperature and carefully poured onto 100 g of ice. The resulting yellow solid was filtered, washed with cold water. and dissolved in the ethyl acetate. After drying over Na_2SO_4, the mixture was purified by column chromatography (petroleum ether and ethylacetate), and compounds **4** (3.8 g) and **5** (410 mg) were obtained. Interestingly, either **4** or **5** can react with secondary amines, and **5** can directly produce the compound **7** in high yield. The resulting precipitate was used in the next step without separation.

4.1.3.1. 3,3-Dichloro-2-oxo-indoline-5-sulfonic acid (4). Pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.42 (s, 1H), 11.50 (s, 1H), 7.77 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 144.4, 139.7, 130.3, 128.5, 122.4, 111.1, 75.4. ESI-MS: m/z 279.74.

4.1.3.2. 2,3-Dioxo-indoline-5-sulfonic acid (5). Yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ14.49 (s, 1H), 11.14 (s, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.59 (s, 1H), 6.89 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.7, 160.0, 150.9, 143.8, 135.9, 121.8, 117.4, 111.9. ESI-MS: m/z 225.87 ([M-H$^+$]).

4.1.4. General procedure for the synthesis of compounds 7a–m

Under nitrogen atmosphere, mixture of **4** and **5** (2.0 mmol) was dissolved in 6 ml of dried THF at 0 °C. Then, a solution of the secondary amine (2.2 mmol) and diisopropylethylamine (3.0 mmol) in 1 ml of THF was slowly added. After stirring the reaction mixture at room temperature overninght, solvents were removed and a brown oil was obtained. The crude product was added to 15 ml of 1:1 (v/v) acetic acid–water solution. The mixture was heated at 90 °C overnight. About 11 g of $NaHCO_3$ was carefully added to the mixture after cooling down. The product was extracted by ethylacetate, washed with water, and dried over $MgSO_4$. Target compound **7** was purified by column chromatography using CH_2Cl_2 and CH_3OH.

4.1.4.1. 5-[(4-Methylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7a). Compound **7a** was prepared according to General Procedure above using 1-methylpiperazine. Pale yellow powder. Yield 57%. mp 173.5–174.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 7.90 (dd, J = 8.4, 2.0 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 2.90 (s, 4H), 2.36 (t, J = 4.4 Hz, 4H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 159.9, 154.3, 137.5, 129.2, 123.9, 118.7, 113.2, 53.6, 45.8, 45.2. IR (KBr, cm^{-1}): 3370, 3090, 2940, 2808, 1747, 1618, 1467, 1356, 1177, 946, 735. ESI-MS: m/z 310.16 ([M+H$^+$]), 308.20 ([M−H$^+$]). Anal. Calcd for $C_{13}H_{15}N_3O_4S$: C, 50.47; H, 4.89; N, 13.58. Found: C, 50.27; H, 4.78; N, 13.44.

4.1.4.2. 5-{[4-(4-Fluorophenyl)piperazinyl-1-yl]sulfonyl}-1*H*-indole-2,3-dione (7b). Compound **7b** was prepared according to General Procedure above by using 1-(4-fluorophenyl)piperazine. Pale yellow powder. Yield 36%. Mp 208.7–210.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.95 (dd, J = 8.4, 2.0 Hz, 1H), 7.72 (d, J = 1.6 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.04 (t, J = 8.4 Hz, 2H), 6.94–6.91 (m, 2H), 3.15 (t, J = 4.0 Hz, 4H), 3.03 (t, J = 4.0 Hz, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 183.3, 160.0, 156.9 (d, J = 229 Hz), 154.4, 147.7, 137.6, 128.9, 123.9, 118.8, 118.6 (d, J = 7.5 Hz), 115.8 (d, J = 21 Hz), 113.3, 49.1, 46.3. IR (KBr, cm^{-1}): 3299, 3237, 2919, 1755, 1614, 1340, 1245, 1155, 952, 819. ESI-MS: m/z 390.24 ([M+H$^+$]). Anal. Calcd for $C_{18}H_{16}N_3O_4S$: C, 55.52; H, 4.14; N, 10.79. Found: C, 55.34; H, 4.27; N, 10.75.

4.1.4.3. 5-{[4-(3-Trifluoromethylphenyl)piperazinyl-1-yl]sulfonyl}-1*H*-indole-2,3-dione (7c). Compound **7c** was prepared according to General Procedure above by using 1-[3-(trifluoromethyl)phenyl]piperazine. Pale yellow powder. Yield 41%. Mp 213.6–215.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 7.95 (dd, J = 8.0, 2.0 Hz, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.20–7.08 (m, 4H), 3.34 (t, J = 4.4 Hz, 4H), 3.05 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 159.9, 154.4, 151.0, 137.6, 130.5, 130.4(q, J = 31 Hz), 128.9, 124.8(q, J = 271 Hz), 123.9, 119.9, 118.8, 115.9(d, J = 4 Hz), 113.3, 111.2 (d, J = 4 Hz), 47.7, 46.1. IR (KBr, cm^{-1}): 3298, 3244, 2834, 1755, 1612, 1453, 1342, 1154, 953. ESI-MS: m/z 440.19 ([M+H$^+$]), 438.22 ([M−H$^+$]). Anal. Calcd for $C_{19}H_{16}N_3O_4S$: C, 51.93; H, 3.67; N, 9.56. Found: C, 51.87; H, 3.61; N, 9.49.

4.1.4.4. 5-{[4-(3-Chlorobenzyl)piperazinyl-1-yl]sulfonyl}-1*H*-indole-2,3-dione (7d). Compound **7d** was prepared according to General Procedure above by using 1-(3-chlorobenzyl)piperazine. Pale yellow powder. Yield 47%. Mp 150.8–152.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 7.90 (dd, J = 8.4, 1.6 Hz, 1H), 7.67 (s, 1H), 7.39 (m, 2H), 7.27 (m, 2H), 7.12 (d, J = 8.4 Hz, 1H), 3.56 (s, 2H), 2.91 (s, 4H), 2.50 (t, J = 9.2 Hz, 4H) (determination in CD_3OD). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 159.9, 154.3, 137.5, 135.6, 133.7, 131.2, 129.7, 129.2, 128.9, 127.5, 123.9, 118.7, 113.2, 58.5, 51.9, 46.5. IR (KBr, cm^{-1}): 3363, 3067, 2850, 1755, 1614, 1456, 1288, 1156, 942, 753. ESI-MS: m/z 440.19 ([M+H$^+$]). Anal. Calcd for $C_{19}H_{18}ClN_3O_4S$: C, 54.35; H, 4.32; N, 10.01. Found: C, 54.02; H, 4.23; N, 9.77.

4.1.4.5. 5-{[4-(3,4,5-Trimethoxybenzyl)piperazinyl-1-yl]sulfonyl}-1*H*-indole-2,3-dione (7e). Compound **7e** was prepared according to General Procedure above by using 1-(3,4,5-trimethoxybenzyl)piperazine dihydrochloride. To neutralize hydrochloric acid from 1-(3,4,5-trimethoxybenzyl)piperazine dihydrochloride, diisopropylethylamine (7.4 mmol) was added in the General Procedure for the synthesis of Compound **7e**. Pale yellow powder. Yield 40%. Mp 192.2–193.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 7.89 (dd, J = 8.4, 2.0 Hz, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 3.75 (s, 3H), 3.71 (s, 6H), 3.38 (s, 2H), 2.89 (s, 4H), 2.43 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 159.9, 154.3, 153.1, 152.4, 142.3, 137.5, 129.3, 125.1, 123.8, 123.6, 118.7, 113.2, 108.1, 61.4, 60.7, 56.3, 55.7, 51.9, 46.5. IR (KBr, cm^{-1}): 3080, 2942, 2834, 1744, 1618, 1466, 1356, 1272, 1176, 1100, 944, 745. ESI-MS: m/z 476.27 ([M+H$^+$]). Anal. Calcd for $C_{22}H_{25}N_3O_7S$: C, 55.57; H, 5.30; N, 8.84. Found: C, 55.44; H, 5.21; N, 9.02.

4.1.4.6. 5-[(4-Phenylethyl-piperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7f). Compound **7f** was prepared according to General Procedure above by using 1-phenylethylpiperazine. Pale yellow powder. Yield 43%. Mp 220.5–222.3 °C. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.00 (dd, J = 8.4, 1.6 Hz, 1H), 7.83 (d, J = 1.6 Hz, 1H), 7.29–7.13 (m, 6H), 3.02 (s, 4H), 2.74 (t, J = 8.4 Hz, 2H), 2.63–2.57 (m, 6H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 182.9, 159.5, 153.8, 140.1, 137.0, 128.6, 128.5, 128.2, 125.9, 123.4, 118.3, 112.7, 58.9, 51.5, 45.9, 32.5. IR (KBr, cm^{-1}): 3025, 2835, 1743, 1616, 1453, 1356, 1177, 952, 735. ESI-MS: m/z 400.10 ([M+H$^+$]). Anal. Calcd for $C_{20}H_{21}N_3O_4S$: C, 60.13; H, 5.30; N, 10.52. Found: C, 60.27; H, 5.34; N, 10.47.

4.1.4.7. 5-[4-(2-Furoyl)piperazin-1-yl-sulfonyl]-1*H*-indole-2,3-dione (7g). Compound **7g** was prepared according to General Procedure above by using 1-(2-furoyl)piperazine. Pale yellow powder. Yield 30%. Mp 261.3–263.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 7.91 (dd, J = 8.4, 2.0 Hz, 1H), 7.82 (s, 1H), 7.69 (d, J = 1.6 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 3.2 Hz, 1H), 6.60 (q, J = 1.6 Hz, 1H), 3.75 (s, 4H), 2.98 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 159.9, 158.7, 154.4, 147.0, 145.5, 137.5, 123.9, 118.8, 116.6, 113.3, 111.9, 100, 57.5, 46.5. IR (KBr, cm^{-1}): 3121, 1755, 1619, 1483, 1351, 1153, 945, 737. ESI-MS: m/z 390.12 ([M+H$^+$]). Anal. Calcd for $C_{17}H_{15}N_3O_6S$: C, 52.44; H, 3.88; N, 10.79. Found: C, 52.29; H, 3.79; N, 10.66.

4.1.4.8. 5-{[4-(Pyridin-2-yl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione (7h). Compound **7h** was prepared according to General Procedure above by using 1-(2-pyridyl)piperazine. Dark yellow powder. Yield 36%. Mp 222.1–223.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 8.07 (dd, J = 4.8, 1.6 Hz, 1H), 7.93 (dd, J = 8.0, 2.0 Hz, 1H), 7.70 (d, J = 2.0 Hz, 1H), 7.51 (td, J = 8.4, 2.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.63 (dd, J = 6.8, 4.8 Hz, 1H), 3.60 (t, J = 4.8 Hz, 4H), 2.98 (t, J = 4.8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 159.9, 158.7, 154.3, 148.0, 138.2, 137.5, 129.1, 123.9, 118.8, 114.0, 113.3, 107.9, 46.0, 44.4. IR (KBr, cm^{-1}): 2857, 1755, 1618, 1438, 1154, 949, 736. ESI-MS: m/z 373.29 ([M+H$^+$]). Anal. Calcd for $C_{17}H_{16}N_4O_4S$: C, 54.83; H, 4.33; N, 15.04. Found: C, 55.01; H, 4.21; N, 14.89.

4.1.4.9. 5-[(Piperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7i). Compound **7i** was prepared according to General Procedure above by using piperidine. Yellow powder. Yield 51%. Mp 199.9–200.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 7.91 (dd, J = 8.4, 1.6 Hz, 1H), 7.67 (d, J = 1.2 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 2.89 (t, J = 5.2 Hz, 4H), 1.55 (m, 4H), 1.36 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 159.9, 154.1, 137.4, 129.9, 123.7, 118.6, 113.2, 47.0, 25.1, 23.3. IR (KBr, cm^{-1}): 3298, 2944, 2863, 1746, 1618, 1471, 1334, 1176, 934, 731. ESI-MS: m/z 295.01 ([M+H$^+$]). Anal. Calcd for $C_{13}H_{14}N_2O_4S$: C, 53.05; H, 4.79; N, 9.52. Found: C, 52.93; H, 4.61; N, 9.55.

4.1.4.10. 5-(Morpholinosulfonyl)-1*H*-indole-2,3-dione (7j). Compound **7j** was prepared according to General Procedure above by using morpholine. Pale yellow powder. Yield 59%. Mp 239.0–240.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.69 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 3.64 (t, J = 4.4 Hz, 4H), 2.88 (t, J = 4.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 156.0, 154.4, 137.6, 128.7, 123.9, 118.8, 113.3, 65.7, 46.3. IR (KBr, cm^{-1}): 3315, 2916, 2855, 1757, 1615, 1470, 1348, 1160, 943, 737. ESI-MS: m/z 295.15 ([M−H$^+$]). Anal. Calcd for $C_{12}H_{12}N_2O_5S$: C, 48.64; H, 4.08; N, 9.45. Found: C, 48.51; H, 3.96; N, 9.22.

4.1.4.11. 5-[(4-Methylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7k). Compound **7k** was prepared according to General Procedure above by using 1-methylpiperidine. Pale yellow powder. Yield 46%. Mp 237.3–239.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 7.90 (dd, J = 8.4, 2.0 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 3.59 (d, J = 11.6 Hz, 2H), 2.22 (dd, J = 12.0, 10.0 Hz, 2H), 1.65 (d, J = 10.8 Hz, 2H), 1.29 (m, 1H), 1.13 (m, 2H), 0.84 (d, J = 10.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 159.9, 154.1, 137.4, 130.0, 123.7, 118.6, 113.2, 46.2, 33.3, 29.8, 21.7. IR (KBr, cm^{-1}): 3228, 2922, 1725, 1610, 1338, 1159, 919, 619. ESI-MS: m/z 309.06 ([M+H$^+$]). Anal. Calcd for $C_{14}H_{16}N_2O_4S$: C, 54.53; H, 5.23; N, 9.08. Found: C, 54.64; H, 5.10; N, 9.01.

4.1.4.12. 5-[(2-Methylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7l). Compound **7l** was prepared according to General Procedure above by using 2-methylpiperidine. Pale yellow powder. Yield 37%. Mp 235.1–237.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 7.98 (dd, J = 8.4, 2.0 Hz, 1H), 7.74 (d, J = 1.2 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 4.10 (m, 1H), 3.59 (d, J = 10.4 Hz, 1H), 2.96 (td, J = 12.8, 2.0 Hz, 1H), 1.55–1.43 (m, 5H), 1.21 (m, 1H), 1.03 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.5, 156.0, 153.7, 136.6, 135.4, 122.9, 118.6, 113.2, 48.7, 30.3, 25.2, 18.1, 15.7. IR (KBr, cm^{-1}): 3195, 2949, 1747, 1614, 1465, 1332, 1134, 993, 719. ESI-MS: m/z 309.01 ([M+H$^+$]). Anal. Calcd for $C_{14}H_{16}N_2O_4$-S: C, 54.53; H, 5.23; N, 9.08. Found: C, 54.51; H, 5.03; N, 8.88.

4.1.4.13. 5-[(3,5-Dimethylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (7m). Compound **7m** was prepared according to General Procedure above by using 3,5-dimethylpiperidine. Yellow powder. Yield 47%. Mp 221.1–222.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.99 (m, 2H), 7.09 (d, J = 8.4 Hz, 1H), 3.72 (d, J = 6.8 Hz, 2H), 1.75 (m, 4H), 1.25 (m, 2H), 0.87 (d, J = 6.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.5, 159.9, 154.1, 137.4, 130.1, 123.6, 118.6, 113.2, 52.7, 41.1, 30.9, 19.2. IR (KBr, cm^{-1}): 3292, 2956, 1762, 1617, 1341, 1150, 796. ESI-MS: m/z 323.11 ([M+H$^+$]). Anal. Calcd for $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69. Found: C, 55.69; H, 5.47; N, 8.77.

4.1.5. General procedure for the synthesis of compounds 8a$_1$–m$_3$

At 0 °C, 10 mg of NaH (60%, 0.25 mmol) was added to a solution of the corresponding **7** (0.25 mmol) in 3 ml of DMF. The mixture was stirred for 15 min, and then 0.5 mmol of R^{3}X was added. The mixture was stirred for 1.5 h at room temperature, added with 30 ml of water, extracted by 50 ml of ethyl acetate, washed with 30 ml of saturated NaCl and dried over Na_2SO_4. After solvent removal, the crude product was purified by column chromatography with CH_2Cl_2 to afford product **8**.

4.1.5.1. 1-Methyl-5-[(4-methylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8a$_1$). Compound **8a$_1$** was prepared according to General Procedure above by using compound **7a** and iodomethane. Yellow powder. Yield 90%. Mp 207.8–209.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 8.4, 1.6 Hz, 1H), 7.96 (d, J = 1.6 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 3.32 (s, 3H), 3.07 (s, 4H), 2.50 (t, J = 5.6 Hz, 4H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.7, 154.3, 137.8, 131.7, 124.8, 117.3,110.2, 53.9, 45.9, 45.7, 26.7. IR (KBr, cm^{-1}): 2926, 2798, 1752, 1610, 1342, 1285, 1153, 960, 748. ESI-MS: m/z 324.33 ([M+H$^+$]). Anal. Calcd for $C_{14}H_{17}N_3O_4S$: C, 52.00; H, 5.30; N, 12.99. Found: C, 51.84; H, 5.15; N, 12.87.

4.1.5.2. 1-Benzyl-5-[(4-methylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8a$_2$). Compound **8a$_2$** was prepared according to General Procedure above by using compound **7a** and benzyl bromide. Yellow powder. Yield 91%. Mp 179.1–181.8 °C. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.87 (dd, J = 8.4, 1.6 Hz, 1H), 7.40–7.31 (m, 5H), 6.91 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H), 3.05 (s, 4H), 2.50 (s, 4H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.8, 153.6, 137.6, 133.5, 131.6, 129.4, 128.6, 127.4, 124.9, 117.5, 111.4, 53.8, 45.7, 45.5, 44.47. IR (KBr, cm^{-1}): 2920, 2807, 1747, 1613, 1455, 1329, 1163, 943, 747. ESI-MS: m/z 400.05 ([M+H$^+$]). Anal. Calcd for $C_{20}H_{21}N_3O_4S$: C, 60.13; H, 5.30; N, 10.52. Found: C, 60.29; H, 5.19; N, 10.71.

4.1.5.3. 5-[(4-Methylpiperazin-1-yl)sulfonyl]-1-β-naphthalenemethyl–1*H*-indole-2,3-dione (8a$_3$). Compound **8a$_3$** was prepared according to General Procedure above by using compound **7a** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 88%. Mp 218.1–220.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.88–7.79 (m, 5H), 7.52 (t, J = 4.0 Hz, 2H), 7.40 (dd, J = 8.4, 1.2 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 5.15 (s, 2H), 3.02 (s, 4H), 2.47 (s, 4H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.8, 153.6, 137.7, 133.3, 133.2, 131.7, 130.9, 129.6, 127.9, 127.8, 126.9, 126.7, 126.6, 124.9, 124.7, 117.5, 111.4, 53.9, 45.9, 45.6, 44.7. IR (KBr, cm^{-1}): 3052, 2945, 2797, 1737, 1615, 1454, 1349, 1129, 935, 754. ESI-MS: m/z 450.35 ([M+H$^+$]). Anal. Calcd for $C_{24}H_{23}N_3O_4S$: C, 64.13; H, 5.16; N, 9.35. Found: C, 63.96; H, 5.02; N, 9.41.

4.1.5.4. 1-Benzyl-5-{[4-(4-fluorophenyl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione (8b$_2$). Compound **8b$_2$** was prepared according to General Procedure above by using compound **7b** and benzyl bromide. Yellow powder. Yield 89%. Mp 219.6–220.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H),7.46 (d, J = 7.2 Hz, 2H), 7.35 (t, J = 7.2 Hz, 2H), 7.29 (t, J = 7.2 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.03 (t, J = 8.8 Hz, 2H), 6.91 (m, 2H), 4.97 (s, 2H), 3.14 (s, 4H), 3.02 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.4, 159.1, 156.0 (d, J = 233 Hz), 153.9, 147.7, 137.3, 135.5, 129.6, 129.1, 128.1, 127.9, 123.4, 118.9, 118.5 (d, J = 7.0 Hz), 115.8 (d, J = 22 Hz), 112.0, 49.1, 46.3, 43.7. IR (KBr, cm^{-1}): 2958, 2854, 1741, 1618, 1508, 1331, 1160, 950, 827, 722. ESI-MS: m/z 480.31 ([M+H$^+$]). Anal. Calcd for $C_{25}H_{22}FN_3O_4S$: C, 62.62; H, 4.62; N, 8.76. Found: C, 62.56; H, 4.33; N, 8.61.

4.1.5.5. 5-{[4-(4-Fluorophenyl)piperazin-1-yl]sulfonyl}-1-β-naphthalenemethyl-1*H*-indole-2,3-dione (8b$_3$). Compound **8b$_3$** was prepared according to General Procedure above by using compound **7b** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 93%. Mp 217.6–219.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.89–7.81 (m, 5H), 7.52 (t, J = 4.0 Hz, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.00–6.89 (m, 5H), 5.15 (s, 2H), 3.19 (d, J = 6.8 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 159.2, 156.9 (d, J = 235 Hz), 154.0, 147.7, 137.3, 133.4, 129.7, 133.0, 132.9, 129.7, 128.8, 128.1(2× C), 126.9, 126.6, 126.3, 126.0, 123.4, 119.0, 118.6 (d, J = 7 Hz), 115.8 (d, J = 22 Hz), 112.0, 49.1, 46.3, 43.9. IR (KBr, cm^{-1}): 3055, 2830, 1741, 1615, 1509, 1328, 1158, 947, 828, 747. ESI-MS: m/z 530.39 ([M+H$^+$]). Anal. Calcd for $C_{29}H_{24}FN_3O_4S$: C, 65.77; H, 4.57; N, 7.93. Found: C, 65.84; H, 4.66; N, 8.07.

4.1.5.6. 1-Benzyl-5-{[4-(3-chlorobenzyl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione (8d$_2$). Compound **8d$_2$** was prepared according to General Procedure above by using compound **7d** and benzyl bromide. Yellow powder. Yield 93%. Mp 129.1–130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 1.6 Hz, 1H), 7.86 (dd, J = 8.4, 1.6 Hz, 1H), 7.41–7.32 (m, 7H), 7.19 (dd, J = 5.6, 3.6 Hz, 2H), 6.92 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 3.64 (s, 2H), 3.06 (s, 4H), 2.63 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.8, 153.6, 137.6, 134.6, 134.5, 133.7, 131.7, 130.9, 129.6, 129.3, 128.7, 128.6, 127.5, 126.7, 124.7, 117.5, 111.4, 51.9, 46.0, 44.5, 30.9. IR (KBr, cm^{-1}): 3052, 2957, 2811, 1751, 1615, 1473, 1350, 1158, 954, 844, 757. ESI-MS: m/z 510.13 ([M+H$^+$]). Anal. Calcd for $C_{26}H_{24}ClN_3O_4S$: C, 61.23; H, 4.74; N, 8.24. Found: C, 61.09; H, 4.54; N, 8.17.

4.1.5.7. 5-{[4-(3-Chlorobenzyl)piperazin-1-yl]sulfonyl}-1-β-naphthalenemethyl-1*H*-indole-2,3-dione (8d$_3$). Compound **8d$_3$** was prepared according to General Procedure above by using compound **7d** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 91%. Mp 193.1–193.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 1.6 Hz, 1H), 7.88–7.80 (m, 5H), 7.52 (t, J = 4.0 Hz, 2H), 7.42 (dd, J = 8.4, 1.6 Hz, 1H), 7.31 (br s, 1H), 7.25 (br s, 1H), 7.16 (br s, 1H), 6.96 (d, J = 8.4 Hz, 1H), 5.14 (s, 2H), 3.58 (s, 2H), 3.01 (s, 4H), 2.57 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.9, 153.6, 137.6, 134.5, 133.3, 133.1, 131.9, 131.0, 130.7, 129.7, 129.5, 128.6, 127.9, 127.8, 126.9, 126.7, 126.6, 124.9, 124.8, 117.5, 111.4, 51.9, 46.1, 44.8, 30.9. IR (KBr, cm^{-1}): 3051, 2832, 1735, 1614, 1478, 1325, 1159, 935, 752. ESI-MS: m/z 560.21 ([M+H$^+$]). Anal. Calcd for $C_{30}H_{26}ClN_3O_4S$: C, 64.34; H, 4.68; N, 7.50. Found: C, 64.11; H, 4.74; N, 7.66.

4.1.5.8. 1-Methyl-5-[(4-phenylethylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8f$_1$). Compound **8f$_1$** was prepared according to General Procedure above by using compound **7f** and iodomethane. Yellow powder. Yield 93%. Mp 183.9–184.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 8.4, 1.6 Hz, 1H), 7.96 (d, J = 1.6 Hz, 1H), 7.26 (t, J = 7.2 Hz, 2H), 7.19 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 3.33 (s, 3H), 3.07 (s, 4H), 2.73 (m, 2H), 2.63–2.60 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 157.8, 154.3, 139.7, 137.8, 131.6, 128.6, 128.5, 126.2, 124.7, 117.3, 110.3, 59.7, 52.1, 46.1, 33.5, 26.7. IR (KBr, cm^{-1}): 3025, 2952 2811, 1752, 1616, 1458, 1321, 956, 748. ESI-MS: m/z 414.07 ([M+H$^+$]). Anal. Calcd for $C_{21}H_{23}N_3O_4S$: C, 61.00; H, 5.61; N, 10.16. Found: C, 60.89; H, 5.56; N, 9.99.

4.1.5.9. 1-Benzyl-5-[(4-phenylethylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8f$_2$). Compound **8f$_2$** was prepared according to General Procedure above by using compound **7f** and benzyl bromide. Yellow powder. Yield 96%. Mp 177.0–178.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.39–7.32 (m, 5H), 7.26 (t, J = 7.2 Hz, 2H), 7.19 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 7.2 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 3.04 (s, 4H), 2.72 (t, J = 8.0 Hz, 2H), 2.61 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 162.5, 157.7, 153. 6, 137.7, 133.6, 131.6, 129.4, 128.6, 128.6, 128.5, 127.4, 126.3, 124.9, 117.5, 111.3, 59.7, 52.0, 46.0, 44.5, 33.4. IR (KBr, cm^{-1}): 3027, 2928, 2815, 1748, 1614, 1454, 1353, 1158, 952, 748, 700. ESI-MS: m/z 490.07 ([M+H$^+$]). Anal. Calcd for $C_{27}H_{27}N_3O_4S$: C, 66.24; H, 5.56; N, 8.58. Found: C, 66.47; H, 5.89; N, 8.78.

4.1.5.10. 1-β-Naphthalenemethyl-5-[(4-phenylethylpiperazin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8f$_3$). Compound **8f$_3$** was prepared according to General Procedure above by using compound **7f** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 92%. Mp 214.4–214.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.87–7.80 (m, 5H), 7.51 (s, 2H), 7.40 (d, J = 8.0 Hz, 1H), 7.25–7.11 (m, 5H), 6.94 (d, J = 8.0 Hz, 1H), 5.13 (s, 2H), 3.00 (s, 4H), 2.68 (d, J = 7.2 Hz, 2H), 2.56 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.7, 157.8, 137.7, 133.2, 133.1, 131.4, 131.4, 130.9, 129.6, 128.6, 128.5, 127.9, 127.8, 126.9, 126.7, 126.6, 126.3, 126.2, 124.9, 124.8, 117.5, 111.4, 59.7, 52.0, 46.0, 44.7, 33.5. IR (KBr, cm^{-1}): 3053, 2952, 2808, 1741, 1619, 1479, 1331, 1161, 943, 751. ESI-MS: m/z 540.21 ([M+H$^+$]). Anal. Calcd for $C_{31}H_{29}N_3O_4S$: C, 69.00; H, 5.42; N, 7.79. Found: C, 68.88; H, 5.31; N, 7.84.

4.1.5.11. 1-Methyl-5-{[4-(pyridin-2-yl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione (8h$_1$). Compound **8h$_1$** was prepared according to General Procedure above by using compound **7h**

and iodomethane. Yellow powder. Yield 91%. Mp 134.9–136.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (dd, J = 4.2, 1.6 Hz, 1H), 8.03 (dd, J = 8.4, 1.6 Hz, 1H), 7.74 (d, J = 1.6 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.69 (t, J = 6.0 Hz, 1H), 3.64 (t, J = 4.8 Hz, 4H), 3.17 (s, 3H), 3.01 (t, J = 4.8 Hz, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 182.3, 158.9, 158.6, 154.9, 148.0, 138.2, 137.4, 129.5, 123.2, 118.4, 114.0, 111.7, 107.9, 46.0, 44.4, 26.8. IR (KBr, cm^{-1}): 3056, 2923, 2854, 1748, 1611, 1466, 1319, 1161, 943, 748. ESI-MS: m/z 387.17 ([M+H$^+$]). Anal. Calcd for $C_{18}H_{18}N_4O_4S$: C, 55.95; H, 4.70; N, 14.50. Found: C, 55.68; H, 4.33; N, 14.86.

4.1.5.12. 1-Benzyl-5-{[4-(pyridin-2-yl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione ($8h_2$). Compound $\mathbf{8h_2}$ was prepared according to General Procedure above by using compound **7h** and benzyl bromide. Yellow powder. Yield 91%. Mp 163.7–165.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (dd, J = 4.8, 1.2 Hz, 1H), 7.99 (s, 1H), 7.61 (dd, J = 8.8, 2.0 Hz, 1H), 7.50 (m, 1H), 7.37 (m, 5H), 6.94 (d, J = 8.4 Hz, 1H), 6.67 (m, 1H), 6.61 (d, J = 9.6 Hz, 1H), 4.97 (s, 2H), 3.67 (t, J = 8.0 Hz, 4H), 3.12 (t, J = 10.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 158.5, 157.7, 153.6, 148.0, 137.8, 137.6, 133.6, 131.7, 129.3, 128.6, 127.5, 124.8, 117.5, 114.2, 111.4, 107.3, 45.7, 44.7, 44.5. IR (KBr, cm^{-1}): 3057, 2924, 1747, 1611, 1475, 1331, 1156, 947, 745. ESI-MS: m/z 463.30 ([M+H$^+$]). Anal. Calcd for $C_{24}H_{22}N_4O_4S$: C, 62.32; H, 4.79; N, 12.11. Found: C, 62.49; H, 4.98; N, 11.92.

4.1.5.13. 1-β-Naphthalenemethyl-5-{[4-(pyridin-2-yl)piperazin-1-yl]sulfonyl}-1*H*-indole-2,3-dione ($8h_3$). Compound $\mathbf{8h_3}$ was prepared according to General Procedure above by using compound **7h** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 96%. Mp 148.2–149.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 4.4 Hz, 1H), 8.01 (s, 1H), 7.93–7.88 (m, 3H), 7.83 (t, J = 4.4 Hz, 1H), 7.80 (s, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.52–7.48 (m, 3H), 7.19 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.63 (t, J = 4.4 Hz, 1H), 5.12 (s, 2H), 3.58 (t, J = 4.8 Hz, 4H), 2.96 (t, J = 4.8 Hz, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.9, 159.2, 158.6, 153.9, 148.0, 138.2, 137.3, 133.3, 133.0, 132.9, 129.8, 129.4, 128.8, 128.1, 126.9, 126.5, 126.3, 126.0, 123.4, 119.0, 114.0, 112.0, 107.9, 46.0, 44.4, 43.9. IR (KBr, cm^{-1}): 3052, 2920, 2853, 1747, 1612, 1477, 1327, 1158, 949, 749. ESI-MS: m/z 513.44 ([M+H$^+$]). Anal. Calcd for $C_{28}H_{24}N_4O_4S$: C, 65.61; H, 4.72; N, 10.93. Found: C, 65.53; H, 4.61; N, 11.88.

4.1.5.14. 1-Benzyl-5-[(piperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione ($8i_2$). Compound $\mathbf{8i_2}$ was prepared according to General Procedure above by using compound **7i** and benzyl bromide.Yellow powder. Yield 94%. Mp 199.1–199.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 1.6 Hz, 1H), 7.89 (dd, J = 8.4, 2.0 Hz, 1H), 7.40–7.32 (m, 5H), 6.92 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 2.99 (t, J = 5.6 Hz, 4H), 1.65 (t, J = 5.6 Hz, 4H), 1.44 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ181.8, 157.8, 153.3, 137.5, 133.8, 132.8, 129.3, 128.6, 127.6, 124.6, 117.5, 111.2, 46.9, 44.5, 25.1, 23.4. IR (KBr, cm^{-1}): 3056, 2959, 2929, 2837, 1752, 1615, 1473, 1370, 1318, 1127, 933, 743. ESI-MS: m/z 385.20 ([M+H$^+$]). Anal. Calcd for $C_{20}H_{20}N_2O_4S$: C, 62.48; H, 5.24; N, 7.29. Found: C, 62.19; H, 5.02; N, 7.09.

4.1.5.15. 1-β-Naphthalenemethyl-5-[(piperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione ($8i_3$). Compound $\mathbf{8i_3}$ was prepared according to General Procedure above by using compound **7i** and 2-(bromomethyl)naphthalene. Dark Yellow powder. Yield 96%. Mp 206.5–207.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.88–7.81 (m, 5H), 7.53 (m, 2H), 7.43 (dd, J = 8.4, 1.6 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 5.14 (s, 2H), 2.96 (t, J = 5.6 Hz, 4H), 1.62 (t, J = 5.6 Hz, 4H), 1.42 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 157.9, 153.3, 137.5, 133.3, 133.1, 132.9, 131.2, 129.5, 127.9, 127.8, 126.9, 126.8, 126.7, 125.0, 124.7, 117.5, 111.3, 46.9, 44.7, 25.1, 23.4. IR (KBr, cm^{-1}): 3050, 2937, 2857, 1737, 1613, 1477, 1340, 1132, 929, 741. ESI-MS: m/z 434.01 ([M+H$^+$]). Anal. Calcd for $C_{24}H_{22}N_2O_4S$: C, 66.34; H, 5.10; N, 6.45. Found: C, 66.67; H, 4.82; N, 6.32.

4.1.5.16. 1-Methyl-5-(morpholinosulfonyl)-1*H*-indole-2,3-dione ($8j_1$). Compound $\mathbf{8j_1}$ was prepared according to General Procedure above by using compound **7j** and iodomethane. Pale yellow powder. Yield 93%. Mp 283.1–283.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 3.76 (t, J = 4.8 Hz, 4H), 3.34 (s, 3H), 3.03 (t, J = 4.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.3, 158.9, 155.0, 137.5, 129.2, 123.3, 118.4, 111.7, 65.7, 46.4, 26.8. IR (KBr, cm^{-1}): 3117, 2918, 2857, 1750, 1615, 1469, 1364, 1113, 942, 749. ESI-MS: m/z 311.42 ([M+H$^+$]). Anal. Calcd for $C_{13}H_{14}N_2O_5S$: C, 50.31; H, 4.55; N, 9.03. Found: C, 50.09; H, 4.35; N, 8.92.

4.1.5.17. 1-Benzyl-5-(morpholinosulfonyl)-1*H*-indole-2,3-dione ($8j_2$). Compound $\mathbf{8j_2}$ was prepared according to General Procedure above by using compound **7j** and benzyl bromide. Yellow powder. Yield 94%. Mp 200.7–202.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.2 Hz, 1H), 7.81 (dd, J = 8.4, 1.6 Hz, 1H), 7.42–7.31 (m, 5H), 6.94 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H), 3.74 (t, J = 4.8 Hz, 4H), 3.00 (t, J = 4.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.7, 153.7, 137.7, 133.6, 131.4, 129.4, 128.7, 127.5, 124.9, 117.6, 111.4, 66.0, 45.9, 44.5. IR (KBr, cm^{-1}): 3083, 2968, 1757, 1617, 1472, 1350, 1159, 1075, 939, 748. ESI-MS: m/z 387.16 ([M+H$^+$]). Anal. Calcd for $C_{19}H_{18}N_2O_5S$: C, 59.06; H, 4.70; N, 7.25. Found: C, 58.89; H, 4.46; N, 7.29.

4.1.5.18. 5-(Morpholinosulfonyl)-1-β-naphthalenemethyl-1*H*-indole-2,3-dione ($8j_3$). Compound $\mathbf{8j_3}$ was prepared according to General Procedure above by using compound **7j** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 97%. Mp 225.6–227.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.88–7.81 (m, 5H), 7.52 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 5.15 (s, 2H), 3.71 (s, 4H), 2.90 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 157.8, 153.7, 137.7, 133.3, 133.2, 131.5, 131.0, 129.6, 127.9, 127.8, 126.7, 124.9, 124.8, 117.6, 111.5, 66.0, 45.9, 44.8. IR (KBr, cm^{-1}): 3051, 2924, 1739, 1615, 1362, 1160, 937, 748; ESI-MS: m/z 437.19 ([M+H$^+$]). Anal. Calcd for $C_{23}H_{20}N_2O_5S$: C, 63.29; H, 4.62; N, 6.42. Found: C, 63.01; H, 4.50; N, 6.37.

4.1.5.19. 1-Methyl-5-[(4-methylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione ($8k_1$). Compound $\mathbf{8k_1}$ was prepared according to General Procedure above by using compound **7k** and iodomethane. Yellow powder. Yield 94%. Mp 195.2–196.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J = 8.4, 1.6 Hz, 1H), 7.96 (d, J = 1.6 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 3.77 (d, J = 11.2 Hz, 2H), 3.32 (s, 3H), 2.30 (t, J = 11.2 Hz, 2H), 1.70 (d, J = 10.0 Hz, 2H), 1.33–1.25 (m, 3H), 0.93 (d, J = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 157.8, 154.1, 137.7, 132.7, 124.6, 117.2, 110.2, 46.5, 33.3, 30.2, 26.7, 21.4. IR (KBr, cm^{-1}): 2935, 2921, 1750, 1614, 1320, 1154, 1067, 925, 746. ESI-MS: m/z 323.02 ([M+H$^+$]). Anal. Calcd for $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69. Found: C, 55.97; H, 5.49; N, 8.78.

4.1.5.20. 1-Benzyl-5-[(4-methylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione ($8k_2$). Compound $\mathbf{8k_2}$ was prepared according to General Procedure above by using compound **7k** and benzyl bromide. Yellow powder. Yield 96%. Mp 162.2–162.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 1.6 Hz, 1H), 7.88 (dd, J = 8.4, 1.6 Hz, 1H), 7.40–7.32 (m, 5H), 6.92 (d, J = 8.4 Hz, 1H), 4.98 (s,

2H), 3.71 (d, J = 11.2 Hz, 2H), 2.28 (t, J = 11.2 Hz, 2H), 1.67 (d, J = 10.0 Hz, 2H), 1.31–1.25 (m, 3H), 0.92 (d, J = 5.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 157.8, 153.3, 137.5, 133.8, 132.8, 129.3, 128.6, 127.6, 124.6, 117.5, 111.3, 46.4, 44.5, 33.3, 30.1, 21.4. IR (KBr, cm^{-1}): 2950, 2929, 1752, 1613, 1475, 1332, 1148, 927, 723. ESI-MS: m/z 399.11 ([M+H$^+$]). Anal. Calcd for $C_{21}H_{22}N_2O_4S$: C, 63.30; H, 5.56; N, 7.03. Found: C, 63.14; H, 5.77; N, 7.31.

4.1.5.21. 5-[(4-Methylpiperidin-1-yl)sulfonyl]-1-β-naphthalenemethyl-1*H*-indole-2,3-dione (8k$_3$). Compound **8k$_3$** was prepared according to General Procedure above by using compound **7k** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 97%. Mp 218.9–221.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.88–7.81 (m, 5H), 7.52 (m, 2H), 7.43 (dd, J = 8.4, 1.6 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 5.14 (s, 2H), 3.69 (d, J = 11.2 Hz, 2H), 2.25 (t, J = 11.2 Hz, 2H), 1.65 (d, J = 10.0 Hz, 2H), 1.30–1.24 (m, 3H), 0.90 (d, J = 5.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 157.9, 153.3, 137.6, 133.3, 133.1, 132.9, 131.2, 129.5, 127.9, 127.8, 126.9, 126.8, 126.7, 125.0, 124.7, 117.5, 111.3, 46.4, 44.8, 33.3, 30.1, 21.4. IR (KBr, cm^{-1}): 2923, 1747, 1612, 1471, 1336, 1178, 928, 747. ESI-MS: m/z 449.39 ([M+H$^+$]). Anal. Calcd for $C_{25}H_{24}N_2O_4S$: C, 66.94; H, 5.39; N, 6.25. Found: C, 66.81; H, 5.24; N, 6.12.

4.1.5.22. 5-[(3,5-Dimethylpiperidin-1-yl)sulfonyl]-1-methyl-1*H*-indole-2,3-dione (8m$_1$). Compound **8m$_1$** was prepared according to General Procedure above by using compound **7m** and iodomethane. Yellow powder. Yield 92%. Mp 209–210.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J = 10.4, 2.0 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 3.73 (d, J = 7.6 Hz, 2H), 3.33 (s, 3H), 1.84–1.73 (m, 5H), 1.27 (m, 1H), 0.87 (d, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 157.8, 154.1, 137.7, 132.8, 124.5, 117.3 110.3, 52.8, 41.3, 31.0, 26.7, 19.0. IR (KBr, cm^{-1}): 3104, 2958, 1743, 1605, 1320, 1159, 793, 629. ESI-MS: m/z 336.99 ([M+H$^+$]). Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C, 57.12; H, 5.99; N, 8.33. Found: C, 57.36; H, 6.17; N, 8.13.

4.1.5.23. 1-Benzyl-5-[(3,5-dimethylpiperidin-1-yl)sulfonyl]-1*H*-indole-2,3-dione (8m$_2$). Compound **8m$_2$** was prepared according to General Procedure above by using compound **7m** and benzyl bromide. Yellow powder. Yield 94%. Mp 171.9–173.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 2.0 Hz, 1H), 7.90 (dd, J = 8.4, 2.0 Hz, 1H), 7.41–7.34 (m, 5H), 6.93 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 3.69 (d, J = 7.2 Hz, 2H), 1.78–1.70 (m, 5H), 1.27 (m, 1H), 0.85 (d, J = 6.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 157.9, 153.3, 137.5, 133.8, 132.9, 129.3, 128.6, 127.6, 124.6, 117.5, 111.3, 52.7, 44.5, 41.3, 31.0, 19.0. IR (KBr, cm^{-1}): 3065, 2952, 1740, 1610, 1474, 1332, 1152, 799, 694. ESI-MS: m/z 413.20 ([M+H$^+$]). Anal. Calcd for $C_{22}H_{24}N_2O_4S$: C, 64.06; H, 5.86; N, 6.79. Found: C, 63.88; H, 5.61; N, 6.97.

4.1.5.24. 5-[(3,5-Dimethylpiperidin-1-yl)sulfonyl]-1-β-naphthalenemethyl-1*H*-indole-2,3-dione (8m$_3$). Compound **8m$_3$** was prepared according to General Procedure above by using compound **7m** and 2-(bromomethyl)naphthalene. Yellow powder. Yield 93%. Mp 234.1–235.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.88–7.82 (m, 5H), 7.53 (t, J = 3.6 Hz, 2H), 7.43 (dd, J = 8.4, 1.6 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 5.14 (s, 2H), 3.67 (d, J = 7.6 Hz, 2H), 1.79–1.67 (m, 5H), 1.25 (s, 1H), 0.83 (d, J = 6.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 157.9, 153.3, 137.5, 133.3, 133.1, 133.0, 131.2, 129.4, 127.9, 127.8, 126.9, 126.8, 126.7, 125.0, 124.6, 117.5, 111.3, 52.7, 44.8, 41.2, 31.0, 18.9. IR (KBr, cm^{-1}): 3053, 2960, 1736, 1614, 1477, 1343, 1156, 796. ESI-MS: m/z 463.25 ([M+H$^+$]). Anal. Calcd for $C_{26}H_{26}N_2O_4S$: C, 67.51; H, 5.67; N, 6.06. Found: C, 67.74; H, 5.99; N, 6.11.

4.2. Biology enzyme assays

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

The SARS CoV 3CL^{pro} inhibition assays were conducted by fluorescence resonance energy transfer (FRET). The natural substrate amino acid sequence (AVLQSGFRKK) of SARS-CoV 3CL^{pro} started with the MCA fluorescent group and connected the Dnp fluorescence quenching group with penultimate K. The screening system was as follows (Table 1): the final concentrations of SARS-CoV 3CL^{pro}, substrate, and compound were 0.5 μM, 16 μM and 1 mM, respectively. The settled concentration of protein, compounds were preheateded at 37 °C and oscillated, and the substrate was added to the mixture above. The excitation/emission light was 320/405 nm, and the test was carried out every 3 s for 60 times. Drawing curves, the maximum value of the negative control curve slope was V_0, and the largest compound curve slope was V_1. The inhibition ratio was defined as $1-V_1/V_0$, and IC_{50} Value was obtained by the equation: $V_0/V = 1 + [I]/IC_{50}$, where V_0 is the initial rate of the reaction without inhibitor, V is the initial rate of reaction with the inhibitor at various concentrations, and [I] is the inhibitor concentration.

4.3. X-ray crystallographic socking studies

The final concentration of purified SARS M^{pro} was 8–10 mg/ml dissolved by ddH_2O. Crystals of SARS M^{pro} belong to space group $P2$ (a = 52.061 Å, b = 95.704 Å, c = 67.587 Å, $\alpha = \gamma = 90°$, β = 102.88°). The buffers used in protein crystallization were 100 mM MES (pH 6.0), 3% (v/v) PEG8000, and 3% DMSO. Crystals were grown at 16 °C for 18 h. Soaking was performed using 1.8 μl of the reservoir in the crystallization well mixed with 0.2–0.4 μl of stock (95% DMSO) solution of the compound at 100 μM for 4 h. The soaking protocol permitted the rapid structural determination of SARS M^{pro}/inhibitor complexes.

Crystals were then transferred to a cryoprotectant solution consisting of 70% crystallization buffer, 20% (v/v) glycol, and 10% (v/v) glycerol. Crystallographic data were routinely collected in a conventional, in-house Rigaku X-ray generator with an R-AXIS 944+ detector at a wavelength of 1.5418 Å. Data were processed and reduced with HKL-2000 package. A total of 360 frames were collected with 0.5° oscillation angle. Exposure times were 30 s/frame. This strategy resulted in >95% completeness in the vast majority of the data sets with reducing R factors between 5% and 9%. Data were processed with HKL2000 and scaled with SCALEPACK. Phase and map calculations were performed using PHASER. The modeling and electron-density fitting software COOT was used to manipulate the models.

Docking studies

The structures (compound geometries) of **7k**, **8k$_1$** and **8k$_2$** were built in Maestro[40] and cleaned by performing short force-field minimization. Compounds **7k**, **8k$_1$**, and **8k$_2$** were subjected to full geometry optimization with DFT at the B3LYP/6-31G (d) level using GAUSSIAN 03.[41] Glide 5.5 (Glide model in Schrodinger software) was used to perform docking simulation of fully optimized **7k**, **8k$_1$**, and **8k$_2$**. The X-ray structure of SARS coronavirus 3CL^{pro} (PDB ID:

1UK4) was used as our initial protein model for docking. The protein structure was prepared using the Schrödinger Suite 2010 Protein Preparation Wizard. Only chain-A of the dimer was used in this simulation. All water molecules were deleted from the protein structure before docking. The standard precision of Glidescore scoring functions was used to rank binding pose.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.11.028.

References and notes

1. Shao, Y. M.; Yang, W. B.; Kuo, T. H.; Tsai, K. C.; Lin, C. H.; Yang, A. S.; Liang, P. H.; Wong, C. H. *Bioorg. Med. Chem.* **2008**, *16*, 4652.
2. Mukherjee, P.; Desai, P.; Ross, L.; White, E. L.; Avery, M. A. *Bioorg. Med. Chem.* **2008**, *16*, 4138.
3. Chen, L. L.; Li, J.; Luo, C.; Liu, H.; Xu, W. J.; Chen, G.; Liew, O. W.; Zhu, W. L.; Puah, C. M.; Shen, X.; Jiang, H. L. *Bioorg. Med. Chem.* **2006**, *14*, 8295.
4. Ramajayam, R.; Tan, K. P.; Liu, H. G.; Liang, P. H. *Bioorg. Med. Chem.* **2010**, *18*, 7849.
5. Ghosh, A. K.; Xi, K.; Grum-Tokars, V.; Xu, X. M.; Ratia, K.; Fu, W. T.; Houser, K. V.; Baker, S. C.; Johnson, M. E.; Mesecar, A. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5876.
6. Ghosh, A. K.; Gong, G. L.; Grum-Tokars, V.; Mulhearn, D. C.; Baker, S. C.; Coughlin, M.; Prabhakar, B. S.; Sleeman, K.; Johnson, M. E.; Mesecar, A. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5684.
7. Chan, J. F.; Li, K. S.; To, K. K.; Cheng, V. C.; Chen, H.; Yuen, K. Y. *J. Infect.* **2012**, *65*, 477.
8. Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J. R.; Hilgenfeld, R. *Science* **2003**, *300*, 1763.
9. Bacha, U.; Barrila, J.; Velazquez-Campoy, A.; Leavitt, S. A.; Freire, E. *Biochemistry* **2004**, *43*, 4906.
10. Zhang, J. M.; Pettersson, H. I.; Huitema, C.; Niu, C. Y.; Yin, J.; James, M. N. G.; Eltis, L. D.; Vederas, J. C. *J. Med. Chem.* **2007**, *50*, 1850.
11. Al-Gharabli, S. I.; Shah, S. T. A.; Weik, S.; Schmidt, M. F.; Mesters, J. R.; Kuhn, D.; Klebe, G.; Hilgenfeld, R.; Rademann, J. *ChemBioChem* **2006**, *7*, 1048.
12. Gan, Y. R.; Huang, H.; Huang, Y. D.; Rao, C. M.; Zhao, Y.; Liu, J. S.; Wu, L.; Wei, D. Q. *Peptides* **2006**, *27*, 622.
13. Zhang, H. Z.; Zhang, H.; Kemnitzer, W.; Tseng, B.; Cinatl, J.; Michaelis, M.; Doerr, H. W.; Cai, S. X. *J. Med. Chem.* **2006**, *49*, 1198.
14. Shao, Y. M.; Yang, W. B.; Peng, H. P.; Hsu, M. F.; Tsai, K. C.; Kuo, T. H.; Wang, A. H. J.; Liang, P. H.; Lin, C. H.; Yang, A. S.; Wong, C. H. *ChemBioChem* **2007**, *8*, 1654.
15. Sydnes, M. O.; Hayashi, Y.; Sharma, V. K.; Hamada, T.; Bacha, U.; Barrila, J.; Freire, E.; Kiso, Y. *Tetrahedron* **2006**, *62*, 8601.
16. Regnier, T.; Sarma, D.; Hidaka, K.; Bacha, U.; Freire, E.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2722.
17. Akaji, K.; Konno, H.; Mitsui, H.; Teruya, K.; Shimamoto, Y.; Hattori, Y.; Ozaki, T.; Kusunoki, M.; Sanjoh, A. *J. Med. Chem.* **2011**, *54*, 7962.
18. Konno, S.; Thanigaimalai, P.; Yamamoto, T.; Nakada, K.; Kakiuchi, R.; Takayama, K.; Yamazaki, Y.; Yakushiji, F.; Akaji, K.; Kiso, Y.; Kawasaki, Y.; Chen, S. E.; Freire, E.; Hayashi, Y. *Bioorg. Med. Chem.* **2013**, *21*, 412.
19. Martina, E.; Stiefl, N.; Degel, B.; Schulz, F.; Breuning, A.; Schiller, M.; Vicik, R.; Baumann, K.; Ziebuhr, J.; Schirmeister, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5365.
20. Chuck, C. P.; Chen, C.; Ke, Z. H.; Wan, D. C. C.; Chow, H. F.; Wong, K. B. *Eur. J. Med. Chem.* **2013**, *59*, 1.
21. Li, Y. Q.; Li, Z. L.; Zhao, W. J.; Wen, R. X.; Meng, Q. W.; Zeng, Y. *Eur. J. Med. Chem.* **2006**, *41*, 1084.
22. Biot, C.; Daher, W.; Chavain, N.; Fandeur, T.; Khalife, J.; Dive, D.; De Clercq, E. *J. Med. Chem.* **2006**, *49*, 2845.
23. Yang, S.; Chen, S. J.; Hsu, M. F.; Wu, J. D.; Tseng, C. T. K.; Liu, Y. F.; Chen, H. C.; Kuo, C. W.; Wu, C. S.; Chang, L. W.; Chen, W. C.; Liao, S. Y.; Chang, T. Y.; Hung, H. H.; Shr, H. L.; Liu, C. Y.; Huang, Y. A.; Chang, L. Y.; Hsu, J. C.; Peters, C. J.; Wang, A. H. J.; Hsu, M. C. *J. Med. Chem.* **2006**, *49*, 4971.
24. Ryu, Y. B.; Jeong, H. J.; Kim, J. H.; Kim, Y. M.; Park, J. Y.; Kim, D.; Naguyen, T. T. H.; Park, S. J.; Chang, J. S.; Park, K. H.; Rho, M. C.; Lee, W. S. *Bioorg. Med. Chem.* **2010**, *18*, 7940.
25. Park, J. Y.; Kim, J. H.; Kim, Y. M.; Jeong, H. J.; Kim, D. W.; Park, K. H.; Kwon, H. J.; Park, S. J.; Lee, W. S.; Ryu, Y. B. *Bioorg. Med. Chem.* **2012**, *20*, 5928.
26. Ryu, Y. B.; Park, S. J.; Kim, Y. M.; Lee, J. Y.; Seo, W. D.; Chang, J. S.; Park, K. H.; Rho, M. C.; Lee, W. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1873.
27. Wen, C. C.; Kuo, Y. H.; Jan, J. T.; Liang, P. H.; Wang, S. Y.; Liu, H. G.; Lee, C. K.; Chang, S. T.; Kuo, C. J.; Lee, S. S.; Hou, C. C.; Hsiao, P. W.; Chien, S. C.; Shyur, L. F.; Yang, N. S. *J. Med. Chem.* **2007**, *50*, 4087.
28. Zhang, J. M.; Huitema, C.; Niu, C. Y.; Yin, J.; James, M. N. G.; Eltis, L. D.; Vederas, J. C. *Bioorg. Chem.* **2008**, *36*, 229.
29. Hoever, G.; Baltina, L.; Michaelis, M.; Kondratenko, R.; Baltina, L.; Tolstikov, G. A.; Doerr, H. W.; Cinatl, J. *J. Med. Chem.* **2005**, *48*, 1256.
30. Ramajayam, R.; Tan, K.-P.; Liu, H.-G.; Liang, P.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3569.
31. Ghosh, A. K.; Xi, K.; Ratia, K.; Santarsiero, B. D.; Fu, W.; Harcourt, B. H.; Rota, P. A.; Baker, S. C.; Johnson, M. E.; Mesecar, A. D. *J. Med. Chem.* **2005**, *48*, 6767.
32. Jacobs, J.; Grum-Tokars, V.; Zhou, Y.; Turlington, M.; Saldanha, S. A.; Chase, P.; Eggler, A.; Dawson, E. S.; Baez-Santos, Y. M.; Tomar, S.; Mielech, A. M.; Baker, S. C.; Lindsley, C. W.; Hodder, P.; Mesecar, A.; Stauffer, S. R. D. *J. Med. Chem.* **2013**, *56*, 534.
33. Ikejiri, M.; Saijo, M.; Morikawa, S.; Fukushi, S.; Mizutani, T.; Kurane, I.; Maruyama, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2470.
34. Cho, J. H.; Bernard, D. L.; Sidwell, R. W.; Kern, E. R.; Chu, C. K. *J. Med. Chem.* **2006**, *49*, 1140.
35. Chen, L.-R.; Wang, Y.-C.; Lin, Y. W.; Chou, S.-Y.; Chen, S.-F.; Liu, L. T.; Wu, Y.-T.; Kuo, C.-J.; Chen, T. S.-S.; Juang, S.-H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3058.
36. Zhou, L.; Liu, Y.; Zhang, W. L.; Wei, P.; Huang, C. K.; Pei, J. F.; Yuan, Y. X.; Lai, L. H. *J. Med. Chem.* **2006**, *49*, 3440.
37. Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P. W.; Camphausen, R. T.; Tam, S.; Tsao, D. H. H.; Keith, J. C.; Nickerson-Nutter, C.; Shilling, A.; Young-Sciame, R.; Wang, Q. *J. Med. Chem.* **2007**, *50*, 21.
38. Jiang, Y.; Hansen, T. V. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1626.
39. Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nuttall, M. E.; Nadeau, D. P.; Kikly, K.; Winkler, J. D.; Sung, C. M.; Ryan, M. D.; Levy, M. A.; Keller, P. M.; DeWolf, W. E. *J. Med. Chem.* **2001**, *44*, 2015.
40. Schrödinger, L.L.C. Maestro, version 9.1, New York, NY, 2010.
41. Gaussian 03, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.