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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4944–4948
Bioorganic & Medicinal Chemistry Letters

Pentacyclic triterpenes. Part 1: The first examples of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases

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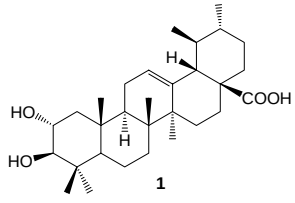
Received 8 June 2005; revised 12 July 2005; accepted 5 August 2005
Available online 16 September 2005

Abstract—The semi-synthesis, in vitro and in vivo biological evaluation of corosolic acid (**1**) and maslinic acid (**2**) are described. Compounds **1** and **2** represent a new class of inhibitors of glycogen phosphorylases. Both **1** and **2** inhibit the increase of fasted plasma glucose of diabetic mice induced by adrenaline. It is therefore proposed that naturally occurring pentacyclic triterpenes **1** and **2** might reduce blood glucose, at least in part, through inhibiting hepatic glycogen degradation.

Corosolic acid (**1**) has recently attracted much attention due to its biological activities, such as anti-diabetes,[1] anti-inflammation,[2] antiproliferation,[3] and protein kinase C inhibition activities.[4] Compound **1** is the primary active principle of a plant extract (commercially named Glucosol), which has been marketed in Japan and the United States for reducing blood glucose levels and weight-loss.[5] Maslinic acid (**2**), which has a similar structure with **1**, has also drawn much interest due to its anti-tumor,[6] anti-HIV,[7] and antioxidant activities.[8] Surprisingly, despite the obvious biological importance of **1** and **2**, little was reported regarding a practical synthetic preparation of both compounds.[9] On the other hand, it is very tedious and expensive to obtain pure compound **1**[10] or **2**[11] by plant extraction. As part of our projects aimed at developing pentacyclic triterpenes as preventive and therapeutic agents, we hoped to establish a practical synthetic preparation of **1** and **2**.

Glycogen phosphorylases (GP), which catalyze the first step of glycogen breakdown, play an important role in glucose metabolism, especially in glycogenolytic pathway. It is well known that liver GP is the major enzyme for controlling hepatic glucose output, and inhibition of this enzyme may afford a useful therapeutic approach for type 2 diabetes.[12a] Moreover, GP inhibitors might also find pharmaceutical applications in treatment of cardiovascular diseases[13] and tumors.[14] Several structural classes of GP inhibitors have been reported, whose binding sites identified in GP include the catalytic site, the purine inhibitory site (also known as I-site), the allosteric site, the glycogen storage site, and a novel allosteric inhibitor site.[12b,12c,12d] Attention attracted in this area remains high as evidenced by the frequent appearance of related patents and publications.



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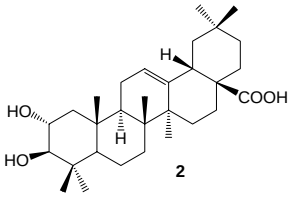

2

Herein, we report an efficient semi-synthesis of **1** and **2**, starting from readily available ursolic acid and oleanolic acid, respectively. Most importantly, we report here, for the first time, that **1** and **2** represent a new class of natural inhibitors of glycogen phosphorylases.

Keywords: Triterpenes; Maslinic acid; Corosolic acid; Glycogen phosphorylase; Inhibitors; Synthesis.
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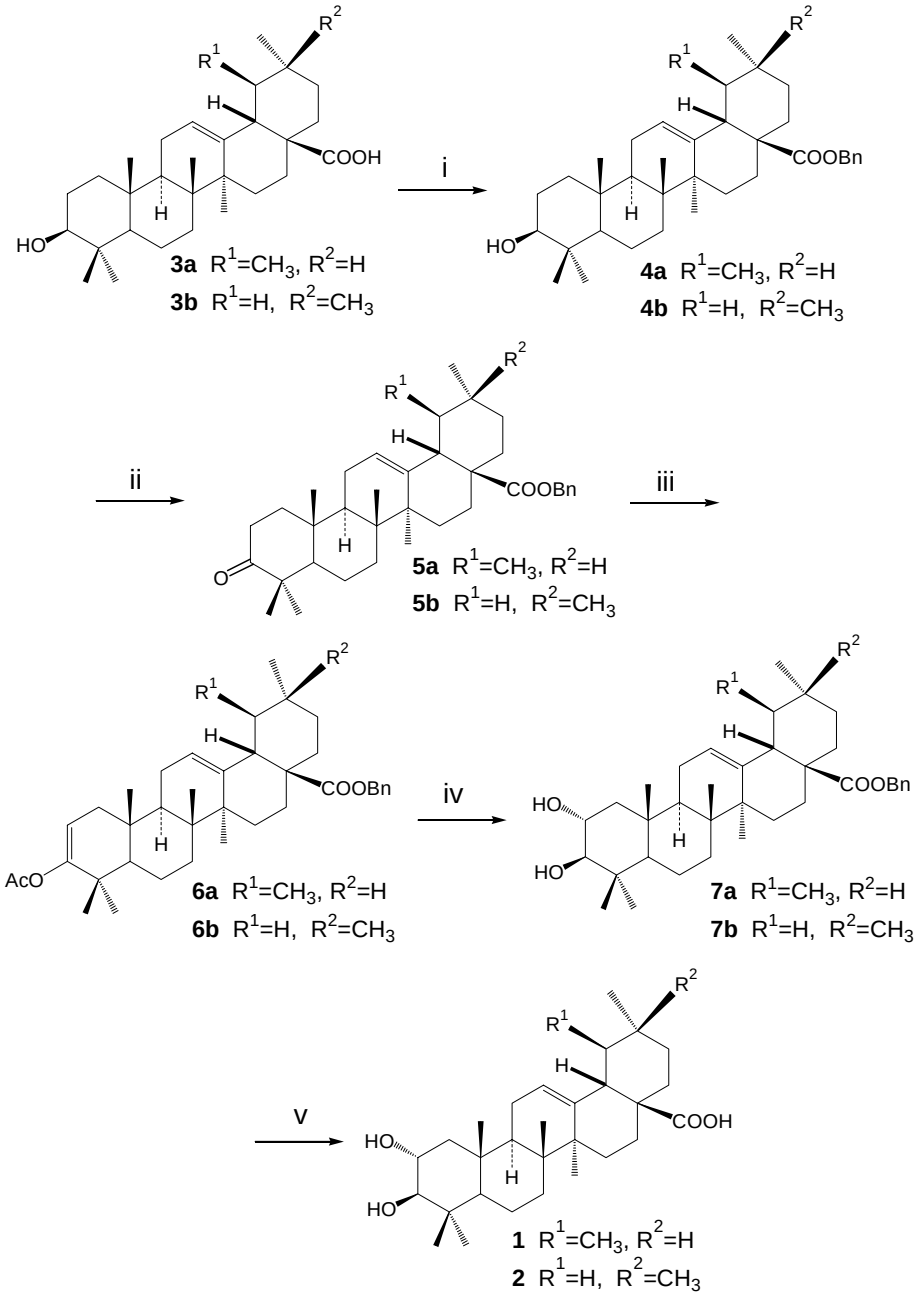
doi:10.1016/j.bmcl.2005.08.026

The syntheses of **1** and **2** are summarized in Scheme 1. Commercially available ursolic acid **3a** was mixed with benzyl chloride and potassium carbonate in DMF at 85 °C for 2 h to give benzyl ester **4a** in 96% yield. Considering that short chain alkyl esters of the C-28 carboxylic acid, such as C-28 methyl ester or ethyl ester, could not be hydrolyzed by conventional hydrolysis methods (e.g., basic or acidic aqueous conditions), we chose benzyl as the protecting group, which can be easily removed later on by catalytic hydrogenolysis without effecting the C12–13 double bond. Oxidation of **4a** with PCC in CH_2Cl_2 at 0 °C to room temperature for 24 h afforded **5a** in 89% yield after simple work-up and crystallization from ethanol. Reaction of **5a** with vinyl acetate or isopropenyl acetate catalyzed by concentrated sulfuric acid gave enol acetate **6a** in 91% yield. Treatment of **6a** with excess borane–THF solution, followed by adding alkaline H_2O_2, afforded 2α,3β-diol **7a** (55%) as the major product after column chromatography purification. The major side products were **5a** (produced by saponification of **6a**) and **4a** (produced by reduction of the reformed **5a**). Another possible isomer (2β,3α) was only trace amount due to the high stereospecificity of the reaction and could be removed by column chromatography. Hydrogenolysis of **7a** over palladium/carbon in THF furnished **1** in quantitative yield.[15] In a similar fashion, **2** was synthesized, starting with oleanolic acid **3b**.[16]



Scheme 1. Reagents and conditions: (i) K_2CO_3, BnCl, DMF, 85 °C; (ii) PCC, CH_2Cl_2; (iii) AcOCH=CH_2, concd H_2SO_4 (cat.), 100 °C; (iv) a—BH_3–THF; b—H_2O_2, NaOH; (v) H_2, Pd/C, THF, rt.

The hypoglycemic mechanism of **1** is not clear,[17] therefore, more research work is still needed to unanimously solve the puzzle. In this regard, we tested **1** in enzyme inhibition assay against GP since we hypothesized that **1** and related triterpenes might lower blood glucose by inhibiting hepatic glycogen degradation. Considering that muscle GP and liver GP share considerable sequence similarity, we first employed rabbit muscle GPa for the enzyme assay since this enzyme was commercially available. As described previously,[18] the activity of rabbit muscle GPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis. The results are outlined in Figure 1. To our delight, **1** exhibited moderate inhibitory activity on rabbit muscle GPa (IC_{50} = 20 μM). Not surprisingly, **2** also effectively inhibited this enzyme (IC_{50} = 28 μM). In our assays, **1** and **2** were at least 4-fold more potent than caffeine (IC_{50} = 114 μM), a proverbial GP inhibitor.[12a]

We also examined the effect of varying glucose concentrations on the IC_{50} values for **1**, **2**, and caffeine, because some GP inhibitors were reported to be more potent in the presence of high glucose concentrations.[19,20] As shown in Figure 2, at high glucose concentrations, the relative IC_{50} values for **1**, **2**, and caffeine were significantly decreased, indicating the increases in potency for the test compounds.

Encouraged by the results obtained with muscle GPa, we further tested **1** and **2** on purified liver GPa obtained by extraction from rat liver as described by Fosgerau et al.[21] The assay results showed that both **1** (IC_{50} = 101 μM) and **2** (IC_{50} = 99 μM) were inhibitors of rat liver GPa (Fig. 3), whereas caffeine (IC_{50} = 648 μM) was a 6-fold less potent inhibitor of rat liver GPa. Although **1** and **2** were less potent as rat liver GPa inhibitors than as rabbit muscle GPa inhibitors in our assays, we could not conclude that **1** and **2** were more selective for muscle GPa than for liver GPa, since the rabbit muscle GPa used for the assay was pure commercial product, while the rat liver GPa used for assays was self-prepared and thus had a different quality from the muscle enzyme. However, in either muscle GPa or liver GPa assays, both **1** and **2** were at least 4-fold more potent than caffeine.

Based on their potency in the enzyme assays, **1** and **2** were evaluated for their hypoglycemic activity in adrenaline-induced diabetic mice since it is well known that adrenaline is thought to induce high blood glucose by indirect stimulating glycogenolysis and therefore increasing hepatic glucose production. Test compounds were administered orally at 100 mg/kg/day for 7 days. Not surprisingly, the preliminary animal study results

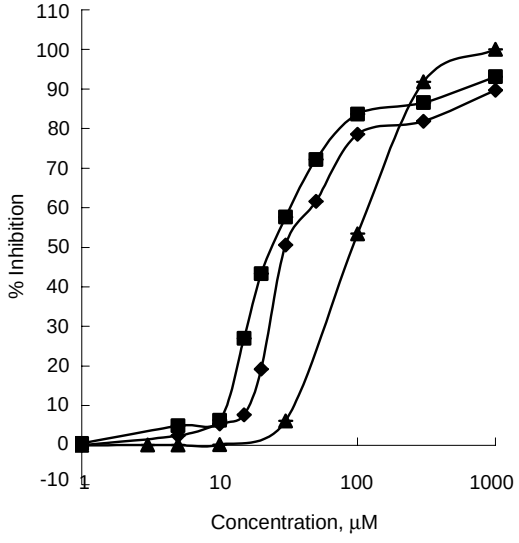


Figure 1. Inhibition of rabbit muscle GPa by **1**, **2**, and caffeine. GPa activity was measured at varied concentrations of 1 (■), 2 (♦) or caffeine (▲). The data are plotted as percent inhibition versus concentration of compound. Results are means ± SEM for three independent experiments.

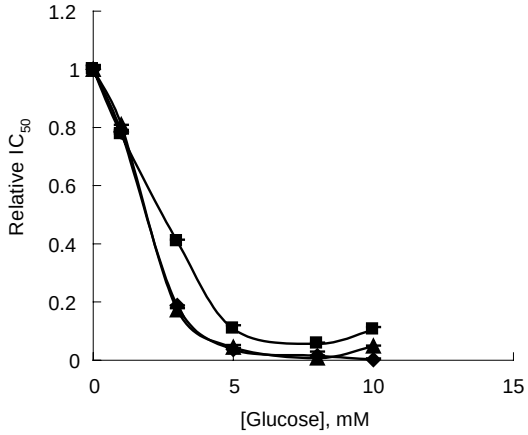


Figure 2. The effect of glucose on the potency of 1 (■), 2 (♦) or caffeine (▲). IC_{50} values for rabbit muscle GPa inhibition were determined at varied glucose concentrations and then normalized by dividing the values by the IC_{50} values obtained in the absence of glucose. The normalized results are plotted as a function of glucose concentration.

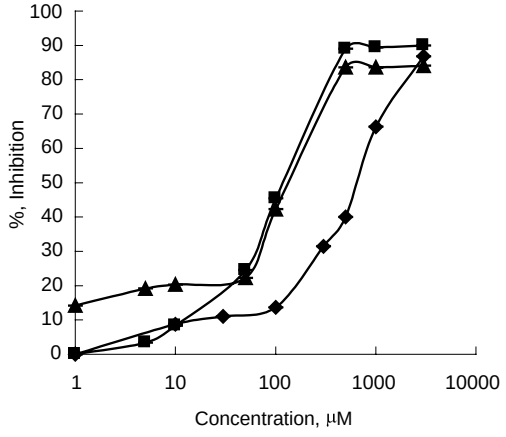


Figure 3. Inhibition of rat liver GPa by **1**, **2**, and caffeine. GPa activity was measured at varied concentrations of 1 (▲), 2 (■) or caffeine (♦). The data are plotted as percent inhibition versus concentration of compound. Results are means ± SEM for three independent experiments.

Table 1. Effect of **1** and **2** on fasted plasma glucose of hyperglycemic mice induced by adrenaline ($n = 10$)

Compound	Dose (mg/kg)	Fasted plasma glucose (OD)		
		0 h	2 h	4 h
Vehicle		0.138 ± 0.028	0.149 ± 0.037	0.124 ± 0.019
1	100	0.111 ± 0.019	0.076 ± 0.026	0.083 ± 0.017
2	100	0.117 ± 0.032	0.081 ± 0.027	0.079 ± 0.023

(Table 1) showed that both compounds significantly inhibited the increase of fasted plasma glucose of diabetic mice induced by adrenaline. To the best of our knowledge, this is the first report that **2** is capable of reducing blood glucose of diabetic mice, while **1** is a well-known hypoglycemic agent. Further in vivo studies on **1**, **2**, and related products as hypoglycemic agents are ongoing.

In summary, we have identified corosolic acid (**1**) and maslinic acid (**2**) as a new class of inhibitors of glycogen phosphorylases. This discovery affords novel lead compounds for developing potent GP inhibitors. As an effort to identify the possible binding sites in GP for **1** and **2**, molecular modeling study is still in process. Based on the in vitro and in vivo studies, it is therefore proposed that naturally occurring pentacyclic triterpenes **1** and **2** might reduce blood glucose, at least in part, through inhibiting hepatic glycogen degradation. The advantages of these natural GP inhibitors as anti-diabetic agents are obvious: (a) they are mild GP inhibitors, and thus side effects caused by strong and nonselective GP inhibition by synthetic GP inhibitors are avoidable; (b) they are nontoxic, for example, **2** is an abundant constituent of olive fruit;[11] (c) except for lowering blood glucose, they also exhibit other therapeutic benefits such as anti-inflammation[2] and antioxidant activities,[8] etc. Extensive research on lead optimization and biological evaluation of pentacyclic triterpenoids as a new class of GP inhibitors is in progress in our laboratory. Further reports will describe structure–activity relationships in a wider series of pentacyclic triterpenoids.

Acknowledgments

We hope to thank Mr. Shanzhi Wang and Ms. Rui Niu for their technical supports in enzyme assay studies.

References and notes

1. Miura, T.; Itoh, Y.; Kaneko, T.; Ueda, N.; Ishida, T.; Fukushima, M.; Matsuyama, F.; Seino, Y. *Biol. Pharm. Bull.* **2004**, *27*, 1103.
2. Banno, N.; Akihisa, T.; Tokuda, H.; Yasukawa, K.; Higashihara, H.; Ukiya, M.; Watanabe, K.; Kimura, Y.; Hasegawa, J.; Nishino, H. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 85.
3. Miyako, Y.; Masahiro, F.; Tsuneatsu, N.; Hikaru, O.; Kazuhisa, M.; Jiro, T.; Yoshiharu, K.; Ryota, T.; Junei, K.; Kunihide, M.; Toshihiro, F. *Biol. Pharm. Bull.* **2005**, *28*, 173.
4. Ahn, K. S.; Hahm, M. S.; Park, E. J.; Lee, H. K.; Kim, I. H. *Planta Med.* **1998**, *64*, 468.
5. Judy, W. V.; Hari, S. P.; Stogsdill, W. W.; Judy, J. S.; Naguib, Y. M. A.; Psswater, R. *J. Ethnopharmacol.* **2003**, *87*, 115.
6. Taniguchi, S.; Imayoshi, Y.; Kobayashi, E.; Takamatsu, Y.; Ito, H.; Hatano, T.; Sakagami, H.; Tokuda, H.; Nishino, H.; Sugita, D.; Shimura, S.; Yoshida, T. *Phytochemistry* **2002**, *59*, 315.
7. Xu, H. X.; Zeng, F. Q.; Wan, M.; Sim, K. Y. *J. Nat. Prod.* **1996**, *59*, 643.
8. Montilla, M. P.; Agil, A.; Navarro, M. C.; Jimenez, M. I.; Garcia-Granados, A.; Parra, A.; Cabo, M. M. *Planta Med.* **2003**, *69*, 472.
9. Caglioti reported a partial synthesis of methyl maslinate based on a hydroboration-oxidation reaction, however, the overall yield was very poor, and moreover, it is very difficult (if possible) to convert methyl maslinate to maslinic acid by conventional acid or base catalyzed hydrolysis Caglioti, L.; Cainelli, G. *Tetrahedron* **1962**, *18*, 1061.
10. Wang, P.; Lei, L. U.S. Patent Application 20030165581, 2003.
11. Noriyasu, N.; Shinohara, G. PCT Patent, WO 0212159, 2002.
12. (a) Oikonomaos, N. G. *Curr. Protein Pept. Sci.* **2002**, *3*, 561; (b) Lu, Z. J.; Bohn, J.; Bergeron, R.; Deng, Q. L.; Ellsworth, K. P.; Geissler, W. M.; Harris, G.; McCann, P. E.; Mckeever, B.; Myers, R. W.; Saperstein, R.; Willoughby, C. A.; Yao, J.; Chapman, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4125; (c) Wright, S. W.; Rath, V. L.; Genereux, P. E.; Hageman, D. L.; Levy, C. B.; McClure, L. D.; McCoid, S. C.; McPherson, R. K.; Schelhorn, T. M.; Wilder, D. E.; Zavadoski, W. J.; Gibbs, E. M.; Treadway, J. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 459; (d) Treadway, J. L.; Mendys, P.; Hoover, D. J. *Expert Opin. Investig. Drugs* **2001**, *10*, 439, and references therein.
13. Tracey, W. R.; Treadway, J. L.; Magee, W. P.; Sutt, J. C.; McPherson, R. K.; Levy, C. B.; Wilder, D. E.; Yu, L. J.; Chen, Y.; Shanker, R. M.; Mutchler, A. K.; Smith, David M.; Flynn, A. H.; Knight, D. R. *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *286*, H1177.
14. Schnier, J. B.; Nishi, K.; Monks, A.; Gorin, F. A.; Bradbury, E. M. *Biochem. Biophys. Res. Commun.* **2003**, *309*, 126.
15. Analytical data for **1**: mp 253–255 °C (mp 255–258 °C, Ref. 6); IR (KBr, cm^{-1}) 3414, 2945, 1695, 1456, 1049; ^1H NMR (pyridine-d_5, 300 MHz): δ 0.94 (3H, s, H-29), 0.96 (3H, s, H-30), 0.97 (3H, s, H-25), 1.02 (3H, s, H-24), 1.05 (3H, s, H-26), 1.19 (3H, s, H-27), 1.25 (3H, s, H-23), 2.60 (1H, d, $J = 11.3$ Hz, H-18), 3.36 (1H, d, $J = 9.4$ Hz, H-3α), 4.06 (1H, ddd, $J = 4.3$, 9.4, 11.1 Hz, H-2β), 5.44 (1H, t, $J = 3.3$ Hz, H-12); ^{13}C NMR (pyridine-d_5, 300 MHz): δ 17.0 (C-25), 17.5 (2C, C-26, C-30), 17.7 (C-24), 18.9 (C-6), 21.4 (C-29), 23.8 (C-11), 23.9 (C-27), 24.9 (C-16), 28.7 (C-15), 29.4 (C-23), 31.1 (C-21), 33.5 (C-7), 37.5 (C-22), 38.5 (C-10), 39.4 (C-19), 39.5 (C-20), 39.8 (C-4), 40.1 (C-8), 42.6 (C-14), 48.0 (C-1), 48.1 (C-17), 53.6 (C-18), 60.0 (C-5), 68.6 (C-2), 83.8 (C-3), 125.5 (C-12), 139.3 (C-13), 179.9 (C-28); MS: 495 $[M+Na]^+$. The spectrum data of **1** were identical with the reported data: see Ref. 6.
16. Analytical data for **2**: mp 269–271 °C (mp 266–269 °C, Ref. 22); IR (KBr, cm^{-1}) 3414, 2943, 1695, 1460, 1051; ^1H NMR (pyridine-d_5, 300 MHz): δ 0.93, 0.98, 0.99, 1.01, 1.06, 1.25, 1.26 (each, 3H, s), 3.28 (1H, dd, $J = 3.9$, 13.6 Hz, H-18), 3.37 (1H, d, $J = 9.3$ Hz, H-3α), 4.07 (1H, ddd, $J = 4.2$, 9.3, 11.0 Hz, H-2β), 5.46 (1H, br s, H-18); ^{13}C NMR (pyridine-d_5, 300 MHz): δ 16.9 (C-24), 17.5 (C-25), 17.7 (C-26), 18.9 (C-6), 23.7 (C-16), 23.8 (C-30), 23.9

(C-30), 26.2 (C-27), 28.3 (C-15), 29.3 (C-23), 31.0 (C-20), 33.2 (C-7), 33.3 (2C, C-22, C-29), 34.3 (C-21), 38.5 (C-10), 39.8 (C-4), 42.0 (C-19), 42.2 (C-14), 46.7 (C-17), 47.8 (C-1), 48.2 (2C, C-8, C-9), 55.9 (C-5), 68.6 (C-2), 83.8 (C-3), 122.5 (C-12), 144.9 (C-13), 180.2 (C-28); MS: 495 $[M+Na]^+$. The spectrum data of **2** were identical with the reported data: see Ref. 6.

17. Miura reported that **1** reduced blood glucose of KK-Ay mice, at least in part, through increasing GLUT4 translocation in muscle and thus stimulating glucose uptake: see Ref. 1.
18. Engers, H. D.; Shechosky, S.; Madsen, N. B. *Can. J. Biochem.* **1970**, *48*, 746.
19. Kasvinsky, P. J.; Shechosky, S.; Fletterick, R. J. *J. Biol. Chem.* **1978**, *253*, 9102.
20. Ercan-Fang, N.; Nuttall, F. Q. *J. Pharmcol. Exp. Ther.* **1997**, *280*, 1312.
21. Fosgerau, K.; Westergaard, N.; Quistorff, B.; Grunnet, N.; Kristiansen, M.; Lundgren, K. *Arch. Biochem. Biophys.* **2000**, *830*, 274.
22. Goswami, A.; Khastgir, H. N. *Indian J. Chem., Sect. B* **1980**, *19*, 315.