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3-HYDROXY-3-CYCLOBUTENE-1,2-DIONE: APPLICATION OF A NOVEL CARBOXYLIC ACID BIOISOSTERE TO AN IN-VIVO ACTIVE NON-TETRAZOLE ANGIOTENSIN-II ANTAGONIST

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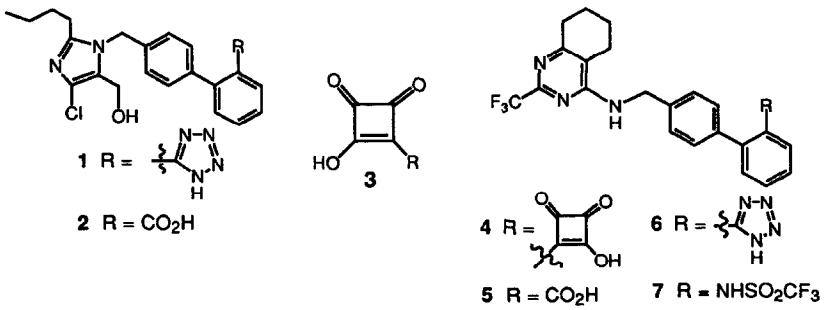
Abstract: Application of the novel carboxylic acid bioisostere 3-hydroxy-3-cyclobutene-1,2-dione to the design of a potent, non-peptidic, non-tetrazole angiotensin II antagonist **4** is described.

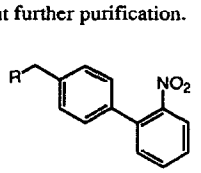
Bioisosterism is one of the most useful tools to the medicinal chemist in drug design.[1] In particular, carboxylic acid bioisosteres have been of special focus by virtue of the important role carboxylic acids occupy in biomolecular recognition. This concept played a pivotal role in the discovery of the non-peptidic angiotensin-II antagonist DUP-753 (losartan; **1**)[2] in which systematic evaluation of bioisosteres of carboxylic acid **2** led to tetrazole **1**, a more potent competitive antagonist *in-vitro* with a more favorable *in-vivo* antihypertensive profile in comparison to its carboxylic acid congener.

In the course of our investigations into novel angiotensin II (AII) antagonists, it became of interest to explore novel carboxylic acid bioisosteres which might have broad application in drug design. Although an array of carboxylic acid bioisosteres have been developed (i.e tetrazole and other acidic heterocycles, hydroxamic acid, and trifluorosulfonamide),[1] little bioisosteric literature has been reported for squaric acid derivatives of general formula **3**, despite the pronounced acidity of such systems.[3] We now report the synthesis of a potent non-tetrazole AII antagonist **4** using this bioisosteric replacement and compare (a) its potency as an AII antagonist *in-vitro* against losartan, carboxylic acid **5**, tetrazole **6**, and trifluorosulfonamide **7**, and (b) its *in-vivo* antihypertensive profile against **6** and losartan in two AII dependent animal models.

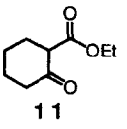


The syntheses of **4** and **7** start from biphenyl **8**,[4] which was converted to amine **10** ((a) NBS, cat. AIBN, CCl_4, reflux; (b) NH_3, EtOH, RT, 18 h) in 44% yield, and then coupled (K_2CO_3, DMF, 100 °C, 12 h)

in 81% yield to tetrahydroquinalozine **13** (prepared in two steps from 2-carboethoxycyclohexanone (**11**) ((a) Na, EtOH, then **12**; (b) $POCl_3$, Me_2NPh, toluene, reflux)) to provide **14**, mp 150 - 151 °C. Catalytic reduction (50 p.s.i. H_2, 10% Pd/C, EtOH / THF) afforded in 83% yield aniline **15** as a foam, which was used without further purification.



8 R = H
9 R = Br
10 R = NH_2



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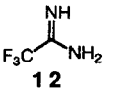

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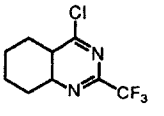

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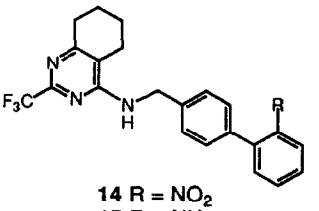
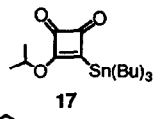


14 R = NO_2
15 R = NH_2
16 R = I

Iodo derivative **16** was prepared from aniline **15** in 91% yield ((a) $NaNO_2$, 6 N HCl, 0 °C; (b) KI) and then, without purification, coupled to the Liebskind stannane **17**[5] (cat. *trans*-benzyl(chloro)-bis(triphenylphosphine)palladium (II), cat CuI, CH_3CN, 70 °C) thereby providing in 71% yield cyclobutenedione **18** as a foam. Acidic hydrolysis (6 N HCl, THF, 45 °C, 12 h) gave **4**, mp 193 °C (dec), in 83% yield. Treatment of **15** with 1.02 equiv of trifluoromethanesulfonic anhydride (CH_2Cl_2, 0 °C) furnished **7**, mp 170 - 173 °C.



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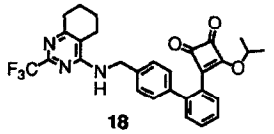

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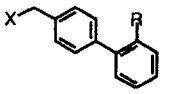
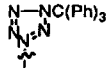
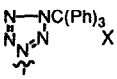
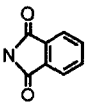
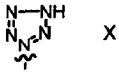
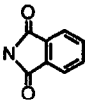


19 R =  X= Br

20 R =  X = 

21 R =  X = NH_3Cl

22 R = CO_2Me X= Br

23 R = CO_2Me X = 

24 R = CO_2H X = NH_3Cl

Tetrazole **6**, obtained as a potassium salt, mp 276 °C (dec), was prepared from **19**[6] according to the sequence: (a) conversion to phthalamide **20** (potassium phthalamide, DMF, 25 °C (76% yield)); (b) hydrazinolysis in 88% yield to **21** ((a) H_4N_2, EtOH, reflux; (b) HCl, EtOH); and (c) coupling to **13** (DMSO, NaOAc, 40 °C, 24 h; 50% yield) followed by potassium salt formation using 1 N ethanolic KOH. Using a similar scheme, **22**[4] was converted to **23** in 48% yield, which upon hydrazinolysis in MeOH and salt formation (HCl, MeOH) gave **24** in 79% yield. Treatment of **24** with **13** (n-BuOH, NaOAc, Δ, 3 days; 59% yield) and salt formation furnished **5** in (mp >300 °C).

Compounds were assessed first for competitive inhibition of AII binding using a rat adrenal gland preparation described previously.[7] As summarized in Table I, the potency of these compounds decreases in

the order: **6 > losartan > 4 > 7 > 5**. Thus, squarate **4** is 11x and 4x more potent than carboxylic acid **5** and N-trifluorosulfonamide **7**, respectively, but is ca. 8x less potent than tetrazole **6**.

Antihypertensive activity of these compounds was evaluated initially in an AII-infused rat model (Table I).[8] Carboxylic acid **5** was screened only at 10 mg/kg, i.d. whereas all others were screened at 3 mg/kg, i.d. All compounds at the doses tested decreased the AII supported portion of mean arterial pressure (MAP). The decrease of AII-supported MAP produced by **4** at 3 mg/kg was smaller than either tetrazole analog **6** or losartan, but larger than N-trifluorosulfonamide **7**. The maximum blood pressure lowering effect of these compounds occurred at different points along the time profile: losartan (15 min), carboxylic acid **5** (30 min), tetrazole **6** (30 min), and cyclobutenedione **4** (4 hr).

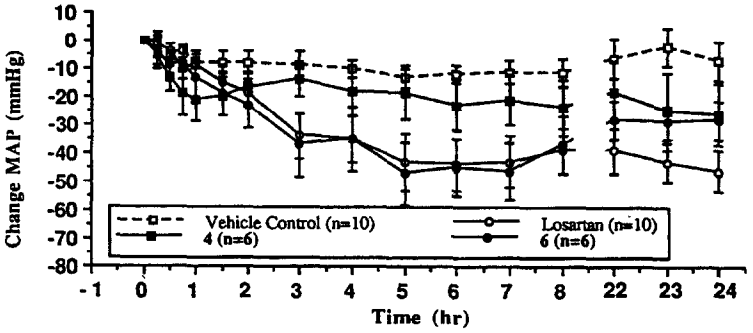
Table I. Inhibition of AII-receptor Binding and Decrease of AII-supported Blood Pressure.

Compound	IC_{50} (nM)[a, 7]	Maximum % Change of AII-supported portion of mean arterial pressure[8]
1 (losartan)	13	-72 ± 8[c] (3 mg / kg, i.d.; n = 8)
4	25	-52 ± 13[c] (3 mg / kg, i.d.; n = 4)
5	275	-62 ± 7[c] (10 mg / kg, i.d.; n = 4)
6	3	-75 ± 7[c] (3 mg / kg, i.d.; n = 7)
7	100	-31 ± 1 (3 mg / kg, i.d.; n = 2)
control[b]		-13 ± 6 (0 mg / kg, i.d.; n = 15)

a. Concentration to inhibit binding of radiolabelled AII to rat adrenal glomerulosa tissue by 50%;[7] b. Control vehicle was 0.5% methyl cellulose; c. $p<0.01$ compared to the control vehicle group as determined by Dunnett's t test.

Selected compounds were tested further for oral activity (10 mg/kg, p.o.) in Goldblatt (2K-1C) hypertensive rats[9] in which hypertension is associated with elevated levels of renin and AII. Tetrazole **6** was approximately equipotent to losartan. Although cyclobutenedione **4** produced a lesser blood pressure lowering effect in comparison to **6**, persistent, long acting activity at 24 hr was still evident with **4**.

Figure 1. Change in MAP in Concious Goldblatt Hypertensive Rats Follwing Administration at 10 mg/kg p.o.



In summary, we have demonstrated carboxylic acid bioisosterism of 3-hydroxy-3-cyclobutene-1,2-dione as applied to a new series of AII receptor antagonists. This relatively unexplored template coupled by its access via Liebskind methodology[5] makes this an attractive piece for further exploitation in medicinal chemistry.

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6. Aldrich, P. E.; Duncia, J. V.; Pierce, M. E. U. S. Patent 4,874,867, 1989.
7. Chiu, A.T.; Duncia, J. V.; McCall, D. E.; Wong, P. C.; Price, W. A.; Thoolen, M. J. M. C.; Carini, D. J.; Johnson, A. L.; Timmermans, P. B. M. W. M. *J. Pharmacol. Exp. Ther.* **1989**; 250, 867.
8. Male, Sprague-Dawley rats weighing 300 - 400g were fasted overnight but allowed water ad libitum prior to the experiment. The rats were anesthetized with Dial-Uretane and the trachea cannulated with PE240 tubing. The left femoral artery and vein were cannulated with PE50 tubing; two cannulas were placed in the femoral vein. A small midline abdominal incision was made to expose the initial portion of the duodenum and a length of PE50 tubing was inserted for the intraduodenal injection of test compounds. Arterial pressure and heart rate were measured from the arterial cannula. Ten to 15 minutes were allowed following surgery for stabilization of arterial pressure. Ganglion blockade was then produced by intravenous administration of mecamylamine at 3 mg/kg (a fall in arterial pressure of ca. 50 mmHg resulted), which was continued every 90 minutes throughout the experiment. AII infusion (0.25 μg/kg/min) was begun on the other cannula. After arterial pressure returned to control levels and were stabilized, baseline values for mean arterial pressure (MAP) were taken. Test compounds, suspended in methylcellulose was administered i.d. in a volume of 1 mL/kg. MAP were taken at 5, 15, 30, 60, 120, 150, 180, 210, and 240 min. after compound administration. The portion of MAP supported by AII was calculated as the difference between baseline MAP (following gangion blockade and AII infusion but prior to administration of test compound) and the minimum value of MAP seen following ganglionic blockade alone (prior to AII infustion). The absolute fall in arterial pressure following the administration of the test compound at the time of interst is the difference between baseline MAP and the pressure at the time of interest. This fall in MAP is then expressed as a percent of the portion of MAP supported by AII. This percentage is the percent inhibition of MAP caused by the test compound at the time of interest.
9. Compounds (10 mg/kg) or vehicle (0.5% methylcellulose) were orally administered to separate groups of tethered, freely moving Goldblatt (2K-1C) hypertensive rats. MAP and heart rate were monitored prior to and then continuously for 24 hr following compound administration.