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Chemistry of ginkgolides: structure–activity relationship as PAF antagonists*

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Abstract: The ginkgolides represent a challenge for the organic chemist owing to their unique cage structure and their high potential biological activity. They were found to be potential PAF antagonists. The results of studies carried out in our laboratory are discussed, leading to conclusions on ginkgolides quantitative structure–activity relationship by using CoMFA and the probable pharmacophore and the interaction fashion between ginkgolides and PAF receptor

INTRODUCTION

Ginkgo biloba L. is a dioecious 'living fossil' tree. It is the only representative of its family Ginkgoaceae. *Ginkgo* plants and relative genera (about 50 species) occupied most of the world's ground surface in the Jurassic and Cretaceous eras some 200–100 million years ago. The great upheavals of the earth during the Quaternary and Tertiary periods and the subsequent Ice Age, most ovule plants vanished from the world. Only *Ginkgo biloba* trees were escaped by sheer luck and still survive in the wild state in China [1]. In China, it is commonly planted and appear to have been cultivated for many centuries, especially used for the embellishment of ground surrounding Buddhist temples. In China, as a famous herbal medicine, it was used in Song Dynasty (960–1127 AD). After that *Ginkgo* was described in many books on Chinese herbal medicine. In Chinese pharmacopoeia, G*inkgo* seed and leaves are reported as 'good for heart and lungs'. The main uses are anti-asthma, anti-tussive, expectorant, analgesic, invigorating circulation, anthelmintic, etc., and most of these actions can be described with modem pharmacology and clinical studies [2].

Recently the preparations of *Ginkgo biloba* extract are widely used in the treatment of cardiac and cerebral vascular and peripheral circulatory disorders [3]. Several European studies report positive results in the treatment of diverse neurological disorder, such as cerebrovascular insufficiency with symptoms of vertigo, tinnitus, short-term memory loss, headache, hearing loss and depression [4]. GBE has certainly risen to the forefront of safe and nontoxic intervention for CNS injury and prevention and treatment of early senile dementia (Alzheimer's disease)[5]. The clinical efficacy of GBE is mainly due to *Ginkgo* terpenes (ginkgolides and bilobalide). *Ginkgo* flavonoids and 6-hydroxykynurenic acid, etc. [2].

Ginkgolides were first isolated by Furukawa in 1932, but their structure was only resolved in 1967 by the pioneering works of Nakanishi and colleagues. They characterized the ginkgolides as 20 carbon cage molecules incorporating a spiro(4,4)-nonane, a tetrahydrofuran cycle and three lactone rings and the structure was confirmed by X-ray crystallography. These noval compounds which were named ginkgolide A **1**, B **2**, C **3**, J **4** and M **5** are only found from *Ginkgo biloba* plant up to date [6].

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ACTIVITY OF GINKGOLIDES

Ginkgolides are specific inhibitor of human platelet aggregation and inhibit the binding of (3H)-PAF-acether to its membrane platelet receptor. The main pharmacological actions are antagonist on PAF induced thrombosis, lung anaphylaxis, cardiac anaphylaxis, transplant rejection, immune disorder, etc. [7]. Ginkgolides derive from one of the most complex natural molecular framework. The *cis*-fused cyclopentanoid F, A, D and C are folded in such a way which a semispherical cavity of a significant size is formed (4 Å wide and 5 Å deep). In relative molecular terms, the cavity is sufficiently large to receive most atoms such as the trimethylammonium group. The two paralleled side of the cavity are defined by the C(11) and C(15) lactone carbonyls induced in the F and C ring, respectively. The head or the cap of the cavity is defined by ring A and D. The tetrahydrofuran ring D occupies a central position in the cage, its etheral oxygen, along with F and C ring ester oxygen and the C(10) hydroxy oxygen constitute a polydentate system similar to that observed in the 'Crown' ether series. This electron-rich cavity is ideally suited to the charged binding of cationic or positive polarized molecules. Another important feature is the t-butyl group which is prominent outside the main framework. Due to the shape of the cavity, if binding does occur, it may well be stereospecific.

Ginkgolides inhibit the binding of PAF-acether to its membrane platelet receptor. This inhibition is competitive. IC_{50} ginkgolide A 7.4×10^{-7}, ginkgolide B 2.5×10^{-7}, ginkgolide C 7.1×10^{-6}, ginkgolide J 5.4×10^{-5}.

The most powerful antagonist ginkgolide A and B are characterized by the presence of two hydroxy groups on C(1) and C(3). Ginkgolide C and J, which have a hydroxy group on C(7) in a α-position of the lipophilic t-butyl moiety are less active. With regard to ginkgolide J, the loss of the hydroxy group on C(1) further decrease the activity. As the ginkgolide becomes less polar its PAF antagonistic activity increase. The 1-methoxy and 1-ethoxy derivatives of ginkgolide B display activity similar to that of natural products [9].

SYNTHETIC GINKGOLIDE ANALOGUES FOR PAF ANTAGONISTS

Corey *et al.* investigated on total synthesis of ginkgolide B analogues [10]. They demonstrated that a number of simpler structural analogs which lack the lactone ring C are almost as potent as their parent compound ginkgolide B, while that lacking the *tert*-butyl substituent on ring B is approximately three order of magnitude less active than the *tert*-butyl substituted compound. The most critical functional group of ginkgolides for anti-PAF activity are possibly rings E and F. The *tert*-butyl group is also essential [11].

ETHERIC AND CARBONATE ANALOGUES OF GINKGOLIDE B

Corey *et al.* found the C(1) and C(10) hydroxys of ginkgolide B are much more readily deprotonated than the C(10) hydroxy of ginkgolide A and were etherized or carbonated on treating with active halogenating reagents under weak base condition [10]. We prepared a series of ginkgolide B O-alkyl and O-acyl products **6–26**.

Compound **6–26** were evaluated as PAF antagonist *in vitro* using an assay involving rabbit platelets. Methyl or alkoxymethyl derivatives **6–8**, **10** and **21** were only slightly less active than ginkgolide B. Considering unsaturated derivatives **9–19**, and **22–23,** we found those involving aromatic ring, carbonyl, and alkoxycarbonyl derivatives were more favored than the allyl analogues **10**. Examination of the substituted group of aromatic ring showed that the electron-donating group (–Cl) is more favored to anti-PAF activity than the electron withdrawing group ($-NO_2$). 1-Substituted and 10-substituted have significant potency, as shown in compounds **7** and **20**, **8** and **21**, **9** and **22**. The oxidative product **25** of ginkgolide B is eight times less active. It can be concluded from this study that modification at 1-, or 10-hydroxyl group of ginkgolide B are tolerated well. In addition to several analogues that showed ginkgolide-like activity, we identified 10-O-p-chlorobenzyl analogues **17** as a derivative possessing about fourfold increased potency compared to ginkgolide B in anti-PAF assay.

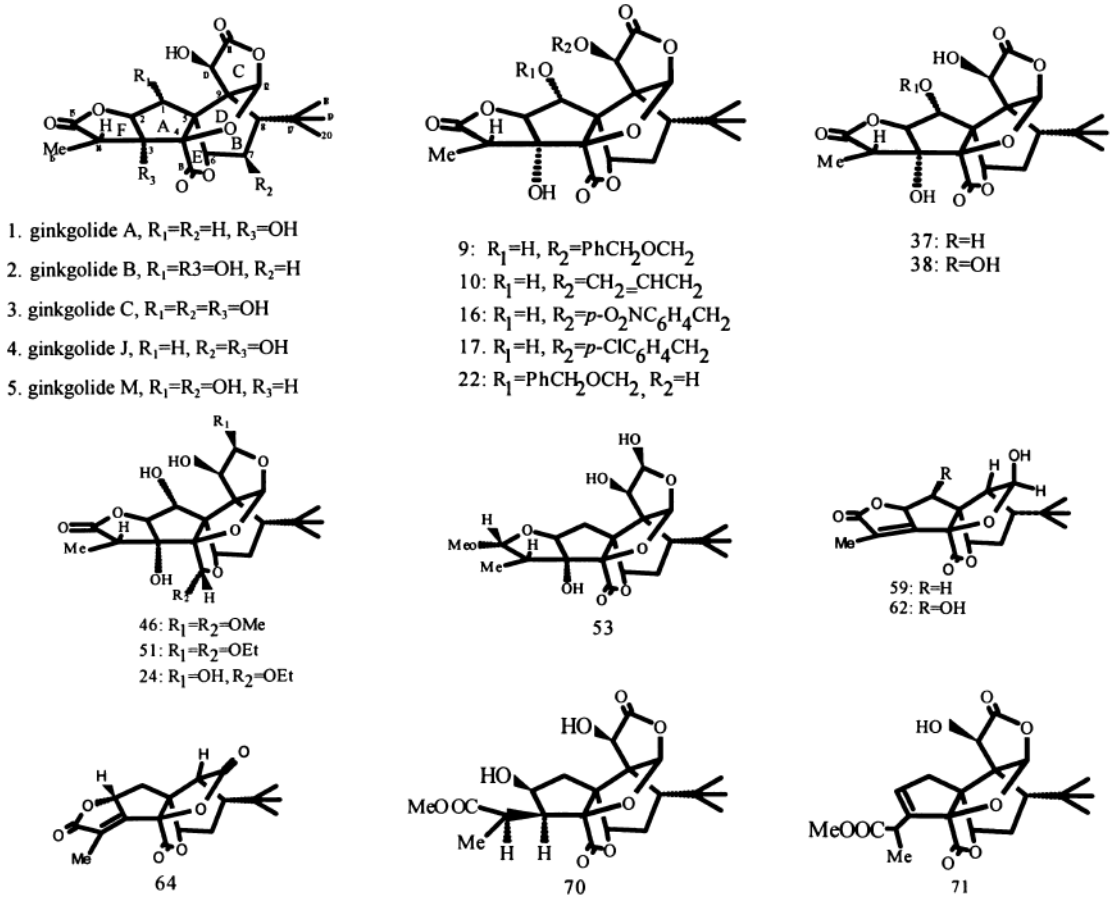

Fig. 1 The ginkgolides.

PREPARATION AND BIOLOGICAL ACTIVITY OF AMIDE DERIVATIVES OF GINKGOLIDE A

Most of alkaloids have strong biological activities and they are usually ingredients of lots of medicinal plants. It is therefore of interest to synthesize and study biological activities of analogues of ginkgolides containing nitrogen atom. Treatment of ginkgolides with Jones' reagent, 10-oxoginkgolides were produced, from which we prepared the amide derivatives by reductive amination to give compounds **27–39.** They are less active than ginkgolide B itself The NMR data show presence of intramolecular hydrogen bond between the 1-hydroxy and 10-hydroxy in ginkgolide B. The intramolecular bond vanished after oxidation of the hydroxy group, thus affect largely the conformation of ginkgolide B, and decrease their anti-PAF activity. Amide analogues of ginkgolide A yield a four to three hundred times decrease in activity due to the great change in backbone.

PREPARATION AND BIOLOGICAL ACTIVITY OF REDUCTIVE ANALOGUES OF GINKGOLIDES

We are also interested to synthesize and study the biological activities of ginkgolides by systematically modifying various functional groups such as lactone carbonyl and hydroxy groups to provide insight regarding the structural features of reductive analogues **37–58** and their anti-PAF activities. They were prepared to examine the role of the lactone ring C, D and F for potency. We found that reduction of the carbonyl groups at C-11, C-13 and C-15 were detrimental and resulted in one to two order of magnitude loss in potency in comparison to their parent compound.

C-NOR AND F-NOR ANALOGUES OF GINKGOLIDES

Ginkgolide A and B were treated with 50% NaOH at 130°C for 30 min to furnish C-Nor derivatives which can be further oxidized to their oxo derivatives using Jones' reagent leading to the C-nor ginkgolide derivatives **59–65**. We found that absence of ring C was detrimental and resulted in two order of magnitude loss in potency in comparison with their parent compounds and the lactone ring C was essential for activity.

Treatment of ginkgolide A with $POCl_3$ in dry pyridine as described by K. Weinges [12] furnished compound 3,14-dehydroginkgolide A **66**. **66** was quantitatively converted into dihydroxy derivative **67** by reaction with $OsO_4/NaClO_3$. Oxidation of 3,14-dihydroxy ginkgolide A with $NaIO_4$ produced F-nor-ginkgolide A **68**. Catalytic hydrogenation of 1,2,3,14-dehydroginkgolide B **69** led to form a mixture of three 15-acid derivatives. The mixed acids were converted into methyl esters and separated by column chromatography to yield F-nor compounds **70–72.** The screening result showed that absence of ring F was also detrimental and resulted in two orders of magnitude loss in potency in comparison with their parent compounds.

3D-QSAR STUDY ON GINLIGOLIDES AND THEIR ANALOGUES WITH COMPARATIVE MOLECULAR FIELD ANALYSIS

Comparative molecular field analysis (CoMFA), a three dimensional quantitative structure–activity relationship (3D-QSAR) paradigm was used to study the correlation between the physicochemical properties and the *in vitro* bioactivities of ginkgolide analogues. The correlation derived from CoMFA analysis has a good predictive capability.

Finally, we undertook a theoretical calculation for these compounds in aqueous and hexadecane quantum chemical method AM1 to modeling the interaction between drug and receptor. The probable pharmacophore and the interaction fashion between the drug and PAF receptor was also proposed.

In this paper we introduce some of our unpublished works. Although most of these ginkgolide analogues are less active on anti-PAF tests, their unusual structural feature seems valuable for further screening on other biological activities.

REFERENCES

1 P. D. Tredici, H. Ling, G. Yang. *Conservation Biol.* **6**, 202 (1992).

2 Z. Chen. *Chinese Pharm. J.* **31**, 326 (1996).

3 F. Y. DeFeudis. *Ginkgo biloba Extract (EGB 761): Pharmacological Activities and Clinical Application*, p. 97. Elsevier (1991).

4 J. Kleijnen, P. Knipschild. *Lancet* **340**, 1136 (1992).

5 P. L. LeBars, M. M. Katz, N. Berman, T. L. Itil, A. M. Freedman, A. F. Schauberg. *JAMA* **278**, 1327 (1997).

6 K. Nakanishi. Ginkgolides. *Chem. Biol. Pharmacol. Clinic. Perspectives* (P. Braquet, ed.), Vol. 1, p. 27. JR Prous Science (1988).

7 P. Braquet, D. Hosford. *J. Ethnopharmacol.* **32**, 135 (1991).

8 E. J. Corey, M. C. Kang, M. C. Desai, A. K. Ghosh, I. N. Houpis. *J. Am. Chem. Soc.* **110**, 649 (1998).

9 E. Buisine, J. Chretien. Ginkgolides. *Chem. Biol. Pharmacol. Clinic. Perspectives* (P. Braquet, ed.), Vol. 2, p. 37 (1989).

10 E. J. Corey, K. S. Rao. *Tetrahedron Lett.* **32**, 4623 (1991).

11 E. J. Corey, A. V. Gavai. *Tetrahedron Lett.* **30**, 6959 (1989).

12 K. Weinges, M. Rummler, H. Schilk, G. Schilling. *Liebigs Ann. Chem.* **287** (1993).

13 J. Chen, L. Hu, H. Jiang, J. Gu, W. Zhu, Z. Chen, K. Chen, R. Ji. *Bioorg. Med. Chem. Lett.* **8**, 1291 (1998).