Plenary Lecture

Medicinal Chemical Studies of Anti-inflammatory and Analgesic Natural Products

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Following the discovery of salicylates and its conversion to aspirin, natural products research has provided many promising leads for further modification as anti-inflammatory and analgesic agents. Recent studies have focused on biosynthesis inhibitors of eicosanoids and receptor antagonists of the platelet activating factor, including a new class of dual functional inhibitors derived from neolignans. The highly potent analgesic alkaloid epibatidine from the frog skin has been synthesized and recharacterized as a very strong acetylcholine nicotinic receptor agonist. Some novel epibatidine analogs have shown promise as potential CNS drugs and research probes for clarifying the anti-addictive property of the African alkaloid ibogaine.

DOCTRINE OF SIGNATURES

The discovery of medicinal plants and the use of natural products to treat illnesses in ancient China has been well documented in various treaties since Shen Nung. In the West, no legendary figures with comparable stature have been recorded as pioneers in the development of herbal or traditional medicine. Nevertheless, the symptoms and characteristics of many human disorders were well recognized in ancient civilizations. For example, the four cardinal signs of inflammation were clearly described by Galen around 170 AD as *Rubor* (redness), *Calor* (heat), *Tumor* (swelling) and *Dolor* (pain), which remain as major symptoms for pharmacological alleviation by anti-inflammatory-analgesic-antipyretic drugs today.[1] In the empirical search for remedies to relieve the sufferings, if not the causes, of various disorders, a rudimentary concept for "drug discovery" gradually evolved and was widely practised. The concept was named "*Doctrine of Signatures*" which meant that "The causes and cures of human illnessess have affinities with one another".[2] A legendary example of this Doctrine is the accidental discovery of "Peruvian barks" (the quinine-containing Cinchona tree barks), which grew in the warm and humid slopes of the Andes mountains in South America, for the treatment of malaria (mal aria, a disease in regions of foul air). Other examples are related to the shape of leaves or roots (similar to the use of ginseng in China) of the medicinal plant.

The use of willow barks for the treatment of pain, fever and arthritis was independently discovered by various ancient civilizations, including the Greek (Hippocrates), Roman, and American Indian. Later, as reported in the Philosophical Transactions of the Royal Society of London in 1763 by Reverend E. Stone, in accordance with the Doctrine of Signatures, he found that the extracts of the bark of willow trees growing in the wetland was effective in relieving the pain and fever of agues in English villagers.[3] Following the advances of chemical sciences, the active ingredient in willow bark was identified as salicylates, and synthetic salicylic acid was then used. In an attempt to reduce the gastrointestinal irritation of sodium salicylate for his ailing father, the *O*-acetyl derivative of salicylic acid (aspirin) was first prepared by Felix Hoffmann in 1899 and found to be superior.[3] As aspirin is still the most widely used antiarthritic drug today, it constitutes a classical example of successful molecular modification of an anti-inflammatory natural product lead.

THE ARACHIDONIC ACID CASCADE

The simple structure of aspirin masked its complex biochemical properties for many decades. By 1971, the principal biochemical mechanism of aspirin, and a new nonsteroidal anti-inflammatory-analgesic drug (NSAID) indomethacin, was found to be the inhibition of the biosynthesis of prostaglandins from cell membrane arachidonic acid (AA, Fig. 1).[4] This observation provided a convenient *in vitro* assay for the development of a large family of NSAIDs. It also facilitated our discovery of sulindac as a reversible prodrug to succeed indomethacin,[5] and diflunisal (Fig. 2) as a more potent, less irritating and longer acting new analog of aspirin.[6] In turn, indomethacin, as a research tool, facilitated the elucidation of the dynamic arachidonic acid cas-

cade, which generates a group of potent lipid mediators of inflammation (Fig. 1), including prostaglandins (PGs), leukotrienes (LTs) and more recently, the platelet activating factor (PAF).

The conversion of AA to PGs and LTs involve the initial hydroperoxidation of specific double bonds in AA by molecular oxygen *via* an iron-catalyzed radical mechanism to form PGH_2 and LTA_4, respectively. Polyphenolic natural products, such as gossypol and the flavonoid quercetin, capable of destroying the radical intermediates have shown moderate inhibitory activities against the biosynthetic enzymes cyclooxygenase (COX) and/or 5-lipoxygenase (5-LO) in many studies. However, in general, redox agents have not been found to be very promising as anti-inflammatory-analgesic leads.

RECEPTOR ANTAGONISTS OF PAF

As shown in Fig. 1, concomitant with the release of AA from membrane phospholipids by the action of phospholipase A_2, the remaining phospholipid fragment, lyso-PAF, is further converted to PAF by an acetyl transferase. PAF is an unique ether phospholipid with profound vascular and cellular activities at nanomolar concentrations.[7] It also potentiates the inflammatory and algesic actions of other mediators like LTs, PGs and bradykinin. In order to regulate this newly characterized potent mediator, a membrane receptor assay was established by my colleague, Dr. San-Bao Hwang to search for antagonists in 1983.[8] Fortuitously, in a collaboration with the laboratory of the late Professor Wang Xu in the Beijing Medical University, a crude extract of the Chinese herbal plant Haifengteng (*Piper futokadsura*) was soon found to contain some effective PAF-antagonists. A principal active ingredient was readily identified as a neolignan, kadsurenone.[9] Kadsurenone is the first non-lipid type of PAF antagonists known and served as a valuable research tool in the biological investigations of PAF. In a more detailed study at the University of Virginia later, several more PAF-antagonists, futoenone, futoquinone, futoxide and (-) galbalgin, were isolated from a batch of Taiwan Haifengteng[10] previously collected by my colleague Dr. Michael N Chang.

Following kadsurenone, a wide variety of lignans were examined for their PAF-antagonism in our laboratory and others. This led to an intensive synthetic modifications of lignans of the 2,5-diaryltetrahydrofuran type, culminating with the discovery of active isosteres at U. Va.[10] and synthesis of a highly potent PAF-antagonist MK-287 (Fig. 3) by the Merck researchers.[11]

The clinical trials of MK-287 and other PAF-antagonists against asthma and various allergic inflammatory disorders have produced some encouraging but less than satisfactory results. In order to increase the efficacy of PAF-antagonists further, we have found that it is chemically feasible to broaden the activity profile of PAF-antagonists by combining it with the pharmacophores for 5-lipoxygenase inhibitors, such as an iron-chelating hydroxyurea side-chain (Fig. 3).[12] Indeed, prototypes of such dual-functional agents inhibit both the action of PAF and the generation of leukotrienes, resulting greater efficacy in several animal models of inflammatory and allergic diseases.[13] Further development of this concept is continuing through a collaboration with the CytoMed Laboratories. In summary, the discovery of neolignans as promising PAF-antagonists from a Chinese herbal plant has rapidly progressed through the clinical evaluation of a potent drug-candidate and the development of second-generation dual-acting congeners as a new class of therapeutic agents.

AN "ANALGESIC" AND NICOTINIC ALKALOID

Frog skins are well known to contain highly potent toxins and pharmacological agents. Epibatidine, a minor alkaloid isolated from the skin extracts of an Ecuadoran poi-

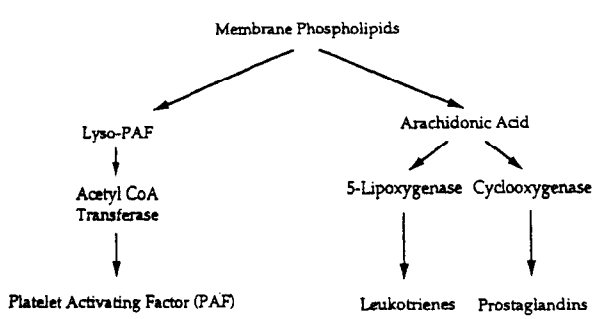


Fig. 1. The cascade of inflammatory lipid mediators.

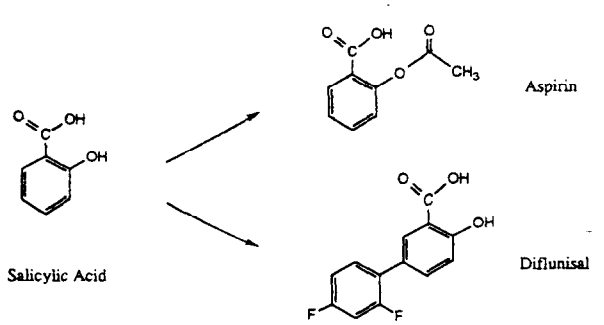
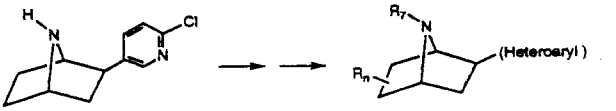


Fig. 2. Structure modifications of salicylic acid.

son frog, *Epipedobates tricolor*, was recently reported by NIH investigators as a novel and very potent "analgesic" agent.[14] In the classical hot-plate and Straub-tail analgesic assays, epibatidine was 500x more potent than morphine, and the action was not blocked by the morphine antagonist naloxone. It has very low affinities for the morphine receptors, and thus the potential of being a non-addicting analgesic. However, further characterization of this intriguing alkaloid was hampered by the scarcity of the natural material. Stimulated by this report, we (with Dr. Daofei Huang) have developed a versatile total synthesis of the 7-azanorbornane epibatidine,[15] *via* a Diels-Alder reaction between *N*-acyl pyrrole and sulfonylacetylene as the dienophile, which enabled us to prepare ample quantities of epibatidine and structural analogs (Fig. 4), including their enantiomers, for extensive pharamcological investigations.[16] In collaboration with CytoMed researchers, we have discovered for the first time that epibatidine is a highly potent and specific agonist of the nicotinic acetylcholine receptor, being 100x more potent than nicotine.[17] The typical binding of epibatidine and analogs to the nicotinic receptor preparation from a specific region of the rat brain are illustrated in Fig. 5. Similar findings have since been reported by other investigators.[18,19]

More than nine communications on the total synthesis of epibatidine have been published today.[20] While epibatidine itself may be too toxic for clinical applications, some active synthetic analogs have shown improved pharmacological profiles in various animal models. Other analogs have shown a partial dissociation of the CNS analgesic effect from affinity for the nicotinic receptor and thus opened up potential applications based on their nicotinic activity, such as in the treatment of Parkinson's and Alzheimer's diseases and cognitive disorders. Further investigation of the clinical utlity of various analogs of this highly potent "analgesic" and/or nicotinic alkaloid from frog skin is in progress.



Epibatidine Analogs

Fig. 4. Synthesis of epibatidine and analogs.

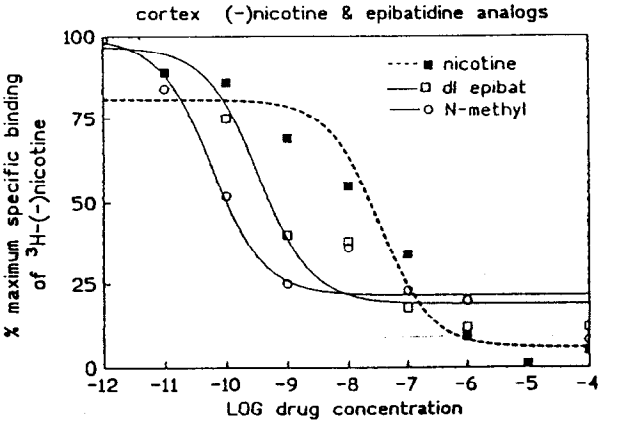
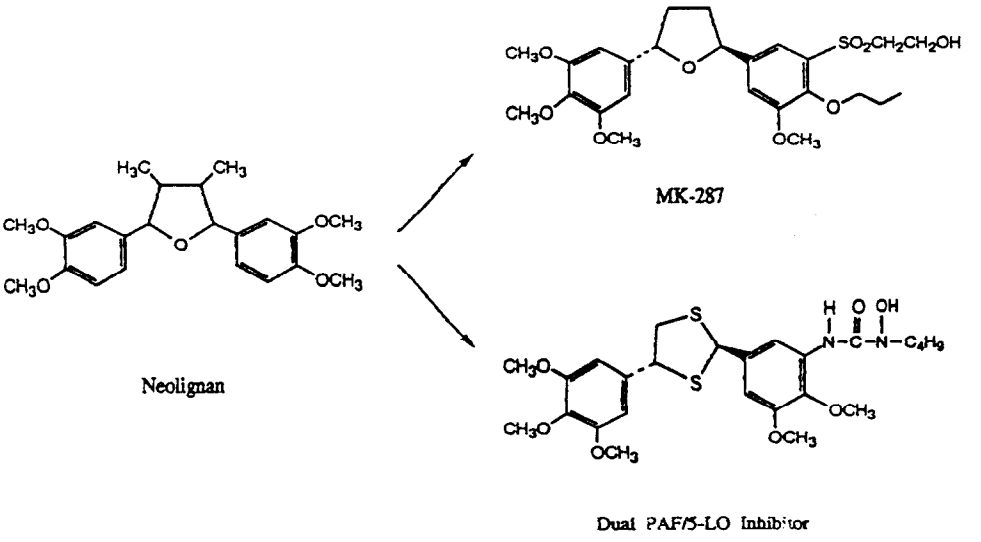


Fig. 5. Binding of epibatidine and an analog to nicotinic receptor in rat brain.



Neolignan MK-287 Dual PAF/5-LO Inhibitor

Fig. 3. PAF antagonists from a neolignan lead.

IBOGAINE - "A DRUG TO END ALL DRUGS"?[21]

Our venture into the synthetic chemistry and CNS activities of the 2-heteroaryl 7-aza[2.2.1]bicyclic congeners of epibatidine broadened our interest in another aryl substituted 2-aza[2.2.2]bicyclic alkaloid, the well studied ibogaine from the African shrub *Tabernathe iboga*. It is of particular interest to note that, facing the serious social problems of illicit drug abuse, ibogaine is currently under clinical evaluation as a potential anti-addictive agent for victims addicted to heroine, cocaine and amphetamine-like agents.[21] Ibogaine, in trained animals and drug users, has been reported to reduce self-administration of drugs and produce a prolonged anti-addictive effect after several doses, but its mechanism of action, for both acute and long-term effects, is not clear.[22,23] It would seem that some long-acting metabolites might contribute to its delayed *in vivo* effect, and we are particularly interested in the potential iminium metabolites of ibgaine produced by an oxidative metabolic transformation of the tertiary amine (Fig. 6). Using an extension of our epibatidine study, in conjunction with some well-established ibogaine chemistry, we have synthesized several aza[2.2.1]bicyclic analogs of ibogaine, including a 2-aza isomer derived from a skeleton rearrangement of the 7-azabicyclic system,[24] as model compounds (Fig. 7). Oxidation to the *N*-oxide followed by a modified

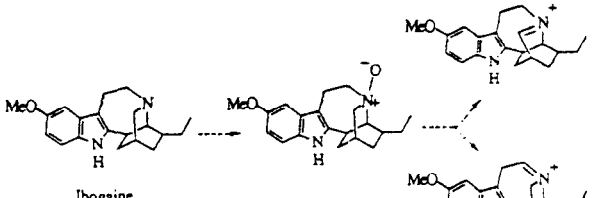


Fig. 6. Putative reactive iminium metabolites of ibogaine.

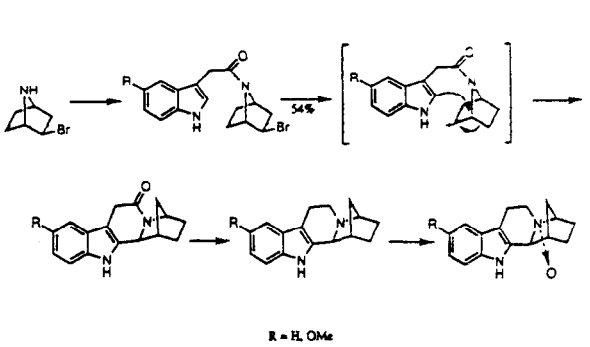


Fig. 7. Synthesis of 2-azanobornane analogs of ibogaine.

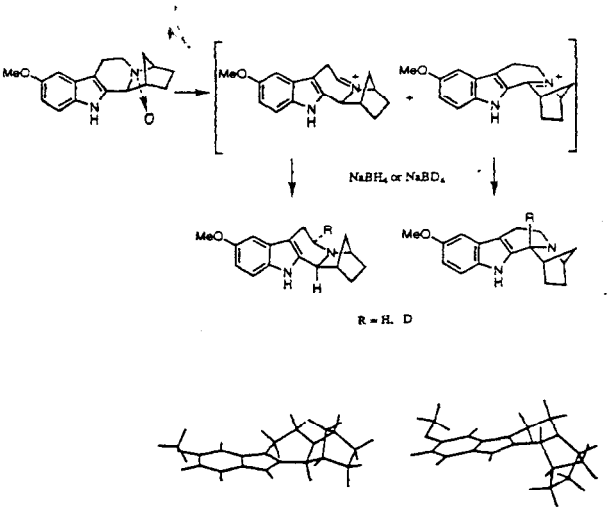


Fig. 8. Characterization of iminium derivatives.

Polonovski reaction produced two isomeric iminium ions, which were characterized spectroscopically and through reduction by sodium borohydride and sodium borodeuteride to their amine precursor and its stereoisomer (Fig. 8). The stability and potential *in vivo* reactivity with its biochemical or neurological targets of such iminium metabolic intermediates are under active investigation.

In conclusion, natural products of plant or animal origins have provided interesting medicinal chemical leads in our study of anti-inflammatory-analgesic agents. Several neolignans were identified as novel receptor antagonists of PAF. Synthetic modifications have increased the potency to yield a clinical candidate as well as broadened the activity profile to form a group of dual functional inhibitors against both PAF and LTs. A higly potent "analgesic" alkaloid from the frog skin, epibatidine, has been synthesized and redefined as an acetylcholine nicotinic receptor agonst which is nearly 100x more potent than nicotine. The structure-activity relationship of novel epibatidine analogs has indicated some promising clinical applications. The synthetic chemistry of epibatidine also enabled us to explore other CNS effects of related structures, including the potential anti-addictive activity of reactive metabolites of the African alkaloid ibogaine.

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Key Words

Natural products; Anti-inflammatory and analgesic agents; Arachidonic acid cascade; Cyclooxygenase inhibitors; 5-Lipoxygenase inhibitors; Platelet activating factor antagonists; Dual functional inhibitors; Neolignan; Epibatidine; Acetylcholine nicotinic receptor agonist; Ibogaine; Antiaddictive effects.

REFERENCES

1. Brune, K. in *Discoveries in Pharmacology* Vol. 2, Parnham, M. J. and Bruinvels, J., Eds.; Elsevier Science Publishers: New York, **1984**; Chapter 10, Part 1.
2. Mann, J. *Murder, Magic and Medicine*, Oxford: New York, **1994**; p 117.
3. Collier, H. O. J. in *Discoveries in Pharmacology* Vol. 2, Parnham, M. J. and Bruinvels, J., Eds.; Elsevier Science Publishers: New York, **1984**; Chapter 10, Part 4.
4. Vane, J. R. *Nature* **1971**, *231*, 232-235.
5. Shen, T. Y.; Winter, C. A. in *Advances in Drug Research* Vol. 12, Simmonds, A. B., Ed.; Academic Press: New York, **1977**; pp 89-245.
6. Hannah, J.; Ruyle, W. V.; Jones, H.; Matzuk, A. R.; Kelly, K. W.; Witzel, B. E.; Holtz, W. J.; Houser, R. W.; Shen, T. Y.; Sarett, L. H. *Brit. J. Clin. Pharmacol* **1977**, *4*, 7S-13S.
7. Braquet, P.; Shen, T. Y.; Touqui, L.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97-145.
8. Hwang, S. B.; Lee, C. S. C.; Cheah, M. J.; Shen, T. Y. *Biochem.* **1983**, *22*, 4756-4763.
9. Shen, T. Y.; Hwang, S. B.; Chang, M. N.; Doebber, T. W.; Lam, M. H.; Wu, M. S.; Wang, X.; Han, G. Q.; Li, R. Z. *Proc. Natl. Acad. Sci.* U.S.A. **1985**, *82*, 672-678.
10. Shen, T. Y. *Lipids* **1991**, *26*, 1154-1156.
11. Sahoo, S. P.; Graham, D. W.; Acton, J.; Biftu, T.; Bugianesi, R. L.; Girotra, N. N.; Kuo, C. H.; Ponpipom, M. M,; Doebber, T. W.; Wu, M. S. *Bioorganic Med. Chem. Lett.* **1991**, *1*, 327-332.
12. Goldstein, D. M.; Shen, T. Y. *Med. Chem. Res.* **1992**, *2*, 451-456.
13. Ip, S. H.; Qian, C.; Libertine-Garahan, L.; Hwang, S. B.; Eckman, J.; Yeh, C. G.; Cai, X.; Killian, D.; Hussoin, S.; Scannell, R.; Biftu, T.; Shen, T. Y. Abstracts, Interntl. Congress Inflammation 93 (Vienna), Oct. **1993**.
14. Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Teh, H. J. C.; Pannell, L.; Daly, J. *J. Am. Chem. Soc.* **1992**, *114*, 3475-3478.
15. Huang, D. F.; Shen, T. Y. *Tetrahedron Lett.* **1993**, *34*, 4477-4480.
16. Li, T.; Qian, C.; Eckman, J.; Huang, D. F.; Shen, T. Y. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2759-2764.
17. Qian, C.; Li, T.; Shen, T. Y.; Libertine-Garahan, L.; Eckman, J.; Biftu, T.; Ip, S. *Eur. J. Pharmacol.* **1993**, *250*, R13-14.
18. Fisher, M.; Fu, D.; Shen, T. Y.; Guyenet, P. G. *J. Pharmacol. Exptl. Therap.* **1994**, *270*, 702-705.
19. Badio, B.; Daly, J. W. *Mol. Pharmacol.* **1994**, *45*, 563-569.
20. Pandey, G.; Bagul, T. D.; Lakshmaiah, G. *Tetrahedron Lett.* **1994**, *35*, 7439-7442 and references cited therein.
21. O'Hearn, E.; Molliver, M. E. *Neuroscience* **1993**, *55*, 303. Touchette, N. *J. NIH Res.* **1993**, *5*, 50-55.
22. Glick, S. D.; Kuehne, M. E.; Raucci, J.; Wilson, T. E.; Larson, D.; Keller, R. W. Jr., Carlson, J. N. *Brain Res.* **1994**, *657*, 14-22.
23. Popik, P.; Layer, R. T.; Skolnick, P. *Psychopharmacol.* **1994**, *114*, 672.
24. Huang, D. F.; Huang, P. Z.; Stepanian, M. W.; Shen, T. Y. unpublished.