Bioorganic & Medicinal Chemistry Letters 21 (2011) 4013–4015

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel xanthone derivatives as xanthine oxidase inhibitors

Lina Hu [a], Honggang Hu [a,*], Weifeng Wu [b], Xiaoyun Chai [a], Jianfei Luo [a], Qiuye Wu [a,*]

[a] Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, China
[b] Fengxian District Central Hospital, Branch Hospital of Shanghai Sixth People's Hospital, Shanghai 201400, China

ARTICLE INFO

Article history:
Received 22 February 2011
Revised 18 April 2011
Accepted 29 April 2011
Available online 6 May 2011

Keywords:
Xanthone
Derivatives
Synthesis
Xanthine oxidase
Molecular modeling

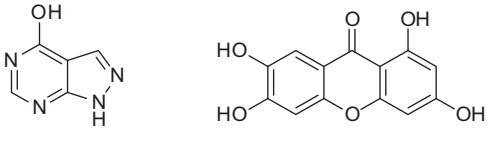
ABSTRACT

Xanthine oxidase is the key enzyme that catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid. In this study, a series of xanthone derivatives were synthesized as effective and a new class of xanthine oxidase inhibitor. Compounds **8a**, **8c**, **8i**, **8g** and **8r** showed good inhibition against xanthine oxidase. The presence of a cyano group at the *para* position of benzyl moiety turned out to be the preferred substitution pattern. Molecular modeling studies were performed to gain an insight into its binding mode with xanthine oxidase, and to provide the basis for further structure-guided design of new non-purine xanthine oxidase inhibitors associated with the xanthone framework.

Xanthine oxidase (XO) is a key enzyme in catabolic sequence of the purine nucleotide metabolism in humans and a few other uricotelic species.[1] It catalyzes the last two steps in the purine degradation pathway prior to formation of uric acid, that is, hydroxylation of hypoxanthine to xanthine and then to uric acid (UA).[2] The increasing of UA concentration eventually leads to the deposition of micro and macroscopic deposits of sodium hydrogen urate monohydrate crystals in joints and kidneys, which in turn causes gouty arthritis and UA nephrolithiasis.[3,4] Since reducing plasma levels of UA can prevent gout, therapy is possible with XO inhibitors that block the production of UA from purine.[5] Allopurinol (Fig. 1), a known XO inhibitor and an analogue of hypoxanthine, has been widely prescribed as a treatment for hyperuricemia and gout. In some cases, however, severe life-threatening side effects have been reported. These include a toxicity syndrome dramatized by eosinophilia, vasculitis, rash hepatitis, and progressive renal failure.[6] Hence, the identification of novel, efficient and less toxic xanthine oxidase inhibitors remains an important and challenging task.

Xanthone is a class of oxygen-containing heterocyclic compounds widely distributed in nature,[7] and exhibits a variety of biological activities.[8–10] In our previous study, compound **5** (Fig. 1) displayed good inhibitory activity aganist xanthine oxidase. However, the solubility of **5** is very poor in most solvents, which inspired us to explore new derivatives with better physicochemical property. Furthermore, it is an effective approach in the search of novel compounds with potent activity by simple chemical-modification on the basis of natural leading compounds. In this Letter, we described the synthesis and preliminary structure–activity relationships of xanthone derivatives as potent XO inhibitors.

The synthesis of the hitherto unreported title compounds **8a–t** was as outlined in Scheme 1. 2,4,5-Trimethoxybenzoyl chloride **2** was obtained by treating 2,4,5-trimethoxybenzoic acid with sulfuryl dichloride, which was then alkylated via Friedel–Crafts reacting with 1,3,5-trimethoxybenzene in absolute ether to give benzophenone **3**. The free hydroxyl group of **3** was formed by demethylation in extra amount of aluminium chloride.[11] Intramolecular cyclization of **3** was carried out with tetrabutylammonium hydroxide in pyridine and water under reflux to provid **4** in high yield,[12] which was demethylated in freshly fused pyridine hydrochloride to form the key intermediate **5**.[13] Chloro(methoxy)methane was added dropwisely into the flask containing **5** and *N*,*N*-diisopropylethylamine at 0 °C to give **6** as light-green powder under the condition of argon protection,[14] and subsequently **6** was benzylated with corresponding benzyl bromine to obtain **7** in quantitative yield. Finally,

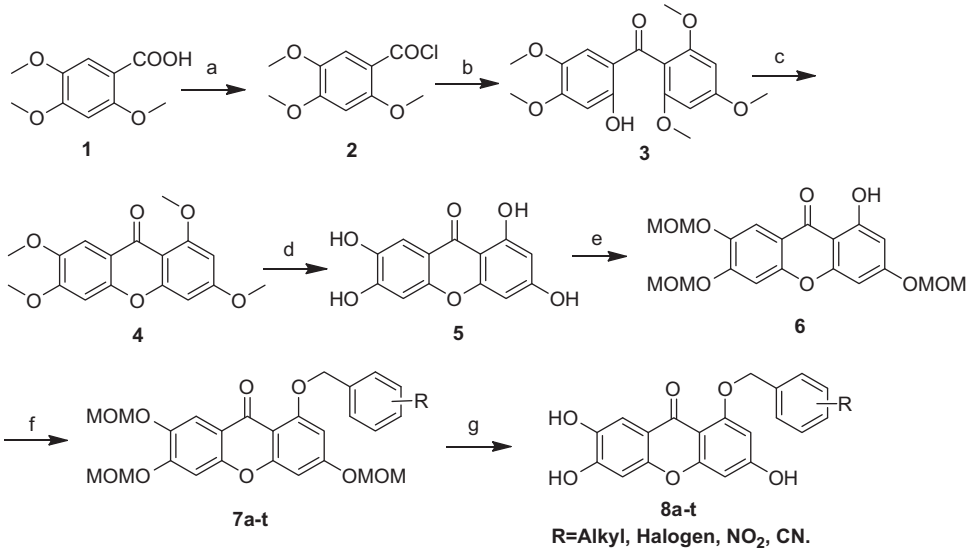


Allopurinol **5**

Figure 1. Chemical structures of allopurinol, compound **5**.

* Corresponding authors. Tel./fax: +86 21 8187 1228 (H.H.); tel./fax: +86 21 8187 1225 (Q.W.).
E-mail addresses: huhonggang_fox@msn.com (H. Hu), wuqy6439@sohu.com (Q. Wu).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.140



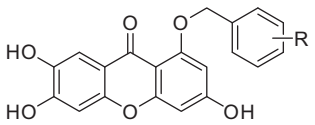
Scheme 1. Synthesis of compounds **8a–t**. Reagents and conditions: (a) $SOCl_2$, reflux, 4–5 h; (b) 1,3,5-trimethoxybenzene, $AlCl_3$, Et_2O, rt, 48 h, 72%; (c) TBAOH (25% in H_2O), Py-H_2O (1:1), reflux, 4 h, 95%; (d) chloro(methoxy)methane, DIEA, dichloromethane, 0 °C–rt, 8 h; (f) benzyl bromides, K_2CO_3, DMF, 50 °C, 6 h; (g) Concentrated hydrocloride, absolute methnol, 55 °C.

the title compounds **8a–8t** were achieved by way of deprotection of **7** using concentrated hydrochloric acid in methanol with yield of 50–65%.[14] The structures of the synthesized target compounds were elucidated by ^{1}H NMR, ^{13}C NMR and MS.[15] All spectral data were in accordance with assumed structures.

The inhibitory activity of the newly synthesized compounds in vitro against XO was measured spectrophotometrically by following uric acid levels at 293 nm.[16,17] Compounds presenting an inhibitory effect higher than 60% at the concentration of 10 μg/mL were further tested at a wide range of concentrations to determine their IC_{50} values, the results were shown in Table 1.

Structure–activity relationships (SARs) were inferred from Table 1. In general, there was no significant influence on biological activity by introducing electron donor or electron withdrawing substituents on benzyl group. In addition, compound **8r** with cyano group at *para* position showed significant inhibition on XO, followed by **8i** with chloride group at *para* position of benzyl, **8c** with methyl group at *para* position, **8g** with chloride group at *ortho* position and **8a** with methyl group at *ortho* position, which suggested that *para* substituted benzyl groups are of benefit to the inhibition rate than that compounds with the same substitutes at *ortho* or *meta* position.

Table 1
In vitro xanthine oxidase inhibitory activity of xanthone derivatives



Compounds	R	IC_{50}[a,b] (μM)	Compounds	R	IC_{50}[a,b] (μM)
5	—	21.73 ± 1.52	**8k**	3-Br	n.a
8a	2-CH_3	7.08 ± 0.65	**8l**	4-Br	20.06 ± 1.37
8b	3-CH_3	13.56 ± 0.28	**8m**	2-NO_2	n.a
8c	4-CH_3	5.73 ± 0.10	**8n**	3-NO_2	n.a
8d	2-F	n.a[c]	**8o**	4-NO_2	n.a
8e	3-F	n.a	**8p**	2-CN	n.a
8f	4-F	n.a	**8q**	3-CN	n.a
8g	2-Cl	6.41 ± 0.15	**8r**	4- CN	4.67 ± 0.35
8h	3-Cl	n.a	**8s**	2,4-*di*-Cl	n.a
8i	4-Cl	4.70 ± 0.12	**8t**	2,6-*di*-Cl	n.a
8j	2-Br	n.a			

[a] IC_{50} values: the concentration of the inhibitor required to produce 50% inhibition of xanthine oxidase.
[b] Each results was performed in triplicate. Allopurinol was used as positive control (IC_{50} = 24.40 ± 0.50 μM) Ref. 18.
[c] n.a.: not active (less than 60% inhibition at 10 μg/mL).

The known X-ray structures of xanthine oxidoreductase with bound molecules show a highly specific binding pocket, presenting a long, narrow cavity leading toward the Mo(IV) complex.[6,19] Molybdenum-pterin sites of both xanthine oxidase and xanthine dehydrogenase are structurally equivalent.[20] To further understand the binding mode of newly synthesized compounds with XO, molecular docking studies of compounds **8i** and **8r** were performed. The crystal structure of xanthine oxidoreductase (PDB entry code 1FIQ) was obtained from the Protein Data Bank (http://www.rcsb.org). The protein was prepared by removing all water molecules and adding all hydrogen atoms using LibDock protocol in Disocovery Studio 2.5 software package,[21] LibDock, developed by Diller and Merz,[22] is based on matching polar and apolar binding site features of the protein-ligand complex. It can get a balance between accuracy and speed.

According to our docking studies, the novel xanthone derivatives **8i** and **8r** indeed inserted into the narrow tunnel towards the Mo(IV) complex. As shown in Figure 2, compounds **8i** and **8r** overlapped properly with Molybdenum-pterin active site with the configuration of 'L'(showed by Fig. 2A and B respectively). Besides, all of them binded within amino residues: Phe798, Gln1194 and Gln112, which were believed to promote the stabilization of the binding positions of the xanthone substrate and might be important for substrate recognition. The presence of these residues made the region closer to the Mo(IV) complex. Both compounds **8i** and **8r** interacted very closely with original ligand docking pose by hydrogen bonds that involved Gln1040, Ser1082, Gln1261, Gly797, Gln767 and Cys150. Moreover, additional hydrogen binding between the carboxyl group of Lys1045 and the nitrogen atom of compound **8r** occurred.

In conclusion, a new class of xanthone derivatives as XO inhibitor were synthesized and evaluated for their inhibitory activities against XO. Compounds **8a**, **8c**, **8g**, **8i** and **8r** demonstrated potent inhibitory activities against XO with IC_{50} values lower than 10 μM, which were much better than allopurinol. Molecular

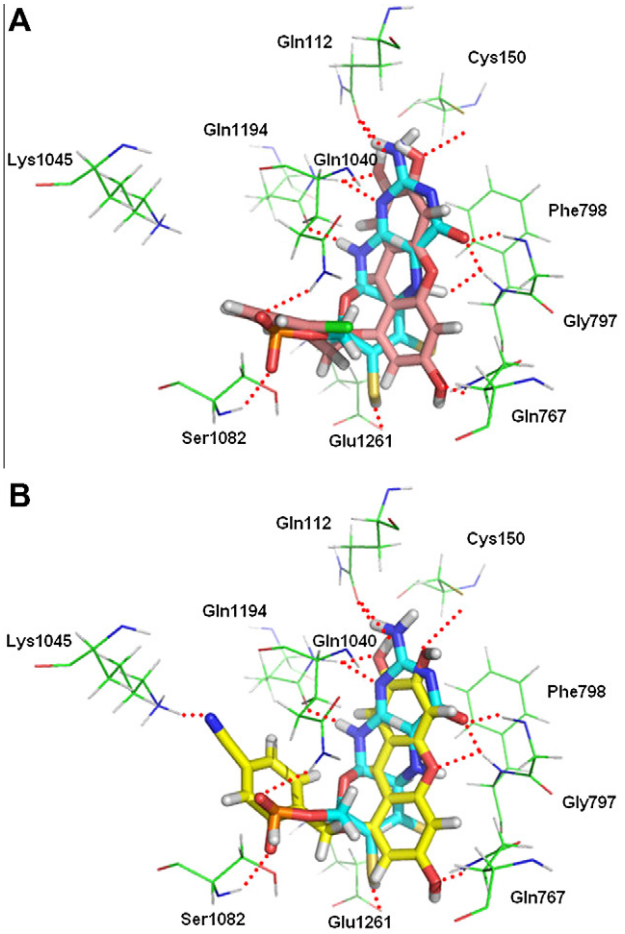


Figure 2. Superposition models of xanthone derivatives and original ligand docking pose (the Molybdenum-pterin active site). (A) Compound **8i** (pink) with original ligand (blue); (B) compound **8r** (yellow) with original ligand (blue). Hydrogen bonds are represented by the dashed red lines.

docking studies provided the molecular basis for rationalizing the activity of the new xanthone derivatives. In particular, both compounds adopted similar binding poses and interacted closely with the active site of XO. Further research in this area is in progress in our laboratory.

Acknowledgments

This work was supported by grants from Science & Technology Commission of Shanghai Municipality (Nos. 054319909 and 08JC1405500) and Shanghai Leading Academic Discipline Project (No. B906).

References and notes

1. Rundles, R. W.; Wyngaarden, J. B. *Annu. Rev. Pharmacol.* **1969**, *9*, 345.
2. Krenitsky, T. A.; Spector, T.; Hall, W. W. *Arch. Biochem. Biophys.* **1986**, *247*, 108.
3. Harris, M. D.; Siegel, L. B.; Alloway, J. A. *Am. Fam. Physician* **1999**, *59*, 925.
4. Abeles, A. M.; Park, J. Y.; Pillinger, M. H.; Cronstein, B. N. *Curr. Pain Headache Rep.* **2007**, *11*, 440.
5. Emmerson, B. T. N. *Engl. J. Med.* **1996**, *334*, 445.
6. Okamoto, K.; Eger, B. T.; Nishino, T.; Kondo, S.; Pai, E. F.; Nishino, T. *J. Biol. Chem.* **2003**, *278*, 1848.
7. Hostettman, K.; Hostettman, M. *Methods Plant Biochem.* **1989**, *1*, 493.
8. Nakatani, K.; Yamakuni, T.; Kondo, N.; Kondo, N.; Arakawa, T.; Oosawa, K.; Shimura, S.; Inoue, H.; Ohizumi, Y. *Mol. Pharmacol.* **2004**, *66*, 667.
9. Iinuma, M.; Tosa, H.; Tanaka, T.; Asai, F.; Kobayashi, Y.; Shimano, R.; Miyauchi, K. I. *J. Pharm. Pharmacol.* **1996**, *48*, 861.
10. Lee, B. W.; Lee, J. H.; Lee, S. T.; Lee, H. S.; Lee, W. S.; Jeong, T. S.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5548.
11. Quillinan, A. J.; Scheinmann, F. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1329.
12. Lin, C. N.; Liou, S. S.; Ko, F. N.; Teng, C. M. *J. Pharm. Sci.* **1992**, *81*, 1109.
13. Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1956**, *78*, 1380.
14. Hall, D. G.; Deslongchamps, P. J. *J. Org. Chem.* **1995**, *60*, 7796.
15. *Representative analytical data for compound* **8r**. Mp: >260 °C; ^{1}H NMR (300 MHz DMSO-d_6, TMS): δ 10.79 (1H, s, phenolic-H), 10.33 (1H, s, phenolic-H), 9.51 (1H, s, phenolic-H), 7.91 (4H, s, Ar-H), 7.37 (1H, s, Ar-H), 6.76 (1H, s, Ar-H), 6.40 (2H, s, Ar-H), 5.29 (1H, s, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.45, 163.29, 160.31, 159.55, 152.87, 149.84, 143.81, 140.84, 134.11, 133.32, 129.05, 127.81, 117.58, 115.22, 113.27, 109.81, 105.23, 102.70, 97.34, 96.20, 68.44. MS (ESI) *m*/*z*: Calcd for $C_{21}H_{13}NO_6$ 375.1. Found 374.21 $[M-H]^+$.
16. Kalra, S.; Jena, G.; Tikoo, K.; Mukhopadhyay, A. K. *BMC Biochem.* **2007**, *8*, 8.
17. Tamta, H.; Kalra, S.; Anand, G. C. S.; Mukhopadhyay, A. K. *J. Biol. Phys. Chem.* **2005**, *5*, 89.
18. Gupta, S.; Rodrigues, L. M.; Esteves, A. P.; Oliveira-Campos, A. M.; Nascimento, M. S.; Nazareth, N.; Cidade, H.; Neves, M. P.; Fernandes, E.; Pinto, M.; Cerqueira, N. M.; Brás, N. *Euro. J. Med. Chem.* **2008**, *43*, 771.
19. Fukunari, A.; Okamoto, K.; Nishino, T.; Eger, B. T.; Pai, E. F.; Kamezawa, M.; Yamada, I.; Kato, N. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 519.
20. Towne, E. B.; Moore, W. H.; Dickey, J. B. U.S. Patent 3, 336,285, 1967, *Chem. Abstr.* **1967**, *68*, 14072.
21. Disocovery Studio 2.5 Software Package AI, San Diego, CA (USA): http://www.accelrys.com.
22. Diller, D. J.; Merz, K. M., Jr. *Proteins* **2001**, *43*, 113.