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MEDICINAL
CHEMISTRY
RESEARCH

GUANIDINES: FROM MOLECULE TO PRIMATE

D.W. Oliver*[1], I.C. Dormehl[2], JES Wikberg[3], M. Dambrova[4]

*[1]Pharmacology, Potchefstroom University for CHE, Faculty of Health Sciences, Potchefstroom, South Africa

[2]AEC Institute for Life Sciences, University of Pretoria, Pretoria, South Africa

[3]Pharmaceutical Biosciences, Pharmacology, Uppsala University, Uppsala, Sweden

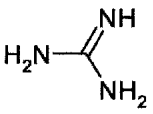
[4]Medicinal Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia

Abstract. Guanidine-like compounds have been investigated since the first observations of their therapeutic potential some 30 years ago in fields of cancer and virology. Guanidine-type compounds that reached clinical status include amongst others the potassium channel opener, pinacidil and the histamine H_2-receptor antagonists (e.g. cimetidine). Recent research on guanidines has focused on enzyme systems such as xanthine oxidase and nitric oxide synthase. Our studies demonstrated an *in vivo* cardioprotection effect of (N-(3,4,-dimethoxy-2-chlorobenzylideneamino)-guanidine: ME10092) in ischaemic reperfusion injury in the rodent. The present investigation in the normal non-human primate, *Papio ursinus* baboons showed cardiovascular negative chronotropic effects and transient decreases in blood pressure, which correspond to those observed in the *in vivo* cardioprotection studies in the rodent.

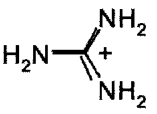
Corresponding author: e-mail: fkldwo@puknet.puk.ac.za

Introduction and Aim

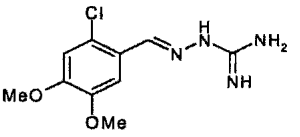
Guanidine and its derivatives had drawn the attention as therapeutic and potential therapeutic agents some 30 years ago in view of the bioactivities of guanidine **1** (see structure 1 and its guanidium cation) in medicinal fields such as cancer and virology [1]. The unique basicity and cationic nature of the guanidine moiety are primarily responsible for interaction and binding to negatively charged counter anion regions present in proteins. The side chain(s) diversity that can be developed for the guanidine-scaffold can lead to the generation of a large number of guanidine libraries with unique pharmacological properties addressing various medicinal targets. Therefore, during the last three decades the guanidine-functionality has been included in numerous pharmacologically active compounds for some of which the clinical status has already been achieved (see below medicinal chemistry background). In view of the promising medicinal potential and vast diverse actions of guanidine-like structures our objective was to explore activities further into the field of cell and organ protection using amongst others *in vivo* approaches. The aim of these studies was to explore *in vivo* the cerebral perfusion effects while monitoring the cardiovasculature. Presently we report the findings in rodents and baboons (*Papio ursinus)* with respect to the cardiovascular effects of ME10092 {N-(3,4-dimethoxy-2-chlorobenzylidenamino)-guanidine} **2**.



1 Guanidine



1(+) Guanidinium



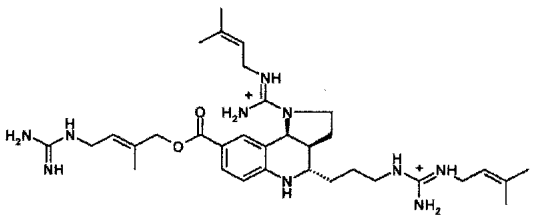
2 ME10092

Historical Background on the Medicinal Chemistry of the Guanidines

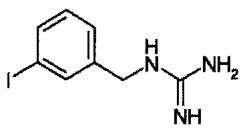
The field of guanidines has since the initial observations evolved significantly as illustrated below with the diversity in structures. Thus the endogenous guanidine–like structures such as the bradykinins **3** have recently drawn the attention of researchers in search of new targets in view of their pathophysiological role amongst others in pain and contribution to inflammation [2]. Promising bradykinin receptor antagonists, i.e, martinelline **4**, have since been developed. The earlier reported medicinal properties have stimulated the design and synthesis of a wide variety of guanidine-like structures in order to explore their bioactivities in virology and cancer (see structures **5**,**6**) [3-6]. The radioactive labeled meta-iodobenzylguanidine **5** (MIBG) has since its

discovery in the 1980s played a major role in the management of neuro-endocrine tumors such as pheochromocytoma [3]. Several subsequent investigations lead to the synthesis of Schiff bases of N-hydroxy-N^1-aminoguanidines that have been explored as antiviral agents (against Rous sarcoma and corona viruses) in view of the activities of guanidine and hydroxy urea [4,5]. After the discovery of the human immune deficiency retrovirus (HIV) as the causative agent in HIV/AIDS in 1981 [6], it became the increasing world health challenge and primary focus of the research in virology. Our studies in the early 1990s on a series of N-hydroxy-N^1-aminoguanidines showed promising *in vitro* activity against the HIV at micromolar range (6 and Scheme 1)[7,8]. Wikberg and Hudson [9] reported the activity of the imidazoline I_2-receptor selective Schiff bases of 1-(benzylidenamino)-3,3-dimethylguanidine 7.

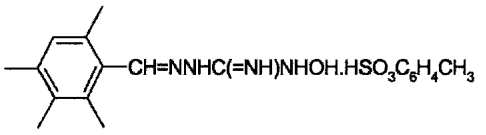
3 Bradykinin



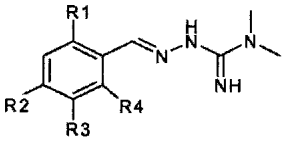
4 Martinelline



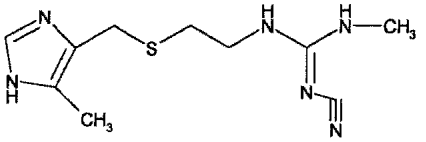
5 MIBG



6 Schiff Base



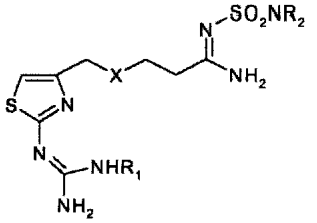
7 Benzylideneamino guanidines



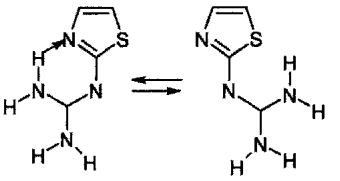
8 Cimetidine

In the treatment of peptic ulcer guanidine structures have found clinical application as histamine, H_2 receptor antagonists. The first clinically approved drug of this structure type was cimetidine 8 followed by famotidine 9, with both drugs having the guanidinylthiazole moiety. Conformational analysis of famotidine and some analogues using AM1 calculations revealed that the guanidium cation (with a conformation orientation that allows a hydrogen-bonding interaction between the

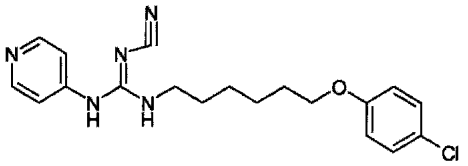
nitrogen of the guanidine chain and the nitrogen of the thiazole ring is the most stable conformation (See conformation equilibrium structures **10** of famotidine) [10]. The authors suggested that this spacial orientation might be the best candidate for histamine H_2 receptor interaction. X-ray diffraction and NMR data showed the existence of this conformer. Pinacidil, a cyanoguanidine (N-cyano-N'-(4-pyridyl)-N"-(1,2,2-trimethylpropyl)guanidine) acts as a potassium channel opener and is currently used in the treatment of hypertension. Recently, CHS 828, a pyridyl cyanoguanidine (N-(6-(4-chlorophenoxy)hexyl)-N'-cyano-N"-4-pyridylcyanoguanidine) **11** was the first candidate drug from a novel group of anti-tumor agents that reached Phase I and Phase II clinical trial status [11,12].



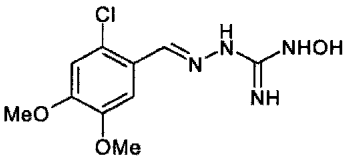
9 Famotidine X=S; R_1=H; R_2= H_2



10 Conformation equilibrium of 2-guanidinylthiazole of famotidine



11 CHS 828



12 PR5 N-hydroxy prodrug of ME10092

Since the turn of the millennium the medicinal chemistry and pharmacology of several known and novel guanidine derivatives have been researched for various new medicinal applications in view of their interactions with enzyme systems such as xanthine oxidase and nitric oxide synthase [13-16]. These activities revealed that their potential to afford neuroprotection in the advent of brain injury [17]. The protein X-ray structures of the rat neuronal heme domain of nitric oxide synthase interacting with N(omega)-hydroxy-L-arginine, propyl- and butylguanidine have recently been reported on by Li *et al.* (2002)[18]. The latter two have been identified as non-amino acid substrates for NOS.

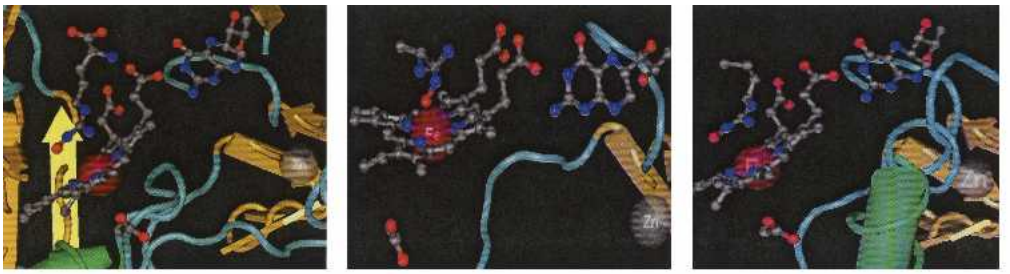
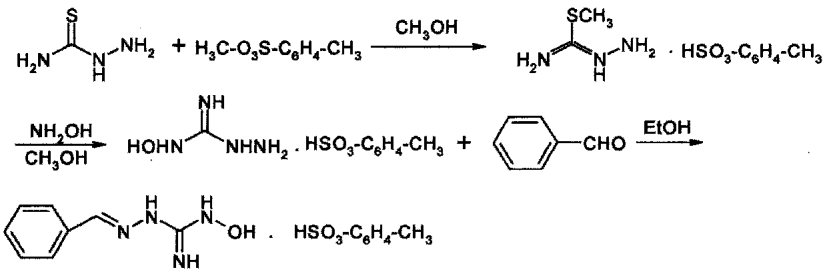


Figure 1: Binding modes of N (omega)-hydroxy-L-arginine (left), isopropylguanidine (middle) and butylguanidine (right) to rat neuronal nitric oxide synthase (from PDB files 1LZX, 1LZZ, 1M00)

The x-ray study revealed two different binding modes in the substrate binding pocket of NOS for propyl- vs. butylguanidine with the binding mode of the latter coinciding with that of N(omega)-hydroxy-L-arginine (Figure 1).

Cardioprotection by guanidines. Investigation of PR5 {N-(3,4-dimethoxy-2-chlorobenzylidenamino)-N^1-hydroxyguanidine} **12** by Veveris *et al.*, (1999) revealed remarkable cardioprotective properties against ischaemia and reperfusion induced myocardial necrosis and life-threatening arrhythmias in rats [19]. A subsequent study indicated that PR5 **12**, acts as a prodrug, and that the active metabolite is the dehydroxy compound N-(3,4,-dimethoxy-2-chlorobenzylideneamino)-guanidine **2**, coded ME10092 [20]. Earlier, it was demonstrated that hydroxy aminoguanidine derivatives decrease the generation of superoxide radicals by xanthine oxidase [15,21]. Xanthine oxidase on release during ischeamia has been found to catalyze the formation of superoxide from oxygen [22,23] and the effects of these guanidines as electron acceptors at the xanthine oxide enzyme was proposed to account for their protection properties. ME10092 was able to dose-dependently reduce the heart rate, reduced the elevation of the ST-segment of the ECGs and attenuated the rebound rise in blood pressure during the reperfusion. These combined effects of ME10092 contributed to the inhibition of the reperfusion-induced arrhythmias and significantly improved the survival of the animals. These results prompted the present study in the baboon, *Papio ursinus*.

Guanidine Chemistry. Typical route for the synthesis of benzylidineamino hydroxyguanidines are presented in Scheme 1. Analogues were synthesized from various starting benzaldehydes.



Scheme 1

Solid-phase synthesis methods have recently attracted the attention of several groups for the synthesis of guanidines [24], and bicyclic guanidines [25, 26]. These methods are applied to generate combinatorial guanidine libraries in the antibacterial and opioid receptor fields.

Results and Discussion

The cerebral perfusion study using Single Photon Emission Computed Tomography (SPECT) following the split-dose method with ^{99m}Tc-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) show no significant effects under current dose (1 and 2 mg/kg) and time schedules. The effects of the ME10092 on the heart rate and blood pressure in the baboons are presented in Tables 1, 2 and 3, and Figures 2, 3 and 4.

Minutes	Procedure A	Procedure B
0	0	0
2	-12.95 ± 3.79	-5.33 ± 3.07
4	-17.10 ± 3.63	-10.77 ± 3.27
6	-18.96 ± 4.93	-14.60 ± 4.03
10	-18.88 ± 7.79	-15.96 ± 4.08
15	-19.68 ± 6.38	-15.47 ± 4.31
20	-19.52 ± 8.71	-15.60 ± 4.42
25	-27.74 ± 9.02	-18.28 ± 4.40

Table 1. Mean (±SD) percentage changes with respect to time t = 0 for heart rates versus time of Procedures A and B after ME10092 administration (2mg/kg and 1mg/kg respectively)

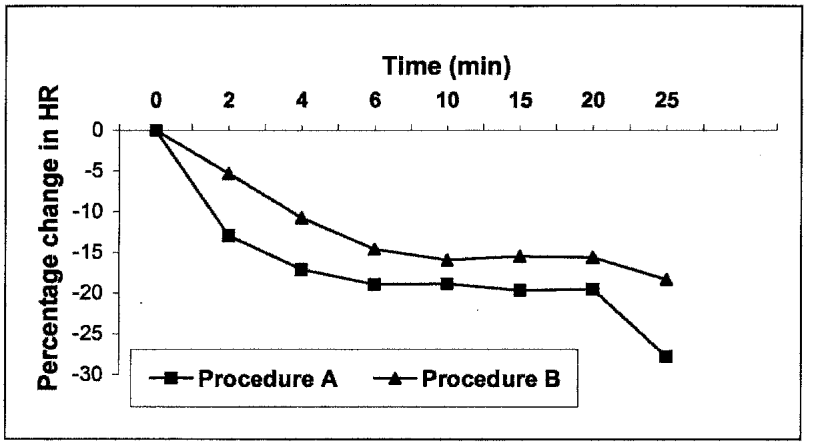


Figure 2. Curves of the mean percentage differences with respect to time t = 0 for the heart rate versus time of Procedures A and B after ME10092 administration (2 mg/kg and 1 mg/kg respectively)

Decreases in heart rate were seen directly after ME10092 administration reaching levels of about -20% for the 2 mg/kg dose (Procedure A) and about -15% for the 1 mg/kg dose (Procedure B) at around 6 min post drug administration (Table 1 and Figure 2). ME10092 therefore induces a negative chronotropic effect for both the 1 and 2 mg doses, with a greater effect from the higher dose. Control untreated animals showed no increase or decrease in heart rate during routine anaesthesia, clearly suggesting that the negative chronotropic effects is drug related. Similarly, decreases were observed in heart rate in the cardioprotective study in rats after ME10992 administration both during normal conditions and during ischaemia and reperfusion. These studies clearly showed that ME10092 exhibits cardiovascular activity both in rodent and primate species.

A transient decrease in both systolic and diastolic blood pressure was observed for the higher dose of 2 mg/kg. No initial decrease was observed for the 1 mg/kg dose (Figures 3 and 4). The increase in blood pressure noted for the 1 mg/kg dose corresponds to increases in blood pressure routinely observed in anaesthesia in control animals, suggesting an anaesthesia effect on blood pressure and not that of the drug intervention [27]. The effect of the 2 mg/kg dose points towards a return to normal blood pressure values after the initial transient decrease. This is a reversal of the routine blood pressure increases noted under anaesthesia. An attenuation of the rebound rise in blood pressure was also observed in with ME10092 in rats during the reperfusion phase of the myocardial ischaemia and reperfusion study [20]. ME10092 clearly exhibits mycocardial effects

in the non-human primate, similar to the effects previously observed in the ischaemia-reperfusion rat model, with strong cardioprotection.

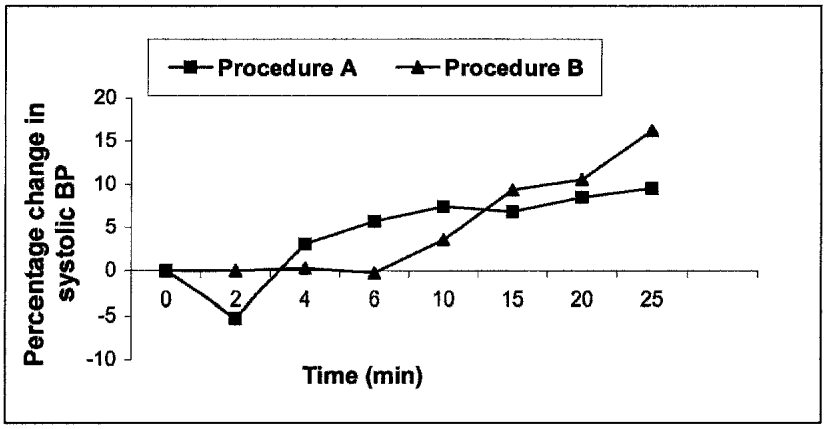


Figure 3. Curves of the mean percentage changes with respect to time t = 0 for the systolic blood pressure versus time of Procedures A and B after ME10092 administration (2 mg/kg and 1 mg/kg respectively)

Minutes	Procedure A	Procedure B
0	0	0
2	-5.24 ± 3.27	0.02 ± 4.08
4	3.10 ± 7.68	0.30 ± 7.29
6	5.72 ± 10.61	-0.26 ± 8.68
10	7.45 ± 12.11	3.62 ± 9.34
15	6.84 ± 11.39	9.36 ± 7.11
20	8.48 ± 16.14	10.56 ± 8.49
25	9.54 ± 18.38	16.22 ± 7.24

Table 2. Mean (±SD) percentage changes with respect to time t = 0 for systolic blood pressure rates versus time of Procedures A and B after ME10092 administration (2mg/kg and 1mg/kg respectively)

Minutes	Procedure A	Procedure B
0	0	0
2	-10.1 ± 8.15	0.02 ± 6.00
4	-4.24 ± 11.08	-2.67 ± 9.65
6	-1.78 ± 16.77	3.80 ± 8.51
10	1.02 ± 16.61	7.58 ± 6.09
15	2.20 ± 19.07	15.16 ± 7.04
20	4.90 ± 15.06	31.36 ± 24.37
25	-2.78 ± 25.87	20.31 ± 8.97

Table 3. Mean (±SD) percentage changes with respect to time t = 0 for diastolic blood pressure rates versus time of Procedures A and B after ME10092 administration (2mg/kg and 1mg/kg respectively)

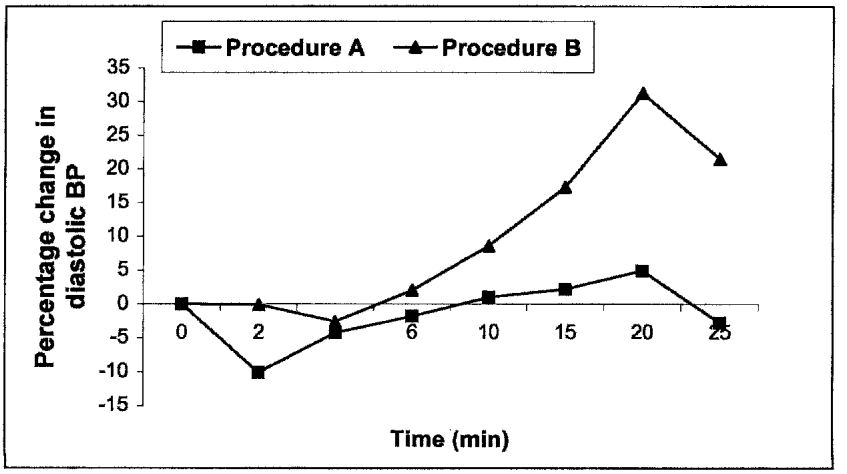


Figure 4. Curves of the mean percentage changes with respect to time t = 0 for the diastolic blood pressure versus time of Procedures A and B after ME10092 administration (2 mg/kg and 1 mg/kg respectively)

Conclusion

In conclusion guanidine-like compounds have over three decades been investigated for pharmacological actions in diverse medicinal fields with a variety of therapeutic applications and potential possibilities ranging from protection against disorders of the brain, heart, and GIT as well as in the fields of virology and cancer. The guanidine structure has proved to be an important scaffold in the design and development of clinically important drugs. Novel guanidine compounds will in the future add to the therapeutic range already in existence. The present study in the normal non-human primate clearly showed significant cardiovascular effects after the administration of N-(3,4,-dimethoxy-2-chlorobenzylideneamino)-guanidine, ME10092, which appear to be dose-dependent. These cardiovascular effects of the ME10092 in the baboon strengthen the previous findings in the rodent where strong cardioprotection was provided against myocardial ischaemia and reperfusion induced myocardial necrosis and life-threatening arrhythmias.

Experimental

Chemistry. ME10092 was synthesized at the Department of Medicinal Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia [15].

Primate studies. Six adult male baboons (*Papio ursinus*, average weight 25 kg) were used for this study. The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

Two different procedures (six animals per group) were carried out which means that each animal was studied twice, with a washout interval of at least six weeks. For the drug intervention studies (Procedures A and B) each baboon was sedated with ketamine hydrochloride (10 mg/kg i.m) (Anaket-V®, Centaur Labs, Bryanston, Gauteng, SA), followed immediately by maintained and controlled infusion of thiopentone sodium (70 ml/h of 0,5% solution) (Intraval®, Rhône-Poulenc Rorer S.A., Midrand, Gauteng, SA). After a 12-min-stabilisation period under thiopentone, Procedures A and B started at t = 0. For the drug interventions with ME10092 (N-(3,4-dimethoxy-2-chlorobenzylideneamino)-guanidine) was administered intravenously for Procedure A (2 mg/kg), whereas for Procedure B 1mg/kg was administered. During all the abovementioned procedures the respiration, heart rate, arterial blood pressure, oxygen saturation (SaO_2) and the % carbon dioxide (CO_2) were monitored. An ECG-device (Life Scope 12, Nihon Kohden) was used to monitor these parameters. To measure the blood pressure a catheter was placed in the femoral artery and connected to a transducer, which is connected to the ECG-device that registers the blood pressure. These parameters were recorded every 2 to 5 min during the studies. Control data originated from routine studies identically performed with out drug intervention. A two-tailed Student's t-test for paired variables was used, with a 5% level of confidence, to establish significant differences between the mean changes of blood pressures and heart rates with respect values at t = 0.

Acknowledgements

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