Bioorganic & Medicinal Chemistry Letters 21 (2011) 1057–1059

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pharmacophore-based design, synthesis, and biological evaluation of novel chloro-pyridazine piperazines as human rhinovirus (HRV-3) inhibitors

Hongliang Wang [a,b], Junhai Xiao [b,*], Dapeng Gao [a,b], Xian Zhang [a,b], Hui Yan [b], Zehui Gong [b], Tinmin Sun [a,*], Song Li [b]

[a] Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[b] Laboratory of Computer-Aided Drug Design & Discovery, Beijing Institute of Pharmacology & Toxicology, Beijing 100850, PR China

ARTICLE INFO

Article history:
Received 8 September 2010
Revised 2 November 2010
Accepted 1 December 2010
Available online 7 December 2010

Keywords:
Pharmacophore
Chloro-pyridazine piperazines
Anti-HRV activity

ABSTRACT

A series of chloro-pyridazine piperazines were developed based on the structure of human rhinovirus (HRV) capsid-binding inhibitors with proven activity using a pharmacophore model. A preliminary evaluation demonstrated potent activity against HRV-3 with low cytotoxicity. A docking analysis indicated that **8a** could fit into, and form tight interactions (e.g., H-bonds, σ–π effect) with the active site in VP1.

Human rhinoviruses (HRV), a genus of Picornaviridae family, is most often associated with common colds. Although rhinovirus infection is self-limited, complications could still occur in patients with asthma, congestive heart failure, bronchiectasis and cystic fibrosis.[1,2] The fact that the US FDA has not approved a single antiviral agent for the treatment or prevention of HRV infection[3] clearly indicates a need to develop novel antiviral agents against HRV.

The capsids of HRV are composed of four structural viral proteins, namely, VP1, VP2, VP3, and VP4.[3] Pleconaril, an antiviral agent, has been shown to have a broad spectrum of activities against rhinovirus by binding to the hydrophobic pocket in VP1, resulting in the inhibition of the virus attachment to the cells and uncoating of viral RNA.[4,5] Subsequent search, spurred by the encouraging results with pleconaril, yielded additional compounds that bind to VP1, including pirodavir, BTA-188, **13** and **5f** (Fig. 1). Pirodavir could inhibit 80% of all HRV strains.[2] BTA-188 has been shown to inhibits 87 of 100 HRV serotypes, and the activity against HRV-14 (EC_{50}: 1.0 ng/ml) was found to be superior to both pleconaril (EC_{50}: 30 ng/ml) and pirodavir (EC_{50}: 3.2 ng/ml).[3] Compound 13 displayed efficient activity against 16 HRV strains and the average EC_{50} is 3.88 ng/ml.[6] Compound 5f was developed in our lab with the equal excellent anti-virus activity against HRV-2 and 14 (EC_{50}: 32 ng/ml).[2]

Based on the features of the aforementioned compounds, we built a four-point pharmacophore model using the Common Feature Pharmacophore Generation within Accelrys Discovery Studio 2.5.[7] The optimal pharmacophore Hypo1 contained an aromatic ring (A), a hydrogen bond acceptor (HBA) and two hydrophobic moieties (HY). All features of Hypo1 model were nicely mapped with BTA-188 (Fig. 2A). Search our in-house chemical database which contains nearly 10,000 compounds using Ligand Pharmacophore Mapping within Accelrys Discovery Studio 2.5 with such a pharmacophore identified one hit (compound **1**) with a chloro-pyridazine piperazine skeleton (Fig. 2C), and the mapping picture was shown in Figure 2B.

Compound **1** had significant anti-HRV activity against HRV-3. The EC_{50} of this lead compound in cell culture cytopathic effect (CPE) assay[8–11] was 25 ng/ml. Using compound **1** as a lead compound, a number of novel compounds were synthesized. Modification included replacing of the methoxy group with other hydrophobic moieties (e.g., ethoxyl, methyl and methoxycarbonyl groups) and varying the number of carbon atoms between the piperazine and benzene ring. Screening of 30 candidate compounds yielded seven compounds (**8a–g**) with promising profiles.

The synthetic route of these compounds (**8a–g**) is illustrated in Scheme 1. The common intermediate piperazin-1-yl-alkylalcohol **4a–b** was synthesized in a two-step reaction by alkylating ethyl piperazine-1-carboxylate **2** with **3a–b** in the presence of K_2CO_3 in acetonitrile and deprotecting the ethoxycarbonyl group in the presence of aq NaOH in ethanol. The 3-chloro group of 3,6-dichloropyridazine **5** was substituted with **4a–b** in the presence

* Corresponding author.
E-mail address: xiaojunhai@139.com (J. Xiao).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.12.001

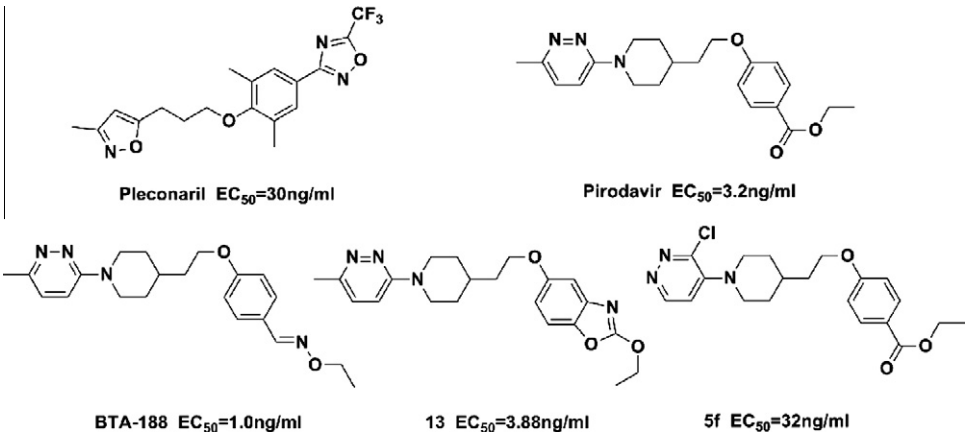


Figure 1. Structure of training set and their biological activity data.

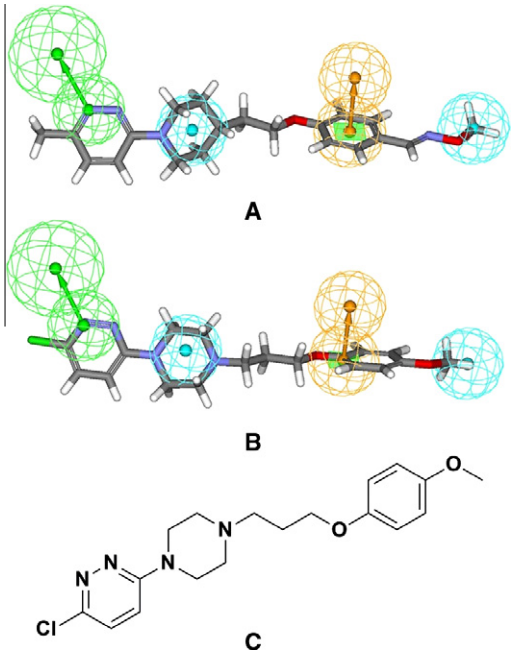
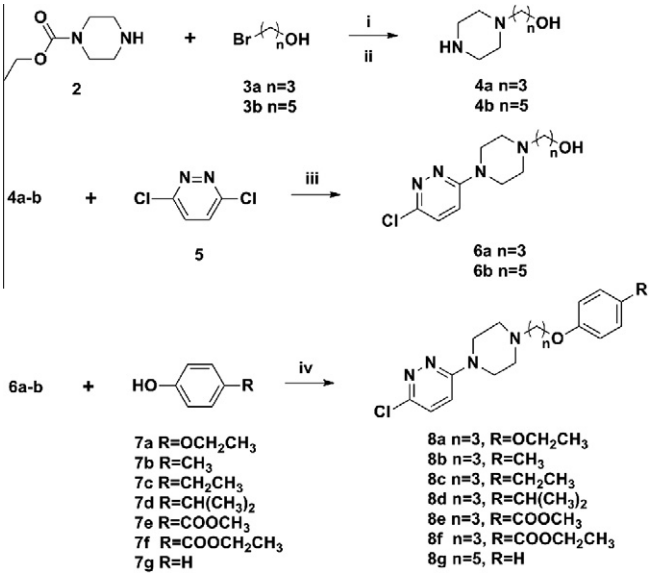


Figure 2. Pharmacophore model of HRV capsid-binding inhibitors generated by Hiphop. (A) Hypo1 mapping with BTA-188. (B) Hypo1 mapping with compound **1**. Pharmacophore features are color-coded with light-blue for hydrophobic feature, orange for ring aromatic feature and green for hydrogen-bond acceptor. (C) The chemical structure of compound **1**.



Scheme 1. Synthesis of compound **8a**–**g**. Reagents and conditions: (i) K_2CO_3, MeCN, reflux; (ii) 10% NaOH(aq), EtOH, reflux; (iii) Na_2CO_3, DMA, rt; (iv) Ph_3P, DEAD, THF.

of Na_2CO_3 in DMA to yield **6a**–**b**. Finally, **6a**–**b** were coupled with 4-substituted phenol **7a**–**g** to produce compounds **8a**–**g** by Mitsunobu reaction.[12]

The anti-HRV activity (EC_{50} in CPE assay) of these compounds is presented in Table 1. Relative to compound **5f** (EC_{50}: 50 ng/ml), these compounds were 2–15 times more potent against HRV-3 (EC_{50}: 3.2–25 ng/ml). Among these, **8a** was the most potent, with an EC_{50} at <3.2 ng/ml. These compounds also had much higher ratio of antiviral potency versus cytotoxicity (CC_{50}/EC_{50}: 4875-624) than **5f** (CC_{50}/EC_{50}: 156).

A docking analysis with LigandFit modular of Discovery Studio 2.5 against crystal structure of HRV-3 VP1 (PDB code: 1RHI) indicated that **8a** could fit into the active site of VP1 in a fashion similar to **5f** (Fig. 3).

In **5f**, the oxygen atom in the carbonyl group is 3.10 Å from the carbamate NH of Val176, indicating H-bond interaction. The N2 of the pyridazine ring and Cl atom are 2.16 and 1.60 Å away from the phenolic hydroxyl group of Tyr197, respectively, also indicating formation of H-bonds. The benzene ring is 3.77 Å from the methylene group of Tyr152, suggesting potential σ–π effect.

In compound **8a**, the benzene ring performs a similar σ–π effect with the methylene group of Tyr152 to **5f** at a distance of 3.64 Å. The ethoxyl group is 3.03 Å from the backbone NH of Ser175, indicating similar H-bond interaction to **5f** (H-bond between oxygen atom and NH of Val176). N1 and N2 of the 3-chloropyridazine

Table 1
The anti-HRV activity and cytotoxicity of compounds **1**, **5f** and **8a**–**g** on HRV-3

Compound	EC_{50} (ng/ml)	CC_{50} (μg/ml)	SI
1	<25	15.6	624
5f	50	7.8	156
8a	<3.2	15.6	>4875
8b	25	15.6	624
8c	25	15.6	624
8d	<12.5	15.6	>1248
8e	6.25	15.6	2496
8f	12.5	7.8	624
8g	6.25	15.6	>2496

SI (selectivity index) = CC_{50} (toxicity)/EC_{50} (antiviral activity).

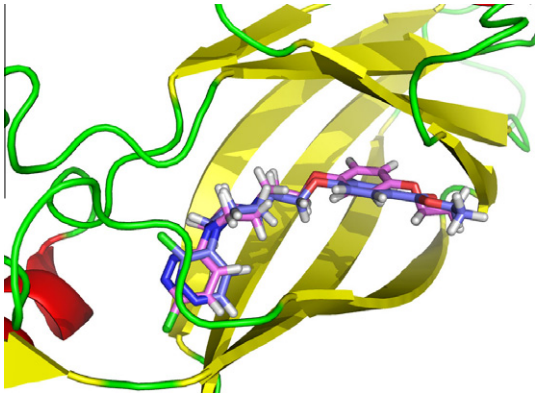


Figure 3. Compounds **5f** (blue) and **8a** (pink) docked in the active site with similar fashion in the structure of HRV-3 VP1.

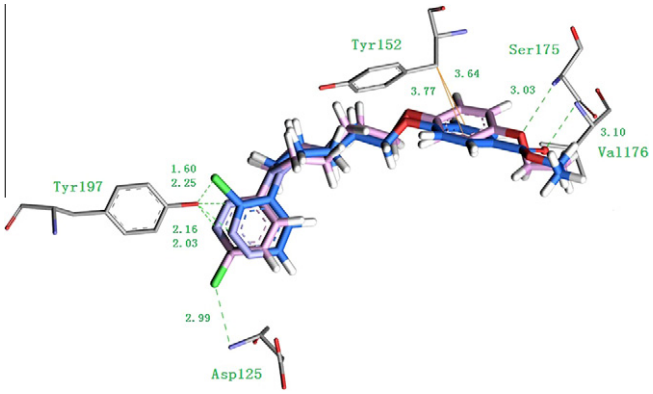


Figure 4. Compounds **5f** (blue) and **8a** (pink) docked in the active site: some important amino acid residues in hydrophobic pocket, H-bonds (blue) and σ–π effect (yellow) are present.

group are 2.25 and 2.03 Å from the phenolic hydroxyl group of Tyr197, respectively, also indicating similar H-bonds interaction to **5f**. The Cl atom is also located in hydrophobic pocket by forming a hydrogen-bond with Asp125 in the distance of 2.99 Å, this additional H-bond interaction may result in the higher activity of **8a** than **5f** (Fig. 4).

Similar types of interactions with the active site of HRV-3 VP1 are noted for compounds **8b–g**. The hydrophobic pocket has sufficient space to accommodate a chain of 3–5 carbon atoms between the piperazine and benzene.

In conclusion, the chloro-pyridazine piperazine derivatives developed in this study interacted with the active site of HRV VP1 in a manner similar to **5f**, a compound with reported activity against HRV. Among these, compounds **8a** and **8g** had excellent anti-HRV activity and low cytotoxicity, and represent promising candidates as anti-HRV agents.

Acknowledgments

This study was supported by the National High-Tech Research and Development Program of China (863 Program; No. 2006AA020601) and Natural Science Foundation (No. 30472093).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.001.

References and notes

1. Palma, A. M. D.; Vlirgen, I.; Clercq, E. D.; Neyts, J. *Med. Res. Rev.* **2008**, *28*, 823.
2. Fan, S. Y.; Zheng, Z. B.; Mi, C. L.; Zhou, X. B.; Yan, H.; Gong, Z. H.; Li, S. *Bioorg. Med. Chem.* **2009**, *17*, 621.
3. Shih, S. R.; Chen, S. J.; Hakimelahi, G. H.; Liu, H. J.; Tseng, C. T.; Shia, K. S. *Med. Res. Rev.* **2004**, *24*, 449.
4. Kaiser, L.; Crump, C. E.; Hayden, F. G. *Antiviral Res.* **2000**, *47*, 215.
5. McKinlay, M. A.; Pevear, D. C.; Rossmann, M. G. *Annu. Rev. Microbiol.* **1992**, *46*, 635.
6. Brown, R. N.; Cameron, R.; Chalmers, D. K.; Hamilton, S.; Luttick, A.; Krippner, G. Y.; Mcconnell, D. B.; Nearn, R.; Stanislawski, P. C.; Tucker, S. P.; Watson, K. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2051.
7. Accelrys Discovery Studio 2.5; Accelrys: San Diego, CA, 2009. http://www.accelrys.com.
8. Arita, M.; Wakita, T.; Shimizu, H. *J. Gen. Virol.* **2008**, *89*, 2518.
9. Cianci, C. C.; Yu, K. L.; Combrink, K.; Sin, N.; Pearce, B.; Wang, A.; Civiello, R.; Voss, S.; Luo, G. X.; Kadow, K.; Genovesi, E. V.; Venables, B.; Gulgeze, H.; Trehan, A.; James, J.; Lamb, L.; Medina, I.; Roach, J.; Yang, Z.; Zadjura, L.; Colonno, R.; Clark, J.; Meanwell, N.; Krystal, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 413.
10. Gaudernak, E.; Seipelt, J.; Triendl, A.; Grassauer, A.; Kuechler, E. *J. Virol.* **2002**, *76*, 6004.
11. Andries, K.; Dewindt, B.; Snoeks, J.; Wouters, L.; Moereels, H.; Lewi, P. J.; Janssen, P. A. J. *J. Virol.* **1990**, *64*, 1117.
12. *Selected data for compound* **8a**: ^{1}H NMR (400 MHz, CD_3Cl) δ = 7.21 (d, 1H, J = 9.6 Hz); 6.90 (d, 1H, J = 9.6 Hz); 6.83 (s, 4H); 3.98 (m, 4H); 3.67 (br, 4H); 2.61 (br, 6H); 2.00 (m, 2H); 1.39 (t, 3H, J = 7.2 Hz). MS (EI) $[M+1]^+$: m/z = 376.2. Mp 122–124 °C.