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Natural and Synthetic Geiparvarins are Strong and Selective MAO-B Inhibitors. Synthesis and SAR Studies

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Abstract—Natural geiparvarin **1** and a number of its analogues were prepared and tested as inhibitors of both monoamine oxidase isoforms, MAO-B and MAO-A. The desmethyl congener **6** of geiparvarin, proved potent and selective MAO-B inhibitor ($pIC_{50} = 7.55$ vs 4.62). X-ray crystallography and molecular modelling studies helped the understanding of the observed structure–activity relationships.

Monoamine oxidase (MAO; amine: oxygen reductase deaminating, flavin-containing, EC 1.4.3.4) is an integral protein of the outer mitochondrial membrane that catalyses the oxidative deamination of biogenic and diet-derived amines in both the central nervous system (CNS) and in peripheral tissues.[1,2] The most important substrates for the enzyme in the CNS are the neurotransmitters dopamine (DA), adrenaline, noradrenaline (NA), serotonin (5-HT) and β-phenylethylamine (PEA). Two MAO isoenzymes have been distinguished on the basis of their substrate preference, sensitivity to inhibitors, tissue and cell distribution, immunological properties and amino acid sequence: MAO-A, which is selectively and irreversibly inhibited by nanomolar concentrations of clorgyline, and is chiefly responsible for the deamination of tyramine in the intestine and of 5HT and NA in the CNS, and MAO-B, which is relatively insensitive to clorgyline and is mainly responsible for the oxidative catabolism of DA and PE.[3]

The involvement of MAO in the catabolism of neurotransmitter amines makes this enzyme particularly attractive for the development of new drugs. Indeed, irreversible and non-selective MAO inhibitors (MAO-Is) were developed many years ago as anti-anxiety and antidepressant agents. Unfortunately, they had to be employed with much care because they could cause lethal hypertensive crises (the *cheese-effect*) by preventing the metabolic inactivation of tyramine present in high concentrations in diary foods and red wine.[4] This severe problem was later overcome with the discovery and development of selective and reversible MAO-A inhibitors[5] which are now safely used as antidepressants (e.g., moclobemide and befloxatone), and selective MAO-B inhibitors like selegeline, (i.e., L-deprenyl) which is currently employed in combination with L-DOPA in the symptomatic therapy of Parkinson's disease.[6]

Renewed interest in the field has come also from the recent findings that MAO-B inhibitors have neuroprotective[7] and antioxidant effects,[8] and play a role in delaying apoptotic neuronal death[9] and in protecting crucial mitochondrial functions.[10] Moreover, the recent, and long awaited, determination of the 3D structure of MAO-B by X-ray crystallography[11] and the thereby easy development of 3D theoretical models of MAO-A now renders feasible the structure-based design of potent and selective MAO inhibitors. However, only indirect modelling studies have been carried out so far

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on numerous and structurally diverse classes of MAO-Is. Significant contributions in this field came also from QSAR and 3D QSAR investigations of MAO-mediated toxication of MPTP analogues[12] and MAO inhibition by isoquinolines,[13,14] indenodiazines,[15,16] xanthones[17] and, more recently, coumarins.[18,19] Coumarins are natural products largely diffused in the plant kingdom, especially in green plants.[20] They display a variety of biochemical and pharmacological activities depending on their substitution pattern.[21,22] Some natural coumarins show a low MAO inhibitory potency[23,24] whereas properly modified natural coumarins have been characterised as potent and selective MAO-Is.[18,19] In particular, it has been observed that a strong modulation of both inhibitory potency and MAO-A/MAO-B selectivity chiefly depends on the size, branching and stereoelectronic properties of the 7-substituent.[18,19]

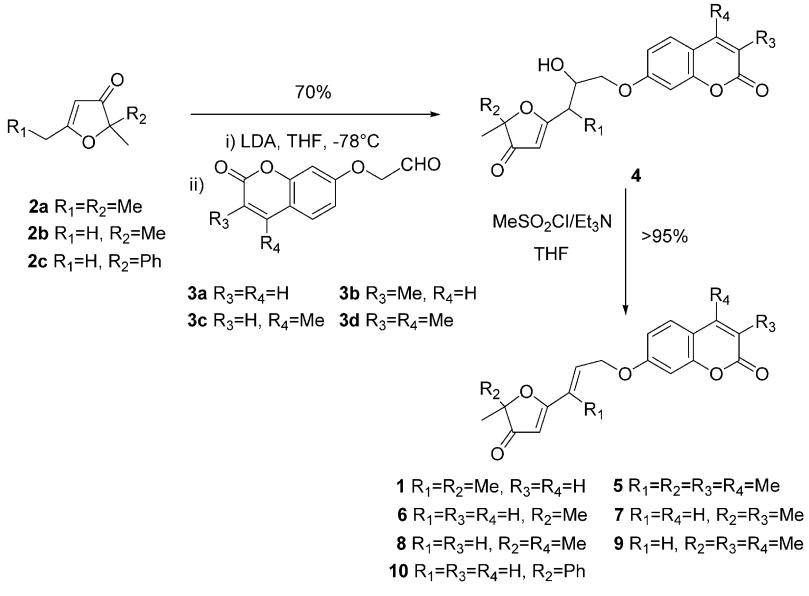
Starting from this important observation, we looked for untested natural coumarins displaying structural analogy with the highly active and selective 7-substituted coumarin derivatives recently synthesised and studied by us and others as potent MAO-Is.[18,19]

A class of coumarins, namely the geiparvarins, drew our attention because of their evident structural similarity with highly potent coumarinic MAO-Is. Geiparvarin **1** (Scheme 1), a natural compound isolated from the leaves of *Geijera parviflora* Lindl (rutaceae), has been characterised and prepared several years ago,[25] and studied in detail for its interesting cytostatic activity along with many of its derivatives and analogues.[26–28] The chemistry and medicinal chemistry of geiparvarins have been reviewed.[29]

In order to demonstrate the validity of our hypothesis on the putative MAO inhibitory activity of geiparvarin **1**, we prepared and tested it as inhibitor of both MAO isoforms. A convenient synthesis of compound **1** is outlined in Scheme 1.

In brief, the reaction between the 2,2-dimethyl-5-ethyl-3(2*H*)furanone (**2a**)[25] and the recently easily available 7-(2-oxoethoxy)coumarin (**3a**),[30] afforded the corresponding aldol **4** (70%) which, via a new application of the Stork–Kraus dehydration protocol, gave geiparvarin **1** in excellent yield (>95%). Satisfactorily, a good inhibitory activity towards MAO-B was detected for **1**, whereas, as expected on the basis of our previous structure–activity relationship (SAR) studies,[15,19] a much lower affinity was shown for the MAO-A isoform (Table 1).

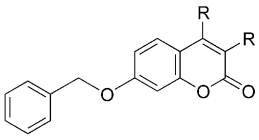
Compared to the activity of the relatively close synthetic coumarinic lead analogue **11** (Chart 1), the MAO-B inhibitory activity of geiparvarin **1** was somewhat lower (pIC_{50} 6.84 vs 7.26). The observation that the introduction of two methyl groups at positions 3 and 4 of the coumarin ring of **11** afforded inhibitor **12** (Chart 1) with greater potency (pIC_{50} 8.36 vs 7.26), prompted us to modify geiparvarin accordingly. Unexpectedly, the resulting 3,4-dimethylgeiparvarin **5** showed no improved MAO-B inhibition over **1** (pIC_{50} 6.89 vs 6.84, Table 1). This surprising result might be ascribed to a slightly different binding mode of the two inhibitors, likely due to the presence at position 7 of geiparvarins of a longer, bulkier and more flexible substituent. Another intrinsic limitation of the geiparvarin skeleton for a higher MAO-B inhibition might be the presence of a methyl group on the double bond, probably responsible for a negative steric effect leading to a weaker affinity. Indeed, previous SAR studies have pointed out that substitution in the linear bridge linking a chromanone ring to a terminal phenyl ring caused a dramatic drop of MAO-B activity. Moreover, *ortho*- and *meta*-substituted 7-benzyloxy coumarins showed an activity lower than the corresponding *para*- and unsubstituted congeners.[19] The molecular superposition of a low energy conformer[31] of inhibitor **12** onto the X-ray crystallographic structure of 3,4-dimethylgeiparvarin **5**,[32] reported in Figure 1, supports our hypothesis that the



Scheme 1. Synthetic pathway leading to geiparvarin (**1**) and its analogues.

Table 1. MAO inhibitory activity[33] for compounds **1**, **5**–**10** and **13**

Compd	pIC_{50} or % of inhibition		
	MAO-A	MAO-B	CLOG P
1	4.57	6.84	3.65
5	37% (10 μM)	6.89	4.60
6	4.62	7.55	3.25
7	4.82	6.23	3.81
8	27% (10 μM)	6.77	3.81
9	4.95	6.20	4.20
10	0% (3 μM)	5.82	4.66
13	26% (10 μM)	5.77	1.87



11 R=H
12 R=Me

Chart 1. Chemical structures of potent coumarin MAO inhibitors.

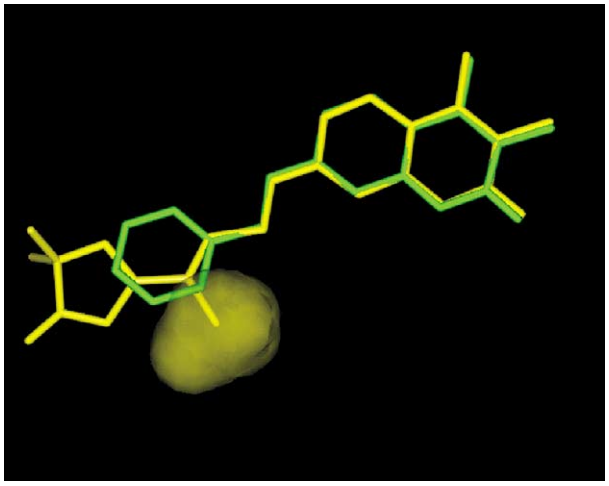


Figure 1. Molecular overlay of 7-benzyloxy-3,4-dimethylcoumarin **12** (green) onto 3,4-dimethylgeiparvarin **5** (yellow). The van der Waals surface of the 3′-methyl substituent of **5** is close to the *ortho*-position of the phenyl ring.

methyl group on the double bond may elicit unfavourable interactions occupying a region close to the forbidden *ortho* positions of the benzyl ring. In addition, the 3(2*H*)furanone ring appears to be close to the *meta* position of the benzyl ring, again an unfavourable region for bulky substituents (the *meta* $NHCOCH_3$ derivative of **12** has a pIC_{50} 6.60).[19]

The coherent and simplest deduction coming from the above findings was to eliminate the methyl group on the double bond.

Thus, the synthesis of 7-{[(*E*)-3-(5,5-dimethyl-4-oxo-4,5-dihydro-2-furanyl)-2-propenyl]oxy}-2*H*-chromen-2-one (**6**)[34] was carried out according to Scheme 1, starting from 2,2,5-trimethyl-3(2*H*)furanone (**2b**).[35]

Gratifyingly, the MAO-B inhibitory activity of compound **6** was significantly enhanced with respect to **1**

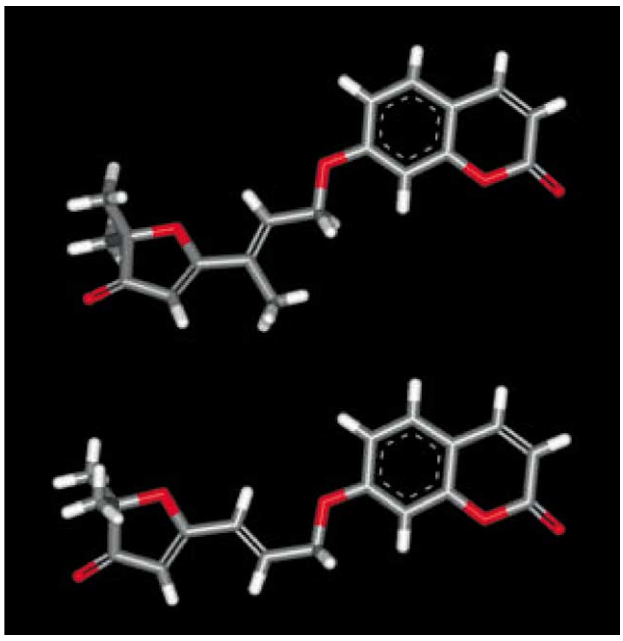


Figure 2. Molecular models, from X-ray crystallographic data, of geiparvarin (**1**) (up) and desmethylgeiparvarin **6** (down) showing two different conformations of the diene system, (*s-trans* and *s-cis*, respectively).

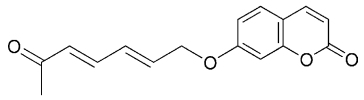
(pIC_{50} 7.55 vs 6.84), whereas the activity against MAO-A was the same (pIC_{50} 4.62 vs 4.57). In order to verify whether the observed higher activity of **6** compared to geiparvarin **1** could depend also on a different binding conformation, we undertook a 3D structural study in the solid state and in solution. The X-ray analysis of **6**, besides confirming the (*E*) configuration of the double bond in the bridge, showed that this compound, unlike geiparvarin **1** crystallises in the *s-cis* conformation (Fig. 2). However, the analysis of the ^1H NMR spectra together with the results from NOESY experiments (data not shown), established that in $CDCl_3$ solution both **1** and **6** assume similar conformations; in particular, they appear to be in the *s-trans* form. Thus, the higher activity of **6** with respect to **1** can be tentatively attributed to the removal of some steric hindrance of the methyl group in the 3′ position of geiparvarin.

As a further step of our investigation, we examined the effect of introducing methyl substituents in positions 3 and 4 of the coumarin ring of compound **6** on their inhibitory potency. Both the monomethyl derivatives **7** and **8** and the dimethyl congener **9** showed an affinity lower than that of the parent compound **6** with pIC_{50} values of 6.23, 6.77 and 6.20, respectively.

These data indicated that substitution at position 3 is the most deleterious for affinity and confirmed that the binding mode of these compounds differs from that of 7-benzyloxycoumarins, for which higher affinities resulted from the introduction of methyl groups at positions 3 and 4 of the coumarin ring.

Finally, to further demonstrate the negative steric effect of the furanone moiety, we carried out two additional drastic structural modifications on **6** by synthesising and

testing the even more sterically crowded 7-{[(*E*)-3-(5-phenyl-5-methyl-4-oxo-4,5-dihydro-2-furanyl)-2-propenyl]oxy}-2*H*-chromen-2-one (**10**) and the 7-{[(2*E*, 4*E*)-6-oxo-2,4-heptadienyl]oxy}-2*H*-chromen-2-one (**13**).



13

The latter can be considered an open analogue of **6**, lacking the bulky *gem*-dimethylfuranone substructure. Compound **10**, as expected, was one of the less active compounds prepared by us, whereas the even lower activity of the open analogue **13** was quite surprising. Evidently, the likely positive effect arising from the removal of the steric crowding of the furanone moiety is cancelled by other structural features, such as a higher conformational flexibility and/or the lack of an sp^3 oxygen atom which seems to be detrimental to efficient binding. Another plausible explanation of the observed rank of activity may be found in the different lipophilic properties of the analysed compounds. Since the influence of lipophilicity on MAO-B inhibitory potency is well documented,[15,16,19] we calculated the partition coefficients of our compounds using the CLOG P program[36] (Table 1). Log P values seem to be linearly related with pIC_{50}, since the inhibitory activity increased with decreasing lipophilicity, reaching a maximum for compound **6** (log P = 3.25). The only exception to this relationship is the open analogue **13** which, despite its lowest lipophilic character, displayed the weakest affinity. It is worth nothing that this result may be indicative also of a biphasic relationship, with a low activity shown by compounds of either high or low lipophilicity. However, the ascending part of the biphasic function is defined by one point only and more inhibitors should be prepared and tested to validate this hypothesis.

In summary, we discovered that geiparvarin and some of its analogues are potent and selective MAO-B inhibitors. These findings may be kept well in mind in the design of new geiparvarin derivatives targeting other important physiopathological conditions.[28]

Structural modifications on either the coumarin or the furanone moiety of geiparvarin **1** are deleterious for MAO activity. In contrast, removal of the methyl group on the alkenoxy bridge afforded derivative **6** which displayed the highest MAO-B inhibitory potency ($pIC_{50} = 7.55$) with an outstanding 850-fold selectivity for the MAO-B isoform.

Acknowledgements

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31. Conformational analysis of inhibitor **3** was performed by means of the CONF module implemented within the QXP software package (Rel.2001, available upon request from C. McMartin at cmcma@ix,netcom.com). 1000 Monte Carlo runs were performed followed by conjugate gradient minimization. The molecular superposition was made as follows: the centroids of the two aromatic rings of the coumarin system

and the ether oxygen, the sp^3 carbon of the oxymethylene group and the first sp^2 carbon atom of the diene moiety of 3,4-dimethylgeiparvarin **5** were fitted onto the corresponding features of inhibitor **12** (the first sp^2 carbon atom of the phenyl ring corresponded to the first sp^2 carbon atom of the diene moiety).

32. X-ray data. Space group: P21/c, unit cell: a = 8.573(1), b = 17.359(2), c = 13.100(3), β = 107.615(4), volume = 1858.3(4),^{3} Z = 4, θ range for data collection = 4.38 to 60.71, (λ = 1.54178), total reflections collected = 3566, independent reflections = 2690, data/restraints/parameters = 2690/0/258, R1 [I > 2sigma(I)] = 0.0464, wR2 = 0.1302, goodness-of-fit on $F^2 = 1.078$.

33. MAO inhibition assays were carried out by the kynuramine spectrophotometric method using clorgyline and selegiline as selective MAO-A and MAO-B inhibitors, respectively, as reported in ref 19.

34. All geiparvarin analogues were characterised by ^{1}H, ^{13}C NMR and IR spectroscopy and analytical data. For example, compound **6** was isolated as a yellow solid (96%, yield); mp 146–147 °C (from methanol); IR (KBr) 1735, 1680, 1611, 1374, 1279, 1174, 1117, 825 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.63 (d, 1H, ^3J = 9.4 Hz, H-4), 7.39 (d, 1H, ^3J = 8.6 Hz, H-5), 6.88 (dd, 1H, ^3J = 8.6 and ^4J = 2.5 Hz, H-6), 6.85 (m, 1H, H-2′), 6.82 (d, 1H, ^4J = 2.5 Hz, H-8), 6.57 (1H, dt, ^3J = 15.9 and ^4J = 1.8 Hz, H-3′), 6.26 (d, 1H, ^4J = 9.4 Hz, H-3), 5.50 (s, 1H, H-3), 4.79 (dd, 2H, ^3J = 4.3 and ^4J = 1.8 Hz, H-1), 1.40 (s, 6H, 2×5-Me); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ: 206.95 (C-4), 179.9 (C-2), 160.9 (C-2), 161.0 (C-7), 155.7 (C-8a), 143.2 (C-4), 134.8 (C-2), 129.0 (C-5), 120.6 (C-3), 113.1 (C-4a), 112.8 (C-6), 113.6 (C-3), 102.3 (C-3), 101.7 (C-8), 88.5 (C-5), 67.2 (C-1), 23.0 (5-Me); MS (EI) *m*/*z* (%) 312 (M^+, 100), 297 (7), 229 (50), 162 (22), 69 (63). Anal. calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.50; H, 5.42.

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