Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Severe acute respiratory syndrome (SARS) has been recognized as a global threat. SARS is characterized by high fever, malaise rigor, headache, chills, cough, and progressive radiographic changes of the chest and lymphophenia. The initial outbreak of SARS was first identified in Guangdong Province, China in November 2002. This outbreak spread to several countries and has had significant health and economic impact. The mortality rate is nearly 10%. With rigorous effort by the world health organization (WHO), researchers found that SARS is caused by a novel coronavirus, SARS-CoV. The SARS-CoV is a positive-strand RNA virus and the genome is ~30 kb (Tor2 strain). The genome is constituted of five major open reading frames namely: replicase polyproteins, nucleocapsid proteins, spike (S), envelope (E), and membrane (M) glycoproteins.

Resulting of structural and functional studies of coronaviral life-cycle has provided a number of significant targets for ceasing the viral replication. During the viral replication, the replicase polyprotein undergoes extensive processing by two viral proteases namely, chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro), reside within the polyprotein. They catalyze their own release from the polyprotein and other non-structural proteins (nsp) from the polyproteins and initiate virus mediated RNA replication. Because of their essential roles in viral replication, both proteases are recognized as attractive targets for development of anti-SARS agents.

To date various SARS-CoV protease inhibitors have been reported from both screened compound libraries and designed compounds based on the substrate structure or active site properties. Their scaffolds are diverse, including C2-symmetric diols, 3-quinolinecarboxylic acid derivatives, thiophene-2-carboxylate derivatives, cinanserin, calmodulin, keto-glutamine analogues, anilide, bifunctional boronic acid compounds, isatin derivatives, benzo triazole as well as glutamic acid and glutamine peptides possessing a trifluoromethyl ketone group, and unsaturated esters, and etacrynic acid derivatives. With metal-conjugated structures, some molecules make a coordinate bond with Cys-145 at the active site of SARS-CoV 3CLpro. However, no effective therapy has been developed so far and recent isolation of strains of SARS-CoV emphasizes the possibility of a reemergence. Therefore, it is still a great challenge to explore new chemical classes of SARS-CoV 3CLpro inhibitors that can be used in anti-SARS therapy in case the disease re-emerges.

In our previous study, from high throughput screening we have identified various heterocycles as novel anti-SARS agents with selective inhibition ranging from IC50 2–10 μM against SARS-CoV 3CLpro (compounds 1–4, Fig. 1). In this paper, as a part of our ongoing efforts to delineate a complete pharmacophore model, we designed several 2-(benzylthio)-6-oxo-4-phenyl-1,6-dihydropyrimidine derivatives as anti-SARS agents (compounds 6a–n, Fig. 1).

From a synthetic point view, the preparation of the target compounds was envisioned following the synthetic routes illustrated in Scheme 1. The synthesis of 6-aryl-5-cyano-2-thiouracils was prepared by reaction between substituted benzaldehydes, ethyl cyanocetate, and thiourea using the literature procedure. The regioselective S-alkylation of 2-thiouracils achieved by slow addition of the respective halides to a solution of DMF
using \( \text{K}_2\text{CO}_3 \) as a base at 0–5°C to yield the corresponding compounds 6a–n. Both analytical and spectral data of all target compounds are accordant with the structures.

The target compounds were tested for anti-SARS activity against SARS-CoV 3CLpro, using previously developed assay method containing 0.05 \( \mu \text{M} \) SARS 3CLpro, 6 \( \mu \text{M} \) fluorogenic substrate Dabcyl-KTSAVLQSFGRKME-Edans, and 50 \( \mu \text{M} \) of test compounds. Enhanced fluorescence of the reactions in the buffer of 20 mM Bis–Tris at pH 7.0 was monitored at 538 nm with excitation at 355 nm using a fluorescence plate reader. The compounds which inhibited more than 50% of the protease activity at 50 \( \mu \text{M} \) were selected for the next assay run at 10 \( \mu \text{M} \) for IC\(_{50} \) calculation. Compound 6m with \( \text{R}^2 \) group of nitro functionality at C-4 position is the most potent inhibitor with an enzyme inhibitory activity against SARS-CoV 3CLpro with an IC\(_{50} \) of 6.1 \( \mu \text{M} \). The structure and IC\(_{50} \) values are given in Table 1. The cytotoxicity of the test compounds was tested by performing the MTT assay and found that all compounds are devoid of cytotoxicity.

To obtain molecular insight into the binding properties of these active compounds, we conducted docking studies in the 3CLpro active site. For modeling analysis, the crystal structure of SARS 3CLpro in complex with a peptidyl inhibitor (PDB code 1UK4) was used. Docking process was performed using an automated ligand-docking subprogram of the Discovery Studio Modeling 1.2 SBD (Accelrys Inc., San Diego, CA), with a set of parameters chosen to control the precise operation of the genetic algorithm. Docking runs were carried out using standard default settings ‘grid resolution’ of 5 \( \AA \), ‘site opening’ of 12 \( \AA \), and ‘binding site’ selected for defining the active site cavity.

| Compound | \( \text{R}^1 \) | \( \text{R}^2 \) | \( n \) | IC\(_{50} \) (\( \mu \text{M} \)) |
|----------|----------------|----------------|------|-----------------|
| 6a       | \( \text{H} \)  | \( \text{H} \)  | 1    | >50             |
| 6b       | \( \text{H} \)  | \( \text{H} \)  | 2    | >50             |
| 6c       | \( \text{H} \)  | 4-\( \text{NO}_2 \) | 1    | 35.2            |
| 6d       | 4-OCH\(_3\)  | \( \text{H} \)  | 1    | >50             |
| 6e       | 4-OCH\(_3\)  | 4-\( \text{NO}_2 \) | 1    | 20.3            |
| 6f       | 4-OCH\(_3\)  | 4-\( \text{NO}_2 \) | 1    | 26.3            |
| 6g       | 4-\( \text{CH}_3 \)  | \( \text{H} \)  | 1    | >50             |
| 6h       | 4-\( \text{CH}_3 \)  | \( \text{H} \)  | 2    | >50             |
| 6i       | 4-\( \text{CH}_3 \)  | 4-\( \text{NO}_2 \) | 1    | >50             |
| 6j       | 3-\( \text{NO}_2 \)  | \( \text{H} \)  | 2    | >50             |
| 6k       | 3-\( \text{NO}_2 \)  | 4-\( \text{NO}_2 \) | 1    | 10.6±1.2        |
| 6l       | 4-\( \text{Cl} \)  | \( \text{H} \)  | 2    | 16.9±1.3        |
| 6m       | 4-\( \text{Cl} \)  | 4-\( \text{NO}_2 \) | 1    | 6.1±1.1         |
| 6n       | 3-\( \text{Cl} \)  | \( \text{H} \)  | 1    | >50             |
Table 1). This result suggests that the substituent R1 can be electron donating groups like methyl and methoxy in the compound (see References and notes). Further investigations revealed by 3D docking give us new directions for a fast development of much more potent inhibitors. Further investigations on this new family of compounds are currently in progress.

References and notes

1. Kissäezek, T. G.; Erdman, D.; Goldsmiths, C.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, L.; Lim, W.; Nicholls, D.; Pei, J.; Shi, L.; Yang, K.; Yuen, K.-Y.; Kuo, T.-H.; Wang, A.-H.; Peng, H.-P.; Huang, H.-J.; Yam, L. Y.-C.; Tong, S.; Yuen, K.-Y.; Kwok, W.-Z.; Li, Y.; Wu, C.; Zhao, G.-P.; Chiu, R. W. K.; Chen, S. C. S.; Tong, Y.-K.; Chan, P. K. S.; Tam, J. S. L. "SARS-CoV-2 eukaryotic cell culture system by a fast development of much more potent inhibitors. Further investigations on this new family of compounds are currently in progress.

References and notes

1. Kissäezek, T. G.; Erdman, D.; Goldsmiths, C.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, L.; Lim, W.; Nicholls, D.; Pei, J.; Shi, L.; Yang, K.; Yuen, K.-Y.; Kuo, T.-H.; Wang, A.-H.; Peng, H.-P.; Huang, H.-J.; Yam, L. Y.-C.; Tong, S.; Yuen, K.-Y.; Kwok, W.-Z.; Li, Y.; Wu, C.; Zhao, G.-P.; Chiu, R. W. K.; Chen, S. C. S.; Tong, Y.-K.; Chan, P. K. S.; Tam, J. S. L. "SARS-CoV-2 eukaryotic cell culture system by a fast development of much more potent inhibitors. Further investigations on this new family of compounds are currently in progress.

References and notes

1. Kissäezek, T. G.; Erdman, D.; Goldsmiths, C.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, L.; Lim, W.; Nicholls, D.; Pei, J.; Shi, L.; Yang, K.; Yuen, K.-Y.; Kuo, T.-H.; Wang, A.-H.; Peng, H.-P.; Huang, H.-J.; Yam, L. Y.-C.; Tong, S.; Yuen, K.-Y.; Kwok, W.-Z.; Li, Y.; Wu, C.; Zhao, G.-P.; Chiu, R. W. K.; Chen, S. C. S.; Tong, Y.-K.; Chan, P. K. S.; Tam, J. S. L. "SARS-CoV-2 eukaryotic cell culture system by a fast development of much more potent inhibitors. Further investigations on this new family of compounds are currently in progress.

References and notes

1. Kissäezek, T. G.; Erdman, D.; Goldsmiths, C.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, L.; Lim, W.; Nicholls, D.; Pei, J.; Shi, L.; Yang, K.; Yuen, K.-Y.; Kuo, T.-H.; Wang, A.-H.; Peng, H.-P.; Huang, H.-J.; Yam, L. Y.-C.; Tong, S.; Yuen, K.-Y.; Kwok, W.-Z.; Li, Y.; Wu, C.; Zhao, G.-P.; Chiu, R. W. K.; Chen, S. C. S.; Tong, Y.-K.; Chan, P. K. S.; Tam, J. S. L. "SARS-CoV-2 eukaryotic cell culture system by a fast development of much more potent inhibitors. Further investigations on this new family of compounds are currently in progress.
**Compound 6m:** Yield: 66%; mp 254–257 °C; IR (KBr): 3000, 2218, 1651, 1517, 1467, 1346, 1244, 1009, 1004, 997, 856, 779 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ: 4.60 (s, 2H, CH₂), 7.51–7.53 (d, J = 8.64 Hz, 2H, ArH), 7.60–7.62 (d, J = 8.76 Hz, 2H, ArH), 7.89–7.91 (d, J = 8.64 Hz, 2H, ArH), 8.13–8.15 (d, J = 8.72 Hz, 2H, ArH), 12.88 (br, 1H, NH); MS (CI) m/z: 399 [M+H]+. Anal. Calcd for C₁₈H₁₁ClN₄O₃S: C, 54.21; H, 2.78; N, 14.05. Found: C, 54.18; H, 2.85; N, 14.01.

25. Kuo, C. J.; Chi, Y. H.; Hsu, J. T. A.; Liang, P. H. *Biochem. Biophys. Res. Commun.* 2004, 318, 862.
26. Yang, H.; Yang, M.; Ding, Y.; Liu, Y.; Lou, Z.; Zhou, Z.; Sun, L.; Mo, L.; Ye, S.; Pang, H.; Gao, G. F.; Anard, K.; Bartlam, M.; Hilgenfeld, R.; Rao, Z. *Proc. Natl. Acad. Sci. U.S.A.* 2003, 100, 13190.