Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 711-716, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

ANTIINFLAMMATORY β-BENZENEETHANAMINES. III

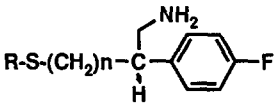
W.W. Wilkerson*, I. DeLucca, W. Galbraith, R. Harris, and J. Kerr
DuPont Merck Pharmaceutical Company, E353/347 Experimental Station, P.O. Box 80353, Wilmington, Delaware 19880-0353

(*Received in USA* 7 *December* 1992; *accepted* 7 *January* 1993)

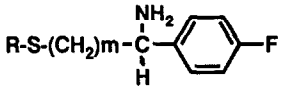
Abstract: A limited series of β-benzeneethanamines (I) was synthesized and evaluated to determine their antiinflammatory activity, and their ability to inhibit rat polymorphonuclear cell phospholipase-A_2, ovine seminal vesicle prostaglandin synthetase, and rat basophilic leukemia cell 5-lipoxygenase.

Introduction

In our efforts to find novel compounds with anti-PLA_2 activity and antiinflammatory activity, we synthesized and evaluated a limited series of β-benzeneethanamines (I) for their ability to inhibit the phospholipase-A_2 from rat polymorphonuclear cells (PLA_2). Previous efforts resulted in a series of α-benzenemethanamines (II) which were moderate to good inhibitors of PLA_2, but weak to moderate oral antiinflammatory agents as measured in the rat carrageenan paw edema assay (CAR) [1,2]. The present work resulted from an attempt to improve on the anti-PLA_2 and antiinflammatory activity through synthetic modifications of **II**.



I



II

DP6711: **R** = 2-naphthyl, **m** = 3
DP6929: **R** = 4-Me-Ph, **m** = 3

Inhibitor Design

Previous studies [1,2] had demonstrated that antiinflammatory PLA_2 inhibitors could have the following general formula:

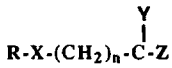
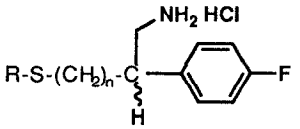


Figure I

where **R** is large and/or lipophilic; **X** is NH, S, O, or CH_2; (CH_2)**n** is a tether of 1 to 4 to link **CYX** and; **Y** is =O, OH, or NH_2 to coordinate with calcium; and **Z** is a relatively small group that might influence the electron

density on **Y**. The present study involved compounds where **X** is S and **Y** is CH_2-NH_2. The first alterations of **II** involved extending the distance between the amine group (**Y**) and the 4-fluorophenyl (**Z**). The result of these efforts was the synthesis of the β-benzeneethanamines (**I**) shown in Table I.

Table I. Physical chemical data for compounds **1-5**



Cmpd	R	n	mp, °C	% Yield	Formula[a]	ClogP[b]	CMR[c]
1C	2-Naph	4	133-136	26	$C_{22}H_{24}NFS$ HCl	6.33	10.97
2	4-Me-Ph	3	113-116	52	$C_{18}H_{22}NFS$ HCl	5.13	9.28
3	4-^{t}Bu-Ph	4	154-156	24	$C_{22}H_{30}NFS$ HCl	6.99	11.14
4C	4-Me-Ph	4	101-103	43	$C_{19}H_{24}NFS$ HCl	5.66	9.75
5	4-Me-Ph	5	166-167	70	$C_{20}H_{26}NFS$ HCl	6.19	10.21
DP6711	2-Naph	3				5.26	9.93
DP6929	4-Me-Ph	3				4.58	8.71

[a] All compounds were analyzed for C, H, N, S; [b] computer generated logP; [c] computer generated molar refractivity

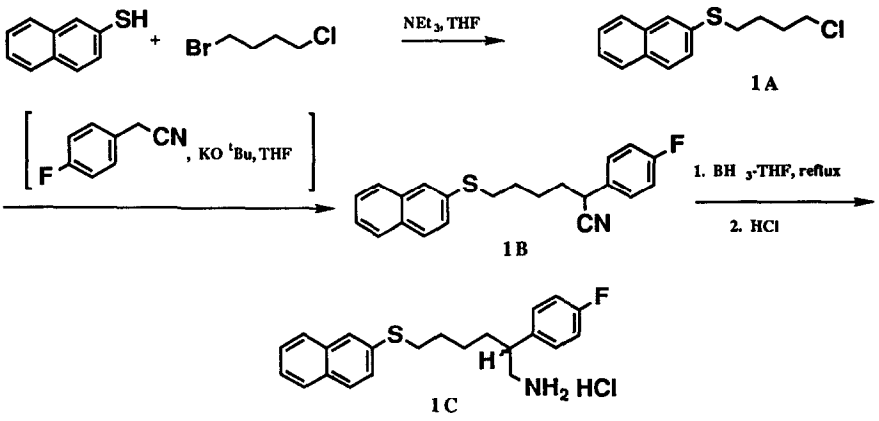
Chemistry

The target compounds were synthesized as illustrated in **Schemes I-II.** Compounds **1C-5** were synthesized by alkylating a thiol with a mixed dihaloalkane under mild basic conditions to give the 'arylthio alkyl halide'(**1A**). The anion of 4-fluorophenylacetonitrile, which was generated with KO^{t}Bu in THF, was then alkylated with **1A** to give the corresponding nitrile (**1B**). Compound **1B** was reduced with refluxing BH_3-THF to give the desired product **1C** as the racemic amine hydrochloride. **Scheme I** illustrates this approach; it is applicable to the other members of the series, and allows greater freedom in synthesizing analogs containing different **Z**-groups.

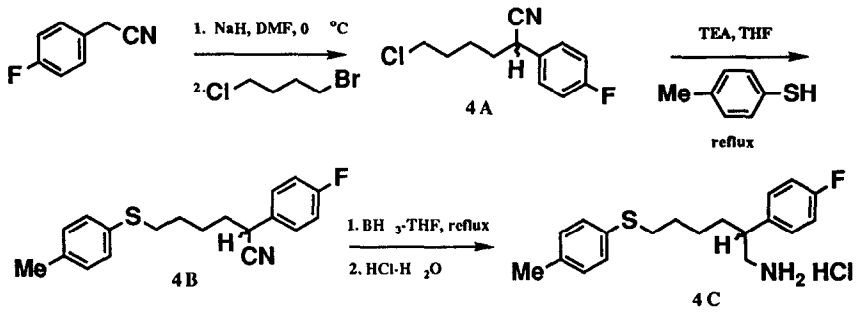
An alternative approach of monoalkylating the anion of 4-fluorophenylacetonitrile with the mixed dihaloalkane, followed by reaction of the 'haloalkylnitrile' (**4A**) with a thiol to give the 'thio-alkylnitrile' (**4B**)

which could be reduced to the 'amine sulfide' (4C) This approach allowed greater freedom in synthesizing analogs useful in determining the importance of R to biological activity. This approach is illustrated in **Scheme II.**

Scheme 1.



Scheme 2.



Pharmacology

Compounds **1C-5** were first evaluated for their ability to inhibit phospholipase-A_2 (PLA_2), 5-lipoxygenase (5LO), and prostaglandin synthetase (PGS). Compounds were then evaluated in the CAR to determine their oral antiinflammatory activity [3]. Because of its activity in the CAR assay, compound **4C** was also tested in the established adjuvant arthritis (ADJ ARTH), and the contact sensitivity (CON SEN) models for other oral antiinflammatory activity [4]. All biological assay procedures have been previously described [1,2,5].

Discussion

The objective was to synthesize an agent that was antiinflammatory and primarily a PLA_2 inhibitor with little or no cyclooxygenase inhibitory activity. Good activity against 5LO was considered a bonus[6]. The initial objectives of this study were met and are illustrated in **Table II.** The desired result of the modifications **DP6711** and **DP6929** were to increase CAR activity (lower ED_{30}'s), and increase PLA_2 and 5LO activities (lower IC_{50}'s, and decrease PGS activity (higher IC_{50}'s). By changing **DP6711** {S-$(CH_2)_3$-CH-N} to **1C** [S-$(CH_2)_4$-CH-CH_2-N] the antiinflammatory activity was not improved. However, PLA_2 activity was improved, PGS activity was essentially unchanged, and 5LO activity was significantly decreased. The changes in naphthyl-thio series (**DP6711**) did not meet our objectives. Converting **DP6929** [S-$(CH_2)_3$-CH-N] to **2** [S-$(CH_2)_3$-CH-CH_2-N] greatly improved CAR activity, modestly improved PLA_2 and 5LO activity, and left PGS activity unchanged. Though the CAR activity was modest, the trend in activities was encouraging. The insertion of a methylene between the amine and the methine carbon in the 4-methylphenyl-thio series appeared to be important. As a result, we synthesized **4C** [S-$(CH_2)_4$-CH-CH_2-N] and **5** [S-$(CH_2)_4$-CH-CH_2-N]. The only difference between **2, 4C**, and **5** is number of methylenes [S-$(CH_2)_n$-CH-CH_2-N] between the sulfur and nitrogen moieties. The change resulted in the following observations: for CAR, $n = 4 > 3 > 5$; for PLA_2, $n = 4 = 5 > 3$; for 5LO, $n = 4 > 3 = 5$; and for PGS, $n = 3 > 4 = 5$. Except for $n = 4$ in **4C**, the changes in **4C** resulted in a far superior antiinflammatory drug as compared to the lead **DP6929**. The increases in calculated logP (ClogP) and calculated molar refractivity (CMR) for the series **2, 4C**, and **5** were anticipated, but do not appear to explain the changes in activities unless there is very small tolerance for lipophilicity and molecular size. For the series **R**-S-$(CH_2)_4$-CH-CH_2-N, we compared **2** (R = 2-naphthyl), **3** (R = 4-^{t}Bu-Ph), and **4** (R = 4-Me-Ph). Clearly 4-Me-Ph produces the best antiinflammatory and 5LO inhibitor, and the change had no significant effect on PLA_2 or PGS inhibition. The trend suggest that the nature of the R-group was important for desirable activity, and suggest an area for future investigation.

All five compounds were considered to be active as PLA_2 inhibitors, with PLA_2 $IC_{50's}$ ranging from 2.4 μM for **1C** to 28.3 μM for compound **3**. Compounds **2-5** were considered active as antiinflammatory agents since they inhibited CAR edema 20% or more at oral doses of 20 mg/kg. Based on standards in these laboratories, none was considered as a "good" inhibitor of PGS or 5LO. All were found to be inactive in the AA Ear and TPA Ear models. Because of its activity in the CAR assay, **4C** was evaluated in other assays associated with our antiinflammatory program. The results in **Table III** would suggest that **4C** is an effective oral antiinflammatory.

Conclusion

On the basis of the results in CAR for the series and the additional data for **4C** in the ADJ ARTH and CON SEN models, we concluded that compounds like **Formula I** are orally active antiinflammatories. The enzyme inhibitory profile for this set of compounds, when compared with activity in the CAR PAW, would

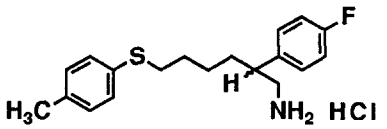
suggest that the antiinflammatory activity resulted from the inhibition of 5LO, and not PGS or PLA_2 as was initially anticipated. However, the *in vivo* antiinflammatory activity could result from "a little inhibition here and a little inhibition there", adding up to an effective inhibition of all three enzymes associated with the arachidonic acid cascade. The compounds could be "mixed" inhibitors. This limited series represent a "lead set" for the discovery and development of novel orally active antiinflammatory drugs whose mechanism of action may be attributed to 5-lipoxygenase and/or PLA_2 inhibition. The above data has provided us with a template describing lipophilicity and size requirements for molecules of this type that are antiinflammatory 5LO/PLA_2 inhibitors. With these results, future studies using x-ray crystallography, computer modeling techniques, drug metabolism, and toxicology should allow us to synthesize safe and efficacious oral antiinflammatory drugs.

Table II. Biological profile for compounds **1-5.**

Cmpd	PMN PLA_2 IC_{50}, μM; (+/- s.e.) n = 3	CAR, po ED_{30}, μM/kg (+/- s.e.) n = 3	OSV PGS, IC_{50}, μM; (+/- s.e.) n = 3	RBL 5LO, IC_{50}, μM (+/- s.e.) n = 3
1C	2.4 (0.1)	171.3	14.5 (3.2)	130.0 (7.3)
2	28.3 (1.4)	50.0	197.0 (5.7)	38.0 (3.7)
3	4.8 (0.1)	153.8	19.1 (0.6)	230.0 (25.3)
4C	5.5 (0.2)	1.4 (0.3)	48.7 (0.3)	9.5 (0.9)
5	6.6 (0.7)	117.7	48.0 (1.6)	35.0 (3.1)
DP6711	14.0 (0.7)	> 153.6	47.0 (0.5)	23.0 (1.0)
DP6929	54.0 (3.0)	> 172.8	215.0 (7.5)	75.0 (6.0)
DEX	>750.0	< 2.5	>25.0	>25.0
BW 755C	>25.0		1.3 (0.2)	1.0 (0.2)
IND	250.0 (14.1)	2.8	17.2 (1.8)	>250.0
MEP	100.0 (5.7)	5.8	130.0 (7.3)	130.0 (11.0)

DEX = dexamethasone, IND = indomethacin, and MEP = mepacrine. ( ) = standard error

Table III. Biological profile of compound **4C.**



Assay	Result[a]
PMN-PLA_2, IC_{50} μM	5.5 +/- 0.17
PAN-PLA_2, IC_{50} μM	4.1 +/- 0.70
OSV-PGS, IC_{50} μM	48.7 +/- 0.3
BSV-PGS, IC_{50} μM	280.0 +/- 13.6
RBL-5LO, IC_{50} μM	9.5 +/- 0.9
CAR PAW, po, % Inh @ 50 mg/kg	59.0 +/- 3.0
CAR PAW, po, ED_{30} mg/kg	0.51 +/- 0.07
EST ADJ ARTH, po, day 18, ED_{50}, mg/kg	50.0 +/- 3.6
CON SEN, po, % Inh @ 50 mg/kg	(51.2 - 36.8)[b]
CON SEN, po, ED_{35} mg/kg	8.9 +/- 0.9
TPA EAR, top, % Inh @ 100 mg/ear	37.0 +/- 4.0

[a] mean +/- standard error for three separate determinations; [b] range of values for three determinations

Abbreviations: PMN-, rat polymorphonuclear cell; PAN-, porcine pancreas; PLA_2, phospholipase-A_2; OSV-, ovine seminal vesicle; BSV-, bovine seminal vesicle; PGS, prostaglandin synthetase; RBL-5LO, rat basophilic leukemia 5-lipoxygenase; CAR PAW, rat carrageenan paw edema; EST ADJ ARTH, rat established adjuvant arthritis paw edema; CON SEN, mouse contact sensitivity; TPA EAR, tetradecanoyl phorbol acetate mouse ear edema.

References

1. Wilkerson, W.; DeLucca, I.; Galbraith, W.; Gans, K.; Harris, R.; Jaffee, B.;Kerr, J. *Eur. J. Med. Chem.* 1991, *26*, 667-676.
2. Wilkerson, W.; DeLucca, I.; Galbraith, W.; Kerr, J. *Eur. J. Med. Chem.* 1992, 27,595-610.
3. Otterness, I. G.; Gans, D. J. *J. Pharm. Sci.* 1988, *77*, 790-794.
4. Hunneyball, I. In *Anti-Rheumatic Drugs*Birkhauser: Basel, 1988; pp 20-30.
5. Wright, S. W.; Harris, R. R.; Collins, R. J.; Corbett, R. L.; Green, A. M.; Wadman, E. A.; Batt, D. G. *J. Med. Chem.* 1992, *35*, 3148-3155.
6. Holsapple, M. P.; Yim, G. K. W. *Inflammation* 1984, *8*, 223-230.