A Highly Selective, Non-Hydantoin, Non-Carboxylic Acid Inhibitor of Aldose Reductase with Potent Oral Activity in Diabetic Rat Models: 6-(5-Chloro-3-methylbenzofuran-2-sulfonyl)-2-*H*-pyridazin-3-one

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Received March 18, 2003

Abstract: We report here on the discovery path that led to a structurally unprecedented non-hydantoin, non-carboxylic acid aldose reductase inhibitor, **24**, which shows remarkably potent oral activity in normalizing elevated sorbitol levels and, more significantly, fructose levels in the sciatic nerve of chronically diabetic rats, with ED_{90} values of 0.8 and 3 mpk, respectively. It is well absorbed in rats (oral bioavailability, 98%) and has a long plasma $t_{1/2}$ (26 ± 3 h).

Several hypotheses have been proposed to discover therapies for the microvascular consequences of long-term diabetes, neuropathy, nephropathy, and retinopathy. One focus of metabolic research has been on hypotheses that pertain to the consequences of high intracellular glucose flux through the polyol pathway (Figure 1), viz., cellular osmotic stress and pseudohypoxia-linked oxidative stress. The first hypothesis is of earlier vintage and has been a target of sustained and extensive research.[1] Very briefly, the osmotic hypothesis has emphasized a central role for the high glucose flux through aldose reductase (AR), resulting in cellular accumulation of sorbitol with subsequent cell swelling caused by influx of water and in the eventual loss of cell integrity. On the other hand, recent studies have emphasized the role of high glucose flux through SDH underlying cytoplasmic NADH/NAD^+ imbalance, vascular dysfunction, and oxidative stress.[2] From a potential therapeutic approach, a robust blockade of excess glucose flux through the polyol pathway, as reflected in the complete normalization of both cellular sorbitol and fructose pools, would address osmotic as well as pseudohypoxia hypotheses. Strong support for this notion is derived from dose titration studies using different aldose reductase inhibitors (ARIs). For example, a nearly full correction of the nerve conduction velocity deficit (NCV) in diabetic rats requires doses of ARIs that not only overnormalize nerve sorbitol levels but also suppress nerve fructose levels by ≥ 90%.[3,4] In view of this, it is quite possible that ARIs tested to date, in phase III studies, have been underdosed because the dose selection was based on the sorbitol end point in laboratory models and/or was limited by clinical side effects. In fact, none of the inhibitors have been shown to effect robust suppression of fructose in human tissues, for example, sural nerve biopsy samples, in a placebo controlled large patient efficacy trial.

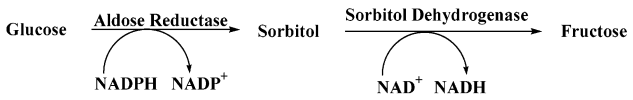
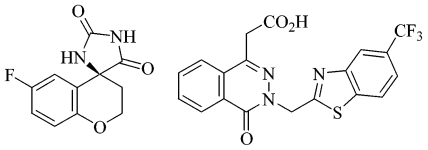
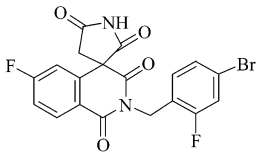


Figure 1. Polyol pathway.

The status quo in the ARI field can be attributed to the dearth of new chemotypes that can powerfully block the flux through the polyol pathway. Only two chemical classes of ARIs, hydantoin and carboxylic acid, have been evaluated in critical phase III trials. Hydantoin ARIs[5] (e.g., sorbinil) are very weakly acidic (pK_a > 8) and hence are largely un-ionized at blood pH, facilitating their efficient tissue penetration and thus being highly potent in vivo with broad spectrum tissue activity. However, members of this chemical class, including sorbinil, are known to cause skin rash and hypersensitivity or liver toxicity. On the other hand, ARIs of the carboxylic acid class[5] (e.g., zopolrestat) with pK_a (between 3 and 4) significantly lower than that of hydantoins are almost completely in the ionized state at blood pH and are markedly less potent in vivo with a narrower spectrum of tissue activity than hydantoins.



The question is whether the polyol pathway approach to diabetic complications merits continued medicinal chemistry attention and whether one can discover an ARI of distinct chemical structure with sufficient potency and duration of action to effectively block the excess flux at safe doses in the clinic. We believe there are strong reasons to be optimistic. It is extremely encouraging that minalrestat, a hydantoin-like spiro-



imide,[6a] in an open label, short-term clinical study has been reported to strongly suppress both sorbitol and fructose in sural nerve biopsy samples, as well as improve NCV, at very low doses.[6b] Additional support comes from results of AR knockout mouse studies[7] and from reports that several allelic variants of the human AR gene that are associated with overexpression of AR are also associated with a significant increase in the risk of development of microvascular complications.[8] These

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10.1021/jm034065z CCC: $25.00
Published on Web 05/10/2003

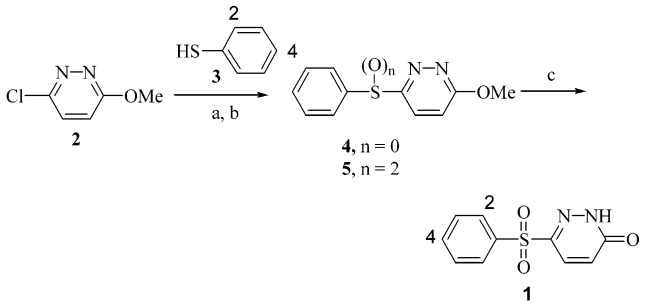
recent developments have lent strong timely support for an initiative that we undertook some years ago to discover an unprecedented chemical class of ARIs with potency clearly superior to the potency of both sorbinil and zopolrestat. More specifically, our target was a non-hydantoin, non-carboxylic acid ARI that suppressed nerve fructose by ≥90% in chronically diabetic rats at low doses and showed evidence of broad tissue penetration. This is the first report in which nerve fructose has been included as a critical part of the primary in vivo criteria for blocking flux through the polyol pathway, rather than merely sorbitol as has routinely been done until now. Herein, we give a sketch of this initiative, including preliminary in vivo characterization of the title compound **24**.

Results and Discussion. Potential ARIs were screened for in vitro activity against human recombinant AR and for oral in vivo activity in two streptozotocin diabetic rat models, acute and chronic, as measured by the ability of the inhibitor to normalize fructose as well as sorbitol that had become elevated in diabetic nerve (see Supporting Information for details).

High-throughput screening of internal libraries of compounds has proved to be an expedient avenue to search for potential new leads. However, the eventual success in delivering potential clinical candidates with satisfactory pharmaceutical properties appears to hinge, in large part, on the initial criteria set for identification of leads and working strategies employed for optimization of structure−activity relationships (SAR). Our target was low molecular weight non-hydantoin, non-carboxylic acid structures with no readily discernible toxic pharmacophore but with good in vitro activity vs human r-AR at ≤1 μM. Surprisingly, our extensive library yielded only one hit with this set credentials, 6-phenylsulfonylpyridazin-2*H*-3-one, **1**.[9] It has low molecular weight (236) and is weakly acidic (pK_a = 7.1). It showed in vitro activity with an IC_{50} of 0.6 μM. Investigators in the field have constantly emphasized that the most critical property of an ARI for in vivo efficacy is how well it penetrates into key target tissues[10] (e.g., peripheral nerve). We initially addressed this issue by measuring the inhibitor's ability to block AR in the sciatic nerve of acutely diabetic rats. Highly potent inhibitors in this test would then be advanced for testing in chronically diabetic rats with established hyperglycemia to simulate the persistent high glucose flux that prevails in the clinical situation. Evidence for broader tissue activity was measured in the same test by measuring the extent of AR blockade in lens. Compound **1** showed attractive AR inhibition activity in vivo. It normalized elevated nerve sorbitol levels by 95% and fructose levels by 89%, albeit at a high dose of 300 mg/kg, in the sciatic nerve of acutely diabetic rats. With this novel lead of desired profile in hand, our goal was to seek an in vivo potent inhibitor, at least 10× better than zopolrestat against the targeted nerve fructose end point (ED_{90}), through SAR around **1**. Because the SO_2-linker and the unsubstituted pyridazinone were found to be optimal for inhibition of AR, we first focused on phenyl substitution, which gave the initial breakthrough.

The synthetic route for the phenyl-substituted analogues is shown in Scheme 1.[11] Substituents were chosen

Scheme 1[a]



[a] Reagents and conditions: (a) Na/MeOH, PhMe; (b) MCPBA/CH_2Cl_2; (c) dioxane/HCL.

Table 1. In Vitro and in Vivo Data for Phenyl-Substituted Pyridazinones

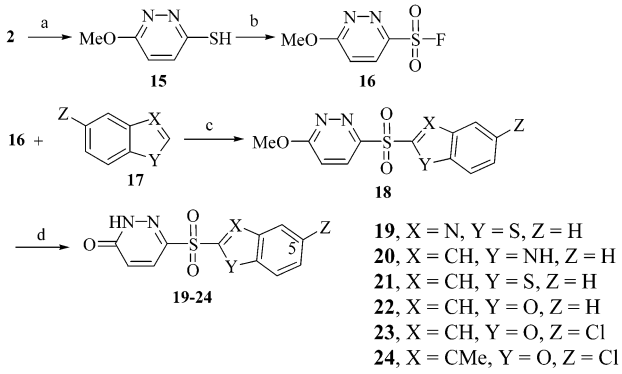
				% inhibition in sciatic nerve[a]	
compd	substituent	IC_{50} (nM)	dose (mpk)	sorbitol	fructose
1		600	50	95	74
6	2-Cl	170	20	96	68
7	2-Br	210	50	55	*b*
8	4-OMe	2.0 μM	*c*		
9	4-Me[d]	1.9 μM	*c*		
10	2-Ph	2.2 μM	*c*		
11	2-Cl, 4-Cl	190	10	95	73
12	2-Cl, 4-F	280	10	91	46
13	2-F, 4-Br	140	10	81	67
sorbinil		140	*e*		
zopolrestat		4	*e*		

[a] Acute test. [b] Not measured. [c] Not tested. [d] *Chem. Abstr.* **1960**, 5714. [e] See Figures 2 and 3 for in vivo data.

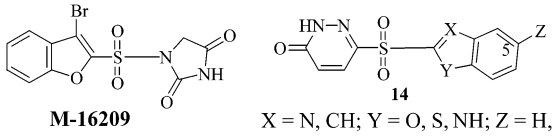
to probe the influence of orientation, lipophilicity, electron withdrawal, electron release, and steric bulk. In general, compact, electron-withdrawing F and Cl substituents that are reasonably lipophilic gave potent compounds both in vitro and in vivo. Compounds with one Cl (**6**), two Cl (**11**), and one Cl with one F (**12**), especially at the 2- and 2,4-positions, showed the best in vivo activity. However, **13**, which features the effective 2F, 4Br substituent pattern present in Ponalrestat,[12] zenarestat,[4] and minalrestat,[6] was no more potent than **11** in vivo. IC_{50} values for this group of compounds were in the range 140−280 nM. Analogues with electron-releasing substituents (e.g., OMe and Me, **8** and **9**) and bulky groups, especially at the 2-position (e.g., Ph, **10**), were less potent than **6**, **11**, or **13** with halogen substituents. Table 1 summarizes the SAR in the phenylpyridazinone series. 6-(2,4-Dichlorophenylsulfonyl)-2*H*-pyridazin-3-one, **11** (IC_{50} = 190 nM), was the most potent inhibitor in chronic test; it was 2× more potent than zopolrestat in normalizing both sciatic nerve sorbitol (ED_{90}, 5 vs 10 mpk) and fructose (ED_{90}, 20 mg/kg vs ∼40 mpk) levels. Also, it was significantly better than zopolrestat in suppressing lens sorbitol levels (94% vs 41% at 20 mpk).

On the basis of previous SAR experience with zopolrestat[13a] and related analogues,[13b] the X-ray structure information from the AR−NADPH ($NADP^+$)−zopolrestat ternary complex,[14] and the literature example M-16209,[15] we targeted a new family of compounds of general formula **14**. We designed a general and versatile synthetic route[16,17] (Scheme 2) to the newly

Scheme 2[a]



[a] Reagents and conditions: (a) thiorurea/MEK; (b) $KHF_2/Cl_2(g)$–MeOH/H_2O; (c) n-BuLi, THF, −78 °C; (d) dioxane/HCl(conc).



targeted family of compounds and prepared benzofuran, benzothiazole, benzothiophene, and indole analogues.

The rank order of in vitro potency (IC_{50}) among the parent heterocycles was benzofuran (**22**) ∼ benzothiophene (**21**) (150 nM) > benzothiazole (**19**) (450 nM) > indole (**20**) (5 μM). None had the desired in vivo potency. However, on the basis of in vitro potency of **22**, literature data for M-16209, and the potential vulnerability of **21** for metabolic oxidation of the sulfur atom, we decided to focus on the SAR around the benzofuran **22**. We thought that we needed to increase lipohilicity for better tissue penetration. There is very sparse knowledge about the metabolism of benzofuran with an electron-withdrawing group at the 5-position. Nevertheless, it appeared that the strategy of blocking the highly electrophillic 5-position with the lipophilic chloro substituent would be a good probe to address both issues. **23** was found to be at least 10× more potent in vitro than **22** (IC_{50} = 25 nM) but was still not very potent in vivo even though it gave good serum levels when administered orally (data not shown).

Faced with an impasse like this, it is not uncommon for medicinal chemists to draw lessons from disparate drug series. We noticed that whenever benzofuran had been employed in other medicinal projects, a 3-substituent had played a remarkable role in enhancing in vivo potency. In addition to the 3-bromo substituent in M-16209, we were particularly impressed by the role of the same substituent in the benzofuran-based AII blocker GR-117289[18] in providing efficacy in blood pressure lowering in rat models.

So we appended a 3-substituent to the best benzofuran in hand, **23**. We preferred to incorporate a methyl group rather than an additional halogen atom, leading to **24**, which is one of the most potent ARIs yet described in the literature. Its IC_{50} vs r-human AR is 840 ± 40 pM (n = 13). In a side-by-side evaluation, its ED_{90} for normalization of fructose in the chronic test (Figure 2) is lower than that of zopolrestat (13×) and of sorbinil (3×). The dose–response of **24** for inhibition of sorbitol and fructose reiterates the greater sensitivity for sorbitol over fructose and should raise caution in targeting just

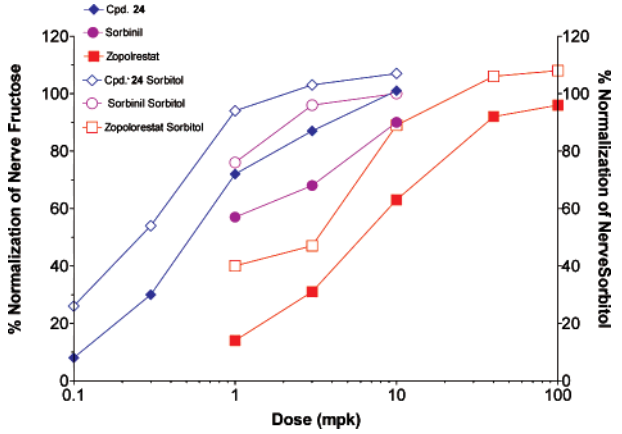


Figure 2. Diabetic rat sciatic nerve sorbitol and fructose dose–response of **24** and comparative inhibitors.

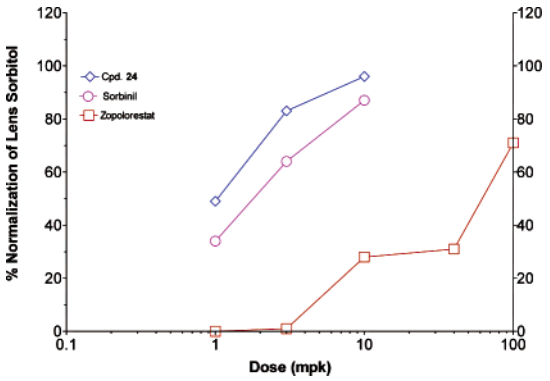


Figure 3. Diabetic rat lens sorbitol dose–response of **24** and comparative inhibitors.

sorbitol as a robust marker of flux through the polyol pathway. Inhibitor **24**, in contrast to zopolrestat, shows remarkable potency in suppressing sorbitol in the lens (Figure 3). It is extremely selective against other reductases and dehydrogenases. For example, it is >1000-fold more selective for AR than aldehyde reductase. It is very well absorbed in rats, with an oral bioavailability of 98% (plasma C_{max} = 9 ± 3 μg/mL at 2 mpk, po), and shows a long plasma $t_{1/2}$ (26 ± 3 h). Its pK_a, log P, and log D (pH 7.4) are 6.9, 3.05, and 2.46, respectively. A higher log D/log P value of 0.81 for **24** is in stark contrast to the value of 0.16 for zopolrestat[13] and is consistent with the more effective penetration of **24** into the lens tissue. These desirable pharmacokinetic and physicochemical properties of **24**, taken together with its stability toward P450 enzymes, and excellent Caco-2 transcellular permeability (>10^{-5} cm/s) bode well for potential once-a-day dosing in the clinic.

Conclusion. High-throughput screening of an internal library of compounds targeted toward a structurally distinct non-hydantoin, non-carboxylic acid ARI with the potential to robustly block the flux through the polyol pathway yielded a weak inhibitor, **1**. Nearly complete normalization of elevated fructose (rather than just sorbitol) levels (ED_{90}) in the sciatic nerve of rats with established diabetes was used as the primary biochemical marker for effective blockade of flux. A systematic SAR pursuit around **1**, with lessons learned from the literature on extant ARIs, including zopolrestat, and the monitoring of the profile of newly prepared analogues for lipophilicity (both log P and log D), movement across the Caco-2 cell monolayer via a transcellular mecha-

nism, and pharmacokinetics in rats led to the title compound 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2*H*-pyridazin-3-one, **24**. It is a novel, highly selective ARI with unprecedented in vitro potency (840 ± 40 pM) and remarkable potency in the sciatic nerve as well as in the lens of chronically diabetic rats, in normalizing polyol metabolites. It is well absorbed in diabetic rats with a sustained plasma $t_{1/2}$ of 26 ± 3 h.

Supporting Information Available: Biological methods and NMR and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kinoshita, J. H. A Thirty Year Journey in the Polyol Pathway. *Exp. Eye Res.* **1990**, *50*, 567−573. (b) Kinoshita, J. H.; Nishimura, C. The Involvement of Aldose Reductase in Diabetic Complications. *Diabetes Metab. Rev.* **1988**, *4*, 323−337.

(2) (a) Tilton, R. G.; Chang, K.; Nyengaard, J. R.; Van den Enden, M.; Ido, Y.; Williamson, J. R. Inhibition of Sorbitol Dehydrogenase. Effects on Vascular and Neural Dysfunction in Streptozotocin-Induced Diabetic Rats. *Diabetes* **1995**, *44*, 234−242. (b) Williamson, J. R.; Chang, K.; Frangos, M.; Hasan, K. S.; Ido, Y.; Kawamura, T.; Nyengaard, J. R.; Van den Enden. M.; Kilo, C.; Tilton, R. G. Hypergylcemic Pseudohypoxia and Diabetic Complications. *Diabetes* **1993**, *42*, 801−813.

(3) Cameron, N. E.; Cotter, M. A.; Basso, M.; Hohman, T. C. Comparison of the Effects of Inhibitors of Aldose Reductase and Sorbitol Dehydrogenase on Neurovascular Function, Nerve Conduction and Tissue Polyol Pathway Metabolites in Streptozotocin-Diabetic Rats. *Diabetalogia* **1997**, *40*, 271−281.

(4) Ao, S.; Shingu, Y.; Kikuchi, C.; Takano, Y.; Nomura, K.; Fujiwara, T.; Ohkubo, Y.; Notsu, Y.; Yamaguchi, I. Characterization of a Novel Aldose Reductase Inhibitor, FR74366, and Its Effects on Diabetic Cataract and Neuropathy in the Rat. *Metabolism* **1991**, *40*, 77−87.

(5) Eggler, J. F.; Larson, E. R.; Lipinski, C. A.; Mylari, B L.; Urban, F. J. In *Advances in Medicinal Chemistry*; Maryanoff, E., Maryanoff, C. A., Eds.; Jai Press Inc.: London, England, 1993; Vol. 2, pp 197−246.

(6) (a) Malamas, M. S.; Hohman, T. C.; Millen, J. Novel Spirosuccinimide Aldose Reductase Inhibitors Derived from Isoquinoline-1,3-diones: 2-[(4-Bromo-2-fluorophenyl)methyl]-6-fluorospiro-[isoquinoline-4(1*H*),3′-pyrrolidine]-1,2′,3,5′(2*H*)-tetrone and Congeners. 1. *J. Med. Chem.* **1994**, *37*, 2043−2058. (b) Hohman, T. C.; Bochenek, W. J.; Meng, X.; Neefe, L.; Beg, M.; Ari, D. T. Nerve Function and Biochemistry in Diabetic Patients Treated with ARI-509, a Novel Aldose Reductase Inhibitor. *Diabetologia* **1997**, *40*, A552.

(7) Ho, E. C. M.; Lam, K. S. L.; Chung, S. S. M.; Chung, S. K. Aldose Reductase Deficient Mice Are Alleviated from the Depletion of GSH in the Peripheral Nerve and MNCV Deficit Associated with Diabetes. *Diabetes* **2001**, *50* (Suppl. 2), A59.

(8) (a) Demaine, A.; Cross, D.; Millward, A. Polymorphism of the Aldose Reductase Gene and Susceptibility to Retinopathy in Type I Daibetes Mellitus. *Invest. Ophthalmol. Visual Sci.* **2000**, *41*, 4064−4068. (b) Heesom, A. E.; Millward, A.; Demaine, A. G. Susceptibility to Diabetic Neuropathy in Patients with Insulin Dependent Diabetes Mellitus Is Associated with a Polymorphism at the 5′ End of the Aldose Reductase Gene. *J. Neurol., Neurosurg. Psychiatry* **1998**, *64*, 213-216.

(9) Takahayashi, N. *J. Pharm. Soc. Jpn.* **1956**, *76*, 1293−1296.

(10) Dvornik, D. A Perspective of Aldose Reductase Inhibitors and Diabetic Complications. *Croat. Chem. Acta* **1996**, *69*, 613−630.

(11) Experimental procedures for phenyl substituted compounds can be found in Patents EP 1236720 and CAN 137:216953.

(12) Stribling, D.; Mirrlees, D. J.; Harrison, H. E.; Earl, D. C. Properties of ICI 128,436, a Novel Aldose Reductase Inhibitor, and Its Effects on Diabetic Complications in the Rat. *Metabolism* **1985**, *34* (4), 336−344.

(13) (a) Mylari, B. L.; Larson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Singleton. D. H. Novel, Potent Aldose Reductase Inhibitors: 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic Acid (Zopolrestat) and Congeners. *J. Med. Chem.* **1991**, *34*, 108−122. (b) Mylari, B. L.; Beyer, T. A.; Scott, P. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Zembrowski, W. J. Potent, Orally Active Aldose Reductase Inhibitors Related to Zopolrestat: Surrogates for Benzothiazole Side Chain. *J. Med. Chem.* **1992**, *35*, 457−465.

(14) Wilson, D. K.; Tarle, I.; Petrash, J. M. Quiocho, F. A. Refined 1.8 Å Structure of Human Aldose Reductase Complexed with the Potent Inhibitor Zopolrestat. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9847−9851.

(15) Kato, K.; Nakayama, K.; Mizota, M.; Miwa, I.; Okuda, J. Properties of Novel Aldose Reductase Inhibitors, M16209 and M16287, in Comparison with Known Inhibitors. ONO-2235 and Sorbinil. *Chem. Pharm. Bull. (Tokyo)* **1991**, *39*, 540−545.

(16) Experimental procedures for heterocyclic substituted pyridazinones can be found in Patents U.S. 2,002,143,017 and CAN 137: 279206.

(17) Indole analogue **20** was more conveniently prepared starting from 2-mercaptoindole and following Scheme 1.

(18) Robertson, M. J.; Barnes, J. C.; Drew, G. M.; Clark, K. L.; Marshall, F. H.; Michel, A.; Middlemiss, D.; Ross, B. C.; Scopes, D.; Dowle, M. D. Pharmacological Profile of GR117289 in Vitro: A Novel, Potent and Specific Non-Peptide Angiotensin AT1 Receptor Antagonist. *Br. J. Pharmacol.* **1992**, *107*, 1173−1180.

JM034065Z