Design and Synthesis of Cinanserin Analogos as Severe Acute Respiratory Syndrome Coronavirus 3CL Protease Inhibitors

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The severe acute respiratory syndrome (SARS) coronavirus 3CL protease is an attractive target for the development of anti-SARS drugs. In this paper, cinanserin (1) analogs were synthesized and tested for the inhibitory activities against SARS-coronavirus (CoV) 3CL protease by fluorescence resonance energy transfer (FRET) assay. Four analogs show significant activities, especially compound 26 with an IC₅₀ of 1.06 μM.

Key words SARS coronavirus 3CL protease; cinanserin analog; inhibitor; fluorescence resonance energy transfer-based assay

Severe acute respiratory syndrome (SARS) is a life-threatening form of atypical pneumonia caused by infection with a novel human coronavirus (SARS-CoV). It rapidly spreaded from southern China to several other countries during late 2002 and early 2003.1,2 By July 31, 2003, a total of 8098 SARS cases and 774 SARS-related deaths were reported around the world.3,4 Although regional preventive measures are being implemented, vaccine and therapeutic drugs are being sought, no effective small molecule antiviral agent has been reported for treating SARS so far.

SARS-CoV is a positive-strand RNA virus that consists of about 29700 nucleotides. It encodes two overlapping polyproteins, ppla (486 kDa) and pplab (790 kDa).4—7 The functional polypeptides are released from each polyprotein through extensive proteolytic processing primarily by 3CL protease.8 Because of this functional importance of SARS-CoV 3CL protease in the viral life cycle, it has been recognized as a key target for drugs designing against SARS.9—11

In our preceding paper, we have reported that cinanserin (1), a well-characterized serotonin antagonist, is a good potential lead compound for designing more active inhibitors of 3CL protease.12 Herein we report the design and synthesis of two series of cinanserin derivatives as novel inhibitors of SARS-CoV 3CL protease.

According to the 3D model of the cinanserin-3CL protease complex,13,14 three series of cinanserin analogs have been designed and synthesized respectively (Table 1). In order to modify the cinnamide moiety of cinanserin, compounds 2—9 were synthesized in which cinnamoyl group is replaced with other phenyl-containing groups. Compounds 10—22 and 26, 27 were designed and prepared to diversify the substituent groups and chains on sulfur atom of cinanserin. Compounds 23—25 were prepared to vary the thioether group of cinanserin into ether and sulfoxide respectively.

Chemical Synthesis Compounds 2—22 were synthesized via a three-step route (Chart 1).13,14 The starting material 2-aminothiophenol (28) was first treated with excessive sodium isopropoxide at room temperature to form sodium salt, which was then reacted with a variety of alkyl halides or aminoalkyl halides, yielding intermediates 29—42. Most of the intermediates are known except 35, 36, 37 and 42. Acylation of the intermediates with the corresponding acyl chloride produced the target compounds 2—22 (Chart 1). Similarly, compounds 23 and 24 were obtained from 2-aminophenol (43) and 2-amino-4-nitrophenol (44) via the same approach respectively (Chart 2). Sulfoxide 25 was prepared by oxidation of cinanserin (1) with sodium periodate in acetonitrile. The synthesis of compounds 26 and 27 also started from 2-aminothiophenol (28), which was reacted with an excess of cinnamoyl chloride (47, R=H) or 2-cyanocinnamoyl chloride (48, R=CN) in the presence of triethylamine at reflux in dichloromethane to give the desired products 29 and 30 respectively (Chart 3). Among the target compounds, 2, 4, 5, 11, 21, 23 and 24 are known, but their 1H-NMR data have not been reported yet.

Biological Results and SAR Discussion The bioactivities of compounds 2—27 were measured by a fluorescence resonance energy transfer (FRET)-based assay using 5-(2-aminoethylamino)naphthethenesulfonic acid (Edans) and 4-(4-dimethylaminophenylazo)benzoic acid (Dabcy) as the energy transfer pair. The peptide substrate is Dabcy-KN-STLQSGLRKE-Edans labeled with Edans and Dabcyl. The inhibition of SARS-CoV 3CL protease slowly increased in the concentration range from 0 to 500 μM of the corresponding compounds. The IC₅₀ value of the compounds in inhibiting the catalytic activity of SARS-CoV 3CL protease was calculated by fitting the dose–response curve using a logistic derivative equation.12 The results are summarized in Table 1.

Of the synthetic derivatives tested, compounds 7, 10, 13, 26 and 27 displayed remarkable inhibitory activity of SARS-CoV 3CL protease, indicating that the cinnamoyl group is crucial for good inhibition of SARS-CoV 3CL protease. Compound 7 is about 2-fold more potent than cinanserin, suggesting that introduction of a cyano substituent on α-position of cinnamoyl group increased the inhibitory activity. Replacement of the dimethylamino group of cinanserin with electron-withdrawing group such as an ester group (13) or elimination of the dimethylamino group (allylthioether 10) enhanced remarkably the inhibitory activity, and the chain length of the thioether is not critical of the inhibitory activity (compound 11 vs. 12; 15 vs. 16). When the phenylthioether moiety in cinanserin was changed to phenylether group (23) or oxidized to sulfoxide (25), no inhibitory activity was observed. Unexpectedly, replacement of the 3-dimethylaminopropyl group in cinanserin with an additional cinnamoyl...
group, giving compound 26 which showed very potent inhibition against 3CL protease, and is 300-fold more potent than cinanserin itself. These observations are in good coincidence with the molecular docking study of the complex of 3CL protease with the ligand.

Docking Study The 3D structure of compound 26 was constructed by the Corina online service (http://www.mol-net.com/online_demos/corina_demo.html). The 3D model of SARS-CoV 3CL protease was retrieved from the Brookhaven Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) (PDB ID: 1UJ1, Chain A). AutoDock Tools (http://autodock.scripps.edu/resources/adt) were used to add polar hydrogen and assign partial charges to both protein and ligand. AutoDock 3.0.5 was employed for the docking of compound 26 to SARS-3CL protease. All the molecular modeling and docking simulations were performed on a Silicon Graphics Origin 3800 (with 128 CPUs).

To address the SARS-CoV 3CL protease inhibitory activity of compound 26, we applied molecular docking to identify the possible binding mode between the compound and the enzyme. The top pose, ranked by the “estimated free energy of binding”, was chosen as the predicted binding mode.

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a) The fluorogenic peptide substrate is Dabcyl-KNSTLQSLRKE-Edans instead of Edans-VNSTLQQLRKE(Dabcyl)M as used in ref. 12.
For the top pose, as shown in Fig. 1, compound 26 locates deep inside the S1 pocket with appropriate steric complement. Forty hydrophobic interaction atom pairs between the compound and the enzyme were detected by using the LIGPLOT. In the light of this binding mode, compound 26 occupies the substrate binding subsite, indicating that it is a competitive ligand for the enzyme’s substrate, and the predicted dissociation constant \( K_d \) is 0.35 M. Compared with the binding mode of cinanserin and SARS-CoV 3CL protease, compound 26 binds tighter with the protease than cinanserin. This might lead to the stronger inhibitory activity against the protease.

**Conclusion**

Three series of cinanserin analogs derived from the modification of cinnametyl, phenylthioether group and sulfur-containing chain of cinanserin have been designed and synthesized as potential inhibitors of SARS-CoV 3CL protease. The inhibitory activities of these compounds were assessed by FRET assay. Some analogs showed improved inhibitory activities against SARS-CoV 3CL protease compared with cinanserin itself. The IC\( _{50} \) values of compounds 10, 13, 26 and 27 are lower than 100 \( \mu \)M. Among them compound 26 is the most potent one. It is much more potent than cinanserin by two orders of magnitude in the inhibition of SARS 3CL protease.

**Experimental**

**General** All starting materials were commercially available and used without further purification. All water-sensitive reactions were carried out in oven-dried glassware with a stirring bar under a nitrogen atmosphere. Toluene was dried over sodium, chloroform and dichloromethane were dried over CaH\(_2\). Melting points were measured in capillary tube on a Buchi 510 melting point apparatus without correction. IR spectra were recorded on a Nicolet Magna IR750 spectrometer with KBr disks or film. NMR spectra were recorded on Brucker AMX-400 (400 MHz). Chemical shifts were reported in parts per million (ppm, \( \delta \) units) downfield from chloroform where solvent peak was used as internal standard. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LR-MS and HR-MS) were given with electric ionization (EI) produced by Varian MAT-711 and Finnigan MAT-95 instrument.

**2-(3-Methoxypropylthio)benzenamine (35)** To a solution of sodium isopropoxide prepared from sodium (0.35 g, 15.2 mmol) in isopropanol (20 ml) was added 28 (1.7 ml, 15 mmol), and the mixture was stirred at room temperature for 30 min. 1-Chloro-3-methoxypropane (1.63 g, 15 mmol) was then added to. The reaction mixture was stirred at reflux for 4 h, and con-
denosed in vacuum. The residue was treated with water and extracted with ether twice. The combined organic layer was washed with water and dried over anhydrous MgSO₄. After removal of the solvent, the residue was purified by flash column chromatography on silica gel eluting with petroether (80): chloroform (13): ethyl acetate (7) to afford 35 (0.63 g, 21%) as a brown oil. 1H-NMR (CDCl₃): δ 1.76—1.85 (2H, m), 2.82 (2H, t, J = 7.1 Hz), 3.31 (3H, s), 3.43 (2H, t, J = 6.4 Hz), 4.33 (2H, s, br), 6.66—6.73 (2H, m), 7.09—7.12 (1H, m), 7.37 (1H, dd, J = 7.7, 1.5 Hz).

2-(4-Methoxybutylthio)benzene-36 (In the same manner as described for 35, it was prepared from 28 and 1-chloro-4-methoxybutane in a yield of 52% as a brown oil. 1H-NMR (CDCl₃): δ 1.62—1.70 (2H, m), 2.76 (2H, t, J = 7.2 Hz), 3.31 (3H, s), 3.63 (2H, s, br), 6.40—6.47 (2H, m), 7.08—7.12 (1H, m), 7.36 (1H, dd, J = 7.7, 1.5 Hz).

2-(2-N-Vinylpyrrolidinyl)ethylthio)benzene (37) In the same manner as described for 35, it was prepared from 28 and 2-(2-chloroethyl)-1-methylpyrrolidin-2-one hydrochloride in a yield of 63% as a brown oil. 1H-NMR (CDCl₃): δ 1.35—1.55 (2H, m), 1.63—1.75 (2H, m), 1.86—1.95 (2H, m), 2.08—2.16 (2H, m), 2.25 (3H, s), 2.63—2.70 (1H, m), 2.78—2.85 (1H, m), 3.00—3.05 (1H, m), 4.34 (2H, t, br), 6.65—6.72 (2H, m), 7.08—7.12 (1H, m), 7.38—7.56 (1H, m).

2-(Phenoxymethylthio)benzene (42) In the same manner as described for 35, it was prepared from 28 and 2-phenoxymethyl bromide in a yield of 94% as a brown oil. 1H-NMR (CDCl₃): δ 3.10 (2H, t, J = 6.5, 0.7 Hz), 3.56 (2H, s, br), 6.92 (1H, d, J = 7.1 Hz), 6.93 (1H, d, J = 7.7 Hz), 7.07—7.12 (1H, m), 1.04 (3H, t, J = 7.1 Hz).

N-[2-(3-Dimethylaminopropylthio)phenyl]-3-phenylpropionamide (28) In the same manner as described for 35, it was prepared from 29 and 3-phenylpropionic acid as a white crystalline solid. 1H-NMR (CDCl₃): δ 3.10 (2H, t, J = 6.5, 0.7 Hz), 3.56 (2H, s, br), 6.92 (1H, d, J = 7.1 Hz), 6.93 (1H, d, J = 7.7 Hz), 7.07—7.12 (1H, m), 1.04 (3H, t, J = 7.1 Hz). In the same manner as described for 28, it was prepared from 30 and cinnamoyl chloride in a yield of 74% as a brown oil. 1H-NMR (CDCl₃): δ 3.10 (2H, t, J = 6.5, 0.7 Hz), 3.56 (2H, s, br), 6.92 (1H, d, J = 7.1 Hz), 6.93 (1H, d, J = 7.7 Hz), 7.07—7.12 (1H, m), 1.04 (3H, t, J = 7.1 Hz). In the same manner as described for 28, it was prepared from 30 and 4-phenylbienzyl chloride in a yield of 22% as a yellow oil. 1H-NMR (CDCl₃): δ 3.10 (2H, t, J = 6.5, 0.7 Hz), 3.56 (2H, s, br), 6.92 (1H, d, J = 7.1 Hz), 6.93 (1H, d, J = 7.7 Hz), 7.07—7.12 (1H, m), 1.04 (3H, t, J = 7.1 Hz).
yield of 53% as a yellowish crystal recrystallized from ethyl acetate–hexane, mp 160–162 °C. IR (KBr) cm⁻¹: 3440, 3268, 1658, 1608, 1576, 1529, 1442, 1348, 1284, 1178, 1148; 1H-NMR (CDCl₃): δ: 1.18—1.20 (4H, t, J = 7.0 Hz), 1.21—1.23 (6H, t, J = 6.8 Hz), 2.10—2.14 (2H, t, J = 15.8 Hz), 2.30—2.40 (2H, t, J = 14.5 Hz), 6.69 (1H, d, J = 7.0 Hz), 6.81 (1H, br, s), 8.13 (1H, s, br). MS m/z: 364 (M⁺), 272, 131 (100%), 103, 77; HR-MS (El) m/z: Calcd for C₁₅H₁₄N₂O₃S (M⁺): 364.0737. Found: 364.0761.

N-[2-(Pyridine-2-yl)ethylthio]phenylcinnamide (20) In the same manner as described for 2, it was prepared from 19 and cinnamoyl chloride in a yield of 86% as a yellowish crystal recrystallized from ethyl acetate–hexane, mp 157.5—158 °C. IR (KBr) cm⁻¹: 3440, 3268, 1658, 1608, 1576, 1529, 1442, 1348, 1284, 1178, 1148; 1H-NMR (CDCl₃): δ: 1.16—1.20 (4H, t, J = 7.0 Hz), 1.21—1.23 (6H, t, J = 6.8 Hz), 2.32—2.37 (2H, t, J = 15.8 Hz), 2.37—2.42 (2H, t, J = 14.5 Hz), 6.68 (1H, d, J = 7.0 Hz), 6.80 (1H, br, s), 8.14 (1H, s, br). MS m/z: 362 (M⁺), 270, 131 (100%), 103, 77; HR-MS (El) m/z: Calcd for C₁₅H₁₄N₂O₂S (M⁺): 362.0783. Found: 362.0785.

N-[2-(Phenylthio)phenylcinnamide (21) In the same manner as described for 21, it was prepared from cinnamoyl chloride and a yield of 80% as a yellow crystal recrystallized from ethyl acetate–hexane, mp 160—162 °C. IR (KBr) cm⁻¹: 3440, 3268, 1658, 1608, 1576, 1529, 1442, 1348, 1284, 1178, 1148; 1H-NMR (CDCl₃): δ: 1.16—1.20 (4H, t, J = 7.0 Hz), 1.21—1.23 (6H, t, J = 6.8 Hz), 2.10—2.14 (2H, t, J = 15.8 Hz), 2.30—2.40 (2H, t, J = 14.5 Hz), 6.69 (1H, d, J = 7.0 Hz), 6.82 (1H, br, s), 8.10 (1H, s, br). MS m/z: 346 (M⁺), 254, 131 (100%), 103, 77; HR-MS (El) m/z: Calcd for C₁₅H₁₄N₂O₂S (M⁺): 346.0737. Found: 346.0761.

N-[2-(2-Chlorophenyl)ethylthio]phenylcinnamide (22) In the same manner as described for 22, it was prepared from 15 and cinnamoyl chloride in a yield of 78% as a yellow crystal recrystallized from ethyl acetate–hexane, mp 151—153 °C. IR (KBr) cm⁻¹: 3440, 3268, 1658, 1608, 1576, 1529, 1442, 1348, 1284, 1178, 1148; 1H-NMR (CDCl₃): δ: 1.16—1.20 (4H, t, J = 7.0 Hz), 1.21—1.23 (6H, t, J = 6.8 Hz), 2.10—2.14 (2H, t, J = 15.8 Hz), 2.30—2.40 (2H, t, J = 14.5 Hz), 6.69 (1H, d, J = 7.0 Hz), 6.83 (1H, br, s), 8.13 (1H, s, br). MS m/z: 344 (M⁺), 252, 130 (100%), 103, 77; HR-MS (El) m/z: Calcd for C₁₅H₁₄N₂O₂S (M⁺): 344.0783. Found: 344.0785.


