solution at room temperature. A reference electrode was placed in contact with the nappy liner and the potential difference between the electrodes recorded on an ABB SE120 chart recorder. Standard and test compounds were dissolved in the superfusion medium.

Acknowledgment. This work has been supported by grants from the Lundbeck Foundation and from the Danish Technical Research Council. The technical assistance of Ms. Lærke Andersen is gratefully acknowledged.

Registry No. **2**, 74341-63-2; **3b**, 83654-14-2; **4a**, 74341-64-3; **4c**·HBr, 143006-73-9; **4c** (free base), 143006-96-6; **4d**·HBr, 143006-74-0; **4d** (free base), 143006-97-7; **4e**·HBr, 143006-75-1; **4e** (free base), 143006-98-8; **4f**, 143006-76-2; **5**, 105-45-3; **6c**, 143006-77-3; **6d**, 143006-78-4; **7c**, 96520-39-7; **7d**, 143006-79-5; **8c**, 107403-07-6; **8d**, 143006-80-8; **9c**, 143006-81-9; **9d**, 143006-82-0; **10c**, 143006-83-1; **10d**, 143006-84-2; **11c**, 143006-85-3; **11d**, 143006-86-4; **12c**, 143006-87-5; **12d**, 143006-88-6; **13**, 143006-89-7; **14**, 143006-90-0; **15**, 143006-91-1; **16**, 143006-92-2; **17**, 76470-10-5; **18**, 106749-22-8; **19**, 143006-93-3; **20**, 143006-94-4; **22**, 143006-95-5; AAMM, 72071-39-7; $Br(CH_2)_7CH_3$, 111-83-1; MeI, 74-88-4.

Flavones. 3. Synthesis, Biological Activities, and Conformational Analysis of Isoflavone Derivatives and Related Compounds

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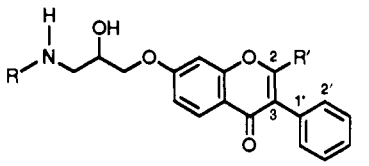
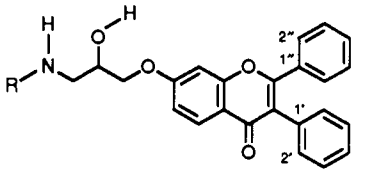
A series of 2-alkylisoflavone derivatives 1 was prepared with the intent to study the importance of the phenyl group (at the 3-position) of the isoflavone in imparting antihypertensive activity and the substitution effects at the 2-position of isoflavone. With the exception of the 2-isopropyl analog, the antihypertensive activity of these compounds appears to have a slow onset and long duration. None of the analogs appears better than the corresponding flavone (3) and 3-phenylflavone (2) analogs. An unsuccessful attempt to correlate the relationship between antihypertensive activity and the calculated torsional angle of C_2–C_3–$C_{1'}$–$C_{2'}$ is discussed. Antiinflammatory activities of these compounds along with 7-(oxypropylamine)flavones were also evaluated and found to be not very potent. The antiinflammatory activity appears to be sensitive to steric effects of the alkyl group on the nitrogen and of substituents at the 2-position of the isoflavones, while the hydroxyl group of the propanolamine side chain is not essential.

As reported in our earlier publications,[1,2a] the 3-phenylflavone analog **2a** or **2b** and the flavone analog **3a** or **3b** (flavodilol), whose antihypertensive activity results, at least in part, from depletion of sympathetic stores of norepinephrine,[2b,c] were both active in reducing blood pressure of spontaneously hypertensive rats, **2a** or **2b** being more active than **3a** or **3b**, respectively. The only difference between the structures of these two compounds is the presence of an additional phenyl group on the 3-position. On the basis of the previous findings[1,2] one can assume that structures for this type of catecholamine depletors require a cationic head such as a positively charged nitrogen atom, an alcoholic group, an aromatic moiety, and a lipophilic cavity for binding.[3] The previous results also suggest that 3-phenyl might merely increase the lipophilicity of the molecule or affinity to receptor binding, hence rendering this molecule more potent and more toxic than **3**.[2a] However, the possibility that a 3-phenyl group might be as important as a 2-phenyl alone cannot be excluded. Assessing the importance of a 3-phenyl group in imparting antihypertensive activity either as a single determinant or a contributor forms the basis of this study. In addition, the torsional angle C_2–C_3–$C_{1'}$–$C_{2'}$ might be dependent upon the steric bulk of substituents at the 2-position. This torsional angle and the lipophilicity of the 2-substituents would, in turn, affect the biological activity of these compounds.

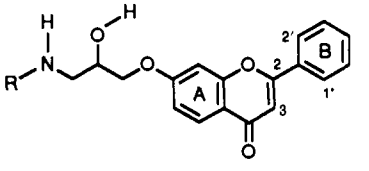
As part of our interests in the area of flavonoids and in expanding our efforts in the area of catecholamine-depleting agents as antihypertensive agents, in this paper we report the synthesis and biological evaluation of a series



1



2



3

a: R = *i*-Pr
b: R = *n*-Pr

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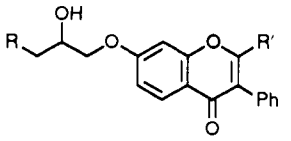
(1) Wu, E. S. C.; Cole, T. E.; Davidson, T. A.; Blosser, J. C.; Borrelli, A. R.; Kinsolving, C. R.; Parker, R. B. Flavones. 1. Synthesis and Antihypertensive Activity of 3-Phenylflavonoxypropanolamines without β-Adrenoceptor Antagonism. *J. Med. Chem.* 1987, *30*, 788–92.

0022-2623/92/1835-3519$03.00/0

Table I. Physical Properties and Pharmacological Activity of the Isoflavone Derivatives

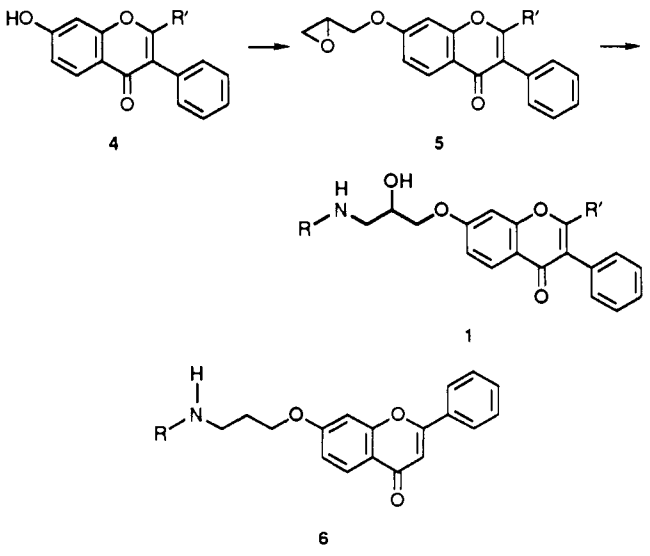


no.	R	R^1	mp,[a] °C	formula	anal.[b]	% yield[c]	antihypertensive activity: % decrease in SBP of SHR[d] 2 h	5 h	7.5 h	24 h	antiinflammatory activity: carrageenan-induced paw edema dose, mg/kg	% inhibn[e] (*n*)
1a	*i*-PrNH	H	128–30[f]	$C_{21}H_{23}NO_4$·HCl	C, H, N, Cl	62	NS	**11**	14	21	50	33* (5)
											30	27*
1b	*i*-PrNH	CH_3	183–5[g]	$C_{22}H_{25}NO_4$·HCl	C, H, N, Cl	40	NS	12	19	17	50	19
1c	*i*-PrNMe	CH_3	169–71[h]	$C_{23}H_{27}NO_4$·HCl	C, H, N, Cl	77	NS	NS	NS	NS	50	19
1d	$^+NMe_2$(*i*-PrNH)	CH_3	211–3[i]	$C_{24}H_{30}NO_4I$	C, H, N, I	62	NS	NS	**11**	NS	50	36
1e	*i*-PrNH	CF_3	220–2[j]	$C_{22}H_{22}F_3NO_4$·HCl	C, H, N, F, Cl	52	NS	16	16	20	50	28 (2)
1f	*n*-PrNH	CF_3	169–71[j]	$C_{22}H_{22}F_3NO_4$·HCl	C, H, N, F, Cl	41	NS	**11**	12	17	50	22
1g	*i*-PrNH	*i*-Pr	187–8[g]	$C_{24}H_{29}NO_4$·$C_4H_4O_4$	C, H, N	94	18	20	25	15	50	28 (2)
1h	*i*-PrNH	2-furyl	187–9[j]	$C_{25}H_{25}NO_5$·HCl	C, H, N, Cl	36	NS	NS	15	NS	50	19
1i	*i*-PrNH	2-c-hex	217–9[j]	$C_{27}H_{33}NO_4$·HCl	C, H, N, Cl	62	NS	NS	NS	NS	50	24
1j	c-OctNH	H	185–6[g]	$C_{26}H_{31}NO_4$·HCl	C, H, N, Cl	43	NS	NS	NS	NS	50	21*
											25	41*
1k	*n*-PrNH[k]	H	glassy solid	$C_{21}H_{23}NO_4$·HCl	C, H, N, Cl	52	NS	–	–	–		
1l	*i*-PrNH	CH_2Ph	215–7[j]	$C_{28}H_{29}NO_4$·HCl[l]	C, H, N, Cl	41	NS	NS	NS	NS	50	−3.4
2a							15	23	30	34	50	24.6*[m]
3a							12	18	23	16	50	14.5
clonidine								15[n]				
indomethacin											4	44.6*

[a] Melting points are not corrected. [b] Elemental analysis were within 0.4% of the original unless otherwise noted. [c] Purified yield from the corresponding epoxide. [d] Five male rats per dosage group. Animals were dosed with 75 mg/kg po except **1k** where 30 mg/kg iv was used. Percentage falls in systolic blood pressure were recorded at the indicated hour after dosing. Values in the table are statistically significant ($p \geq 0.05$) relative to control values; asterisks indicate percentage falls in systolic blood pressure that were not statistically significant. [e] Groups of six male Sprague–Dawley rats were orally given the test compounds 2 h prior to challenge with the edemagen. Usually one experiment was performed on each compound unless otherwise noted. "*n*" is the number of experiments. *$p < 0.05$. [f] Recrystallized from EtAc–EtOH–Ether. [g] Recrystallized from i-PrOH. [h] Recrystallized from i-PrOH–ether. [i] Recrystallized from EtOH. [j] Recrystallized from *i*-PrOH–MeOH. [k] The side chain is on the 8-position instead of the 7-position. [l] This compound contained 1.7% of *i*-PrOH and 0.52% of MeOH, as determined by GC. [m] There was 32% inhibition at a dose of 25 mg/kg. The corresponding *n*-propyl isomer, **2b**, produced 27.4%* inhibition at 50 mg/kg. [n] Clonidine (150 mg/kg po) was administered.

of isoflavone oxypropanolamines **1**. In this series the emphasis was centered on modification of substituents at the 2-position of the chromone ring and evaluation of their structure–activity relationship. Substituents such as alkyl,

Scheme I



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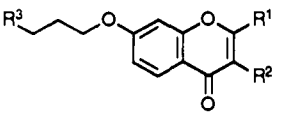
(4) Synthesis of 7-hydroxyisoflavone: (a) Mahal, H. S.; Rai, H. S.; Venkataraman, K. J. Synthetic Experiments in the Chromone Group. XII. Synthesis of 7-Hydroxyisoflavone and of α- and β-naphthoisoflavone. *J. Chem. Soc.* **1934**, 1120–2. (b) Karmarker, S. S. Chromones. XXXIII. Further Applications of the Ethyl Orthoformate Method for the Synthesis of Isoflavones. *J. Sci. Ind. Res.* **1961**, *20b*, 334–8.

(5) Synthesis of 2-isopropyl, 2-cyclohexyl, and 2-(2-furyl)-7-hydroxyisoflavones: Szabo, V.; Farkas, E.; Levai, A. Synthesis of C-2-substituted Isoflavones. *Acta Phys. Chim. Debrecina* **1970**, *15/16*, 191–9.

(6) Synthesis of 2-methyl-7-hydroxyisoflavone: Baker, W.; Robinson, R. Synthetical Experiments in the Isoflavone Series. *J. Chem. Soc.* **1925**, 1981–6.

(7) (a) Rossi, M.; Cantrell, J. S.; Farber, A. J.; Dyott, T.; Carrell, H. L.; Glusker, J. P. Molecular Structures of 5,6- and 7,8-benzoflavones, Inhibitors of Aryl Hydrocarbon Hydroxylase. *Cancer Res.* **1980**, *40*, 2774–84. (b) Ting, H.-Y.; Watson, W. H.; Dominguez, X. A. Molecular Structure of 3′,5,5′,6-tetramethoxyflavone, $C_{19}H_{18}O_6$. *Acta Crystallogr.* **1972**, *B28*, 1046–51. (c) Vleggaar, R.; Kruger, G. J.; Smalberger, T. M.; van Den Berg, A. J. *Flavonoids* from Tephrosia-XI: the Structure of Glabratephrin. *Tetrahedron* **1978**, *34*, 1405–8.

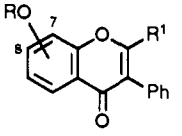
Table II. Physical Properties and Antiinflammatory Activity of the Desoxyflavone Derivatives



no.	R^1	R^2	R^3	mp,[a] °C	solvent	formula	anal.[b]	% yield[c]	carageenan-induced paw edema: % inhibn[b] (n)
6a	Ph	H	NH-*i*-C_3H_7	248–9	MeOH	$C_{21}H_{23}NO_3$·HCl	C, H, N, C	66	41* (5)
6b	Ph	H	NH-*n*-C_3H_7	254–5	*i*-PrOH	$C_{21}H_{23}NO_3$·HCl	C, H, N, Cl	55	18 (2)
6c	Ph	H	NH-*t*-C_4H_9	229–30	*i*-PrOH	$C_{22}H_{25}NO_3$·HCl	C, H, N, Cl	49	34* (3)
6d	Ph	H	$N(CH_3)_2$	210–1	*i*-PrOH	$C_{20}H_{21}NO_3$·HCl	C, H, N, Cl	53	13 (2)
6e	Ph	H	NH-*n*-C_5H_{11}	264–5	MeOH	$C_{23}H_{27}NO_3$·HCl	C, H, N, Cl	48	−9
6f	Ph	H	NH-*c*-C_5H_9	234–6	MeOH	$C_{23}H_{25}NO_3$·HCl·$^1/_3H_2O$[e]	C, H, N, Cl	33	21*
6g	Ph	H	NH-*c*-C_8H_{15}	244–6	MeOH	$C_{26}H_{31}NO_3$·HCl	C, H, N, Cl	30	26*
6h	Ph	OCH_3	NH-*i*-C_3H_7	189–90	*i*-PrOH	$C_{22}H_{25}NO_4$·HCl	C, H, N, Cl	36	−2
6i	H	Ph	NH-*t*-C_4H_9	189–91	*i*-PrOH	$C_{22}H_{25}NO_3$·HCl[f]	C, H, N, Cl	47	6

[a] Melting points are not corrected. [b] Elemental analyses were within 0.4% of the theoretical value unless otherwise noted. [c] Purified yield based on the starting mesylate. [d] See footnote *e* of Table I. The dose used was 50 mg/kg. [e] This compound contained 1.5% water. C: calcd, 67.96; found, 67.29. [f] C: calcd, 68.12; found, 67.52.

Table III. Physical Properties of Some Intermediates



no.	position	R	R^1	mp,[a] °C	solvent	formula	anal.[b]	% yield[c]
4a	7	H	CF_3	222–3	MeOH	$C_{16}H_9F_3O_3$·$^1/_4$MeOH	C, H, F	75
5a	7	$CH_2\overline{CH-O-CH_2}$	CF_3	146–8	CH_2Cl_2	$C_{19}H_{13}F_3O_4$	C, H, F	61
5b	7	$CH_2\overline{CH-O-CH_2}$	*i*-Pr	139–40	CH_2Cl_2	$C_{21}H_{20}O_4$	C, H	27
5c	8	$CH_2\overline{CH-O-CH_2}$	H	141–3	*i*-PrOH	$C_{18}H_{14}O_4$	C, H	81

[a] Melting points are not corrected. [b] Elemental analyses were within 0.4% of the theoretical value unless otherwise noted. [c] Purified yield.

cycloalkyl, arylalkyl, and heterocyclic were used.

During its in-depth pharmacological evaluation, **3b** was found to exhibit a marginal inhibitory action in the carrageenan-induced paw edema assay. Antiinflammatory action of flavonoids including isoflavone analogs have been reported in the literature.[9] In search of a potential antiinflammatory agent, these isoflavone derivatives and related compounds were synthesized and tested.

Chemistry

The general synthesis of the isoflavone oxypropanolamines 1 was carried out as shown in Scheme I. The parent and 2-substituted 7-hydroxyisoflavones[4-6] were prepared according to published methods. The hydroxyflavone **4** was allowed to react with epichlorohydrin in the presence of alcoholic sodium hydroxide. The resulting epoxide **5** was heated under reflux with amines to afford the target (aryloxy)propanolamine 1 (Scheme I). The desoxy flavone analogs **6** were prepared from the corresponding mesylate **7** using the procedures as described in our previous publication.[2]

Pharmacology

The antihypertensive activity and the antiinflammatory activity of these compounds were evaluated in male spontaneously hypertensive rats (SHR) of the Wistar–Kyoto strain and male Sprague–Dawley rats, respectively. Arterial systolic blood pressure was measured by indirect tail cuff methods.[1,2] The antiinflammatory activity was determined by the carrageenan-induced rat paw edema test. The results are summarized in Tables I, II, and III.

Results and Discussion

Antihypertensive Activity. As shown in Table I, the parent isoflavone analog **1a** exhibited the antihypertensive activity. Generally the activity of **1a**[13] was weaker and its duration was longer than the corresponding flavone and 3-phenylflavone derivatives **3a** and **2a**, respectively. This observation indicates that elimination of the phenyl group at the 2-position of **2a** or reposition of the phenyl of **3a** at the 3-position results in attenuation but not abolition of the activity.

Most of the isoflavone derivatives exhibited moderate oral antihypertensive activity at the dose of 75 mg/kg in spontaneously hypertensive rats. The onset of the activity for this series of compounds was generally slow except for **1g** (R^1 = *i*-Pr), but their duration was long (>24 h) as compared with that of the flavone and diphenylchromone

(8) (a) Cantrell, J. S.; Stalzer, R. A. Structure of 3-bromo-2-phenyl-4H-1-benzopyran-4-one (3-bromoflavone). *Acta Crystallogr.* **1982**, *B38*, 983–4. (b) Wallet, J. C.; Gaydou, E. M.; Fadlane, A.; Baldy, A. Structure of 3-methoxy-2-phenyl-4H-1-benzopyran-4-one (3-methoxyflavone). *Acta Crystallogr.* **1988**, *C44*, 357–9.

(9) (a) Gabor, M. Anti-inflammatory and Anti-allergic properties of Flavonoids. *Prog. Clin. Biol. Res.* **1986**, *213*, 471–80. (b) *Jap. Pat.* 85199396.

(10) The ring-opening of flavone by amines to the corresponding β-aminochalcone and the acid-catalyzed cyclization of the β-aminochalcone to flavone have been reported, see ref 2 and therein.

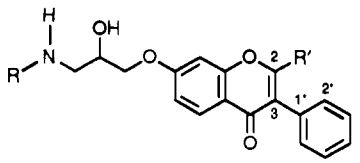
(11) The torsional angle was calculated using conformational analysis in Sybyl[15] as described in this manuscript.

(12) Pauling, L. *The Nature of the Chemical Bond and the Structure of Molecules and Crystals. An Introduction to Modern Structural Chemistry.* 3rd ed.; Cornell University Press: Ithaca 1960, 237.

(13) This compound was found to deplete catecholamines in heart of SHR.[2a]

(14) Unpublished results.

Table IV. Torsional Angles and Bond Lengths Calculated by Sybyl and Mopac



no.	R	torsional angle[a] C_2–C_3–$C_{1'}$–$C_{2'}$, deg	bond length C_3–$C_{1'}$, Å	λ_{max}[b]
1a	H	44.0 (−38)	1.515 (1.460)	304.0
1b	CH_3	55.4 (−49.3)	1.514 (1.4623)	301.0
1e	CF_3	57.3 (−56.8)	1.513 (1.4621)	303.0
1g	*i*-Pr	62.3 (−56.6)	1.513 (1.4635)	303.0
1h	2-furyl	57.6 (−51.6)	1.516 (1.4625)	330.0
		−158.2 (132.0)[c] (C_3–C_2–$C_{2''}$–$C_{3''}$)[d]	1.487 (1.4503) (C_2–$C_{2''}$)	
1i	*c*-hex	61.4	1.516	301.5
1l	CH_2Ph	−60.0	1.513	302.8
3b (or **3a**)		0–3	1.492 (C_2–$C_{1'}$)	305.0
		11.9[e]	1.491[e] (C_2–$C_{1'}$)	
2a	Ph	−51.0 (C_3–C_2–$C_{1'}$–$C_{2'}$)	1.514	306.0
		−43.3 (C_2–C_3–$C_{1''}$–$C_{2''}$)	1.515 (C_2–$C_{1''}$)	
3-Br-flavone		53.2	1.513 (C_2–$C_{1'}$)	
		45.7[f]	1.492[f] (C_2–$C_{1'}$)	

[a] The lower number was usually used in this table with the exception of **1h** (see footnote *b*). All the data in parentheses were calculated by Mopac using isoflavone derivatives substituted with a 7-methoxy group instead of 7-(3-amino-2-hydroxypropoxy). [b] λ_{max} was measured by a Varian UV-visible recording spectrophotometer Model Cary 219. Methanol was used as solvent. [c] See ref 16. [d] This is the torsional angle for the C_2-furan. [e] These results were obtained from the X-ray study; unpublished results. [f] X-ray data. See ref 8a.

analogs **2a** and **3a**, respectively. Some compounds, **1a**, **1b**, **1e**, and **1f**, appeared to reach peak activity in between 7.5 and 24 h after dosing. The most active compound was **1g**, where R^1 is an isopropyl group.

The effect of N-substitution was studied. The cyclooctyl analog **1j** was inactive at the dose of 75 mg/kg while its corresponding isopropyl congener **1a** was active (see Table I). The branched N-substituent, for example **1e** (R = *i*-PrNH), appeared to be slightly more active than the nonbranched N-substituent, for example **1f** (R = *n*-PrNH). The difference was small and was within the experimental error. The tertiary amine, **1c**, was inactive at the tested dose, while the secondary amine, **1b**, was active. The quaternary ammonium salt, **1d**, was marginally active. These results of N-substitution effects are similar to what has been observed with the flavone and diphenylchromone oxypropanolamines; however compounds with a nonbranched *N*-alkyl usually displayed better antihypertensive activity than those with a branched *N*-alkyl in the previous reports.[1,2]

To examine the effects of the oxypropanolamine side chain at the 8-position of isoflavone, **1k** was prepared and found to be devoid of antihypertensive activity. This result is in agreement with our earlier report that the oxypropanolamine side chain substituted at the 7-position of the chromone residue produced the optimal activity.[2]

The result of **1a** being active in lowering the blood pressure of SHR led us to further explore the effect of replacing the hydrogen at the 2-position with an alkyl or a heterocyclic group. The rationale for this study is based on the following facts: (a) the 2-phenyl residue for **3a** freely rotates around the C_2–$C_{1'}$ bond, and the torsional angle between C_3–C_2–$C_{1'}$–$C_{2'}$ for simple flavone derivatives has been shown by X-ray studies to be 5–30°,[7] and this torsional angle of flavone with the minimum energy configuration was calculated to be 33.1°;[11] (b) contrary to flavone, the two phenyl groups of **2a** have restricted rotations around C_2–$C_{1'}$ and C_3–$C_{1''}$ bonds because of the steric interaction between the two phenyl rings; (c) an alkyl or a heterocyclic group substituted at the 2-position of **1a** would restrict the free rotation of the 3-phenyl group around the bond C_3–$C_{1'}$ and, in turn, increase the torsional angle of C_2–C_3–$C_{1'}$–$C_{2'}$. This situation is similar to what has been observed in 3-bromoflavone[8a] and 3-methoxyflavone,[8b] where the phenyl ring at C_2 is rotated 45.9° and 37.2°, respectively, with respect to the remainder of the molecule; (d) furthermore, a simple substituent effect might be also expected due to changes in electronic or steric factors. It was hoped that there would be a correlation between the torsional angle and antihypertensive activity which would provide additional information enabling the design of a new generation of antihypertensive agents.

To achieve this goal derivatives substituted with 2-methyl; 2-trifluoromethyl, which is a slightly larger group with a strong electron-withdrawing effect than methyl; 2-isopropyl, which is a bulky group; 2-cyclohexyl, which is a cyclic derivative of isopropyl; 2-benzyl, which is also a bulky group; and 2-furyl, which is a heterocyclic aromatic, were synthesized and tested in the SHR. Introduction of a 2-methyl (**1b**) or 2-(trifluoromethyl) (**1e** and **1f**) did not potentiate nor reduce the antihypertensive activity. However the activity was enhanced when an isopropyl group (**1g**) was substituted on the 2-position. Having a fast onset (within 2 h) and a long duration of action (>24 h), this analog proved to be the most active compound in this series. Substitution with a 2-cyclohexyl (**1i**) or a benzyl (**1l**) residue produced a loss of the activity, whereas a marginal activity was observed with the 2-furanyl analog (**1h**).

Conformational Analysis. (a) **By SYBYL.** SYBYL molecular modeling software program[15] was employed to ultimately determine the torsional angle between the phenyl ring and the pyran double bond (C_2=C_3). Molecules were constructed using the X-ray structure of flavodilol and modified by the BUILD command. MAXIMIN2 was used to configurationally minimize the structures. SEARCH was then used for conformational analysis determination. The rotatable bonds are C_3–$C_{1'}$ and/or C_2–R; R = substituent at the 2-position. The final SEARCH run was done at 1° increments. Lowest energy conformers thus obtained were then minimized by

(15) Tripos Associates, Inc., St. Louis, MO, Sybyl versions 5.31, 5.4 and 5.41c.

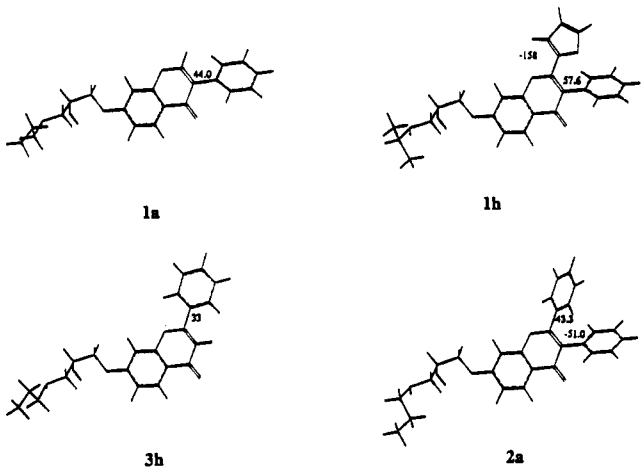


Figure 1. Energy-minimized structures of **1a**, **1h**, **3b**, and **2a**. The respective torsion angles are also indicated.

MAXIMIN2. The results are illustrated in Table IV.

To validate our conformational analysis, 3-bromoflavone was used to compare calculated torsional angle and bond length with the X-ray data. The calculated torsional angle (53.2°) of C_3–C_2–$C_{1'}$–$C_{2'}$ and bond length (1.513 Å) of C_2–$C_{1'}$ were close to those obtained from the X-ray study (45.7° and 1.492 Å, respectively). As shown in Table IV, calculated torsional angles of C_2–C_3–$C_{1'}$–$C_{2'}$ for all C-2-substituted isoflavone analogs were in the range of ±51 to ±62.3° and bond lengths of C_3–$C_{1'}$ were in the range of 1.515–1.492 Å, which are generally in agreement with the expected 1.504 Å for an sp^2–sp^2 single bond.[12]

The calculated torsional angle of (C_2–C_3–$C_{1'}$–$C_{2'}$) for the parent isoflavone analog **1a** was 44° (see Figure 1) and the bond length of C_3–$C_{1'}$ was 1.515 Å. The isopropyl analog (**1g**) had a slightly larger torsional angle than the methyl (**1b**) and the trifluoromethyl (**1e**) analogs. The torsional angles for the cyclohexyl (**1i**) and the benzyl (**1l**) analogs (61.4° and −60.0°, respectively) were slightly larger than that of the methyl (**1b**) or the trifluoromethyl (**1e**) and similar to that of the isopropyl analog (**1g**) while the size of the cyclohexyl or the benzyl group is relatively bigger than that of the methyl, trifluoromethyl, or isopropyl group. This may be attributed to the fact that the most severe nonbonded interactions reside between the $C_{2'}$ or $C_{6'}$ hydrogen of the 3-phenyl ring and substituents on the $C_{1''}$ atom of C_2–$C_{1''}R^1R^2R^3$. This explanation is further supported by the fact that the furyl (**1h**) and 2-phenyl (**2a**) analogs have torsional angles of 57.6° and −51°, respectively, despite an anticipated severe steric congestion between the 2-furyl or 2-phenyl and the 3-phenyl ring of isoflavone.

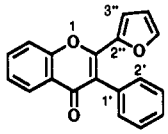
The torsional angle for the furyl group of the furyl analog (**1h**) with respect to the chromone ring (C_3–C_2–$C_{2''}$–$C_{3''}$) was calculated to be −158.2° (Figure 1). This angle indicates that the furan ring is close to coplanar to the chromone ring.[16] This steric arrangement is further supported by the much shorter C_2–$C_{2'}$ bond (1.487 Å) than the C_3–$C_{1'}$ bond (1.516 Å) and by a large bathochromic shift of the absorption maximum to 330 nm as compared to the λ_{max} at 304 nm of **1a** or 305 nm of **3b**. The C_2–Ph of **2a** is not coplanar with respect to the chromone nucleus, as evidenced from its torsional angle (−43.3°) and λ_{max} (306 nm).

The flavone analogs **3a** and **3b** had a calculated torsional angle of 33.08° (C_3–C_2–$C_{1'}$–$C_{2'}$). The X-ray study of **3b** indicated that the torsional angle was 11.9°.[14] The λ_{max} (305 nm) for **3b** was the same as that of 7-[3-(isopropylamino)-2-hydroxypropoxy]-2-methylchromone (λ_{max} = 300 nm)[17] or that of the isoflavone analog **1a**, indicating that the B-ring of **3b** did not lie in the same plane as the remainder of the molecule. Although the solid-state conformations obtained from the X-ray studies are not always the same as those observed in the solution phase, the UV data lends support toward the X-ray result that the B-ring of **3b** is not in the same plane as that of the chromone ring.

(b) By Mopac. Semiempirical calculations were done using QCPE program No. 455, Mopac (4.0). The AMI Hamiltonian was used in the electronic part of the calculation to obtain the molecular geometries. The geometry was specified using internal coordinates (Z-matrix), and during optimization all of the bond lengths, angles and dihedrals were allowed to change. A 7-methoxy group was employed instead of the 2-hydroxy-3-(alkylamino)propoxy side chain, assuming the substitution at this position would not affect the calculation. The results are shown in Table IV. The torsional angles obtained from this method are comparable with those from the SYBYL analysis, however the bond lengths (1.46 Å) are generally shorter than those from the latter (~1.51 Å).

Discussion and Conclusions. The 2-phenyl analog **2a** was the most potent antihypertensive compound as shown in Table I. **3a**, similar to **3b** with the *N*-*i*-Pr group replacing *N*-*n*-Pr, and **1g** (R = *i*-Pr) were moderately active compounds. The furyl analog (**1h**) is marginally active. These facts suggest that the torsional angle of a phenyl group at the 2- or 3-position of chromone with respect to the chromone ring is not important for their antihypertensive activity and the relative potency of these compounds might depend upon other factors such as steric factors. They further indicate that the tolerance with steric bulkiness is rather limited as the isopropyl analog (**1g**) was active while the cyclohexyl (**1i**) and benzyl (**1l**) derivatives were inactive. The result that the 2-phenyl compound (**2a**) is the most active, the furyl (**1h**) marginally active, and the cyclohexyl (**1i**) inactive might imply that drug–receptor interaction in the lipophilic pocket might prefer a flat moiety or is quite sterically sensitive. Furthermore, the size of a substituent, but not the lipophilicity of the substituent, at the 2-position of isoflavone might also be important as shown in the case of **1i** and **1l**, which were inactive in the SHR assay. Interestingly the UV results from Table IV also provided additional support that the presence of a phenyl group at the 2- or 3-position of chromone did not affect the absorption maximum of the chromone ring as absorption maxima of all the compounds except **1h** were approximately the same as that of 7-[3-(isopropylamino)-2-hydroxypropoxy]-2-methylchromone (λ_{max} = 300 nm). This study clearly indicates that there is no correlation between the torsional angle of 3-phenyl or 2-phenyl with respect to chromone and the antihypertensive activity, and the optimal size of substituents at C_2 might be important in the interaction of ligands with active sites.

(16) An ab initio calculation at the 3-21 G basis set level of approximation on the fragment as shown below, with fixed bond lengths C_3–C_1 = 1.463 Å and C_2–$C_{2''}$ = 1.450 Å, gave the final torsional angles −85.7° and 7.05° for C_2–C_3–$C_{1'}$–$C_{2'}$ and C_3–C_2–$C_{2''}$–$C_{3''}$, respectively. This supports the view that the 2-furyl ring can delocalize better than the phenyl ring and thence prefers to be coplanar.



In summary the present results coupled with our earlier findings[1,2a,17] suggest that the 3-phenyl group of these chromone derivatives is as essential as the 2-phenyl in imparting the antihypertensive activity.

Antiinflammatory Activity. Of eleven isoflavone compounds tested, only **1j** exhibited good activity, 41% inhibition at 25 mg/kg. Weak inhibitory activity was observed with **1a** (33% at 50 mg/kg and 27% at 25 mg/kg). The remainder of the isoflavone derivatives produced either no significant or marginal inhibition on the carrageenan-induced paw edema. It appeared that the inhibitory activity of **1j** was biphasic—21% inhibition at 50 mg/kg and 41% at 25 mg/kg. Substitution at the 2-position of isoflavone with an alkyl (**1b**, **1e**, **1f**, and **1g**), alicyclic (**1i**), or heterocyclic group (**1h**) reduced the inhibitory activity. The 2-benzyl analog (**1l**) was essentially devoid of the activity. N-Substitution seemed to potentiate the inhibition. For example, **1d**, where R = $^{+}NMe_2$(*i*-PrNH), exhibited 36% inhibition at 50 mg/kg, although not statistically significant, and **1j**, where R = *c*-OctNH, 41% inhibition at 25 mg/kg.

While the borderline inhibitory activity was observed with the flavone analog **3b**, the corresponding *N*-isopropyl analog **3a** was inactive. The 3-phenylflavone analogs **2a–c** showed marginal activity.

In conjunction with this study on isoflavones, efforts were devoted to examine the importance of the 2-hydroxy group on the side chain of **3**. **6a**, a desoxy compound, was synthesized, tested, and found to have a moderate inhibitory action at 50 mg/kg (41% inhibition, as compared to 15% inhibition of **3a**). This result led to the further investigation of the effect of N-substitution. A straight alkyl chain on the nitrogen atom, for example, **6b** (R^3 = *n*-PrNH) and **6e** (R^3 = *n*-PenNH; Pen = pentyl), attenuated the action. Cyclic analogs **6f** (R^3 = c-PenNH) and **6g** (R^3 = c-OctNH) exhibited marginal activity. Compounds with more hindered groups such as *t*-Bu (**6c**) retained their activity. The tertiary amine **6d** was devoid of activity. Introduction of a methoxy group at the 3-position of flavone such as **6h** resulted in a loss of activity. The desoxy analog of isoflavone (**6i**), where R^3 = *t*-BuNH, was also inactive.

Experimental Section

The melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were determined in the indicated solvent on a Varian EM360A NMR spectrometer at the ambient operating temperature with tetramethylsilane as internal standard for proton spectra unless otherwise stated. Chemical shifts are given in ppm units, and coupling constants are in Hz. Splitting patterns are designated as follows: s, singlet, br s, broad singlet; d, doublet; t, triplet; q, quarter; m, multiplet. Infrared spectra were recorded on a Nicolet MX-1 Fourier transform infrared spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical value when indicated by symbols of the element, unless otherwise noted.

7-Hydroxy-2-(trifluoromethyl)isoflavone (4a). A suspension of ω-phenylresacetophenone[1] (50 g, 0.22 mol), trifluoroacetic anhydride (150 g, 0.714 mol), and sodium trifluoroacetate (60 g, 0.441 mol) was heated in the presence of nitrogen at 140 °C to a light tan solution. The reaction mixture was maintained at 140 °C overnight (14 h) and cooled. A light brown solid was formed, acidified with 10% aqueous hydrochloric acid, and stirred. The light tan solid, (62.3 g, 92% crude yield, mp 217–221 °C) was collected. Recrystallization of 16.4 g of the crude product from methanol (250 mL) afforded 13.5 g of white prisms: mp 222–223 °C; IR (KBr) 3200, 1638 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90–8.20 (m, 8 H, aromatic H), 10.00–10.50 (br s, 1 H, exchangeable, OH). Anal. ($C_{16}H_9F_3O_3 \cdot {}^1/_4MeOH$) C, H, F.

7-(2,3-Epoxypropoxy)-2-(trifluoromethyl)isoflavone (5a). To a stirred solution of sodium hydroxide (6.0 g, 0.15 mol) in 50% of aqueous ethanol (300 mL) and crude 7-hydroxy-2-trifluoroisoflavone (45.9 g, 0.15 mol) was added epichlorohydrin (277.5 g, 3 mol) and the mixture was heated at 70 °C for 2.5 h. The reaction mixture was cooled and evaporated to give a pink solid. The solid was collected, washed with water, and air-dried. The dried solid was further stirred with anhydrous ether (100 mL), filtered, and washed with anhydrous ether (20 mL) and isopropyl alcohol (25 mL) to afford 33.3 g (61% yield) of a white solid, mp 135–138 °C; TLC (2% MeOH in CH_2Cl_2) indicated that the solid contained traces of the starting epoxide and one impurity, which was suspected to be the corresponding chlorohydrin. The product was used for next reaction without further purification. A portion (13.1 g) of the crude product was purified by column chromatography (silica gel) eluting with 5% ether in methylene chloride. A pure white solid (11 g), mp 146–148 °C, was obtained: IR (KBr) 1651, 1611, 1575, 1500 cm^{-1}; ^{H} NMR ($CDCl_3$) δ 2.60–3.05 (m, 2 H, CH_2 of the epoxide ring), 3.20–3.60 (m, 1 H, CH of the epoxide ring), 3.75–4.50 (m, 2 H, OCH_2), 6.80–8.25 (m, 8 H, aromatic H). Anal. ($C_{19}H_{13}F_3O_4$) C, H, F.

7-[3-(Propylamino)-2-hydroxypropoxy]-2-(trifluoromethyl)isoflavone (1f). To 39.1 mL (476 mmol) of *n*-propylamine were added 3.46 g (9.51 mmol) of 7-(2,3-epoxypropoxy)-2-(trifluoromethyl)isoflavone and 18 mL of isopropyl alcohol. The mixture was stirred and heater under reflux for 1.5 h. The reaction mixture was evaporated on a rotovap, under reduced pressure, to a yellowish green oil (4.52 g) which was stirred with anhydrous ether overnight to give a white solid. The product was filtered and washed with ether, yielding 1.7 g of a white solid.

The crude base was dissolved in isopropyl alcohol (14 mL) and treated with hydrogen chloride saturated ethanol to obtain a solution of pH 1–2. The crystals formed were filtered and washed with isopropyl alcohol to yield 1.8 g of white crystals: mp 172–174 °C; IR (KBr) 3100–2300, 1651, 1209 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (t, 3 H, CH_3), 1.40–2.30 (bs, 2 H, CH_2CH_3), 2.80–3.66 (m, 4 H, CH_2NCH_2), 4.00–4.90 (2 br s, 3 H, OCH_2CH), 6.80–8.40 (m, 8 H, aromatic protons), 7.52–7.54 (m, 3 H, aromatic protons). Anal. ($C_{22}H_{23}ClF_3NO_4$), C, H, N, Cl, F.

7-[3-(Cyclopentylamino)propoxy]flavone Hydrochloride (6f). A mixture of 7-[3-[(methylsulfonyl)oxy]propoxy]flavone[2] (10.86 g, 29 mmol), cyclopentylamine (25 mL, 290 mmol), and DMSO (78 mL) was heated at 80 °C for 20 h and cooled to room temperature. The reaction mixture was poured into 30 mL of an ice–water mixture. The resulting oil precipitate was dissolved in ether, then evaporated under reduced pressure to an oil which was shown by NMR to be a mixture of the desired product and the corresponding β-cyclopentylaminochalcone.[10] The mixture was dissolved in isopropyl alcohol (300 mL), acidified with saturated HCl/EtOH, and then heated under reflux until the chalcone disappeared.[10] The reaction mixture was cooled to room temperature. The crude hydrochloride precipitate was filtered off and recrystallized from MeOH yielding 3.87 g (33%), mp 234–236 °C. Anal. ($C_{23}H_{25}NO_3Cl \cdot {}^1/_3H_2O$) H, N, Cl; C: calcd, 67.96; found, 67.29.

Indirect Blood Pressure Measurement in Unanesthesized Rats. Arterial systolic blood pressure was measured with an indirect tail cuff method.[2]

Antiinflammatory Activity. Acute antiinflammatory activity was determined in the carrageenan-induced rat paw edema test.[18] Male Sprague–Dawley rats (Blue Spruce Farm) weighing 124–140 g were housed for 1 week, and allowed food and water ad libitum. At the time of the experiments, only rats weighing 160–200 g were used.

All compounds were dissolved or suspended in a 0.5% water solution of methocel and orally administered to groups of six rats. Control rats received methocel only. Indomethacin (4 mg/kg)

(17) 7-[3-(isopropylamino)-2-hydroxypropoxy]-2-methylchromone was reported previously to be inactive in SHR test.[2a]

(18) Winter, C. A.; Risley, E. A.; Nuss, G. W. Carageenin-induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs. *Proc. Soc. Exp. Bio. Med.* **1962**, *111*, 544–7.

was used as the positive reference standard. Two hours later, paw edema was induced by the subcutaneous injection into the plantar surface of the right hind paw of 0.1 mL of a 1.0% homogenized suspesnion of carrageenan.

Immediately, the swelling of the paw was measured by immersing it in a mercury plethysmomeric system to above the lateral mateolus. The mercury plethysmometer was constructed as follows: a glass cylinder 22 mm in diameter and 60 mm deep was connected at the bottom of the cylinder by a column of water to a Statham transducer (model P23BB), of pressure range 0–5 cm. The displacement was recorded electronically on a Beckman R511 recorder. Three hours later, the inflamed paw volume was measured again, and the change in displacement was recorded for each group. The percent inhibition of edema was calculated using the control group paw volume as 100% edema, i.e.

$$\frac{\Delta \text{ control group edema} - \Delta \text{ test group edema}}{\Delta \text{ control group edema}} \times 100 = \text{inhibn of edema}$$

Drug effects on paw volumes were evaluated by comparing the extent of edema with that produced in the corresponding control group. Using an analysis of variance test followed by a Newman–Keuls t-test.

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Renin Inhibitors Containing New P_1–P_1' Dipeptide Mimetics with Heterocycles in P_1'

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A series of renin inhibitors containing new P_1-P_1' dipeptide mimetics are presented. The P_1-P_1' mimetics were obtained from (4*S*,5*S*)-3-(*tert*-butoxycarbonyl)-4-(cyclohexylmethyl)-5-[(ω-mesyloxy)alkyl]-2,2-dimethyloxazolidines **5b**, **9**, and 11**b** by nucleophilic substitution of the mesylate groups with the sodium salts of mercapto- and hydroxyheterocycles. Removal of the protecting groups and stepwise acylations with amino acid derivatives provided renin inhibitors with a length of a tripeptide. Replacement of P_2 histidine by other amino acids maintained or enhanced renin inhibitory potency. By alteration of P_3 phenylalanine, compounds with IC_{50} values in the nanomolar range and stability against chymotrypsin were obtained. Finally, the effect of the C-terminal heterocycle on the renin inhibition was studied. Compound XVII was examined in vivo for its hypotensive effects. In salt-depleted cynomolgus monkeys, XVII inhibited plasma renin activity and lowered blood pressure after oral administration of a dose of 10 mg/kg.

Introduction

The renin–angiotensin system (RAS) is a complex enzymatic–hormonal system controlling electrolyte homeostasis, fluid volume, and arterial blood pressure by the production of the potent vasopressor and aldosteronogenic octapeptide angiotensin II. The great success of angiotensin converting enzyme inhibitors in the treatment of hypertension and congestive heart failure[1] provided the impetus to look for alternate approaches to interfering with the RAS, by inhibition of renin[2] and antagonism of angiotensin II at the receptor level.[3] Renin is the enzyme that catalyzes the first and rate-limiting step in the ultimate production of angiotensin II. The high substrate specificity of renin led to an intensive search for effective inhibitors. Most of the potent renin inhibitors have been developed by starting from peptides which correspond to the sequence around the cleavage site of angiotensinogen and by replacing the scissile Leu^{10}-Val^{11} dipeptide by nonhydrolyzable transition-state mimetics.

These inhibitors remain partly peptidic and their therapeutic efficacy is limited due to proteolytic instability, poor resorption, and rapid biliary excretion.[4] Therefore, we initiated a synthesis program to overcome these limitations by reducing the peptide character and molecular size of the inhibitors.

As one aspect of our renin inhibitor strategy, we chose to prepare modified compounds of small peptides, such as I and II,[5] containing the statine analogue ACHPA (4-

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