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Techniques used for the discovery of therapeutic compounds: The case of SARS

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Severe acute respiratory syndrome coronavirus (SARS-CoV) is the etiological agent of SARS disease, which has ever severely menaced humans from the end of 2002 to June 2003. To date, great efforts have been made for the discovery of therapeutic compounds by using various technologies. In this report, we present a survey of these techniques and their applications in the development of promising anti-SARS agents.

Introduction

With rapid theoretical and technical progress across the biochemical and biophysical fields, extensive assay technologies have been developed for the discovery of effective therapeutic compounds in the post-genomic era. These assay formats can be divided into three main groups: in silico screening that can largely enrich the hit rate compared with the random screening; in vitro biochemical assays that evaluate the biological activity of compounds against the target proteins and cell-based assays that monitor the biological response to compounds in the cell. The outbreak of SARS epidemic in 2003 led to a worldwide threat to human health, and the novel severe acute respiratory syndrome coronavirus (SARS-CoV) was identified as the etiological agent [1]. SARS-CoV genome-encoded major proteins such as 3C-like protease (3CLₚ₅₉), are attractive targets for the development of anti-SARS agents due to their functional importance in the viral life cycle [2] (see Box 1). Some assay platforms for discovering effective inhibitors against SARS-CoV have been established. In this review, we outline the existing and emerging techniques used in anti-SARS drug discovery and discuss their potential promise, advantages and limitations.

Key technologies used in the discovery of anti-SARS agents

In the drug discovery pipeline, developing new and efficient strategies to distinguish active from inactive substances among large numbers of natural or synthetic compounds is critical at the primary screening stage [3]. Subsequent evaluation of the appropriate biochemical or cellular effects of the compounds is also needed in more effective and less expensive ways [4]. To date, much progress has been made in the discovery of techniques aiming at these goals (Fig. 1). In the following section, we summarize the reliable assay technologies used in the anti-SARS agent research.

In silico techniques

In silico techniques have clearly become one of the most powerful approaches to drug design, and they can provide
especially useful structural information on high-quality candidates to be followed up by biological evaluation. As a complementary approach to experimental high-throughput screening (HTS), virtual screening is a typical in silico technique used for screening databases of compounds [3]. This technique involves rapidly screening the compound database to dock into the active sites of the three-dimensional protein structures. Docking is a recognition procedure between the target protein and the compounds determined by geometric and energetics matching and the prerequisite for the success is the detailed understanding of the structural properties of the target protein and the criteria that determines the binding of the ligands [3,5]. In recent years, successful cases have revealed that structure-based virtual screening can hugely enrich the hit rate compared with random screening, and it has emerged as a reliable, inexpensive method for identifying lead compounds [6], when varied complementary docking algorithms are applied to improve the quality of the scoring function and decrease the relatively high false-positive rate of virtual screening [6].

Virtual screening has successfully been applied to the discovery of SARS-CoV inhibitors [7]. On the basis of the 3D model of SARS-CoV 3CL\textsuperscript{pro}, Xiong et al. reported a virtual screening study of a database of 73 protease inhibitors and discovered that several available protease inhibitors could be used for further anti-SARS agent research [7]. Along with the release of the crystal structures of SARS-CoV 3CL\textsuperscript{pro} and its complex with a specific inhibitor (PDB ID: 1Q2W, 1UK4)
[8,9], more virtual screening studies of structure-based anti-SARS drug design and discovery were performed [10]. The exploration of the available drugs through virtual screening technology against the small molecules database [11,12] and the identification of the available potential inhibitors [13] have provided an effective strategy to accelerate the SARS-CoV 3CL\textsuperscript{pro} inhibitor discovery process and improved the success rate.

**In vitro biochemical assays**

Usually in vitro biochemical assays may be employed for measuring the inhibitory activities of the tested compounds against an enzyme or determining the binding affinities of the compounds to the target protein. In most cases, a relatively high purity of the target protein is needed in the screening system. At present, the recombinant SARS-CoV 3CL\textsuperscript{pro} can be obtained at a high purity by expression in Escherichia coli [14] and the mammalian COS-7 cells [15]. According to its specific substrate activity [16], the quenched fluorescence resonance energy transfer (FRET) technology based assay, high performance liquid chromatography (HPLC) technique, enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) techniques were developed for potent inhibitors screening against SARS-CoV 3CL\textsuperscript{pro} [12,17–21].

**FRET technology: detection of proteolytic activity and screening of inhibitors**

FRET technology based assay is usually commonly used to monitor protease activity and identify inhibitors by an efficient and robust HTS mode [22]. During the assay, a pair of fluorophores as the fluorescent donor and acceptor (quencher) is attached to the terminus of the peptide substrate (one at each end). When the substrate is intact, the excited state energy of the donor is transferred to the acceptor and the fluorescence is quenched. Once the substrate is cleaved by the protease, the donor and acceptor are spatially separated and the subsequent fluorescence from the donor can be detected. To ensure an efficient internal quenching, commercially available donor/quencher pairs are available [22–27]. So far, many potential inhibitors of SARS-CoV 3CL\textsuperscript{pro} have been identified in vitro by the FRET-based assay, including C2 symmetric peptidomimetic compounds [28], metal-conjugated compounds [23], thiophenecarboxylated compounds [22], bifunctional aryl boronic acid compounds [18], a quinolinecarboxylate derivative [29], phthalhydrizide-substituted ketoglutamine analogues [19], dipeptidomimetic α,β-unsaturated esters [30] and a peptide anilide [25]. The major advantages of this technique are its sensitivity, speed and high-throughput, but it also has several disadvantages. Firstly, the fluorogenic substrate is relatively expensive for the special substrate preparation. Secondly, since the absorption spectra of many synthetic and natural products may often partly overlay the emission spectra of the fluorogenic donor (λem = 415~538 nm) [19,20,22,23,27,31], they could quench the fluorescence, thus generating false positives. Moreover, any trace contamination of the peptide substrate with free fluorogenic donor (e.g. EDANS) would result in a high fluorescence background [31].

**HPLC technique: detection of proteolytic activity and screening of inhibitors**

HPLC technique has been successfully used for detecting the proteolytic activity of SARS-CoV 3CL\textsuperscript{pro} in some cases [16,26], and it can be also applied to inhibitor screening. The inhibitory activities of the compounds against SARS-CoV 3CL\textsuperscript{pro} could be tested in the substrate-analog peptide cleavage assay. During the assay, the cleaved substrates or the cleavage products could be separated by reversed-phase HPLC, and the inhibitory efficiencies of the tested compounds were thus calculated by comparing the peak areas of the cleaved substrates or the products with those of the control substrates without the protease. Chen et al. discovered several potent SARS-CoV 3CL\textsuperscript{pro} inhibitors by screening a natural product library involving 720 compounds based on the HPLC method [21]. Because many compounds in this library are chromogenic, the FRET-based assay could not be used instead [21]. HPLC technique is thereby suitable for testing chromogenic compounds. However, it cannot be applied to screen enormous compounds in a high-throughput format because of the time-consuming (separation) and complicated experimental processes, and relatively poor sensitivity [20].

**ELISA technique: screening of inhibitors**

ELISA represents another recently developed method for screening inhibitors against SARS-CoV 3CL\textsuperscript{pro} [32]. The cleavage substrate (TVRLQAGNATE) was generated as a fusion protein with the N-terminal S-Tag and the C-terminal HSV-Tag. In the assay system, the substrate fusion protein was captured by anti-HSV-Tag antibodies in wells, and then incubated with SARS-CoV 3CL\textsuperscript{pro}. The non-cleavage substrate protein was detected by ELISA using the peroxidase-conjugated S protein and ABTS/H\textsubscript{2}O\textsubscript{2} substrates. The inhibitory efficiencies of the tested compounds were thus evaluated according to the cleavage activities with or without the compounds. This technique is generally more sensitive than the previously mentioned methods, whereas the reagents used are expensive and the experimental procedure is tedious. Moreover, incomplete wash of the non-captured substrate fusion protein will lead to increased false positives.

**SPR technique: determining binding properties**

Besides the inhibitory activity determination, analysis of the interactions between the compounds and the target protein is also a key part of the drug discovery process [33]. As a method to screen compounds for receptor binding in vitro, SPR...
biosensor technique can provide detailed information on the binding affinity and binding kinetics [33]. The technique requires immobilizing the target protein on the surface of a sensor chip and monitoring its binding to the candidate compounds. The binding of the compounds to the target protein will cause changes in the refractive index at the surface layer of the sensor chip, which are detected as changes in the signal of an optical phenomenon termed surface plasmon resonance (SPR) [33]. To date, there are many successful examples for drug screening by SPR-based assay [34,35]. We have recently reported the discovery of low molecular SARS-CoV 3CL\textsuperscript{pro} inhibitors by using SPR-based biosensor technology [12]. Compared with other techniques in measuring molecular interactions, SPR-based assay exhibits several distinct advantages, such as label-free, sensitive, real-time, noninvasive measurements, low sample consumption and high throughput. This technique could help a rapid selection and a following optimization of a lead compound based on the detailed understanding of how the compound interacts with the target protein. However, in some cases immobilization of the target protein on the sensor chip might affect its structural conformation, and some hits obtained by SPR-based assays are probably nonspecific binding molecules that will show no inhibitory activities. To solve this problem, the positive compounds must be used for corroborating the validity of the target protein, and the hits should be further tested in enzymatic inhibitory assays. As such, SPR technology is more applicable to primary screening ahead of enzymatic inhibitory assays.

In addition, several other new \textit{in vitro} techniques have recently been reported in the literature [18,36,37]. For example, immobilized metal affinity chromatography (IMAC) and microarray techniques were applied to prepare a protein microarray for the recombinant His-Tagged SARS-CoV 3CL\textsuperscript{pro} [36]. The SARS-CoV 3CL\textsuperscript{pro}-immobilized chip could be thus used for corresponding high-throughput inhibitors screening and coupled with other methods for detecting the binding affinity or enzymatic inhibitory activity. Affinity capillary electrophoresis (ACE) is another reported method, which can be developed as an effective and simple way of large-scale drug screening and evaluation [37]. This method mainly determines the binding constants of the tested compounds by evaluating the changes in the migration time of the inhibitors at different concentrations of SARS-CoV 3CL\textsuperscript{pro}.

In principle, these two methods are drug discovery techniques with high efficiency, short analysis time, ease of automation, and further in-depth inhibitors investigation should be carried out. Furthermore, isothermal titration calorimetry (ITC) technology affords a promising platform for studying inhibitor-protein interactions, which can determine the binding affinity, stoichiometry, and thermodynamic parameters in a single injection experiment [18]. However, this method is unsuitable for large-scale inhibitors screening due to a relatively high sample consumption and long experimental duration.

\textbf{Cell-based assays}

After compounds have been identified as potential inhibitors against the target protein \textit{in vitro}, it is necessary to establish relevant cellular or physiological systems to test their inhibitory efficacy \textit{in vivo}. Cell-based assay systems (thoroughly reviewed in [38]) have significant advantages over \textit{in vitro} assays. Compared with \textit{in vitro} screening methods, cell-based assays do not require the purification of the target protein, and are more close to the normal physiological situation. Moreover, cell-based assays can provide valuable insights into the bioavailability and cytotoxicity of the tested compounds. However, we must realize that the experimental period of cell-based assays is relatively longer than \textit{in vitro} assays.

For the discovery of anti-SARS agents, the most popular method in cell-based assays is to directly test the antiviral activities of the compounds. Several groups [12,13,28,29] have reported the use if mammalian cells for anti-SARS inhibitors screening. Vero cells (a cell line with the hypodiploid chromosome count and initiating from the kidney of a normal adult African green monkey, extensively used for detection of virus infection) were incubated with different concentrations of the tested compounds, and then infected with SARS-CoV. The degree of protection offered by the tested compounds against SARS-CoV infection was then measured by Vero cells: cytopathic effect, plaque reduction assay [29] or the virus RNA concentration in the supernatant [12]. The cytotoxicity of the compounds could be determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) [12,29] or MTS (the tetrazolium salts) assay [23,28]. Moreover, western blot analysis by anti-spike (S) protein monoclonal or polyclonal antibody, immunofluorescence assay (IFA), flow cytometry assays, and ELISA techniques were applied to further characterize the action mechanism of the active compounds [23,28].

The cell-based \textit{cis}-cleavage assay is another cell-based method for directly testing the inhibitory efficacy of the compounds against SARS-CoV 3CL\textsuperscript{pro} \textit{in vivo} [32]. As reported, the gene of SARS-CoV 3CL\textsuperscript{pro}, its specific substrate sequence, and the luciferase gene were co-constructed into the vector pcDNA3.1, subsequently transfected into Vero cells, and the stable cell line expressing the 3CL\textsuperscript{pro}-S-Luc fusion protein was selected. Since SARS-CoV 3CL\textsuperscript{pro} (more than 30 kDa) fused at the N-terminus of the luciferase resulted in a dramatic decrease of luciferase activity [39], the increase of luciferase activity could be considered to represent \textit{in vivo} \textit{cis}-cleavage activity of SARS-CoV 3CL\textsuperscript{pro}. The inhibitory activities were calculated by measuring the luciferase activities in the presence of the tested compounds at varied concentrations. In considering the fact that sometimes the inhibitory activity against SARS-CoV 3CL\textsuperscript{pro} might show a poor correlation to
effective antiviral activity, the cell-based assay for directly detecting the antiviral activities of the compounds is more superior to the cell-based cis-cleavage assay.

Conclusions
Since the outbreak of SARS, great efforts have been devoted to anti-SARS treatment. Over the past 3 years we have gained valuable knowledge of SARS-CoV, including its phylogenetic property and genome organization, as well as the structural and functional characters of major proteins involved in viral life cycle. Although the disease has been controlled by conventional measures such as rapid detection, infection control, isolation, quarantine, no efficacious therapy and drugs against SARS are available to date. This review gives a brief summary of the existing and emerging technologies being applied to the discovery of anti-SARS agents (Table 1).

Up to now, there are mainly two feasible strategies for therapeutic compounds identification against the SARS disease. One is the target-based drug discovery by *in vitro* biochemical assay, for example screening the inhibitors that can inhibit the activity or bind to the substrate-binding pocket of SARS-CoV encoded enzyme, for example 3CLpro. Most reported inhibitors discovery studies have adopted this pattern. The other is cell-based assay, measuring the antivirus effects of the compounds firstly and then determining the possible target protein of the active compound. While the target-based drug discovery is more timesaving and convenient compared with the latter method, which must be processed in biosafety level 3 laboratory and the screening throughput is relatively low. Despite all that, experimental screening is always a slow and laborious procedure. However, virtual screening approach based on the 3D structure of SARS-CoV encoded major proteins can reliably narrow down the number of potential candidates

| Techniques | Stage of discovery used | Advantages | Disadvantages | Refs |
|------------|-------------------------|------------|---------------|------|
| **In silico techniques** | | | | |
| Virtual screening | Discovery of compounds for target protein binding | Enrich the hit rate, reliable, inexpensive and rapid | Relatively high false-positive rate | [7,10,11] |
| **In vitro biochemical assays** | | | | |
| FRET<sup>a</sup> based assay | Detection of proteolytic activity; screening of inhibitors, measurement of IC50, Ki values and inhibition type | Sensitive, less time-consuming and high-throughput | Relatively expensive, unsuitable for some synthetic and natural compounds | [18,19,22,23,25,28–30] |
| HPLC<sup>b</sup> technique | Detection of proteolytic activity, screening of inhibitors, primary inhibitory activities determination | Suitable for testing the chromogenic compounds | Time-consuming, complicated experimental processes and relatively poor sensitivity | [20,21] |
| ELISA<sup>c</sup> method | Screening of inhibitors | Very sensitive | Expensive, tedious experimental procedure, high false-positive rate | [32] |
| SPR<sup>d</sup> technique | Discovery of compounds for target binding, measurement of binding affinity | Label-free, sensitive, noninvasive, low sample consumption and high throughput. | Nonspecific binding, no inhibitory activities available | [12,34,35] |
| **Other techniques** | | | | |
| IMAC<sup>e</sup> and microarray, ACE<sup>f</sup> method | Discovery of compounds for target binding, measurement of binding affinity | High efficiency, short analysis time, ease of automation | No inhibitory activities available | [36,37] |
| ITC<sup>g</sup> technique | High contents (the binding affinity, stoichiometry, thermodynamic parameters | High sample consumption and long experimental duration | n/a | |
| **Cell-based assays** | | | | |
| Direct determination of antiviral activity | Discovery of inhibitors against SARS-CoV infection directly | *In vivo*, close to the normal physiological situation, available for bioavailability and cytotoxicity | Time-consuming, lack of the detailed action mechanism of the active compounds | [12,13,28,29] |
| Cis-cleavage assay | Screening of inhibitors | Target specific, available for bioavailability and cytotoxicity | Long experimental duration, no antiviral activities available | [32] |

<sup>a</sup> Fluorescence resonance energy transfer.  
<sup>b</sup> High performance liquid chromatography.  
<sup>c</sup> Enzyme-linked immunosorbent assay.  
<sup>d</sup> Surface plasmon resonance.  
<sup>e</sup> Immobilized metal affinity chromatography.  
<sup>f</sup> Affinity capillary electrophoresis.  
<sup>g</sup> Isothermal titration calorimetry.
before experimental testing. After finishing the virtual screening by setting kinds of filters, the hits obtained by purchase or synthesis can be used for further enzymatic or cell-based inhibitory activities determination. At the same time, structural optimization of the lead compound and quantification of the structure-activity relationship may be considered in the drug discovery strategy by further structural alteration and biological evaluation. Therefore, virtual screening is a good choice at the beginning for the discovery of inhibitors ahead of the target-based or cell-based assays.

For in vitro biochemical assays, SPR technology can determine the binding of the tested compounds against the target proteins, this method could be applied before the enzymatic inhibitory assays thus to reduce the screening scale. In the following enzymatic inhibitory assays, FRET method is practical to identify potent inhibitors in an efficient and robust HTS mode, but it has some limitations from the chromogenic compounds. While HPLC method can make up this weakness. Therefore, the two complementary techniques may be integrated for the effective discovery of inhibitors against SARS-CoV. At the same time, ELISA, ACE and other techniques may also provide useful information about the binding affinities or inhibitory activities of the tested compounds. In addition, cell-based assays, which assess antiviral activities of the compounds in a biological system close to the physiological environment and allow for an earlier indication of potential compound toxicity, can offer an attractive alternative to in vitro biochemical assays.

With the development of modern combinatorial chemistry and HTS approaches, the drug discovery process has been much accelerated, and integration of different complementary techniques may be the optimal drug discovery strategy. The successful identification of extensive anti-SARS inhibitors has proven an interdisciplinary paradigm for the discovery of therapeutic compounds by combining different techniques. A similar approach could be applied to the discovery of therapeutics against other viruses such as the highly pathogenic Avian Influenza Virus.

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References
1 Rota, P.A. et al. (2003) Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300, 1394–1399
2 Anand, K. et al. (2003) Coronavirus main protease (3CLpro) structure: basis for design of anti-SARS drugs. Science 300, 1763–1767
3 Jenkins, J.L. et al. (2003) Virtual screening to enrich hit lists from high-throughput screening: a case study on small-molecule inhibitors of angiogenin. Proteins 50, 81–93
4 Smith, A. (2002) Screening for drug discovery: the leading question. Nature 418, 453–459
5 Kraemer, O. et al. (2004) Virtual screening for inhibitors of human aldose reductase. Proteins 55, 814–823
6 Lyne, P.D. (2002) Structure-based virtual screening: an overview. Drug Discov. Today 7, 1047–1055
7 Xiong, B. et al. (2003) A 3D model of SARS_CoV 3CL proteinase and its inhibitors design by virtual screening. Acta. Pharmacol. Sin. 24, 497–504
8 Yang, H. et al. (2003) The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc. Natl. Acad. Sci. U S A 100, 13190–13195
9 Bonanno, J.B. et al. (2003) X-ray crystal structure of the SARS Coronavirus main protease. Available from: http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=1q2w
10 Liu, Z. et al. (2005) Virtual screening of novel noncovalent inhibitors for SARS-CoV 3C-like proteinase. J. Chem. Inf. Model 45, 10–17
11 Rajnarayanan, R.V. et al. (2004) Teaching old drugs to kill new bugs: structure-based discovery of anti-SARS drugs. Biochem. Biophys. Res. Commun. 321, 370–378
12 Chen, L. et al. (2005) Cinanserin is an inhibitor of the 3C-like protease of severe acute respiratory syndrome coronavirus and strongly reduces virus replication in vitro. J. Virol. 79, 7095–7103
13 Dooley, A.J. et al. (2006) From genome to drug lead: identification of a small-molecule inhibitor of the SARS virus. Bioorg. Med. Chem. Lett. 16, 830–833
14 Sun, H. et al. (2003) Molecular cloning, expression, purification, and mass spectrometric characterization of 3C-like protease of SARS coronavirus. Protein Expr. Purif. 32, 302–308
15 Lu, J.H. et al. (2005) Variation analysis of the severe acute respiratory syndrome coronavirus putative non-structural protein 2 gene and construction of three-dimensional model. Chin. Med. J. (Engl.) 118, 707–713
16 Fan, K. et al. (2004) Biosynthesis, purification, and substrate specificity of severe acute respiratory syndrome coronavirus 3C-like proteinase. J. Biol. Chem. 279, 1637–1642
17 Lin, C.W. et al. (2004) Characterization of trans- and cis-cleavage activity of the SARS coronavirus 3CLpro protease: basis for the in vitro screening of anti-SARS drugs. FEBS Lett. 574, 131–137
18 Bacha, U. et al. (2004) Identification of novel inhibitors of the SARS coronavirus main protease 3CLpro. Biochemistry 43, 4906–4912
19 Jain, R.P. et al. (2004) Synthesis and evaluation of keto-glutamine analogues as potent inhibitors of severe acute respiratory syndrome 3CLpro. J. Med. Chem. 47, 6113–6116
20 Kuo, C.J. et al. (2004) Characterization of SARS main protease and inhibitor assay using a fluorogenic substrate. Biochem. Biophys. Res. Commun. 318, 862–867
21 Chen, C.N. et al. (2005) Inhibition of SARS-CoV 3C-like Protease Activity by Theaflavin-3,3’-digallicate (TF3). Evid Based Complement Alternat. Med. 2, 209–215
22 Blanchard, J.E. et al. (2004) High-Throughput Screening Identifies Inhibitors of the SARS Coronavirus Main Proteinase. Chem. Biol. 11, 1445–1453
23 Hsu, J.T. et al. (2004) Evaluation of metal-conjugated compounds as inhibitors of 3CL protease of SARS-CoV. FEBS Lett. 574, 116–120
24 Hsu, W.C. et al. (2005) Critical assessment of important regions in the subunit association and catalytic action of the severe acute respiratory syndrome coronavirus main protease. J. Biol. Chem. 280, 22741–22748
25 Shie, J.J. et al. (2005) Discovery of potent anilide inhibitors against the severe acute respiratory syndrome 3CL protease. J. Med. Chem. 48, 4469–4473
26 Chou, C.Y. et al. (2004) Quaternary structure of the severe acute respiratory syndrome (SARS) coronavirus main protease. Biochemistry 43, 14958–14970
27 Kuang, W.F. et al. (2005) Mutational and inhibitive analysis of SARS coronavirus 3C-like protease by fluorescence resonance energy transfer-based assays. Biochem. Biophys. Res. Commun. 331, 1554–1559
28 Wu, C.Y. et al. (2004) Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc. Natl Acad. Sci. U S A 101, 10012–10017
29 Kao, R.Y. et al. (2004) Characterization of SARS-CoV main protease and identification of biologically active small molecule inhibitors using a continuous fluorescence-based assay. FEBS Lett. 576, 325–330
30 Shie, J.J. et al. (2005) Inhibition of the severe acute respiratory syndrome 3CL protease by peptidomimetic alpha, beta-unsaturated esters. Bioorg. Med. Chem. 13, 5240–5252
31 Sittampalam, G.S. et al. (1997) High-throughput screening: advances in assay technologies. Curr. Opin. Chem. Biol. 1, 384–391
32 Lin, C.W. et al. (2005) Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. Antiviral Res. 68, 36–42
33 Cooper, M.A. (2002) Optical biosensors in drug discovery. Nat. Rev. Drug Discov. 1, 515–528
34 Fagerstam, L.G. et al. (1992) Biospecific interaction analysis using surface plasmon resonance detection applied to kinetic, binding site and concentration analysis. J. Chromatogr. 597, 397–410
35 Markgren, P.O. et al. (1998) Screening of compounds interacting with HIV-1 protease using optical biosensor technology. Anal. Biochem. 265, 340–350
36 Sinchaikul, S. et al. (2005) Protein microarray using alpha-amino acids as metal tags on chips. Bioorg. Med. Chem. Lett. 15, 1413–1416
37 Ding, L. et al. (2005) The interaction between severe acute respiratory syndrome coronavirus 3C-like proteinase and a dimeric inhibitor by capillary electrophoresis. Anal. Biochem. 343, 159–165
38 Silverman, L. et al. (1998) New assay technologies for high-throughput screening. Curr. Opin. Chem. Biol. 2, 397–403
39 Joubert, P. et al. (2000) Identification of a new cleavage site of the 3C-like protease of rabbit haemorrhagic disease virus. J. Gen. Virol. 81 (Pt 2), 481–488
40 Han, Y.S. et al. (2005) Papain-Like Protease 2 (PLP2) from Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV): Expression, Purification, Characterization, and Inhibition. Biochemistry 44, 10349–10359
41 Ratia, K. et al. (2006) Severe acute respiratory syndrome coronavirus papain-like protease: Structure of a viral deubiquitinating enzyme. Proc. Natl. Acad. Sci. U S A 103, 5717–5722
42 Cheng, A. et al. (2005) Expression, purification, and characterization of SARS coronavirus RNA polymerase. Virology 335, 165–176
43 Wong, S.K. et al. (2004) A 193-Amino Acid Fragment of the SARS Coronavirus S Protein Efficiently Binds Angiotensin-converting Enzyme 2. J. Biol. Chem. 279, 3197–3201