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Quinoline-4-acetamides as sPLA$_2$ Inhibitors

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Abstract—Quinoline-4-acetamides were designed as potential phospholipase A_2 inhibitors by structural based method and synthesized. The chemical structures of the obtained compounds were confirmed by elemental analyses, ^{1}H NMR and MS. Preliminary bioassay study shows that quinoline-4-acetamides display certain inhibition to sPLA$_2$. © 2001 Elsevier Science Ltd. All rights reserved.

Phospholipase A_2 (PLA$_2$) specifically hydrolyzes the fatty acyl ester bond at the *sn*-2 position of glycerophospholipid, producing rate-limiting precursors of inflammatory free fatty acids and lysophospholipids. PLA$_2$ plays an important pathophysiological role in arthritis,[1] septic shock,[2] acute pancreatitis,[3] and other inflammatory diseases. It is clear that a potent and selective inhibitor of PLA$_2$ would have great potential for the treatment of a wide range of common clinical disorders.

Two major groups of PLA$_2$ related to arthritis have been reported, cytosolic PLA$_2$ (cPLA$_2$) and the secretory PLA$_2$ (sPLA$_2$). Currently, the most widely studied PLA$_2$ is the latter, low molecular weight (14 kDa), Ca^{2+} dependent, extra-cellular enzymes which have been isolated from such sources as bee venom, snake venom, and mammalian pancreas and which are also secreted by other mammalian cell types such as platelets.[4] Human non-pancreatic secretory phospholipases A_2 (hnps-PLA$_2$) is considered to be a member of this class.

We focus on designing and synthesizing non-analogues of the phospholipidic substrate of sPLA$_2$ in order to find new inhibitors. Herein, the quinoline ring was selected and novel quinoline-4-acetamides were synthesized and tested as sPLA$_2$ inhibitors.

Chemistry

Our general synthetic approach to the title compounds is depicted in Scheme 1.

With reference to the literature,[5,6] we obtained 2-chloro-4-methyl-6-methoxyquinoline **3** from *p*-methoxyaniline via three-step reactions of ethyl acetoacetate, oil of vitriol and phosphoryl chloride. Compound **4**, 2-phenoxy-4-methyl-6-methoxyquinoline, was obtained by nucleophilic substitution of **3** with phenol in the presence of potassium hydroxide. Condensation of **4** with benzaldehyde in the presence of anhydrous zinc chloride gave 2-phenoxy-4-cinnamyl-6-methoxyquinoline **5**. Compound **5**, when treated with osmium tetroxide in moderate conditions, yielded 2-phenoxy-6-methoxyquinoline-4-caraldehyde **6**. Oxidation of **6** with silver nitrate at room temperature gave 2-phenoxy-6-methoxyquinoline-4-carboxylic acid **7**. Compound **7** when treated with oxalyl chloride at room temperature gave 2-phenoxy-6-ethoxyquinoline-4-carbonyl chloride **8**. Compound **8** was used as the intermediate to synthesize the target,[7] when **8** reacted with ammonia, the title compound **12**, 2-phenoxy-6-methoxyquinoline-4-carboxamide, was obtained in 93.2%, mp 202–204 °C; while **8** reacted with diazomethane (diazo-reaction) to give 2-phenoxy-6-methoxyquinoline-4-carbonyl iazoparaffinyl **9**, followed by Arndt–Eistert reaction to give the other title compound 2-phenoxy-6-methoxyquinoline-4-acetamide **10** in 76.4% yield, mp 186–188 °C, demethylation of **10** with boron tribromide yielded the third title compound 2-phenoxy-6-hydroxyquinoline-4-acetamide **11** in 28.4% yield.

Biological Evaluation

The SIBLINKS[8,9] method was used to evaluate bioactivity of the compounds. *Naja naja* sPLA$_2$ was used as the target. The reaction was monitored by the absorbance at 405 nm with a BIO-RAD plate reader. Test compounds were added to each well as DMSO solution

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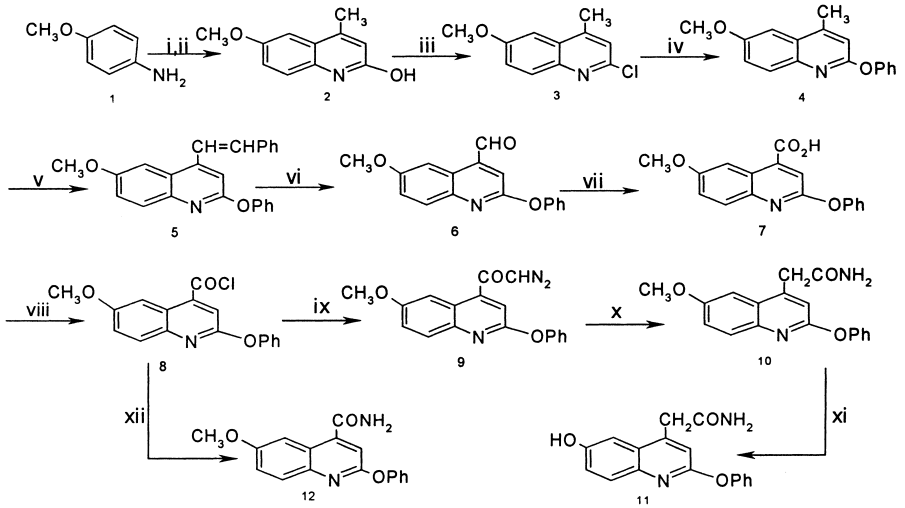

Scheme 1. Synthesis of quinoline-4-acetamide derivatives: (i) $CH_3COCH_2CO_2C_2H_5$ (4 eq), 140 °C, 30 min, 73.8%; (ii) H_2SO_4, 90–110 °C, 2 h, 62.4%; (iii) $POCl_3$, 110–120 °C, 30 min, 89.3%; (iv) PhOH/KOH, 160 °C, 4 h, 80.8%; (v) PhCHO, $ZnCl_2$, 160 °C, 3 h, 20.1%; (vi) $OsO_4/NaIO_4$, 24–26 °C, 24 h, 74.6%; (vii) $AgNO_3$/NaOH, rt, 12 h, 95.6%; (viii) $(COCl)_2$, rt, 24 h, ~100%; (ix) CH_2N_2/Et_2O, 0–15 °C, 3 h, 92.0%; (x) $AgNO_3/NH_3$, 100–110 °C, 2 h, 76.4%; (xi) BBr_3/CH_2Cl_2, rt, 4 h, 28.4%; (xii) NH_3, rt, 6 h, 93.2%.

with pure DMSO in the control wells. Reaction was initiated by addition of the enzyme. Enzyme inhibition data are the mean of two samples at each concentration. Reported IC_{50} values, determined by plotting log concentration versus inhibition values, are the mean of three or four separate experiments.[10]

Discussion

Our study is based on the structure of hnps-PLA_2. The crystal structure of hnps-PLA_2 was obtained from the Brookhaven Protein Data Bank (PDB). The ligand associated to the enzyme was a phosphonate transition state analogue (TSA) (PDB code: 1POE).[11] The programs DOCK[12,13] and SCORE[14] were used to predict how these compounds bind with the enzyme. After analyzing known inhibitors and using pocket module in LigBuilder,[15] the pharmacophore was derived and a series of heterocyclic compounds was designed. All compounds had similar characteristics according to the pharmacophore—one hydrophobic center which was suggested to be present at the pocket of hnps-PLA_2; one hydrogen bond (HB) acceptor and one HB donor. In the model, the quinoline ring was adopted as molecular framework at the pocket, 2-phenoxy was selected to be the hydrophobic center, 4-acetamide and 6-substituent used to HB acceptor and HB donor, respectively. The superimposition of pharmacophore and one quinoline inhibitor is shown in Figure 1.

We encountered some difficulties in the experiments of synthesis: (a) The 2-substituent of quinoline influenced the reactive activity of the 4-substituent. Benzoxy at the 2-position which directs the quinoline ring N atom to the *ortho* position, not only was itself a very strong leaving group, but also had electron-withdrawing ability. It deactivated the reactive activity of the methyl at the 4-position of the quinoline ring. The activation of 4-methyl was the key step for the target. Through trial and error, using the condensation with benzaldehyde and the subsequent breaking of the double bond under the mild conditions, the 4-methyl was activated successfully. (b) How to change 2-phenoxy-6-methoxyquinoline-4-carboxylic acid into 2-phenoxy-6-methoxyquinoline-4-acetamide? In our experiment, only using Arndt–Eistert reaction,[16] 4-carboxyl could be translated to 4-acetamide.

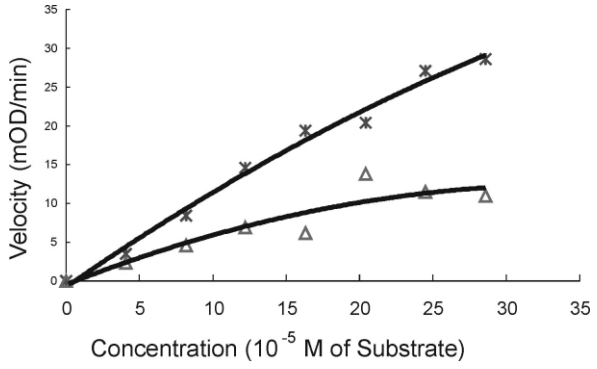

Figure 2. The concentration–velocity curve of the compound **10**: ∗ standard; △ inhibitor (10^{-6} M).

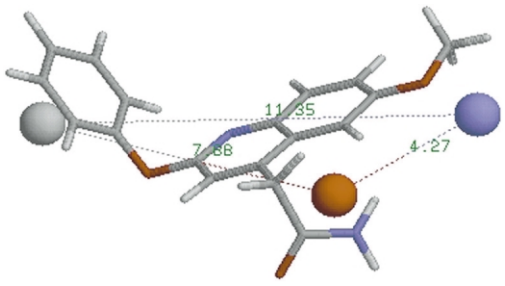

Figure 1. Superimposition of pharmacophore and one quinoline inhibitor, compound **10**. The pharmacophore elements are shown in gray, red and blue for hydrophobic moiety, HB acceptor and HB donor, respectively.

We obtained the designed title compounds, but the synthetic approach was rather long and had a 'bottle-neck' step [compound **5** was obtained in lower yield (almost 20%)]. Further improvements are underway.

A preliminary bioassay study in vitro showed that compounds **11** and **12** displayed inhibition to *Naja naja* $sPLA_2$ with the IC_{50} of 10 μM in the system we used. The IC_{50} of compound **10** was 1 μM, and it was also shown to be active on an animal model (data not shown). The concentration–velocity (maximum) curve of compound **10** compared to a standard curve is shown in Figure 2.

Conclusions

We have designed and synthesized novel quinoline-4-acetamide compounds as non-structural analogues of the phospholipidic substrate of PLA_2s. Bioassay results indicate that the compounds are potential inhibitors of $sPLA_2$.

Acknowledgements

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References and Notes

1. Bomalaski, J. S.; Clark, M. A. *Arthritis Rheum*. **1993**, *36*, 190.
2. Vadas, P. *Biochim. Biophys. Acta* **1997**, *1346*, 193.
3. Büchler, M.; Malfertheiner, P.; Schädlich, H.; Nevalainen, T. J.; Friess, H.; Berger, H. G. *Gastroenterology* **1989**, *97*, 1521.
4. Kramer, R. M.; Johansen, B.; Hession, C.; Pepinsky, R. B. *Adv. Prostaglandin Thromboxane Leuktriene Res.* **1990**, *20*, 79.
5. Conrad, M.; Limpach, L. *Ber*. **1887**, *20*, 944.
6. Kaslow, C. E.; Lauer, W. H. *Org. Synth. Coll.* **1955**, *3*, 194.
7. New compounds gave satisfactory structural data. Selected physical data and ^1H NMR data are as follows: **10**: MS: *m*/*z* 308 (M^+) calcd for $C_{18}H_{16}N_2O_3$ 308. 34; ^1H NMR (400 MHz, CD_3COCD_3): 3.94 (s, 3H, $-OCH_3$), 4.05 (s, 2H, $-CH_2-$), 6.52 (br, 1H, –NH, disappears after D_2O exchange), 7.15 (br, 1H, –NH, disappears after D_2O exchange), 7.22–7.28 (m, 4H, 3-H, Ph-H), 7.32 (dd, 1H, $J=9.4$, 2.0 Hz 7-H), 7.44–7.52 (m, 2H, Ph-H), 7.54 (d, $J=2.0$ Hz, 5-H), 7.59 (d, $J=9.4$ Hz, 8-H); Anal (calcd) %: C: 70.11 (70.13); H: 5.23 (5.19); N: 9.11 (9.09). **12.** *m*/*z* 294 (M^+) calcd for $C_{17}H_{14}N_2O_3$ 294.32; ^1H NMR (400 MHz, CD_3COCD_3): 3.84 (s, 3H, $-OCH_3$) 7.22–7.28 (m, 3H, Ph-H), 7.29 (s, 1H, 3-H), 7.35 (dd, 1H, $J=8.2$, 1.9 Hz, 7-H), 7.45–7.49 (m, 2H, Ph-H), 7.57 (d, 1H, $J=1.9$ Hz, 5-H), 7.60 (d, 1H, $J=8.2$ Hz, 8-H), 7.89 (br, 1H, –NH weakens after D_2O exchange), 8.29 (br, 1H, $-NH_2$, weakens after D_2O exchange); Anal (calcd)%: C: 69.48 (69.39); H: 4.71 (4.76); N: 9.48 (9.52).
8. Washburn, W. N.; Dennis, E. A. *J. Am. Chem. Soc*. **1990**, *112*, 2040.
9. Washburn, W. N.; Dennis, E. A.. *J. Biol. Chem*. **1991**, *266*, 5042.
10. Test condition in vitro: Re = 1.6; CMC = 0.29; [SIBLINKS] = 0.768 M; [Triton] = 1.49 mM; $T=22\,^{\circ}C$; $[PLA_2]=7.5$ μg/mL.
11. Scott, D. L.; White, S. P.; Browning, J. L.; Rosa, J. J.; Gelb, M. H.; Sigler, P. B. *Science* **1991**, *254*, 1007.
12. Kuntz, I. D.; Blaney, J. M.; Oatley, S. J.; Langridge, R.; Ferrin, T. E. *J. Mol. Biol*. **1982**, *161*, 269.
13. Meng, E. C.; Kuntz, I. D.; Abraham, D. J.; Kellogg, G. E. *J. Comput.-Aided. Mol. Des*. **1994**, *8*, 299.
14. Wang, R. X.; Liu, L.; Lai, L. H.; Tang, Y. Q. *J. Mol. Model*. **1998**, *4*, 379.
15. Wang, R. X.; Gao, Y.; Lai, L. H. *J. Mol. Model*. **2000**, *6*, 498.
16. Arndt, F.; Eistert, B. *Ber*. **1935**, *1*, 38.