Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 7, pp. 793-798, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00110-8

DICARBOXYLIC ACID INHIBITORS OF PHOSPHOLIPASE A_2

Dane M. Springer,* Bing-Yu Luh, Stan V. D'Andrea, Joanne J. Bronson, and Muzammil M. Mansuri[§]
Central Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute
5 Research Parkway, P.O. Box 5100, Wallingford, CT 06492

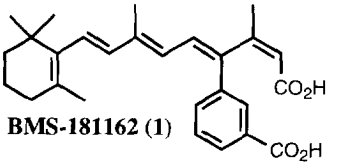
James R. Burke, Kurt R. Gregor, Paul L. Stanley, and Kenneth M. Tramposch
Dermatology Discovery Research, Bristol-Myers Squibb Pharmaceutical Research Institute
100 Forest Avenue, Buffalo, NY 14213

Abstract: Ten diacids were synthesized via a simple regio- and stereoselective aldol reaction. All of these compounds were good to excellent inhibitors of 14 kDa human platelet PLA_2, and many of these derivatives displayed activity in a phorbol-ester induced mouse ear edema assay. One of the new compounds reported here was selected for further development as a potential antipsoriasis agent. © 1997 Elsevier Science Ltd.

Arachidonic acid released from membrane phospholipids by the action of phospholipases A_2 serves as a biosynthetic precursor for the production of proinflammatory prostaglandins and leukotrienes.[1] The lysophospholipid product of PLA_2 lipid hydrolysis can be converted to platelet activating factor, a known mediator of inflammation. Therefore, the inhibition of phospholipases A_2 by chemical agents could lead to an antiinflammatory response.

Three distinct PLA_2 enzymes have been isolated from human sources; an 85 kDa protein found in the cytosol,[2] a 14 kDa protein found in the pancreas,[3] and a 14 kDa enzyme found in platelets and the synovial fluid of arthritic joints.[4] Due to the overabundance of the nonpancreatic 14 kDa protein in inflammatory conditions, we decided to develop inhibitors of this enzyme in the hopes of discovering novel antiinflammatory agents.

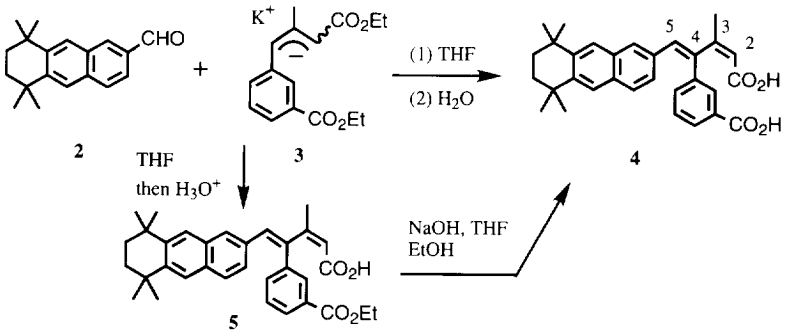
We developed a screen utilizing human platelet PLA_2 (HP-PLA_2) and discovered **BMS-181162** (**1**) a dicarboxylic acid that became a lead structure in our PLA_2 efforts. Many types of carboxylic acids are known inhibitors of PLA_2s.[5] Diacid **1** inhibits HP-PLA_2 in vitro with an IC_{50} = 40 μM. It is an effective antiinflammatory agent in a phorbol-ester induced acute inflammation assay, reducing mouse ear edema with an ED_{50} = 160 μg/ear.[6] Diacid **1** has been subsequently shown to act as an active-site directed competitive inhibitor of HP-PLA_2 at the lipid/enzyme interface.[7]



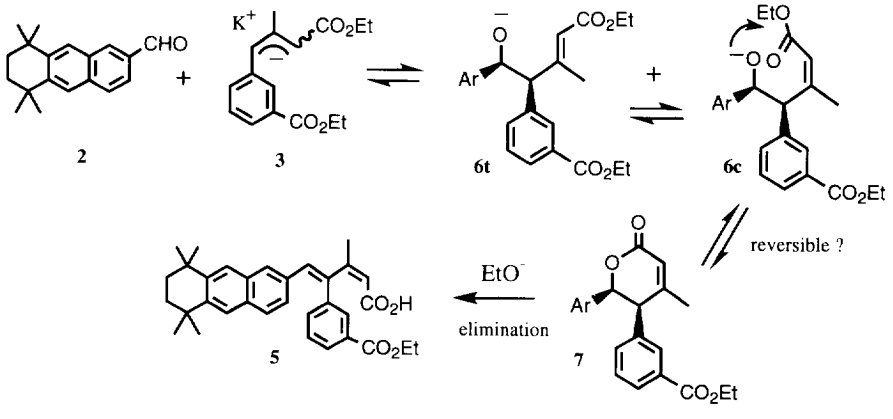
BMS-181162 (1)

[§]Current address: Mitotix Inc., One Kendall Square, Bldg. 600, Cambridge, MA 02139.

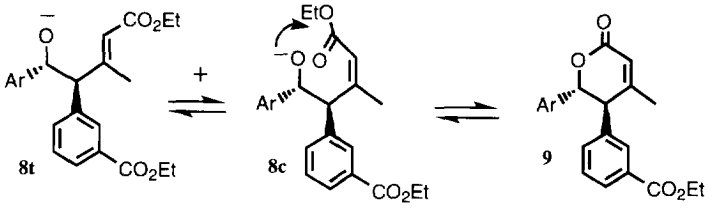
We wished to synthesize analogues of **1** that were more potent, and more chemically stable than the polyolefinic **1**. One tactic we employed for analogue synthesis utilized the convergent route to **1** reported previously.[5d] As shown below, the anion of ethyl 4-(3-carboethoxyphenyl)-3-methyl-2-butenoate (**3**) is formed with potassium *t*-butoxide in THF, and is then condensed with aldehydes (such as **2**). The addition of water causes hydrolysis of any esters to the final carboxylic acids. In the reaction, regioselective γ-alkylation of anion **3** was observed, and stereoselective formation of the $\Delta^{2,3}$ and $\Delta^{4,5}$ olefins occurred to provide **4** (from aldehyde **2**) as the only isolated product.[8,9] We further observed that when the reaction is quenched with mild aqueous acid before the basic hydrolysis, the mono-carboxylic acid **5** is obtained as the only isolated product. Subsequent basic hydrolysis of **5** then provided diacid **4**.



An explanation for the observed *cis-cis* olefin geometry in the production of monoester **5** is illustrated by the events shown in the scheme below. The anion mixture **3** reacts with aldehyde **2** to afford many possible adducts, two of which are the γ-alkylation adducts **6t** and **6c**. The *trans* alkoxide isomer **6t** cannot intramolecularly cyclize upon the ester and either reverts to a mixture of **2** and **3**, or is isomerized to **6c**. Alkoxide **6c** can cyclize to afford lactone **7**, which is subsequently eliminated in situ to afford the product of observed stereochemistry.



Models indicate that lactone **7** can easily attain the required *trans*-antiperiplanar transition state in the elimination of the carboxylate group. The dihedral angle between the C-O bond that fragments and the methine proton that is removed is ~180°. The alternative γ-alkylation adducts **8t** and **8c** may produce lactone **9** (below); however, this lactone cannot easily eliminate the carboxyl group via a stereoelectronically favorable pathway. In order to obtain a favorable dihedral angle for elimination to occur, the two aryl groups on the lactone ring must both assume pseudoaxial positions. This high energy transition state appears to preclude elimination from lactone **9**. The intermediates **8t**, **8c**, and **9** (if formed) must revert back to aldehyde **2** and anion **3**. All the possible intermediate adducts of the condensation reaction between **2** and **3** must eventually equilibrate to lactone **7**. The elimination of lactone **7**, to afford the carboxylate salt of **5**, serves to drive the reaction to completion.



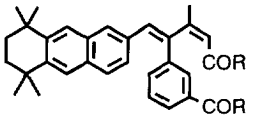
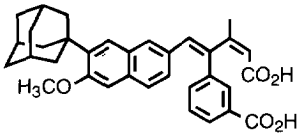
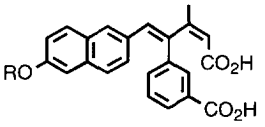
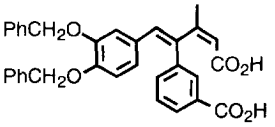
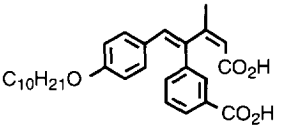
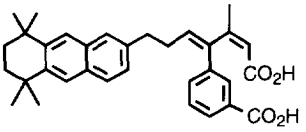
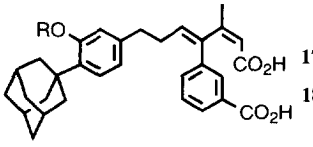
We subsequently found that anion **3** reacted with a variety of aldehydes to yield products containing the *cis-cis* olefin geometry (as observed in the case of **4**). These analogues are shown in Table 1.[9] As the biological data in Table 1 indicate, all of these compounds are good to excellent inhibitors of HP-PLA_2.[10] A comparison of the data for **4** and bis-amide **10** indicates that there is often a poor correlation between the effect on activity in vitro and in vivo arising from a particular change in the structure of a compound. The bis-amide **10** is clearly more active in vitro than **4**, yet markedly less active in vivo. Similarly, compound **16** is much more active in vitro than **4**, but less active in vivo. Pharmacological factors inherent in the in vivo model, such as bioavailability and metabolism, may explain these discrepancies. Another possible explanation is that any inhibitor may exhibit differential activity between human platelet PLA_2 and the corresponding mouse PLA_2.[11]

In summary, we have reported a series of inhibitors of HP-PLA_2 that have displayed good activity as anti-inflammatory agents in a mouse model of chronic inflammation. As in the case of the lead compound **1**, diacid **4** has been subsequently found to be an active-site directed competitive inhibitor of HP-PLA_2.[12] Compound **4** was selected for further pharmaceutical development based on its excellent antiinflammatory activity in the mouse chronic ear edema assay.

Acknowledgments

We thank the members of the Chemical Process Research group at Bristol-Myers Squibb in Regensburg, Germany for providing us with intermediate **3** used in our work. We also thank the Department of Analytical Research in Wallingford, CT, for analytical data in support of this work.

Table 1: Biological Data for Compounds Synthesized[a]

Structure		% Inhibition of HP-PLA$_2$ at 100 μM/at 10 μM	IC_{50} (μM)[b]	ED_{50} (μg/ear per dose)[c]
	**4** R = OH	98/48	17	9, 37[d]
	10 R = NH_2	98/68	4	186
	**11**	97/40	–	–
	**12** R = n-$C_{10}H_{21}$-	100/45	14	31
	13 R = $Ph(CH_2)_3$-	100/44	17	30, 90[d]
	**14**	85/21	–	208
	**15**	99/57	7	–
	**16**	99/88	2	89
	**17** R = 3,5-di-F-$Ph(CH_2)$-	99/48	–	–
	18 R = H	96/55	8	–

[a]See references 9 and 10.

[b]HP-PLA_2

[c]Phorbol-ester induced mouse ear chronic inflammation assay. For details see ref 6b.

[d]Values for two separate determinations.

References and Notes

1. (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.; Woglichi, W. B. *J. Lipid Res.* **1978**, *19*, 18.
2. (a) Clark, J. D.; Milona, N.; Knopf, J. L. *Proc. Nat. Acad. Sci. U.S.A.* **1990**, *87*, 7708. (b) Kramer, R. M.; Roberts, E. F.; Manetta, J.; Putnam, J. E. *J. Biol. Chem.* **1991**, *266*, 5268. (c) Clark, J. D.; Lin, L. L.; Kriz, R. W.; Ramesha, C. S.; Sultzman, L. A.; Lin, A. Y.; Milona, N.; Knopf, J. L. *Cell* **1991**, *65*, 1043. (d) Sharp, J. D.; White, D. L.; Chiou, X. G.; Goodson, T.; Gamboa, G. C.; McClure, D.; Burgett, S.; Hoskins, J.; Skatrud, P. L.; Sportsman, J. R.; Becker, G. W.; Kang, L. H.; Roberts, E. F.; Kramer, R. M. *J. Biol. Chem.* **1991**, *266*, 14850.
3. Nishijima, J.; Okamoto, M.; Ogawa, M.; Kosaki, G.; Yamano, T. *J. Biochem.* **1983**, *94*, 137.
4. (a) Hara, S.; Kudo, I.; Chang, H. W.; Matsuta, K.; Miyamoto, T.; Inoue, K. *J. Biochem.* **1989**, *105*, 395. (b) Seilhamer, J. J.; Pruzanski, W.; Vadas, P.; Plant, S.; Miller, J. A.; Kloss, J.; Johnson, L. K. *J. Biol. Chem.* **1989**, *264*, 5335. (c) Kramer, R. M.; Hession, C.; Johansen, B.; Hayes, G.; McGray, P.; Chow, E. P.; Tizard, R.; Pepinsky, R. B. *J. Biol. Chem.* **1989**, *264*, 5768.
5. (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. *Biochemical Pharmacology* **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.
6. (a) This is a phorbol-ester induced mouse ear *acute* inflammation assay. In the acute assay the test drug is dosed to the mouse ear concurrent with the application of the irritating phorbol ester. The ED_{50} is therefore a measure of protection of the mouse ear from inflammation caused by the irritant. For the experimental details of this assay see ref 5d. (b) The ED_{50}s reported in Table 1 in this work are for the performance of these compounds in a phorbol-ester induced mouse ear *chronic* inflammation assay (for details of the chronic assay see ref 5e and: Stanley, P. L.; Steiner, S.; Havens, M.; Tramposch, K. M. *Skin Pharmacol.* **1991**, *4*, 262). In the chronic assay ten day study, a solution of the phorbol ester is applied once on days 0, 2, 4, 7, and 9. The test compound is dosed to three different groups of animals at three different molar concentrations twice daily on days 7, 8, and 9, followed by one dose on day 10 (7 total doses). The animals were sacrificed at the end of day 10. The ears were evaluated for myeloperoxidase activity (a marker for polymorphonuclear leukocyte influx) and an ED_{50} determined by linear regression. The application of irritant alone over the first seven days establishes a chronic state of inflammation, therefore the ED_{50} is a measure of a compound's ability to reduce edema in an entrenched inflammatory condition. For comparison purposes compound **1** has an ED_{50} = 180 μg/ear per dose in the chronic assay. The ED_{50} of lonapalene (a known 5-lipoxegenase inhibitor and antipsoriasis agent) in this assay is 1000 μg/ear (for details see ref 5e).
7. Burke, J. R.; Gregor, K. R.; Tramposch, K. M. *J. Biol. Chem.* **1995**, *270*, 274. Diacid **1** was originally synthesized as part of a retinoic acid analog program, and discovered as a hit in a HP-PLA_2 screen of proprietary compounds. Compounds **1**, **4**, **10**, **12**, and **17** were evaluated for binding and activation of retinoic acid receptors (RARs) α, β, γ. All the compounds tested failed to activate RARs α, β and γ.

8. Experimental procedure for the synthesis of **4**: Potassium *tert*-butoxide (1.40 g, 12.5 mmol) was suspended in THF (40 mL) under nitrogen at -5 °C. A solution of ethyl 4-(3-carboethoxyphenyl)-3-methyl-2-butenoate (see ref 5d) (1.66 g, 6.00 mmol) in THF (5 mL) was added. The mixture was stirred at 0 °C for 30 min. A solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenaldehyde (1.33 g, 5.00 mmol) in THF (5 mL) was added over 5 min. After stirring at 0 °C for 30 min, 4 mL of water was added. After 3 h of additional stirring, the solution was acidified to pH 2 with 1 N HCl. The solution was extracted with ether, and the organic phase was washed with water and brine. The organic layer was dried ($MgSO_4$), and concentrated. The product was chromatographed on silica using methylene chloride, then 5% methanol/methylene chloride as eluent. An oil was obtained that was then dissolved in acetonitrile to precipitate **4** as a white solid (1.34 g, 2.86 mmol, 57%): mp 186-7 °C; ^{1}H NMR (300 MHz, $CDCl_3$ with one drop DMSO-d_6) δ 7.95 (s, 1H, ArH), 7.88 (d, J = 8 Hz, 1H, ArH), 7.54 (d, J = 13 Hz, 2H, ArH), 7.42-7.39 (m, 2H, ArH), 7.31 (d, J = 8 Hz, 1H, ArH), 7.20 (dd, J = 8, 8 Hz, 1H, ArH), 6.85 (dd, J = 8, 1 Hz, 1H, ArH), 6.64 (s, 1H, ArCH=C), 5.82 (d, J = 8 Hz, 1H, C=$CHCO_2$), 1.84 (s, 3H, C=CCH_3), 1.68 (s, 4H, CH_2CH_2), 1.29 (s, 12H, 4 × CH_3); Anal. calcd for $C_{31}H_{32}O_4$: C, 79.48; H, 6.84. Found: C, 79.30; H, 7.08. The crude ^{1}H NMR spectrum of **4** obtained prior to chromatography was very clean for structure **4**. There are only a few trace peaks in the baseline, and these could not be assigned unequivocally to another isomer. The yields reported for compound **4** and the other compounds shown in Table 1 (see ref 9) are unoptimized. The yields appear low due to losses from chromatography (yield for center cuts reported) and crystallization. We believe, however, that the actual yield of these reactions from starting aldehyde to product diacid is generally >80%.

9. All new compounds gave appropriate 300 MHz ^{1}H NMR, 75 MHz ^{13}C NMR, IR, MS, and CHN or HRMS data consistent with their structures. The structures of **4** and compound **11** were determined by ^{1}H NMR NOE studies. The olefinic geometries of the remaining compounds in Table 1 were not rigorously determined, but follow logically from the mechanistic discussion advanced in the text of this work. Yields for the remaining compounds shown in Table 1 (aldehyde to diacid) are as follows (purified by normal phase flash chromatography using MeOH/CH_2Cl_2 unless otherwise indicated): **10**, 56% (obtained from **4** via reaction with DCC (2.5 equiv), N-hydroxysuccinimide (2.5 equiv), and 1-hydroxybenzotriazole (catalytic) in CH_2Cl_2, 23 °C, 20 h); **11**, 54% (chromatographed on C-18 silica using MeOH/water, then crystallized from CH_2Cl_2/pentane); **12**, 41%; **13**, 36%; **14**, 35%; **15**, 31%; **16**, 19% (chromatographed on C-18 silica using MeOH/water, then crystallized from MeOH/CH_2Cl_2/pentane); **17**, 58%; **18**, 45% (chromatographed using EtOAc/hexane).

10. For a detailed description of the PLA_2 assay protocols used in this work see references 5d and 5e. Data determined at inhibitor concentrations of 100 μM and 10 μM were obtained in a high throughput screen and are the mean of two samples at each concentration. The IC_{50}s reported for the compounds in Table 1 are the mean of three separate determinations. For comparison purposes, the natural product manoalide (a potent inhibitor of PLA_2s) was found to have an IC_{50} = 6 μM under our assay conditions. The IC_{50} of manoalide was found to be 20 nM against the identical 14 kDa PLA_2 isolated from human synovial fluid (Marshall, L. A.; Bauer, J.; Sung, M. L.; Chang, J. Y. *J. Rheumatol.* **1991**, *18*, 59), and 3.2 μM against the same enzyme isolated from human polymorphonuclear leukocytes (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).

11. It is of interest to consider that a truly specific inhibitor of human PLA_2 would only show in vivo activity in man. For a recent paper describing an extremely selective human PLA_2 inhibitor see: Schevitz, R. W.; Bach, N. J.; Carlson, D. G.; Chirgadze, N. Y.; Clawson, D. K.; Dillard, R. D.; Draheim, S. E.; Hartley, L. W.; Jones, N. D.; Mihelich, E. D.; Olkowski, J. L.; Snyder, D. W.; Sommers, C.; Wery, J. P. *Nature Struct. Biol.* **1995**, *2*, 458.

12. Unpublished results obtained for **4** from a "scooting-mode" assay as described for compound **1** in ref 7. The equilibrium dissociation constant for **4** bound to the enzyme at the lipid-enzyme interface (K_i*) was determined to be 0.0076 mol fraction. This indicates that **4** binds approximately twice as tightly to the enzyme under the assay conditions than does **1** (K_i* = 0.013 mol fraction). For a comparison of the K_i* values of **1** and three other known inhibitors of the human 14 kDa PLA_2 see ref 7.

(*Received in USA* 16 *December* 1996; *accepted* 19 *February* 1997)