Tetrahedron Letters,Vol.30,No.50,pp 6959-6962,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

SIMPLE ANALOGS OF GINKGOLIDE B WHICH ARE HIGHLY ACTIVE ANTAGONISTS OF PLATELET ACTIVATING FACTOR

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Summary: A number of simple synthetic analogs of ginkgolide B (**1**) are described which are even more potent as antagonists of platelet activating factor, for example (±)-**5** (IC_{50} = 0.3 μM) and (±)-**7** (IC_{50} = 0.2 μM).

Platelet activating factor (PAF) is a potent bioregulator which appears to play a key role in acute allergy, inflammation, asthma, ischemic injury, and tissue rejection through its action at high affinity receptors ($EC_{50} \sim 10^{-10}$ *M*).[1] Consequently, the development of PAF antagonists which are suitable for therapeutic use has assumed considerable importance.[2] Among the known types of PAF antagonists ginkgolide B (**1**) is especially interesting because of its long history of human use (in the form of extracts of leaves of the ginkgo tree, *Ginkgo biloba*), its notable lack of toxicity, and its total resistance to metabolism.[3] In view of the therapeutic potential of ginkgolide B, the limited amounts of ginkgolide available from the ginkgo tree, and the poor absorption (*ca.* 15%) of orally administered ginkgolide B, we have investigated the possibility that simpler and smaller molecular analogs of **1** might be more suitable for medical use by taking advantage of the chemical process which led to the first successful total synthesis of **1**.[5-7] In this paper we report on three synthetic ginkgolide analogs which are easily available by synthesis, simpler and less polar than **1** (and hence likely to be better absorbed after oral administration), and even somewhat more active than **1** as inhibitors of PAF. Studies of a range of synthetic analogs have also provided insights regarding the structural features of **1** which enhance anti-PAF activity.

The starting point for the construction of new molecules with anti-PAF activity was the tetracyclic lactone **2**, a key intermediate in the total synthesis of **1**.[5,6] The racemic form of **2** was employed since a sizeable quantity of this compound was available from earlier work;[5] all of the analogs of **2** reported herein were obtained as racemates. Initial studies of the anti-PAF activity of early-stage, tetracyclic synthetic intermediates lacking the oxygen bridge between C(4) and C(12) had indicated very low biological potency (IC_{50} > 100 μM). In contrast, the lactone subunit attached to C(2) and C(3) of **1** is *not* essential to biological activity, as indicated by the information which follows.

Lactone **2** was transformed in five steps via **3** to the chlorohydrin bis lactone **4**, by the following sequence: (1) stereospecific α-epoxidation of the C(1) - C(2) olefinic linkage (*m*-chloroperoxybenzoic acid in CH_2Cl_2 - pH 8 aqueous phosphate buffer at 23°C, 92%); (2) oxirane ring opening to form chlorohydrin **3** (3 equiv of BCl_3 and 4 equiv of benzyltriethylammonium chloride in CH_2Cl_2 at -45°C to 23°C, 79%); (3) elimination of methanol to convert methyl acetal **3** to the corresponding dihydrofuran (heating with 5 equiv of each pyridinium tosylate and pyridine in chlorobenzene at 135°C for 16 h, 83%);

(4) dihydroxylation of the C(10) - C(11) olefinic linkage (osmium tetroxide-pyridine, 55°C for 36 h, 69%); and (5) oxidation of lactol to lactone (I_2, aqueous MeOH containing $CaCO_3$ at 23° for 0.5 h, 82%).[8] Bis lactone diol **4** was also converted to the corresponding bis methoxymethyl (MOM) ether, **5** (excess $CH_2(OMe)_2$, P_2O_5 in $ClCH_2CH_2Cl$ at 23°C, 69%). The anti-PAF activity of **5** was measured to be IC_{50} = 0.3 μM as compared to IC_{50} = 0.6 μM (±0.2) measured for ginkgolide B (**1**) as control.[9,10] The diol **4**, IC_{50} 1.1 μM, was somewhat less active than the bis MOM derivative **5**, indicating that free hydroxyl groups are not necessary for anti-PAF function of ginkgolides. Assuming that only one enantiomer of **5** is active, it follows that chiral **5** is about four times more potent as an anti-PAF agent as ginkgolide B.

The 2-bromo analogs of **4** and **5** were synthesized from **2** in a parallel fashion, and their anti-PAF IC_{50} values were determined as 14 μM and 0.6 μM, respectively.

The 1α,2β-dichloro derivatives **6** and **7** were also synthesized from **2** by a sequence consisting of 1α,2β-dichlorination (chlorine and benzyltriethylammonium chloride in CH_2Cl_2-CF_3CH_2OH at 0°C, 65%) and then functional group modification at C(10) and C(11) as described above for the synthesis of **4** and **5**. The anti-PAF IC_{50} values of **6** and **7** were determined to be 0.4 μM and 0.2 μM, respectively. Thus, the active enantiomer **7** is expected to be *ca.* six times as active as ginkgolide B with IC_{50} = 0.1 μM. Since **7** is considerably less polar than ginkgolide B, it is expected to be much better absorbed after p. o. administration, and possibly more efficacious.

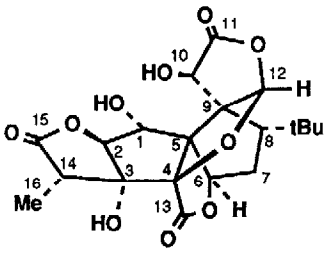
The C(10) epimer of **6** was synthesized by oxidation of **6** to the corresponding α-keto lactone (Jones' reagent, acetone-water, 23°C for 1 h) and subsequent reduction using excess aluminum amalgam in 20 : 1 THF-H_2O at 23°C for 2 h.[11] The anti-PAF IC_{50} value for the C(10)-epimer of **6** was found to be 1.3 μM. The isomer of this dichloride having an oxygen bridge between C(4) and C(8), compound **8**, was synthesized from the related C(1) - C(2) - olefin[5] and found to be considerably less active, IC_{50} = 38 μM.

A carbonyl function at C(11) is beneficial for anti-PAF activity, but not essential; thus, the IC_{50} for **2** was 120 μM as compared to 80 μM for the corresponding structure having a carbonyl group at C(11).

The effect of substituents at C(10) was evaluated for the series **9** - **14**, having no substituents at C(1) and C(2). The following IC_{50} values were measured: **9**, 76 μM; **10**, 13 μM; **11**, 9.4 μM; **12**, 13 μM; **13**, 11 μM; **14**, 21 μM. The α-keto lactone obtained by oxidation of the 10-hydroxyl function of **9** or **12**, which showed an IC_{50} of 18 μM, upon irradiation produced the photoproduct **15**, IC_{50} = 9.2 μM.[12] For comparison the IC_{50} values for ginkgolide A, its 10-keto analog, and the photoproduct of the 10-keto analog[12] were found to be 1.9 μM, 3.9 μM and 0.7 μM, respectively.

Another interesting active polycyclic compound which is readily available is the hexacyclic bromo ether **16**, prepared simply by reaction of **2** with bromine in CH_2Cl_2 - HOAc at 0-23°C (78% yield). The IC_{50} value determined for **16** was 2.9 μM. This result provides further evidence that a lactone carbonyl at C(11) is not essential for anti-PAF activity.

The most important conclusion which emerges from the above described results is that simpler analogs of ginkgolide B can be made which are even more active as PAF antagonists. The most critical functional groups of ginkgolide B for anti-PAF activity are the C(4) - C(12) ether bridge and possibly the C(4) - C(6) lactone bridge. The latter might serve as a mimic of the crucial acetyl function of PAF. It is not unreasonable to expect that still more active anti-PAF compounds will be discovered in the ginkgolide series with the help of the studies reported herein.[13]



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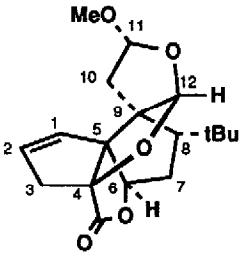

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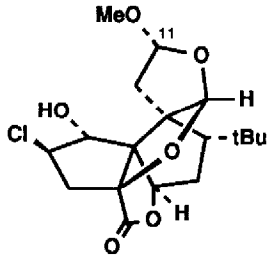

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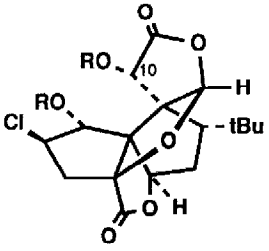

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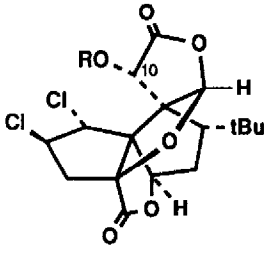
5 R = CH_2OCH_3



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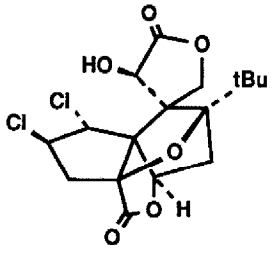

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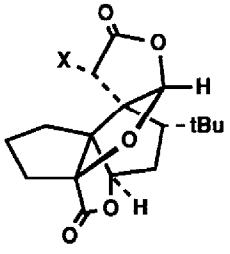
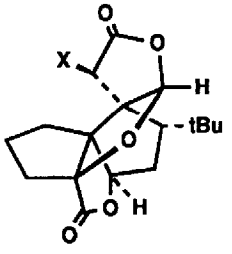
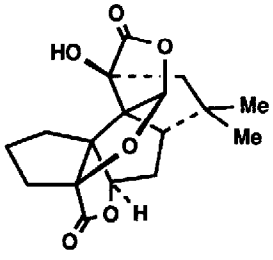


9 X = OH
10 X = OAc
11 X = OCH_2OCH_3



12 X =OH
13 X = OAc
14 X = OCH_2OCH_3



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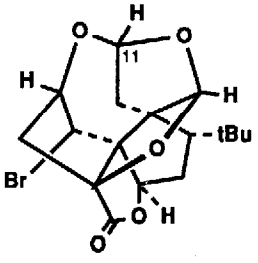

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References and Notes

1. D. J. Hanahan, *Ann. Rev. Biochem.*, **55**, 483 (1986).
2. See, for example, K. Cooper and M. J. Parry, *Ann. Rept. Med. Chem.*, **24**, 81 (1989).
3. P. Braquet, *Drugs of the Future*, **12**, 643 (1987).
4. P. Braquet, Ed., *The Ginkgolides, Chemistry, Pharmacology and Clinical Perspectives Vol. I* (J. R. Prous Science Publishers, Barcelona, 1988).
5. E. J. Corey, M.-C. Kang, M. C. Desai, A. K. Ghosh, and I. N. Houpis, *J. Am. Chem. Soc.*, **110**, 649 (1988).
6. E. J. Corey and A. V. Gavai, *Tetrahedron Letters*, **29**, 3201 (1988).
7. E. J. Corey, *Chem. Soc. Rev.*, *17*, 111 (1988).
8. Structural assignments were supported by 500 MHz ^{1}H NMR, infrared and mass spectral data obtained on chromatographically purified and homogeneous samples. All stereochemical assignments were confirmed by NOE data.
9. The assay of anti-PAF activity utilized rabbit platelets which were prepared as follows. Rabbits were anesthetized by pentobarbital sodium injection ("Nembutal"; 1 ml/kg of rabbit) and 42.5 ml of blood was taken directly from the heart into a syringe containing 7.5 ml of acid citrate dextrose (ACD) as anti-coagulant. The blood was centrifuged at 260 g for 10 min to remove the erythrocytes by sedimentation. The supernatent platelet rich plasma (PRP) was brought to pH 6.5 using ACD and centrifuged at 1950 g for 10 min. The platelets thus obtained in the form of a pellet were gently resuspended in the same volume of the washing buffer (4 mm KH_2PO_4, 6 mM Na_2HPO_4, 100 mM NaCl; 56 mM glucose, 0.1% bovine serum albumin, 2 mM EGTA; pH 6.5) and recentrifuged at 1950 g for 10 min. The platelets were resuspended gently in the final buffer solution (4 mM KH_2PO_4, 6 mM Na_2HPO_4, 100 mM NaCl, 56 mM glucose, 0.1% bovine serum albumin; pH 7.25) to give a final platelet count of between 3.25 and 4.75 x 10^8 per ml using a Coulter counter. The platelets were always handled in plastic or siliconized glass tubes and were stored at room temperature for no more than 2-3 hours.
10. The anti-PAF activity of the synthetic analogs of **1** was measured by their ability to protect rabbit platelets from aggregation in the presence of known amounts of PAF. The aggregation studies were performed in a Biodata Corporation (model PAP-4) four-channel aggregometer using siliconized glass cuvettes in which the platelets were stirred at 1100 rpm at 37°C. Washed platelets were preincubated for 2 min in the aggregometer with 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 2.5 µl of the antagonist (dissolved in DMSO). Stirring was initiated and 20 µl of 1% bovine fibrinogen (dissolved and diluted in the final buffer) was added. This was followed by 5 µl of PAF stock solution in 0.15 M NaCl containing 0.35% bovine serum albumin (final concentration of PAF = 0.28 nM). The aggregation was monitored until it reached its saturation value. The concentration of the antagonist required for 100% inhibition was determined and the aggregation studies were carried out with at least five lower concentrations. A plot of % inhibition versus the concentration of the antagonist provided the concentration corresponding to 50% inhibition, i.e. IC_{50} for the test compound. Each IC_{50} determination was accompanied by control determinations using **1** as the standard anti-PAF agent; over many runs the measured IC_{50} value for **1** was 0.6 ± 0.2 µM.
11. Thin layer chromatographic R_f values for **6**, the corresponding 10-ketone, and the 10-epimer of **6** on silica gel plates using 3 : 2 EtOAc-hexane were 0.44, 0.45 and 0.43, respectively.
12. Y. Nakadaira, Y. Hirota, and K. Nakanishi, *J. Chem. Soc. Chem. Commun.*, 1469 (1969).

13. This research was assisted financially by grants from the National Science Foundation and the National Institutes of Health.

(Received in USA 11 September 1989)