Design, Synthesis and Antiviral Activity of 2-(3-Amino-4-piperazinylphenyl)chromone Derivatives

Mi Kyoung Kim,[a] Hyunjun Yoon,[a] Dale Lynn Barnard,[b] and Youhoon Chong*,[a]

[a] *Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University; Hwayang-dong, Gwangjin-gu, Seoul 143–701, Korea: and* [b] *Institute for Antiviral Research, Department of Animal, Dairy and Veterinary Science, Utah State University; 5600 Old Main Hill, Logan, UT 84322, U.S.A.*

Received December 7, 2012; accepted January 23, 2013

Previously, we have confirmed that the antiviral activities of the chromone derivatives were controlled by the type as well as the position of the substituents attached to the chromone core structure. In the course of our ongoing efforts to optimize the antiviral activity of the chromone derivatives, we have been attempting to derivatize the chromone scaffold *via* introduction of various substituents. In this proof-of-concept study, we introduced a 3-amino-4-piperazinylphenyl functionality to the chromone scaffold and evaluated the antiviral activities of the resulting chromone derivatives. The synthesized 2-(3-amino-4-piperazinylphenyl)-chromones showed severe acute respiratory syndrome-corona virus (SARS-CoV)-specific antiviral activity. In particular, the 2-pyridinylpiperazinylphenyl substituents provided the resulting chromone derivatives with selective antiviral activity. Taken together, this result indicates the possible pharmacophoric role of the 2-pyridinylpiperazine functionality attached to the chromone scaffold, which warrants further in-depth structure–activity relationship study.

Key words piperazinylphenylchromone; antiviral activity; hepatitis C virus; severe acute respiratory syndrome (SARS); SARS-corona virus

1,3-Diketoacid (DKA) (Fig. 1) is one of the well-known antiviral scaffold with anti-human immunodeficiency virus (HIV),[1] anti-hepatitis B virus (HBV),[2] and anti-severe acute respiratory syndrome-corona virus (SARS-CoV)[3] activity. Previously, we discovered a chromone scaffold (Fig. 1) as a novel pharmacophore for antiviral agents against HCV[4] as well as SARS-CoV,[5] and reported structure–activity relationship studies on a series of chromone derivatives.[6–8] Interesting antiviral activities against HCV and/or SARS-CoV were observed from various chromone derivatives.[6–8] Also, through structure–activity relationship study, we confirmed that a substituent could play a key role in controlling the antiviral activity of the chromone derivatives against HCV and SARS-CoV.[9] Thus, in the course of our ongoing efforts to optimize the antiviral activity of the chromone derivatives, we have been attempting to derivatize the chromone scaffold *via* introduction of various substituents. In this context, a 2-pyridinylpiperazine moiety which was used to potentiate the anti-HCV activity of the acridone scaffold[10] (Fig. 1) drew our attention. In particular, structural similarity between the chromone and the acridone scaffold led us to design novel chromone derivatives with a piperazinylphenyl substituent (Fig. 1).

Herein, we report synthesis and antiviral evaluation of 2-(3-amino-4-piperazin-1-yl-phenyl)chromone derivatives (Fig. 1).

Chemistry The chromone scaffold was constructed by condensation of 3-nitro-4-piperazinylbenzoic acid **9** and 2-hydroxyacetophenone **11** followed by ring closure (Chart 1). The piperazinylbenzoic acid **9a**–**d** were prepared by nucleophilic aromatic substitution reaction of the commercially available 4-chloro-3-nitrobenzoic acid **7** with variously substituted piperazines. However, direct substitution of **7** with piperazine did not work due to the acidic nature of **7**. Thus, **7** was transiently converted to the corresponding *tert*-butyl ester **8a**–**d**, which smoothly underwent nucleophilic substitution reaction. Treatment of the resulting *tert*-butyl piperazinylbenzoate with trifluoroacetic acid (TFA) provided the desired piperazinylbenzoic acids **9a**–**d** as white powder, which was used for the next step without further purification. On the other hand, the two phenolic hydroxyl groups of 2,4,6-trihydroxyacetophenone **10** were protected with either methyl or benzyl group to give the corresponding 2-hydroxy-4,6-dialkoxy acetophenone **11a** or **b** in 88% and 94% yield, respectively. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated coupling of **9** with **11** provided the corresponding ester which underwent cyclization followed by dehydration upon sequential treatment with *tert*-BuOK and sulfuric acid to give the chromone scaffold. Finally, reduction of the nitro functionality to the amino group was accomplished upon treatment with H_2 in the presence of $Pd(OH)_2$–C with concomitant removal of the benzyl protecting groups to furnish the desired compounds **1**, **4**, **5**, and **6**, in 84%, 83%, 84%, and 88% yield, respectively. Deprotection of

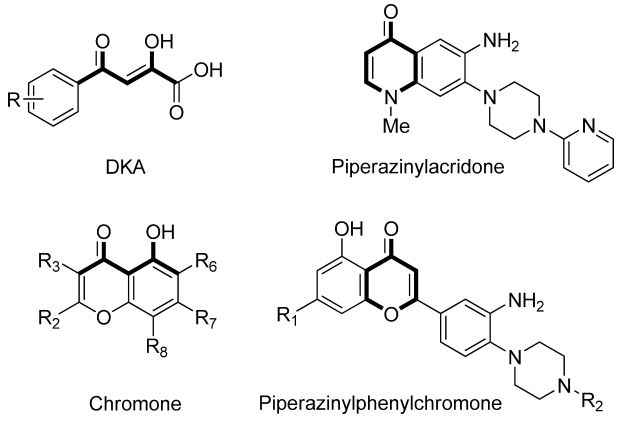

Fig. 1. Structures of DKA, Chromone, Piperazinylacridone, and the Title Compound (Piperazinylphenylchromone)

Bold lines indicate structural similarities between these structures.

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: chongy@konkuk.ac.kr

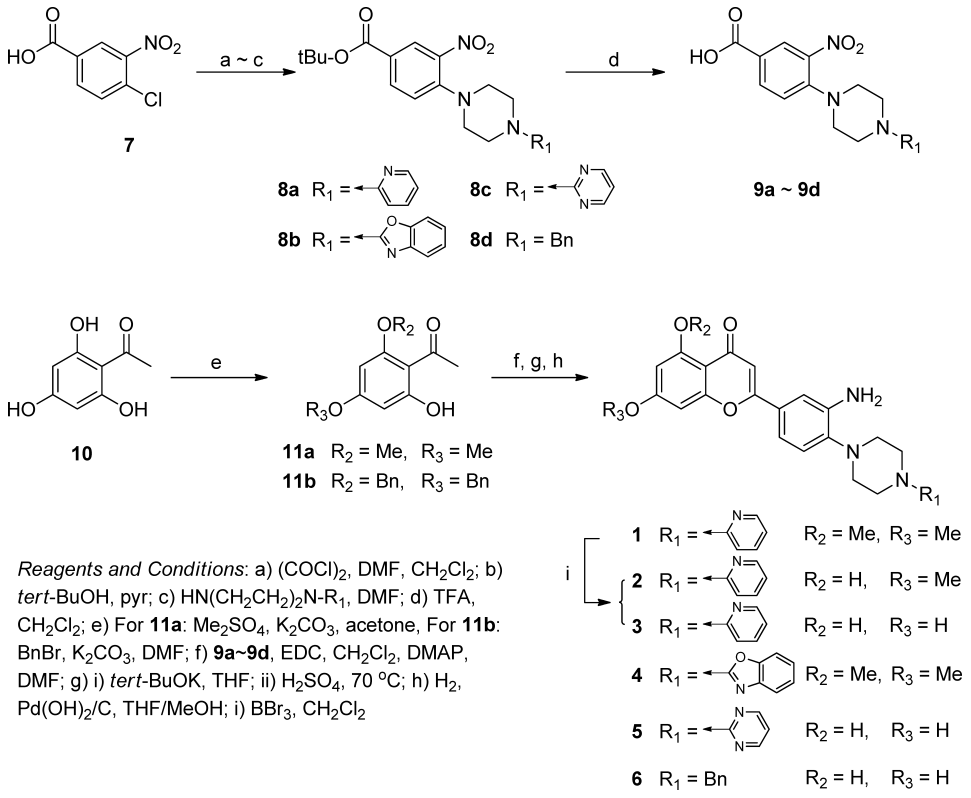

Chart 1. Synthesis of the Title Compounds **1**–**6**

the methyl group of **1** was attempted with BBr_3 to give a mixture of **2** (28% yield) and **3** (43% yield), which were readily separated by column chromatography on silica gel.

Anti-HCV Activity The synthesized compounds **1**–**6** were evaluated for their antiviral activity in the Huh-Luc/neo cell line harboring a genotype 1b/strain Con-1 HCV subgenomic replicon.[11,12] The level of HCV RNA replication was quantified with luciferase reporter assay, and the results were summarized in Table 1. Except compound **4**, no other compound prepared in this study showed antiviral activity against HCV. The compound **4** with a piperazinylbenzoxazole substituent showed significant anti HCV-activity (EC_{50}=1.5 μM) but with low selectivity (CC_{50}=3.8 μM, SI=2.6).

Anti-SARS-CoV Activity The potential for all the synthesized compounds to inhibit the SARS-CoV in cell culture was also evaluated. Using a modified protocol of Barnard *et al.*,[13] cytopathic effect (CPE) reduction assays were performed in Vero 76 cells, which were visually assessed (visual assay, Table 1) and then verified spectrophotometrically by neutral red (NR) uptake assay[14,15] (neutral red assay, Table 1) in the same plate.

In the visual assay, three compounds (**1**, **3**, **4**) showed moderate but selective antiviral activity (EC_{50}=34–42 μM, CC_{50}>100 μM). However, in the NR uptake assay only compound **1** maintained antiviral activity. In comparison, compound **2** showed improved activity and selectivity in the NR uptake assay compared to those values obtained in the visual assay. The activity/selectivity profiles of **5** and **6** were similar in both assays in that **5** showed moderate antiviral activity with low selectivity while **6** was neither active nor cytotoxic.

Table 1. Antiviral Activity of the Synthesized Compounds against HCV and SARS-CoV

Compound	Anti-HCV activity (μM)			Anti-SARS-CoV activity (μM)					
				Visual assay			Neutral red assay		
	EC_{50} [a]	CC_{50} [b]	SI [c]	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI
1	>20	>20	1.0	34	>100	>2.9	52	>100	>1.9
2	>20	>20	1.0	68	68	1.0	51	>100	>2.0
3	>20	>20	1.0	38	>100	>2.6	>45	45	<1.0
4	1.5	3.8	2.6	42	>100	>2.4	>100	>100	1.0
5	>20	13.8	<0.7	32	42	1.3	38	38	1.0
6	>20	>20	1.0	>100	>100	1.0	>89	89	<1.0
rIFNα-2b [d]	0.07	>2	>28.6	—	—	—	—	—	—
M128553 [e]	—	—	—	0.7	>100	>150	0.58	>100	>170

a) EC_{50}: compound concentration that reduces viral replication by 50%. *b*) CC_{50}: compound concentration that reduces cell viability by 50%. *c*) SI=CC_{50}/EC_{50}. *d*) Recombinant human interferon α-2b was used as a positive control for anti-HCV activity assay. *e*) SARS-CoV M(pro) protease inhibitor (Maxim Pharmaceuticals) was used as a positive control for the anti-SARS-CoV activity assay.

It is noteworthy that compounds **1**–**3** showed selective anti-SARS-CoV activity in both the visual and the NR assay, which indicates the possible pharmacophoric role of the 2-pyridinylpiperazine functionality.

In this proof-of-concept study, we introduced a 3-amino-4-piperazinylphenyl functionality to the chromone scaffold, which showed SARS-CoV-specific antiviral activity. In particular, the 2-pyridinylpiperazinylphenyl substituents provided the resulting chromone derivatives with selective antiviral activity. Based on this result, an extensive structure–activity relationship study of the piperazinylphenyl-substituted chromone derivatives is in progress that will be reported elsewhere.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0008260), by a Grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A08-4628-AA2023-08N1-00010A), by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824), and by a Grant from Next Generation Bio-Green 21 PJ007982 (Korea Rural Development Administration). This project has been funded in part with Federal Funds from the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institute of Health, Department of Health and Human Services, U.S.A., under contract numbers HHSN272201100019I/HHSN27200002/B05 (SARS-CoV) and HHSN272201100012I (HCV).

References

1) Summa V., Petrocchi A., Pace P., Matassa V. G., De Francesco R., Altamura S., Tomei L., Koch U., Neuner P., *J. Med. Chem.*, **47**, 14–17 (2004).
2) Sergio A., Tomei L., Koch U., Neuer P. J. S., Summa V., EP1100763A1 (2001).
3) Lee C., Lee J. M., Lee N. R., Jin B. S., Jang K. J., Kim D. E., Jeong Y. J., Chong Y., *Bioorg. Med. Chem. Lett.*, **19**, 1636–1638 (2009).
4) Lee C., Park K. S., Park H. R., Park J. C., Lee B., Kim D. E., Chong Y., *Bull. Korean Chem. Soc.*, **31**, 3471–3473 (2010).
5) Kim M. K., Yu M. S., Park H. R., Kim K. B., Lee C., Cho S. Y., Kang J. H., Yoon H., Kim D. E., Choo H., Jeong Y. J., Chong Y., *Eur. J. Med. Chem.*, **46**, 5698–5704 (2011).
6) Lee C., Lee J. M., Lee N. R., Kim D. E., Jeong Y. J., Chong Y., *Bioorg. Med. Chem. Lett.*, **19**, 4538–4541 (2009).
7) Lee H. S., Park K. S., Lee C., Lee B., Kim D. E., Chong Y., *Bioorg. Med. Chem. Lett.*, **20**, 5709–5712 (2010).
8) Lee H. S., Park K. S., Lee B., Kim D. E., Chong Y., *Bioorg. Med. Chem.*, **18**, 7331–7337 (2010).
9) Yoon H., Kim M. K., Mok H. J., Chong Y., *Bull. Korean Chem. Soc.*, **33**, 2803–2805 (2012).
10) Manfroni G., Paeshuyse J., Massari S., Zanoli S., Gatto B., Maga G., Tabarrini O., Cecchetti V., Fravolini A., Neyts J., *J. Med. Chem.*, **52**, 3354–3365 (2009).
11) Lohmann V., Körner F., Koch J., Herian U., Theilmann L., Bartenschlager R., *Science*, **285**, 110–113 (1999).
12) Vrolijk J. M., Kaul A., Hansen B. E., Lohmann V., Haagmans B. L., Schalm S. W., Bartenschlager R., *J. Virol. Methods*, **110**, 201–209 (2003).
13) Barnard D. L., Stowell V. D., Seley K. L., Hegde V. R., Das S. R., Rajappan V. P., Schneller S. W., Smee D. F., Sidwell R. W., *Antivir. Chem. Chemother.*, **12**, 241–250 (2001).
14) Barnard D. L., Sidwell R. W., Xiao W., Player M. R., Adah S. A., Torrence P. F., *Antiviral Res.*, **41**, 119–134 (1999).
15) Barnard D. L., Xu Z. Q., Stowell V. D., Yuan H., Smee D. F., Samy R., Robert W. S., Nielsen M. K., Sun L., Cao H., Li A., Quint C., Deignan J., Crabb J., Flavin M. T., *Antivir. Chem. Chemother.*, **13**, 39–59 (2002).