



A SIMPLE SYNTHESIS OF BIARYL PHOSPHOLIPASE A₂ INHIBITORS: PROBING HYDROPHOBIC EFFECTS

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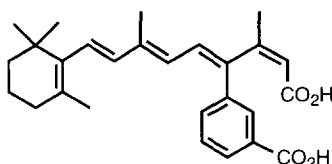
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Abstract: A series of biaryl acids has been synthesized by treating diphenic anhydride with a variety of amines. The one step strategy allows the rapid interchange of groups for evaluating the hydrophobic constraints of inhibitors of phospholipase A₂. The most active inhibitor synthesized displayed an IC₅₀ = 6 μ M against human platelet phospholipase A₂. Copyright © 1996 Elsevier Science Ltd

Phospholipases A₂ are a ubiquitous class of enzymes that have received considerable recent attention due to the premise that they initiate/mediate inflammatory processes.¹ Many classes of compounds have been shown to be inhibitors of human non-pancreatic PLA₂.² These include hydrophobic carboxylic acids,³ phospholipid analogs,⁴ natural products,⁵ and others.⁶

Recently, we described the synthesis and activity of a novel inhibitor of human platelet PLA₂ (HP-PLA₂).^{3d,e} The compound, BMS-181162, has an IC₅₀ = 40 μ M against HP-PLA₂, and effectively reduces mouse ear edema in a phorbol ester induced inflammation assay (ED₅₀ = 160 μ g/ear). Kinetic studies, obtained in the "scouting-mode",⁷ have determined that BMS-181162 is a tight binding competitive inhibitor of HP-PLA₂.⁸

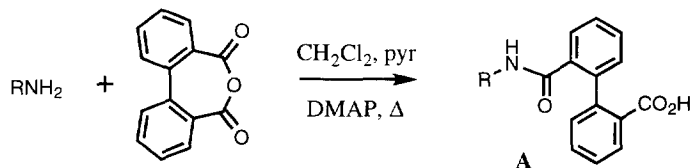


BMS-181162

BMS-181162 became a lead compound in a chemistry program to design more potent inhibitors of 14 kDa human platelet PLA₂. In the case of BMS-181162, the gross structural features that comprise the pharmacophore could be summarized as a hydrophobic (polyene) tail linked to a polar (dicarboxylic acid) head group. Concurrent with a traditional program to determine structure-activity relationships of close analogs of this lead, we desired to

explore more expansive structural changes in the hopes of discovering new lead compounds. Analysis of the literature lead to the observation that phospholipases A₂ are inhibited by a large variety of structures, of vastly varying molecular size. Since mono- and dicarboxylic acids containing various hydrophobic groups have been reported as excellent inhibitors of phospholipases A₂,³ we reasoned that any molecule containing one or two carboxylate groups linked to a hydrophobic moiety may be a potential inhibitor of PLA₂s.

We desired a synthetic strategy that would allow the rapid evaluation of many hydrophobic groups while retaining a non-variant polar head group comprised of a mono- or dicarboxylic acid. Utilizing this strategy we might more rapidly discover an optimal hydrophobic group, allowing its future incorporation into compounds more closely resembling the original lead structure BMS-181162. One tactic that we employed for this purpose can be illustrated by the reaction shown below. Treating diphenic anhydride with amines in the presence of pyridine and DMAP in refluxing methylene chloride provided biaryl carboxylic acids of type **A**.⁹ Molecules **A** contain a monocarboxylic acid head group, and an amide-linked hydrophobic group R. Since many amines are commercially available or easily synthesized, access to an unlimited amount of potential inhibitors of PLA₂ was possible.



We were pleased to observe that the biaryl carboxylic acids of type **A** synthesized in this manner proved capable of inhibiting human platelet PLA₂. The biological data for a series of biaryl acids synthesized by this method are shown below in Table 1.¹⁰ As the data in the table indicates, compounds with larger hydrophobic groups are better inhibitors of human platelet PLA₂. The n-decyl containing compounds **5** and **6** are the most active of the group. A comparison of the data for inhibitor **5**, with that for **3** and **4**, clearly demonstrates that the enzyme binds molecules with flexible alkyl chains better than those of similar size (number of carbon atoms) that have more rigid structures. This agrees with the fact that the natural substrates for the enzyme are phospholipids which contain esters of flexible fatty acids. Molecules which contain polar groups in the hydrophobic region, such as **7** and **8**, are poor inhibitors of HP-PLA₂.

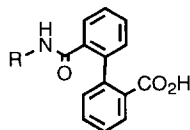
The IC₅₀ of inhibitor **6** was determined in a subsequent experiment to be 6 μM.¹¹ For comparison purposes, the natural product manoalide (a potent inhibitor of PLA₂s) was found to have an IC₅₀ = 6 μM under our assay conditions.

In summary, we have developed a simple strategy to rapidly assess structural requirements in the hydrophobic region of a new class of biaryl acid inhibitors of human platelet PLA₂. Compound **6** was found to be a potent inhibitor of HP-PLA₂. The method reported here should prove useful for discovering improved inhibitors of this important enzyme.

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Table 1: Biological Data for Compounds of Structure



R	Structure Number	% Inhibition of PLA ₂ at 100 μM	% Inhibition of PLA ₂ at 10 μM
	1	8	2
	2	15	2
	3	35	14
	4	63	6
	5	92	26
	6	98	85
	7	23	2
	8	0	0

References and Notes:

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 § Current address: Mitotix Inc., One Kendall Square, Bldg. 600, Cambridge, MA 02139.
 † Current address: Pfizer, Inc., Eastern Point Road, Groton, CT 06340.
- (a) Pruzanski, W.; Vadas, P. *Immunology Today* **1991**, *12*, 143. (b) Hope, W. C.; Patel, B. J.; Fieldler-Nagy, C.; Wittreich, B. H. *Inflammation* **1990**, *14*, 543. (c) Vishwanath, B. S.; Fawzy, A. A.; Franson, R. C. *Inflammation* **1988**, *12*, 549. (d) Vadas, P.; Pruzanski, W. *Laboratory Investigation* **1986**, *55*, 391. (e) Forster, S.; Ilderton, E.; Norris, J. F. B.; Summerly, R.; Yardley, H. J. *Br. J. Derm.* **1985**, *112*, 135. (f) Pruzanski, W.; Vadas, P.; Stefanski, E. *J. Rheumatol.* **1985**, *12*, 211. (g) Vadas, P.; Urowitz, M. B. *J. Lab. Clin. Med.* **1984**, *104*, 873. (h) Franson, R.; Dobrow, R.; Weiss, J.; Elsbach, P.; Woglich, W. B. *J. Lipid Res.* **1978**, *19*, 18.
 - For an early review of inhibitors of PLA₂ from various sources see: Wilkerson, W. W. *Drugs Of The Future* **1990**, *15*, 139.

3. (a) Musser, J. H.; Kreft, A. F.; Failli, A. A.; Demerson, C. A.; Shah, U. S.; Nelson, J. A. *International Patent Publication* **1991**, WO91/06537; *Chem. Abstr.* **1991**, *115*, 135935. (b) Failli, A. A.; Kreft, A. F.; Musser, J. H.; Banker, A. L.; Nelson, J. A.; Shah, U. S. *US Patent* **1991**, 5,071,988; *Chem. Abstr.* **1992**, *116*, 151752. (c) Djuric, S. W.; Haack, R. A.; Miyashiro, J. M. *International Patent Publication* **1992**, WO92/21644; *Chem. Abstr.* **1993**, *119*, 8502. (d) Tramposch, K. M.; Steiner, S. A.; Stanley, P. L.; Nettleton, D. O.; Franson, R. C.; Lewin, A. H.; Carroll, F. I. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 272. (e) Tramposch, K. M.; Chilton, F. H.; Stanley, P. L.; Franson, R. C.; Havens, M. B.; Nettleton, D. O.; Davern, L. D.; Darling, I. M.; Bonney, R. J. *J. Pharmacol. Expt. Therapeut.* **1994**, *271*, 852. (f) Kohler, T.; Heinisch, M.; Kirchner, M.; Peinhardt, G.; Hirschelmann, R.; Nuhn, P. *Biochem. Pharmacol.* **1992**, *44*, 805. (g) LeMahieu, R. A.; Carson, M.; Han, R.-J.; Madison, V. S.; Hope, W. C.; Chen, T.; Morgan, D. W.; Hendrickson, H. S. *J. Med. Chem.* **1993**, *36*, 3029. (h) Beaton, H. G.; Bennion, C.; Connolly, S.; Cook, A. R.; Gensmantel, N. P.; Hallam, C.; Hardy, K.; Hitchin, B.; Jackson, C. G.; Robinson, D. H. *J. Med. Chem.* **1994**, *37*, 557. (i) Carson, M.; Han, R.-J.; LeMahieu, R. A.; Madison, V. S. *US Patent* **1994**, 5,298,652; *Chem. Abstr.* **1994**, *121*, 231362.
4. (a) Pisabarro, M. T.; Ortiz, A. R.; Palomer, A.; Cabre, F.; Garcia, L.; Wade, R. C.; Gago, F.; Mauleon, D.; Carganico, G. *J. Med. Chem.* **1994**, *37*, 337. (b) Wheeler, T. N.; Blanchard, S. G.; Andrews, R. C.; Fang, F.; Gray-Nunez, Y.; Harris, C. O.; Lambert, M. H.; Mehrotra, M. M.; Parks, D. J.; Ray, J. A.; Smalley, Jr., T. L. *J. Med. Chem.* **1994**, *37*, 4118.
5. (a) For an early review see: Potts, B. C. M.; Faulkner, D. J. *J. Nat. Prod.* **1992**, *55*, 1701. (b) Tanaka, K.; Matsutani, S.; Matsumoto, K.; Yoshida, T. *J. Antibiotics* **1992**, *45*, 1071. (c) Tanaka, K.; Matsutani, S.; Kanda, A.; Kato, T.; Yoshida, T. *J. Antibiotics* **1994**, *47*, 631. (d) Barnette, M. S.; Rush, J.; Marshall, L. A.; Foley, J. J.; Schmidt, D. B.; Sarau, H. M. *Biochem. Pharmacol.* **1994**, *47*, 1661.
6. (a) Carganico, G.; Mauleon, D.; Garcia, M. L. *International Patent Publication* **1993**, WO93/21211; *Chem. Abstr.* **1994**, *121*, 134802. (b) Marki, F.; Breitenstein, W.; Beriger, E.; Bernasconi, R.; Caravatti, G.; Francis, J. E.; Paioni, R.; Wehrli, H. U.; Wiederkehr, R. *Agents Actions* **1993**, *38*, 202. (c) Pruzanski, W.; Greenwald, R. A.; Street, I. P.; Laliberte, F.; Stefanski, E.; Vadas, P. *Biochem. Pharmacol.* **1992**, *44*, 1165. (d) Garst, M. E. *Curr. Opin. Ther. Pat.* **1992**, 400. (e) Schiehser, G. A.; Von Burg, G. F. *US Patent* **1991**, 5,070,207; *Chem. Abstr.* **1992**, *116*, 128366. (f) Marshall, L. A.; Steiner, K. E.; Schiehser, G. A. *US Patent* **1990**, 4,933,365; *Chem. Abstr.* **1990**, *113*, 126604.
7. Gelb, M. H.; Berg, O.; Jain, M. K. *Curr. Opin. Struc. Biol.* **1991**, *1*, 836.
8. Burke, J. R.; Gregor, K. R.; Tramposch, K. M. *J. Biol. Chem.* **1995**, *270*, 274.
9. All new compounds gave appropriate 300 MHz ^1H NMR, 75 MHz ^{13}C NMR, IR, MS, and CHN or HRMS data consistent with their structures. The unoptimized yields of pure product from the acylation reaction ranged between 39–87%. The products can be recrystallized from EtOAc/hexane (structures **1–4**, and **7**) or chromatographed on reverse-phase silica (C-18, methanol as eluent; structures **5**, **6**, and **8**). The acylation reaction leading to product **7** was performed on the tert-butyl ester of the acid in the amine fragment. After acylation (87%), the ester was cleaved with TFA in CH_2Cl_2 , and the product was recrystallized from EtOAc/hexane to give a 20% yield of **7**.
10. For a detailed description of the PLA₂ assay protocol used in this work see reference 3d. Data determined at 100 μM and 10 μM was obtained in a high throughput screen and is the mean of two samples at each concentration.
11. The IC₅₀ reported for compound **6** is the mean of three separate determinations. The IC₅₀ of manoalide has been determined to be 20 nM against the 14 kDa PLA₂ isolated from human synovial fluid (Marshall, L. A.; Bauer, J.; Sung, M. L.; Chang, J. Y. *J. Rheumatol.* **1991**, *18*, 60), and 3.2 μM against the same enzyme isolated from human polymorphonuclear leukocytes (ref. 6b).

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