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.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 57–62
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Generation of predictive pharmacophore model for SARS-coronavirus main proteinase

Xue Wu Zhang *, Yee Leng Yap, Ralf M. Altmeyer

Department of Bioinformatics, HKU-Pasteur Research Center, 8 Sassoon Road, Pokfulam, Hong Kong

Received 14 June 2004; accepted 15 September 2004

Available online 05 November 2004

Abstract

Pharmacophore-based virtual screening is an effective, inexpensive and fast approach to discovering useful starting points for drug discovery. In this study, we developed a pharmacophore model for the main proteinase of severe acute respiratory syndrome coronavirus (SARS-CoV). Then we used this pharmacophore model to search NCI 3D database including 250, 251 compounds and identified 30 existing drugs containing the pharmacophore query. Among them are six compounds that already exhibited anti-SARS-CoV activity experimentally. This means that our pharmacophore model can lead to the discovery of potent anti-SARS-CoV inhibitors or promising lead compounds for further SARS-CoV main proteinase inhibitor development.

Keywords: SARS-CoV; Pharmacophore; Virtual screening; Drug design

1. Introduction

The infection of the newly emerged severe acute respiratory syndrome coronavirus (SARS-CoV) is characterized by acute flu-like symptoms that progress to acute lung injury or acute respiratory distress syndrome with over 10% of mortality [1]. To date there are no universally recommended therapy for the disease. Many scientists are now making efforts to develop effective drugs against SARS. The combination therapy of corticosteroid with lopinavir, ribavirin and ritonavir can improve clinical response and reduce mortality rates apparently [2,3]. Cinatl et al. [4] found that ribavirin, azauridine, pyrazofurin and glycyrrhizin are active against SARS-CoV. Barnard et al. [5] reported that calpain inhibitors and β-D-N^4-hydroxycytidine exhibit inhibitory effects on SARS-CoV.

Structure-based drug design focuses on two important approaches: one is receptor-based docking technique, another is pharmacophore-based virtual screening technique. A pharmacophore is the 3D arrangement of atoms or functional groups essential for the compound to bind to a specific receptor [6]. The power of a pharmacophore model is to discover new leads by using 3D database pharmacophore searching and guide chemists to synthesize new compounds [7]. Such a pharmacophore-based method has been successfully applied to many drug development programs [8–18]. The main proteinase of SARS-CoV plays an important role in virus replication and is the primary target for drugs. The aim of this study is to develop 3D pharmacophore models for SARS-CoV main proteinase and expect to provide useful knowledge for anti-SARS drug design.

2. Material and methods

There are two methods to derive a reasonable pharmacophore model. One is from the crystal structure of protein–ligand complex, another is based on molecular modeling of enzyme with its potential inhibitors. Here we used the experimental structure of SARS-CoV main proteinase complexed with its peptide inhibitor CMK (PDB ID 1UK4) [19] and the predicted structures of SARS-CoV main proteinase with six drugs/compounds [20] for establishing pharmacophore models. The structures of CMK peptide and six compounds are shown in Fig. 1.

* Corresponding author. Tel.: +852 2816 8407; fax: +852 2872 5782.
E-mail address: xwzhang@hkucc.hku.hk (X.W. Zhang).

0223-5234/$ - see front matter © 2004 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.09.013

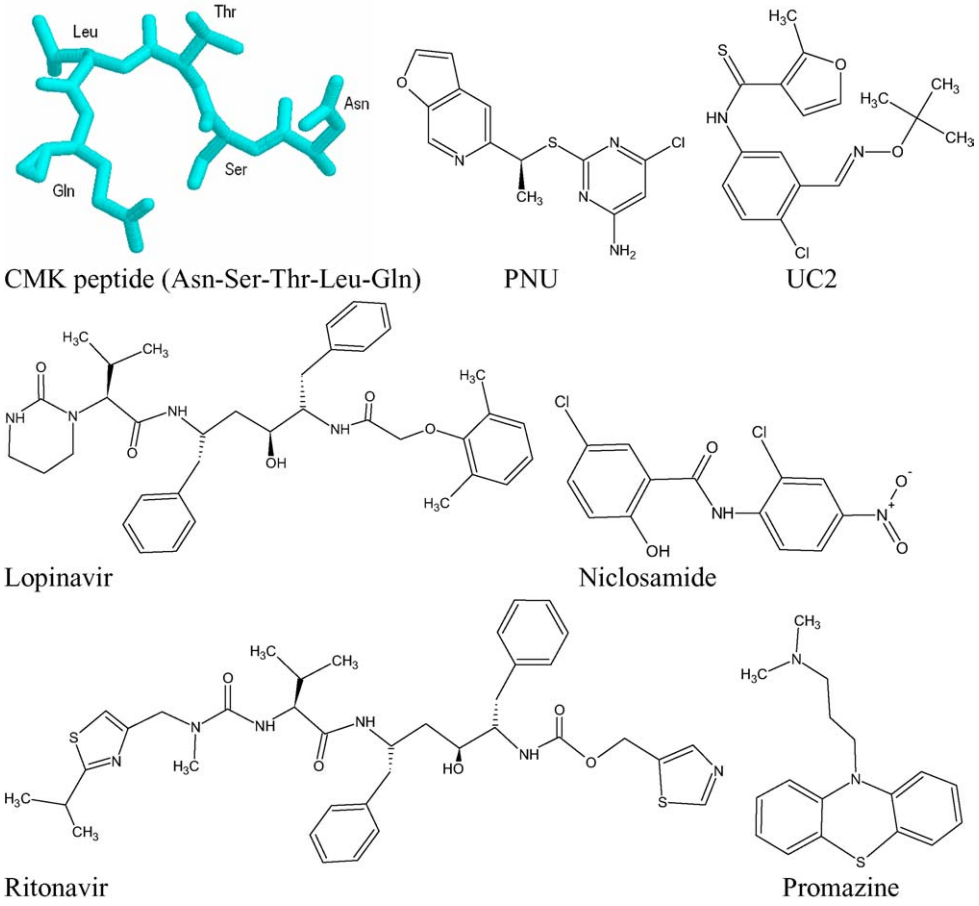

Fig. 1. The peptide and compounds used for pharmacophore generation.

The POCKET module in LigBuilder program [21] was employed to obtain the pharmacophore models of SARS-CoV main proteinase. This approach was successfully applied to the identification of novel inhibitors for alanine racemase [22]. The proposed pharmacophore model is a binding-site-derived pharmacophore model, which includes the following pharmacophore features of ligands binding to the enzyme's active site: a positively charged nitrogen atom (ammonium cation) to represent a hydrogen bond donor (HBD), a negatively charged oxygen atom (as in a carboxyl group) to represent a hydrogen bond acceptor (HBA), and a carbon atom (methane) to represent a hydrophobic center (HPC). A pharmacophore model is generated for each protein–ligand complex.

3. Results and discussion

Using CMK peptide and six compounds in Fig. 1, we generated a set of seven eight-point pharmacophore models for SARS-CoV main proteinase, which is listed in Table 1. These hypotheses exhibit different features due to the diversities of the compounds involved. Based on these models, we extracted a common four-point pharmacophore distance pattern shown in Fig. 2, P1 is HBA, HBD and HPC, P2 is HBA and HPC, P3 is HBA and HBD, P4 is HBA and HBD. Such a pharmacophore distance pattern was subsequently used for the 3D database search.

The pharmacophore searching in 3D database was conducted in the 3D NCI database, which has 250, 251 open structures ready for searching. Table 2 summarizes the results for similarity search of above-mentioned four-point pharmacophore model with constraints: (1) the distance ranges for P1P2, P1P3, P2P3, P1P4, P2P4 and P3P4 (Fig. 2) are 2–3, 2–3, 1.5–2.5, 5–6, 3–4 and 3–4 Å, respectively; (2) the compound is drug; (3) the antiviral probability is over 70%; (4) the compounds labeled as "No Name" is not in-

Table 1
The eight-point pharmacophore models obtained by LigBuilder from seven peptide and compounds

Pharmacophore	Peptide/compounds	Features
1	CMK peptide	HBD HBD HPC HBD HPC HBD HPC HBA
2	Lopinavir	HBA HBD HBA HBD HBA HBA HBA HBA
3	Ritonavir	HPC HPC HBD HBD HBD HBA HBD HBA
4	Niclosamide	HBA HBD HBA HBA HBA HBD HPC HBA
5	Promazine	HBA HBD HBA HPC HBA HBA HBA HBA
6	PNU	HBA HPC HBA HBD HBD HBA HBA HBA
7	UC2	HPC HBD HBD HBA HBD HPC HPC HBA

HBA = hydrogen bond acceptor, HBD = hydrogen bond donor, HPC = hydrophobic center.

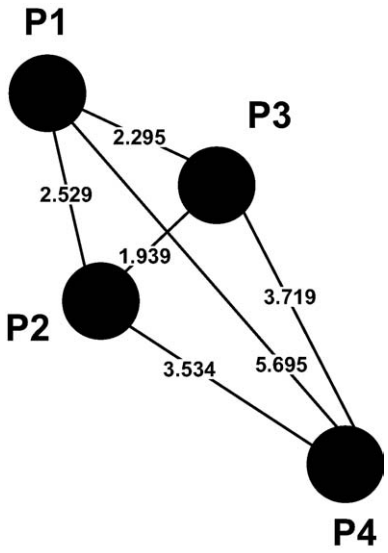

Fig. 2. Four-point pharmacophore distance pattern for SARS-CoV main proteinase. Here, P1 is HBA, HBD and HPC, P2 is HBA and HPC, P3 is HBA and HBD, P4 is HBA and HBD.

Table 2
Summary of NCI database search by four-point pharmacophores

Pharmacophore features in Fig. 2				Hits
P1	P2	P3	P4	
HBD	HBA	HBD	HBD	987
HBA	HBA	HBA	HBA	794
HPC	HBA	HBA	HBD	286
HPC	HPC	HBA	HBD	305
HPC	HBA	HBD	HBD	298
HBA	HBA	HBD	HBD	881
HBA	HBA	HBA	HBD	895

HBA = hydrogen bond acceptor, HBD = hydrogen bond donor, HPC = hydrophobic center.

cluded. After review of these hitlists, 30 drugs were selected for further analysis, their chemical structures and bioactivities (documented in anti-HIV and anti-opportunistic infection chemical compound database, which contains approximately 100,000 compounds, http://www.apps1.niaid.nih.gov/struct_search/an/an_search.htm) are shown in Fig. 3 and Table 3, respectively. It is noted that almost all drugs exhibited anti-HIV activity, and some of them have activities against *Mycobacterium tuberculosis* (puromycin), influenza

Table 3
Thirty drugs obtained by four-point pharmacophore search in NCI 3D database

Name	NSC number	Formula	Bioactivity documented in HIV/OI therapeutics database (http://www.apps1.niaid.nih.gov/struct_search/an/an_search.htm)
PD-ADI	218321	$C_{11}H_{16}N_4O_4$	HIV
Coformycin	277817	$C_{11}H_{16}N_4O_5$	HIV
Zidovudine	602670	$C_{10}H_{13}N_5O_4$	HIV, HSV, human cytomegalovirus, vaccinia virus, cowpox virus
Vira-A	404241	$C_{10}H_{13}N_5O_4$	HIV, HSV, human cytomegalovirus, varicella-zoster virus, vaccinia virus, cowpox virus
Angustmycin C	53104	$C_{11}H_{15}N_5O_5$	
ARA-AMP	259272	$C_{10}H_{14}N_5O_7P$	HIV, HSV, vaccinia virus
Cordycepin	63984	$C_{10}H_{13}N_5O_3$	HIV
Triciribine	154020	$C_{13}H_{16}N_6O_4$	HIV, human cytomegalovirus, HSV
5-AZCR	102816	$C_8H_{12}N_4O_5$	HIV
Puromycin	3055	$C_{22}H_{29}N_7O_5$	HIV, *M. tuberculosis*
CHETOMIN	289491	$C_{31}H_{30}N_6O_6S_4$	HIV
Vengicide	99843	$C_{12}H_{13}N_5O_4$	Human cytomegalovirus
Spongothymidin	68929	$C_{10}H_{14}N_2O_6$	HIV, HSV, varicella-zoster virus
Arauridine	68928	$C_9H_{12}N_2O_6$	
P-Ara-C	135962	$C_{25}H_{43}N_3O_6$	HIV
Pyrazofurin	143095	$C_9H_{13}N_3O_6$	HIV, vaccinia virus, West Nile virus
Thymidin	21548	$C_{10}H_{14}N_2O_5$	HIV, varicella-zoster virus
Radibud	38297	$C_9H_{11}BrN_2O_5$	
Alexan	63878	$C_9H_{13}N_3O_5$	HIV, HSV, human cytomegalovirus, varicella-zoster virus, measles virus
Floxuridin	27640	$C_9H_{11}FN_2O_5$	HIV, HSV, vaccinia virus
Gemcitabine	613327	$C_9H_{12}ClF_2N_3O_4$	HIV, cowpox virus, vaccinia virus
Dideoxyguanosine	619072	$C_{10}H_{13}N_5O_3$	HIV, HBV
2′,3′-Dideoxycytidine	606170	$C_9H_{13}N_3O_3$	HIV, HBV
5-Bromo-2′-deoxycytidine	61765	$C_9H_{12}BrN_3O_4$	HSV
Ribavirin	163039	$C_8H_{12}N_4O_5$	HIV, HSV, HCV, influenza virus, dengue virus, measles virus, respiratory syncytial virus, rhinovirus, polio virus, vaccinia virus, cowpox virus
Azauridine	32074	$C_8H_{11}N_3O_6$	West Nile virus, cowpox virus, vaccinia virus, dengue virus, Japanese encephalitis virus, Yellow fever virus
Fialuridine	678514	$C_9H_{10}FIN_2O_5$	Cowpox virus, vaccinia virus, HSV, varicella-zoster virus
Emanil	39661	$C_9H_{11}IN_2O_5$	HIV, HSV, cowpox virus, vaccinia virus, varicella-zoster virus
Tubercidin	56408	$C_{11}H_{14}N_4O_4$	HIV, vaccinia virus, human cytomegalovirus
Viroptic	75520	$C_{10}H_{11}F_3N_2O_5$	HSV, cowpox virus, vaccinia virus

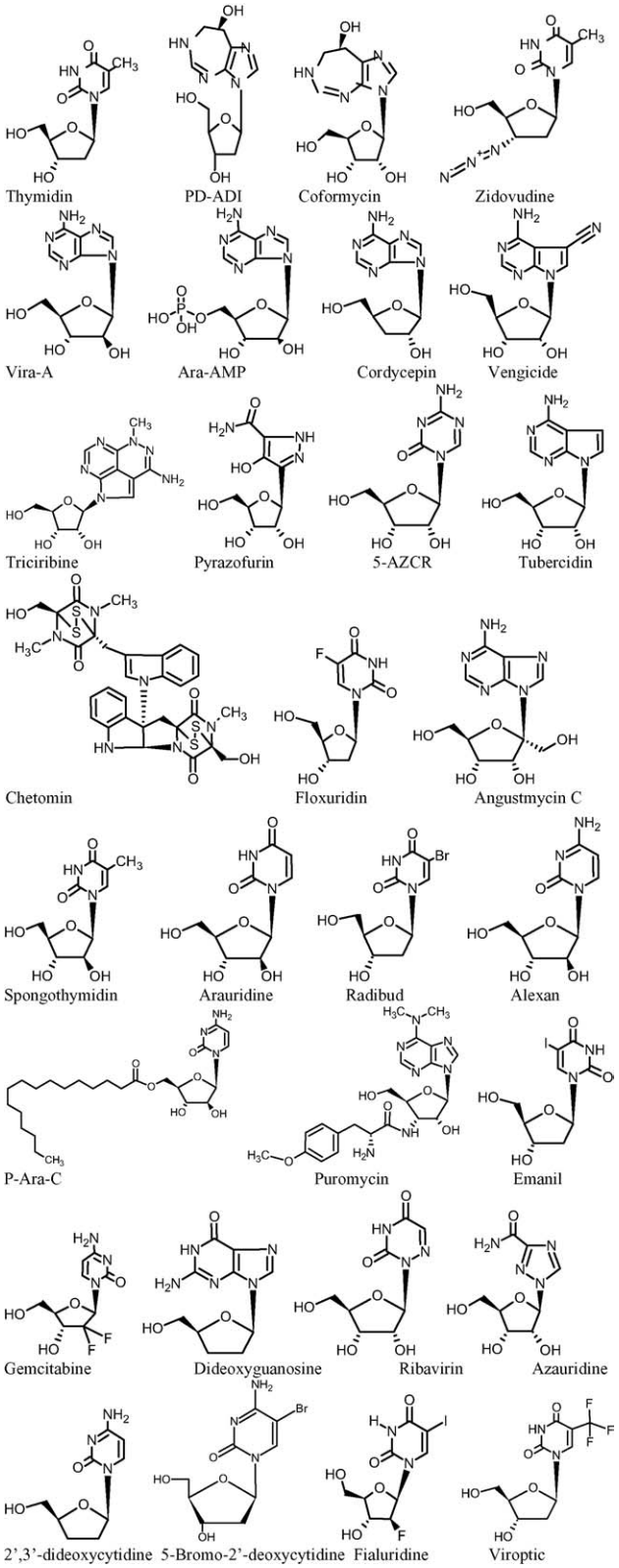

Fig. 3. Chemical structures of 30 drugs obtained by four-point pharmacophore search in NCI 3D database.

virus (5-bromo-2′-deoxycytidine), hepatitis virus (dideoxyguanosine, 2′,3′-dideoxycytidine and ribavirin), dengue virus (azauridine and ribavirin), rhinovirus and poliovirus (ribavirin). This should be very meaningful in consideration of

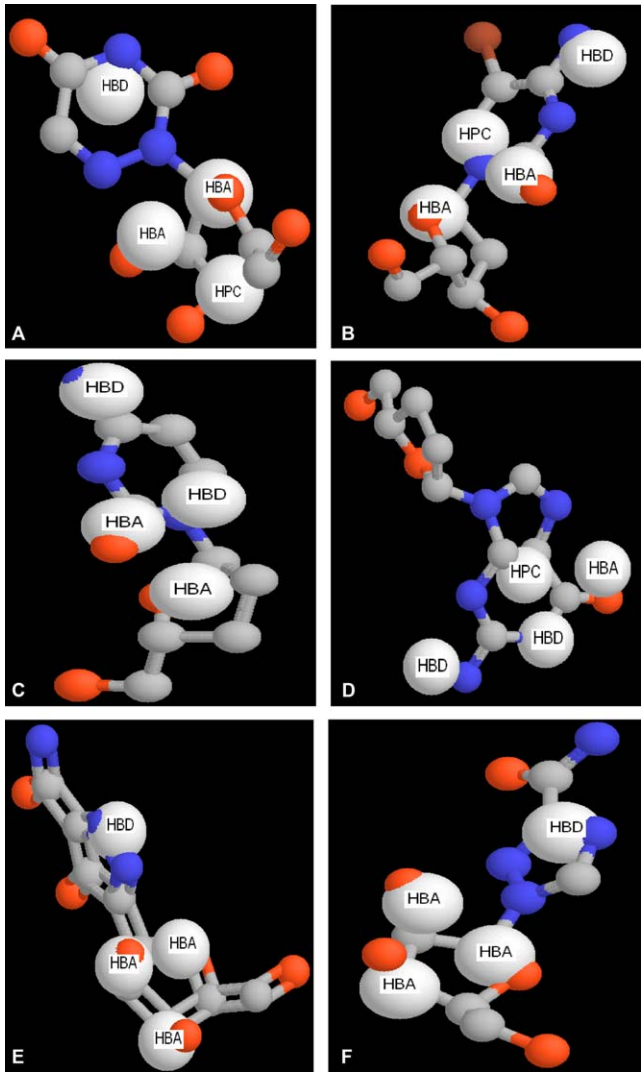

Fig. 4. The mappings of six compounds that experimentally exhibited anti-SARS-CoV activity into the four-point pharmacophore model: (A) azauridine, (B) 5-bromo-2′-deoxycytidine, (C) dideoxycytidine, (D) dideoxyguanosine, (E) pyrazofurin, (F) ribavirin.

the following facts: (1) there are some links between SARS-CoV and HIV and HBV [23–25]; (2) similar structure patterns exist in SARS-CoV main proteinase with rhinovirus 3c protease, poliovirus 3c proteinase, HAV 3c protease, HCV Ns3 protease and dengue virus Ns3 protease [26]; (3) SARS-CoV has clinically similar symptoms with influenza virus/*M. tuberculosis*, such as fever, cough, pains, pneumonia and death [27].

Indeed, among the 30 drugs are six compounds that already exhibited anti-SARS-CoV activity experimentally: azauridine, pyrazofurin, ribavirin, 2′,3′-dideoxycytidine, dideoxyguanosine, and 5-bromo-2′-deoxycytidine [4,5]. This shows that our pharmacophore model can lead to the discovery of potent anti-SARS-CoV inhibitors or at least provide some useful clues. Fig. 4 shows the mappings of the six compounds into the four-point pharmacophore model, which are mapped to 1–3 HBA, 1–2 HBD and 0–1 HPC. In addition, most of the remaining compounds have remarkable

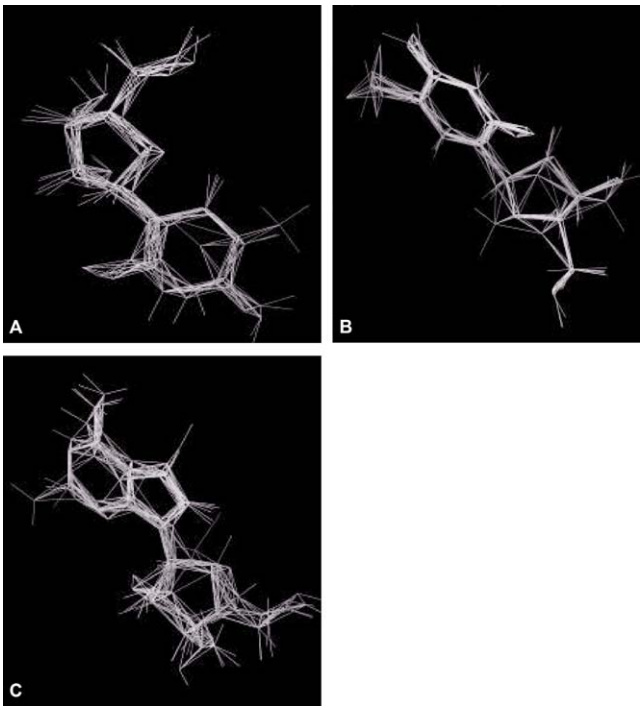

Fig. 5. Superpositions of anti-SARS-CoV compounds with other compounds. (A) azauridine with 5-AZCR, alexan, arauridine and spongothymidin; (B) 5-bromo-2′-deoxycytidine with email, fialuridine, floxuridin, radibud, thymidin and viroptic; (C) dideoxyguanosine with angustmycin C, cordycepin, tubercidin, vengicide and vira-A.

similarities with one of the above six compounds, for example, azauridine with 5-AZCR, alexan, arauridine and spongothymidin; 5-bromo-2′-deoxycytidine with emanil, fialuridine, floxuridin, radibud, thymidin and viroptic; dideoxyguanosine with angustmycin C, cordycepin, tubercidin, vengicide and vira-A. The superpositions for these compounds are shown in Fig. 5. In summary, our results indicate that the existing 30 drugs identified by our pharmacophore model could be potential inhibitors against SARS-CoV, or at least good lead compounds for anti-SARS-CoV drug design.

Acknowledgements

We wish to thank the Hong Kong Innovation and Technology Fund for supporting the present research.

References

[1] T.W. Lew, T.K. Kwek, D. Tai, A. Earnest, S. Loo, K. Singh, et al., Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome, J. Am. Med. Assoc. 290 (3) (2003) 374–380.

[2] K.S. Chan, S.T. Lai, C.M. Chu, E. Tsui, C.Y. Tam, M.M. Wong, et al., Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study, Hong Kong Med. J. 9 (6) (2003) 399–406.

[3] C.M. Chu, V.C. Cheng, I.F. Hung, M.M. Wong, K.H. Chan, K.S. Chan, et al., HKU/UCH SARS Study Group, Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings, Thorax 59 (3) (2004) 252–256.

[4] J. Cinatl, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, H.W. Doerr, Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus, Lancet 361 (2003) 2045–2046.

[5] D.L. Barnard, V.D. Hubbard, J. Burton, D.F. Smee, J.D. Morrey, M.J. Otto, et al., Inhibition of severe acute respiratory syndrome-associated coronavirus (SARSCoV) by calpain inhibitors and beta-d-N4-hydroxycytidine, Antiviral Chem. Chemother. 15 (1) (2004) 15–22.

[6] J.S. Mason, A.C. Good, E.J. Martin, 3-D pharmacophores in drug discovery, Curr. Pharm. Des. 7 7 (2001) 567–597.

[7] O. Dror, A. Shulman-Peleg, R. Nussinov, H.J. Wolfson, Predicting molecular interactions in silico: I. A guide to pharmacophore identification and its applications to drug design, Curr. Med. Chem. 11 (1) (2004) 71–90.

[8] N. Neamati, H. Hong, A. Mazumder, S. Wang, S. Sunder, M.C. Nicklaus, et al., Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching, J. Med. Chem. 40 (6) (1997) 942–951.

[9] H. Hong, N. Neamati, S. Wang, M.C. Nicklaus, A. Mazumder, H. Zhao, et al., Discovery of HIV-1 integrase inhibitors by pharmacophore searching, J. Med. Chem. 40 (6) (1997) 930–936.

[10] H. Hong, N. Neamati, H.E. Winslow, J.L. Christensen, A. Orr, Y. Pommier, et al., Identification of HIV-1 integrase inhibitors based on a four-point pharmacophore, Antiviral Chem. Chemother. 9 (6) (1998) 461–472.

[11] M.C. Nicklaus, N. Neamati, H. Hong, A. Mazumder, S. Sunder, J. Chen, et al., HIV-1 integrase pharmacophore: discovery of inhibitors through three-dimensional database searching, J. Med. Chem. 40 (6) (1997) 920–929.

[12] O.O. Clement, C.M. Freeman, R.W. Hartmann, V.D. Handratta, T.S. Vasaitis, A.M. Brodie, et al., Three dimensional pharmacophore modeling of human CYP17 inhibitors. Potential agents for prostate cancer therapy, J. Med. Chem. 46 (12) (2003) 2345–2351.

[13] A.K. Debnath, Generation of predictive pharmacophore models for CCR5 antagonists: study with piperidine- and piperazine-based compounds as a new class of HIV-1 entry inhibitors, J. Med. Chem. 46 (21) (2003) 4501–4515.

[14] P.A. Keller, C. Birch, S.P. Leach, D. Tyssen, R. Griffith, Novel pharmacophore-based methods reveal gossypol as a reverse transcriptase inhibitor, J. Mol. Graph Model. 21 (5) (2003) 365–373.

[15] J. Faragalla, J. Bremner, D. Brown, R. Griffith, A. Heaton, Comparative pharmacophore development for inhibitors of human and rat 5-alpha-reductase, J. Mol. Graph Model. 22 (1) (2003) 83–92.

[16] M.R. Doddareddy, H.K. Jung, J.Y. Lee, Y.S. Lee, Y.S. Cho, H.Y. Koh, et al., First pharmacophoric hypothesis for T-type calcium channel blockers, Bioorg. Med. Chem. 12 (7) (2004) 1605–1611.

[17] G.I. Mustata, A. Brigo, J.M. Briggs, HIV-1 integrase pharmacophore model derived from diverse classes of inhibitors, Bioorg. Med. Chem. Lett. 14 (6) (2004) 1447–1454.

[18] H. Sun, D.N. Greeley, X.J. Chu, A. Cheung, W. Danho, J. Swistok, et al., A predictive pharmacophore model of human melanocortin-4 receptor as derived from the solution structures of cyclic peptides, Bioorg. Med. Chem. 12 (10) (2004) 2671–2677.

[19] H. Yang, M. Yang, Y. Ding, Y. Liu, Z. Lou, Z. Zhou, et al., The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor, Proc. Natl. Acad. Sci. USA 100 (2003) 13190–13195.

[20] X.W. Zhang, Y.L. Yap, Old drugs as lead compounds for a new disease? Binding analysis of SARS coronavirus main proteinase with HIV, psychotic and parasite drugs, Bioorg. Med. Chem. 12 (10) (2004a) 2517–2521.

[21] R. Wang, Y. Gao, L. Lai, LigBuilder: a multipurpose program for structure-based drug design, J. Mol. Model. (Online) 6 (2000) 498–516.

[22] G.I. Mustata, J.M. Briggs, A structure-based design approach for the identification of novel inhibitors: application to an alanine racemase, J. Comput. Aided Mol. Des. 16 (12) (2002) 935–953.

[23] Y. Kliger, E.Y. Levanon, Cloaked similarity between HIV-1 and SARS-CoV suggests an anti-SARS strategy, BMC Microbiol. 3 (2003) 20–26.

[24] X.W. Zhang, Y.L. Yap, Structural similarity between HIV-1 gp41 and SARS-CoV S2 proteins suggests an analogous membrane fusion mechanism, J. Mol. Struct. (Theochem.) 677 (2004b) 73–76.

[25] M. Robertson, FgI2: link between hepatitis B and SARS? Drug Discov. Today 8 (2003) 768–770.

[26] X.W. Zhang, Y.L. Yap, Exploring the binding mechanism of the main proteinase in SARS-associated coronavirus and its implication to anti-SARS drug design, Bioorg. Med. Chem. 12 (2004c) 2219–2223.

[27] J. Oxford, S. Balasingam, R. Lambkin, A new millennium conundrum: how to use a powerful class of influenza anti-neuraminidase drugs (NAIs) in the community, J. Antimicrob. Chemother. 53 (2) (2004) 133–136.