Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1033–1037
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological activity evaluation of lignan lactones derived from (−)-cubebin

Rosangela da Silva,[a,*] Gustavo H. B. de Souza,[a] Ademar A. da Silva,[a] Vanessa A. de Souza,[a] Ana C. Pereira,[a] Vanesa de A. Royo,[a,b] Marcio L. A. e Silva,[b] Paulo M. Donate,[c] Ana L. S. de Matos Araújo,[a] José C. T. Carvalho[d] and Jairo K. Bastos[a]

[a]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Avenida do Café, s/n°, 14040-903 Ribeirão Preto, SP, Brazil*
[b]*Núcleo de Pesquisa em Ciências Exatas e Tecnológicas da Universidade de Franca, 14404-600 Franca, SP, Brazil*
[c]*Departamento de Química da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 Ribeirão Preto, SP, Brazil*
[d]*Laboratório de Fitofármacos, Universidade de Alfenas, 37130-000 MG, Brazil*

Received 14 May 2004; revised 10 December 2004; accepted 13 December 2004
Available online 12 January 2005

Abstract—The anti-inflammatory and analgesic effects of three dibenzylbutyrolactone lignans, (−)-hinokinin (**2**), (−)-6,6′-dinitrohinokinin (**3**), and (−)-6,6′-diaminohinokinin (**4**), obtained by partial synthesis from (−)-cubebin (**1**), were investigated using different animal models. It was observed that compounds (**1**) and (**2**) inhibited the edema formation in the rat paw edema assay at the same level and that all responses were dose dependent. Also, at the dose of 30 mg/kg, compounds **1**, **2**, **3**, and **4** inhibited the edema formation by 53%, 63%, 54%, and 82%, respectively, at the third hour of the experiment. In the acetic acid-induced writhing test in mice, compounds **2** and **4** produced inhibition levels of 97% and 92%, respectively, while **3** displayed lower effect (75%), which was still higher than **1**. The assayed compounds neither displayed activity in the cell migration test nor in the hot plate test.

Prostaglandins (PGs) are potent mediators of inflammation and non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting its production. The pharmacological target of NSAIDs is cyclooxygenase (COX, also known as PGH synthase), which catalyses the first committed step in arachidonic-acid metabolism. Two isoforms of the membrane protein COX are known: COX-1, which is constitutively expressed in most tissue and is responsible for the physiological production of prostaglandins; COX-2, which is induced by cytokines, mitogens, and endotoxins in inflammatory cells, and is responsible for elevated production of prostaglandins during inflammation. Hence, differences in the pharmacological profiles of various NSAIDs might be accounted for their different degrees of selectivity for COX-1 and COX-2. Also, the potency and selectivity of NSAIDs seems to be directly correlated with their gastric, renal, and hepatic toxicities.[1]

The search for new compounds bearing analgesic and anti-inflammatory activities, with specificity for the isoform 2 of cyclooxygenase, has been intense and many classes of compounds have been investigated.[2] Thus, tricyclic compounds composed of two aromatic rings and one five-membered heterocycle displayed promising results.[3] The most important compound of this class is the COX-2 inhibitor rofecoxib (Vioxx®) developed by Merck Research Laboratories.[4] This compound bears a furanone ring with an aromatic group at position 2 and another aromatic ring bearing a methylsulfone at position 3.

The similarities between the basic structure of 2,3-aryl-heterocyclic compounds (Vioxx and others) and dibenzylbutyrolactone lignans (Fig. 1) encouraged our research group to biologically evaluate dibenzylbutyrolactone derivative compounds obtained by partial synthesis from (−)-cubebin (**1**), which was previously

Keywords: Cubebin; Anti-inflammatory and analgesic activities; Lignan lactones.
* Corresponding author. Tel.: +55 16 602 3879; fax: +55 16 633 8151; e-mail: rosilva@usp.br

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.035

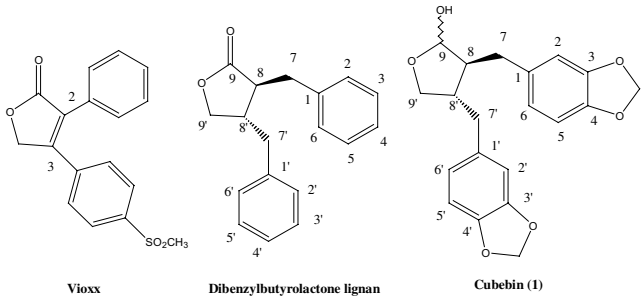
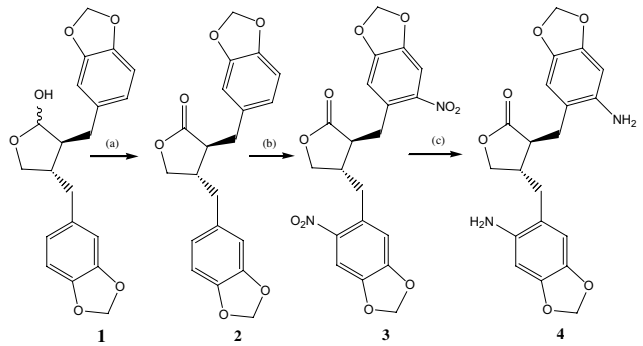


Figure 1. Comparison of the basic structure of 2,3-arylheterocyclic compounds (Vioxx and others) with the structure of dibenzylbutyrolactone lignans.

evaluated and displayed moderated anti-inflammatory and analgesic activities.[5]

Cubebin belongs to the dibenzylbutyrolactone lignans, which are widely distributed in the plant kingdom[6] and have been investigated by researchers for their different biological activities.[7] Natural products obtained in screening programs are often good leads for the development of drugs and through the synthesis of derivatives it is often possible to increase their biological activities. The work described here attempts to identify new compounds displaying mechanism of action similar to the non-steroidal anti-inflammatory drugs, and with reduced side effects. (−)-Cubebin (**1**) was used as the starting compound for synthesis of (−)-hinokinin (**2**), which was submitted to nitration reaction to furnish (−)-6,6′-dinitrohinokinin (**3**). Reduction of the nitro groups of compound **3** furnished (−)-6,6′-diaminohinokinin (**4**) (Scheme 1).

Cubebin (**1**) was isolated from the seeds of *Piper cubeba* as described in the literature.[8] (−)-Hinokinin (**2**) was obtained by oxidation of **1** with 2 equiv of PCC (CH_2Cl_2, room temperature, 24 h, 98%).[9] After oxidation, **2** was submitted to nitration by dropping nitric acid into a solution of **2** in chloroform, furnishing the dinitro derivative **3** (6 equiv HNO_3, $CHCl_3$, −10 °C, 4 h, 90%) functionalized at positions 6 and 6′ of the aromatic rings.[10] Compound **4** was obtained by reduction of the nitro groups of **3** using H_2/palladium/carbon/20 atm. (CH_3OH, room temperature, 24 h, 78%),[11] as showed in Scheme 1. The introduction of NH_2 groups to the aromatic rings was undertaken aiming not only to enhance the polarity of the compound, but also to allow stronger interactions, through hydrogen bonds, with the amino acid residues present in the enzymes.[2]



Scheme 1. Reagents and conditions: (a) PCC, CH_2Cl_2, rt, N_2, 12 h; (b) 6 equiv HNO_3, 2 h; (c) H_2/Pd/C 20 atm. overnight.

All spectral and characterization data are in accordance with the assumed chemical structures.[12] Analysis of the ^{1}H NMR spectra indicate that all hydrogens displayed the expected chemical shifts and integration values. Methylenic and aromatic hydrogens of cubebin were seen at 6.10 and 6.40–6.80 ppm, respectively. The presence of the hydrogen H-9 at 5.20 ppm confirms the presence of the cubebin lactol group. The absence of H-9 hydrogen at 5.20 ppm and presence of a signal at 178.4 ppm in the ^{13}C NMR spectrum indicate the presence of a carbonyl group, which confirms the transformation of the lactol group of the (−)-cubebin (**1**) into the lactone group of the (−)-hinokinin (**2**). The funtionalization of the aromatic ring with NO_2 group can be confirmed by both integration of all the aromatic hydrogens (4H) and displacement of the methylenedioxy hydrogens chemical shift, from 5.90 to 6.10 ppm. Positions of nitro groups at the aromatic rings can be confirmed by the absence of the coupling between the aromatic protons, which indicate that these groups are linked at positions 6 and 6′ at the aromatic rings.

The chemical structure of compound **4** was confirmed by ^{1}H NMR chemical shift at 8.3 ppm, which corresponds to the hydrogen bond between the hydrogen of the NH_2 group at position 6 with the oxygen of the carbonyl group. It was confirmed by the analysis of the HMBC spectrum by observing the correlation between a hydrogen at 8.3 ppm with carbons C-9 and C-8, which brings its signal far away from its expected place in the ^{1}H NMR. The other chemical shifts of the hydrogens of the NH_2 groups appear as broad singlet at 3.0–2.5 ppm.

(−)-Cubebin (**1**), (−)-hinokinin (**2**), (−)-6,6′-dinitrohinokinin (**3**), (−)-6,6′-diaminohinokinin (**4**), non-steroidal, and steroidal reference drugs were screened for anti-inflammatory activities by using both carrageenan hind–paw edema test and acute carrageenan-induced inflammatory reaction into the peritoneal cavity of rats.[13] The analgesic activity screening was undertaken by using both the acetic acid writhing test in mice and the hot plate test in rats, by using indomethacin and morphine as reference drugs, respectively.[14,15]

The administration of carrageenan into the rat paws produced a high edematogenic response, with the peak observed at the third hour. The treatment of the animals with cubebin (**1**) and its derivatives **2**, **3**, and **4** inhibited significantly the edema formation ($p < 0.05$). It was observed that cubebin (**1**) and hinokinin (**2**) inhibited the edema formation at the same level and that all the responses were dose dependent. At the dose of 30 mg/kg, compounds **1**, **2**, **3**, and **4** inhibited the edema formation by 53%, 63%, 54%, and 82%, respectively, at the third hour of the experiment (Fig. 2).

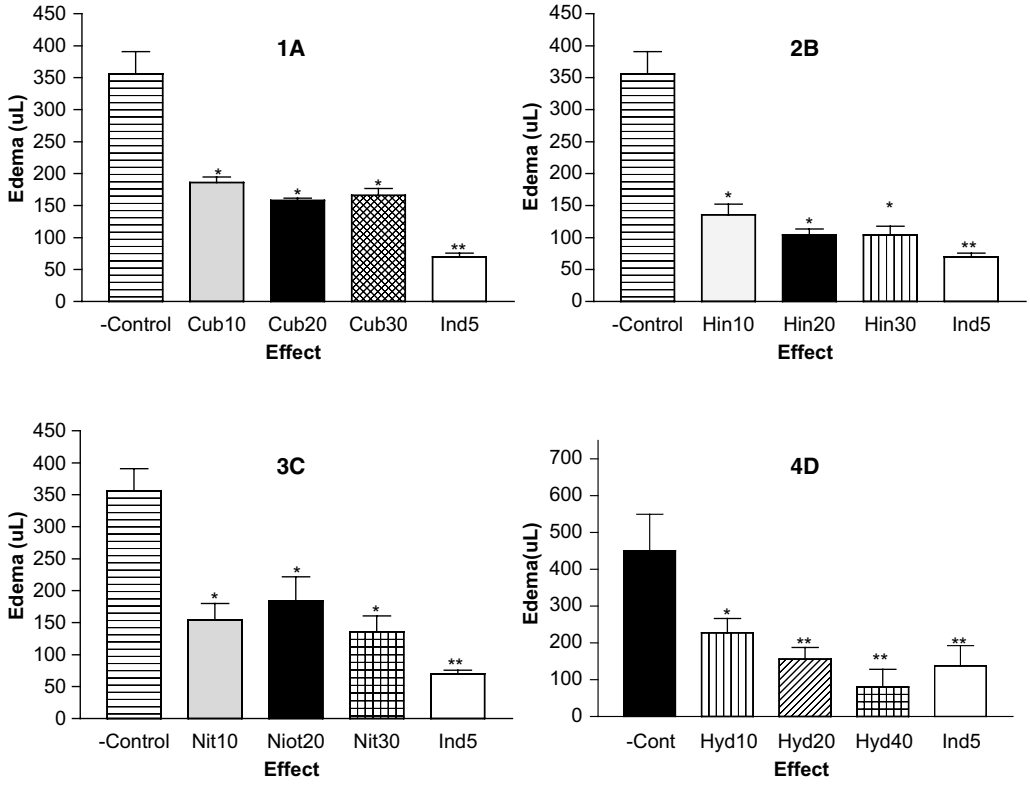


Figure 2. Effect of the oral administration of **1** (**A**), **2** (**B**), and **3** (**C**) at 10, 20, and 30 mg/kg dosages; **4** (**D**) at 10, 20, and 40 mg/kg dosages and indometacin at 5 mg/kg dosage on carrageenan (100 μg/paw) induced rat paw edema (3 h), after carrageenan injection. The data were analyzed by one-way ANOVA and Dunnett's Multiple Comparison Test, and were significant in the level set at $p < 0.05$.

In the anti-inflammatory assay, compound **2** and **3** were more active than cubebin (**1**), and compound **3** was less active than **2**. Besides, compound **4** was the most active one among the obtained cubebin derivatives, displaying the highest inhibition level of edema formation. Raz et al. reported that carrageenan has a dose dependent pro-tumorigenic effect and can trigger at any time tumor growth, which is mediated by prostaglandins.[16] Therefore, cyclooxygenase inhibitors, such as indomethacin, can hinder this process. Multiple mechanisms regulate the induction of COX-2, especially in drastic animal models. However, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism.[17] Therefore, it is suggested that the mechanism of action of these compounds (**1**, **2, 3**, and **4**) may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of indomethacin in the inhibition of the inflammatory process induced by carrageenan. In addition, the classification of anti-nociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system.[18] There is a high correlation between the effects produced by carrageenan injection with the ones observed for the writhing test induced by acetic acid injection. The acetic acid nociceptive response involves the liberation of endogenous compounds, such as bradikinin and prostanoids.[19] Therefore, this is a standard sensitive test for both the opioids and non-opioids analgesics.[20]

In the writhing test in mice, compounds **2** and **4** produced the highest inhibition levels of the algogenic process (97% and 92%, respectively), while compound **3** was less active (75%). However, all the obtained derivatives were more active than (−)-cubebin (**1**) (Fig. 3).

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, like local anesthetics and narcotics do.[21] Nevertheless, the hot plate test was undertaken for **1**, **2**, **3**, and **4** to verify if these compounds could show any central analgesic effect. As expected, the obtained results did not show any significant activity. On the other hand, the results obtained for the group treated with morphine were highly significant. Thus, these compounds were considered not to have analgesic effect on the central nervous system that would contribute to their peripheral analgesic effect.

Also, the cell migration into the damaged tissue is an important step in the inflammatory process. Hence, using carrageenan as a stimulus, it was possible to produce an acute inflammatory response after 4 h into the peritoneal cavity of rats, with a large number of polymorphonuclear cells in the exudates. However, the administration of **1**, **2**, **3**, and **4** did not reduce the cell migration compared to the negative control group (5% tween saline solution), while dexamethasone inhibited 80%.

In conclusion, we have shown that compounds possessing a lactone ring bearing two methylendioxyaryl groups

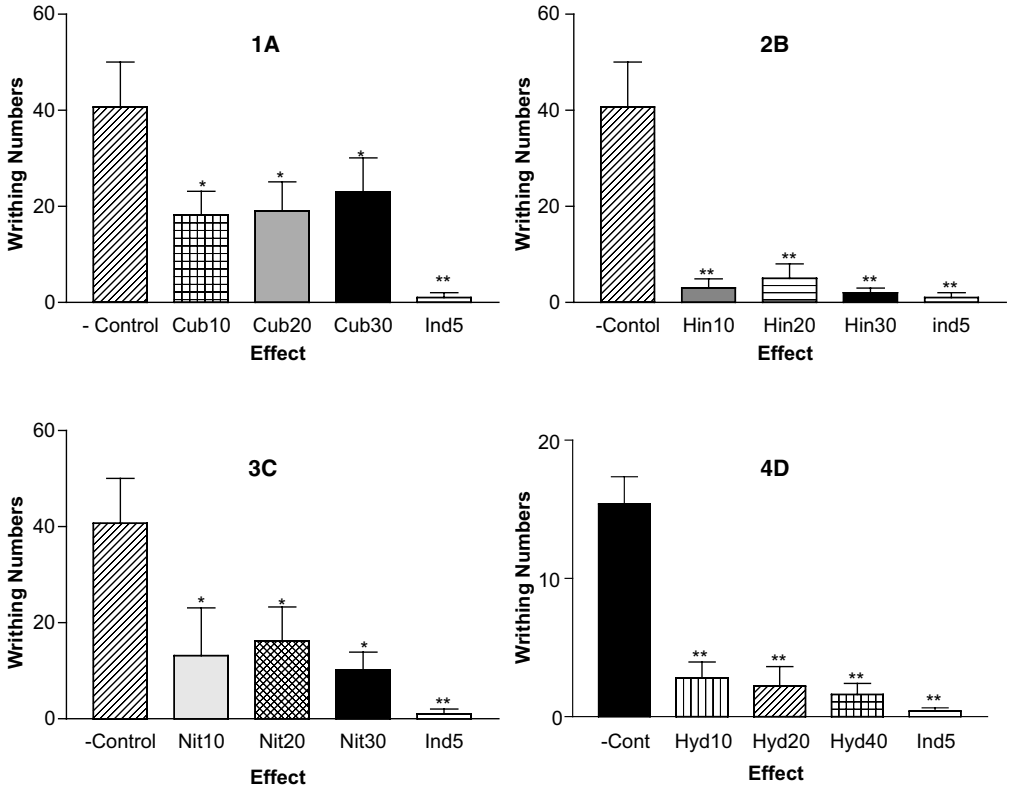


Figure 3. Effect of the oral administration of **1** (**A**), **2** (**B**), and **3** (**C**) at 10, 20, and 30 mg/kg dosages; **4** (**D**) at 10, 20, and 40 mg/kg dosages and indometacin at 5 mg/kg dosage, on acetic acid-induced writhing test in mice. Each point represents the average ± SEM ($n = 6$) of the total writhing number in 20 min for the different dosages. The data were analyzed by one-way ANOVA and Dunnett's Multiple Comparison Test, and were significant in the level set at $p < 0.05$.

display significant anti-inflammatory and analgesic activities, and that the introduction of polar groups such as NH_2 are beneficial for activity. SAR studies are in progress to determine which groups and positions of the aromatic rings furnish better results and how they affect COX-2 selectivity.

Acknowledgements

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), the Coordenadoria de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. We are Also thankful to Dr. Regina Sparrapan of the Thomson Laboratory of Mass Espectrometry of Unicamp for running the HRMS analyses.

References and notes

1. Plaska, E.; Sahin, G.; Pelin, K.; Durlu, N. T.; Altinok, G. *Fármaco* **2002**, *57*, 101.
2. Garavito, R. M.; DeWitt, L. D. *Biochim. Biophys. Acta* **1999**, *1441*, 278.
3. Leblanc, Y.; Gauthier, J. Y.; Ethier, D.; Guay, J.; Manzini, J.; Riendeau, D.; Tagari, P.; Vickers, P.; Wong, E.; Prasit, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2123.
4. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Léger, S.; Mancini, J.; O'Neil, J. P.; Ouellet, M.; Percival, M. D.; Perrier, H.; Rindeau, D.; Rodger, I.; Tagari, P.; Thérien, M.; Vickers, P.; Wong, E.; Xu, L. J.; Young, R. N.; Zamboni, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
5. Bastos, J. K.; Carvalho, J. C. T.; Souza, G. H. B.; Pedrazzi, A. H. P.; Sarti, S. J. *J. Ethnopharmacol.* **2001**, *75*, 279.
6. Rao, C. B. C. *Chemistry of Lignans*; Andhara University Press: Andhra Pradesh, India, 1978; p 95.
7. Ayres, D. C.; Loike, J. D. *Chemistry and Pharmacology of Natural Products: Lignans; Chemical, Biological and Clinical Properties*; Cambridge University Press: New York, 1990.
8. Bastos, J. K.; Gottlieb, O. R.; Sarti, J. S.; Filho, D. S. *Nat. Prod. Lett.* **1996**, *9*, 65.
9. Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *31*, 2647.
10. Barreiro, E. J.; Fraga, C. A. M. *Química Nova* **1999**, *22*, 744.
11. Hudlický, M. *Reductions in Organic Chemistry*; John Wiley & Sons: Chichester, 1984; p 69.
12. (−)-Hinokinin (**2**): $[\alpha]_D^{26}$ −30 (*c* 0.99, $CHCl_3$); ^1H NMR δ ($CDCl_3$) 6.8–6.4 (m, 1H), 5.9 (broad s, 2H), 4.15 (dd, 1H, $J = 7.1$ and 9.3 Hz), 3.85 (dd, 1H, $J = 7.1$ and 9.1 Hz), 3.0 (dd, 1H, $J = 5.1$ and 14.2 Hz), 2.85 (dd, 1H, $J = 7.3$ and 14.2 Hz), 2.6 (d, 1H, $J = 7.1$ Hz), 2.55 (m, 1H), 2.45 (d, 1H, $J = 8.6$ Hz), 2.4 (m, 1H); ^{13}C NMR δ ($CDCl_3$) 178.4, 147.9, 147.8, 146.5, 146.4, 131.6, 131.3, 122.2, 121.55, 109.4, 108.8, 108.4, 108.3, 101.0, 71.2, 46.4, 41.3, 38.4,

34.8. (−)-6,6′-Dinitrohinokinin (**3**): mp 191–193 °C; $[\alpha]_D^{26}$ −29 (*c* 0.008, $CHCl_3$); ^1H NMR δ ($CDCl_3$) 7.5 (s, 1H), 7.48 (s, 1H), 6.8 (s, 1H), 6.6 (s, 1H), 6.1 (m, 2H), 4.35 (dd, 1H, *J* = 7.1 and 9.1 Hz), 4.0 (dd, 1H, *J* = 7.3 and 9.1 Hz), 3.26 (d, 2H, *J* = 7.1 Hz), 3.2 (dd, 1H, *J* = 6.3 and 13.6 Hz), 3.0 (dd, 1H, *J* = 7.8 and 13.6 Hz), 2.8 (m, 2H); ^{13}C NMR δ ($CDCl_3$) 178.0, 152.3, 152.2, 147.6, 143.1, 142.9, 130.9, 130.7, 112.5, 111.2, 106.6, 106.2, 103.6, 103.5, 71.4, 45.7, 41.7, 37.2, 34.2; HRMS $(M+H)^+$ calcd for $C_{20}H_{17}N_2O_{10}$: 445.0884; found: 445.0890. Anal. Calcd for $C_{20}H_{16}N_2O_{10}$: C, 54.09; H, 3.63; N, 6.31; O, 35.97. Found: C, 53.92; H, 3.55; N, 6.21; O, 36.32.

(−)-6,6′-Diaminohinokinin (**4**): mp 133–136 °C; $[\alpha]_D^{26}$ −36 (*c* 0.0015, $CHCl_3$); ^1H NMR δ (C_6D_6) 8.3 (s, H–NH), 6.45 (s, 1H), 6.3 (s, 1H), 6.1 (s, 1H), 5.8 (s, 1H), 5.4 (d, 2H, *J* = 9.8 Hz), 3.45–2.5 (broad singlet 3H), 3.4 (dd, 1H, *J* = 11.2 and 4.5 Hz), 3.4 (dd, 1H, *J* = 11.2 and 7.6 Hz), 2.7 (ddd, 1H, *J* = 4.5, 5.8 and 12.6 Hz), 2.5 (m, 2H), 2.2–2.0 (m, 5H); ^{13}C NMR δ ($CDCl_3$) 172.8, 145.7, 145.5, 142.4, 138.7, 138.6, 129.3, 115.0, 114.8, 109.4, 107.3, 100.1, 99.3, 97.1, 96.3, 60.9, 40.0, 37.7, 28.9, 25.4; HRMS $(M+H)^+$ calcd for $C_{20}H_{21}N_2O_6$: 385.1321; found: 385.1101; Anal. Calcd for $C_{20}H_{20}N_2O_6$: C, 62.54; H, 5.25; N, 7.29; O, 24.92. Found: C, 62.28; H, 5.28; N, 7.26; O, 25.19.

13. (a) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 453; (b) Souza, G. E. P.; Ferreira, S. H. *Agents Actions* **1985**, *17*, 97.
14. Koster, R.; Anderson, M.; Beer, E. J. *Fed. Proc.* **1959**, *18*, 412.
15. (a) Woolfe, G.; MacDonald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300; (b) Yaksh, T. L.; Yeung, J. C.; Rudy, T. A. *Brain Res.* **1976**, *114*, 83.
16. Raz, A.; Levin, G.; Khomiak, Y. *Cancer Lett.* **2000**, *148*, 115.
17. Nantel, F.; Denis, D.; Gordon, R.; Northey, A.; Cirino, M.; Metters, K. M.; Chan, C. C. *Braz. J. Pharmacol.* **1999**, *128*, 853.
18. Planas, E.; Sanchez, S.; Rodriguez, L.; Pol, O.; Puig, M. M. *Pharmacology* **2000**, *60*, 121.
19. Berkenkopf, J. W.; Weichman, B. M. *Prostaglandins* **1988**, *36*, 693.
20. Steranka, L. R.; Dehaas, C. J.; Vavrek, R. J.; Stewart, J. M.; Enna, S. J.; Snyder, S. H. *Eur. J. Pharmacol.* **1987**, *136*, 261.
21. Ferreira, S. H.; Lorenzetti, B. B.; Castro, M. S. A.; Correa, F. M. A.; Dumonde, D. C.; Jasani, M. K. *The Recognition of Anti-Rheumatic Drugs*; Lancaster, MTP Press Limited, St. Leonard House: Lancaster, 1978; p 25.