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Preliminary communication

Guanabenz-related amidinohydrazones: potent non-azole inhibitors of aldosterone biosynthesis

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Summary — A new series of potent, guanabenz-derived, non-steroidal aldosterone biosynthesis inhibitors are presented. Salient features of the structure–activity relationship indicate the requirement of a hydrophobic core, presence of a hydrophilic (or basic) peripheral appendage, and, in some cases, profound dependence on hydrazone stereochemistry. The most potent compound of the series, **29**, was 2 orders of magnitude more potent than guanabenz as an aldosterone biosynthesis inhibitor.

aldosterone / biosynthesis / inhibition / guanabenz

Introduction

Aldosterone **6**, the most potent mineralocorticoid hormone responsible for volume expansion through water retention and sodium retention, contributes to the pathophysiology of congestive heart failure [1]. In excess, aldosterone leads to hypertension and produces oedema formation. Accordingly, antialdosterone therapy in patients with secondary hyperaldosteronism is directed to one or more of 3 goals: a) antagonism of aldosterone at its receptor site; b) inhibition of aldosterone secretion stimuli; and c) reduction of aldosterone levels through synthesis inhibition. To explore further the potential of identifying an aldosterone biosynthesis inhibitor, we have prepared and evaluated a series of guanabenz-related, non-azole, non-steroidal inhibitors, which forms the basis of this paper.

The biosynthesis of the mineralocorticoid aldosterone **6** proceeds from cholesterol **1** by a series of sequential oxidations (fig 1) that is stimulated physiologically by peptide hormones (*eg* angiotensin II,

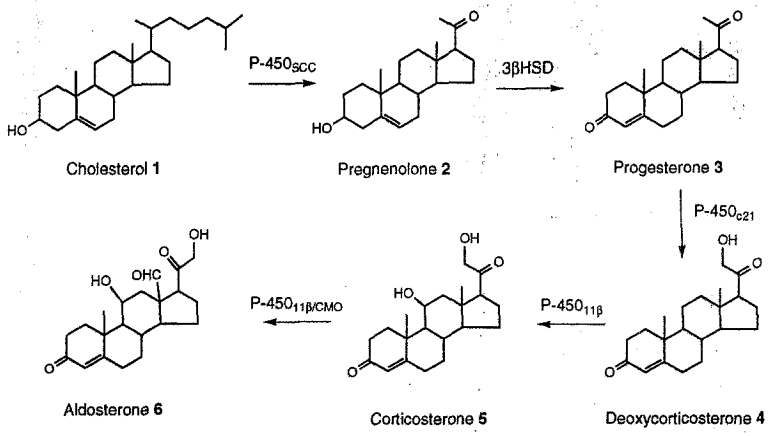


Fig 1. Aldosterone biosynthesis from cholesterol.

ACTH), monovalent cations (*eg* K^+), amines (*eg* serotonin, histamine), and prostaglandins [2]. One natural endogenous inhibitor of aldosterone biosynthesis is the 28 amino-acid polypeptide atrial natiuretic factor (ANF) [3], which potently inhibits the formation of pregnenolone **2** and the conversions of progesterone **3** to deoxycorticosterone **4**, **4** to corticosterone **5**, and **5** to **6** as summarized in table I. The synthetic pathway is also inhibited by the α_2-adrenergic receptor agonist guanabenz **7** (fig 2). The effects of guanabenz occur at much higher concentrations than those necessary for inhibition by ANF and appear to be independent of its α_2-adrenergic receptor agonist properties since clonidine **9** fails to inhibit aldosterone secretion as determined in a rat adrenal preparation [4].

We wished to amplify further the aldosterone secretion inhibitory activity of **7**. Through systematic modifications about the hydrazone moiety, we have identified a series of potent inhibitors of aldosterone secretion and have compared *in vitro* activity to other inhibitors of aldosterone secretion.

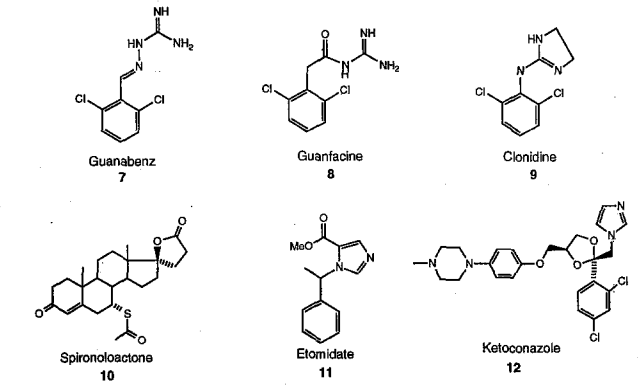
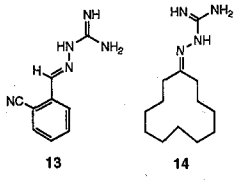


Fig 2. Structures of standards used in this study.



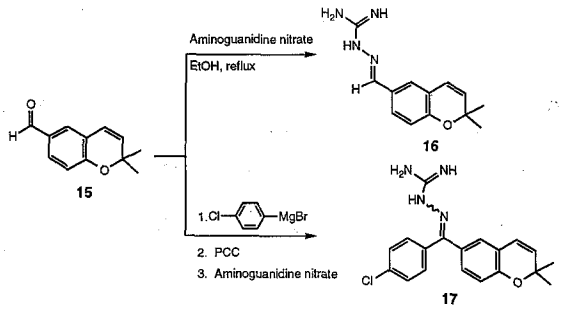


Fig 3. Syntheses of **13**, **14**, **16** and **17**.

Chemistry

The syntheses of compounds for this study are shown in figures 3–5. In general, hydrazone derivatives of aldehydes were obtained as single isomers (*E*) whereas those of non-symmetrical ketones were obtained as mixtures of *E* and *Z* isomers of varying compositions. In selected cases, the isomers were separated by either selective crystallization of the corresponding salt or by flash chromatography of the free base. Stereochemical assignments were based on NOE experiments on the corresponding HCl salts.

Compounds **13** and **14** were prepared from commercially available carbonyl precursors. Analog **16** was derived from the known chromene **15** [6, 7]. Addition of *p*-chlorophenylmagnesium bromide to **15**, oxidation with pyridinium chlorochromate (PCC), and hydrazone formation provided **17**.

Trifluoromethylphenyl isomers **21** and **22** were prepared from **18** [5] by the sequence: a) metal-halogen exchange of **18** with *t*-butyllithium at –78°C followed by addition of freshly prepared **19** provided alcohol **20**; b) oxidation with PCC; c) hydrazone formation with aminoguanidine nitrate; and d) selective crystallization of the HCl salts. Compound **24**, a

Table I. Inhibition of aldosterone biosynthesis by atrial natiuretic factor and guanabenz [3].

Conversion steps	*Inhibition by atrial natiuretic factor (ED_{50})*	*Inhibition by guanabenz (ED_{50})*
1 to **2**	114 pM	66 μM
3 to **4**	199 pM	1.6 μM
4 to **5**	14 pM	3.3 μM
5 to **6**	92 pM	29 μM

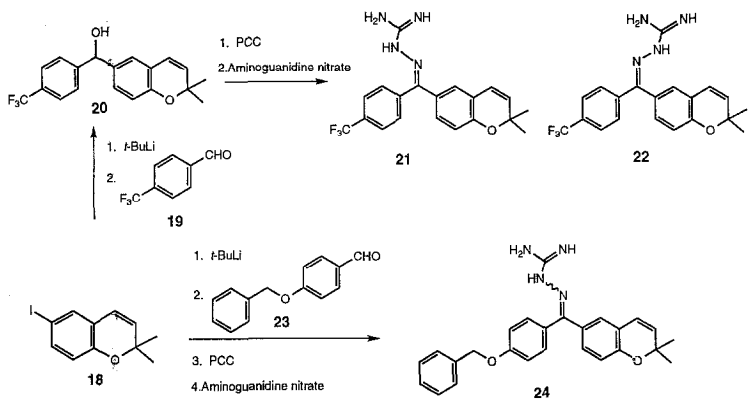


Fig 4. Syntheses of **21**, **22** and **24** from chromene **18**.

mixture of isomers, was prepared in a similar fashion using aldehyde **23**. This approach was also employed for the preparation of pyridinyl isomers **29** and **30** except that oxidation of **27** to **28** was effected with MnO_2 and the individual isomers were separated by flash chromatography prior to salt formation. This sequence was also used to prepare isomers **34** and **35**. Compounds **32** and **37** were prepared as mixtures. The known aldehydes **26**, **31**, and **33** [8] were conveniently prepared by the Mitsunobu reaction [9] from the corresponding heterocyclic carbinols and hydroxybenzaldehydes.

Imidazole analog **38** was prepared *via* Mitsunobu reaction [8] of **27** with imidazole (fig 6). Imidazoline **39** was synthesized from ketone **28** as was semicarbazone **40**; both compounds were obtained as isomeric mixtures.

Results and discussion

The aldosterone biosynthesis inhibitory activity of the compounds in this study were evaluated in an isolated rat adrenal glomerulosa cell preparation in which

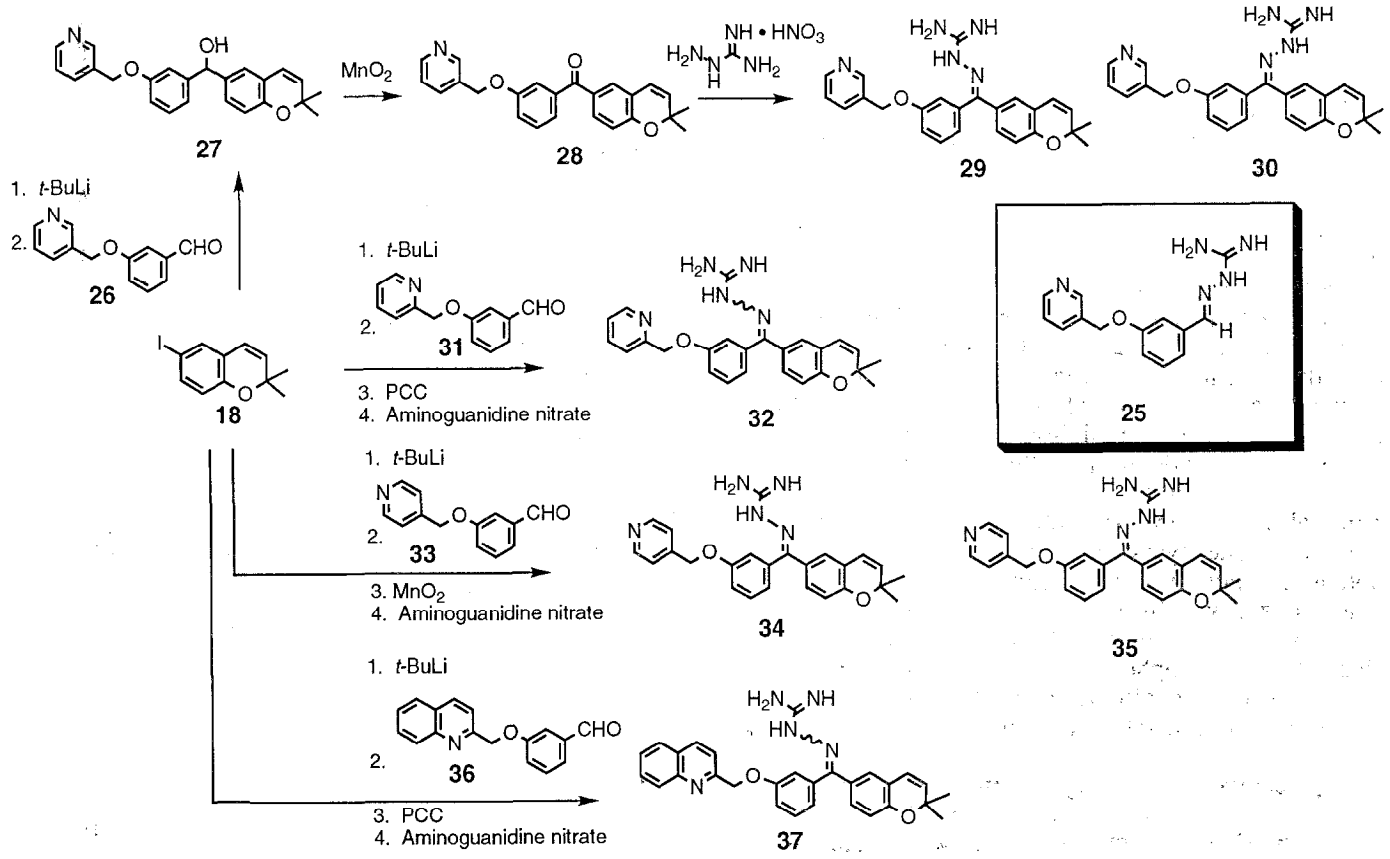


Fig 5. Preparation of compounds **25**, **29**, **30**, **32**, **34**, **35** and **37** from chromene **18**.

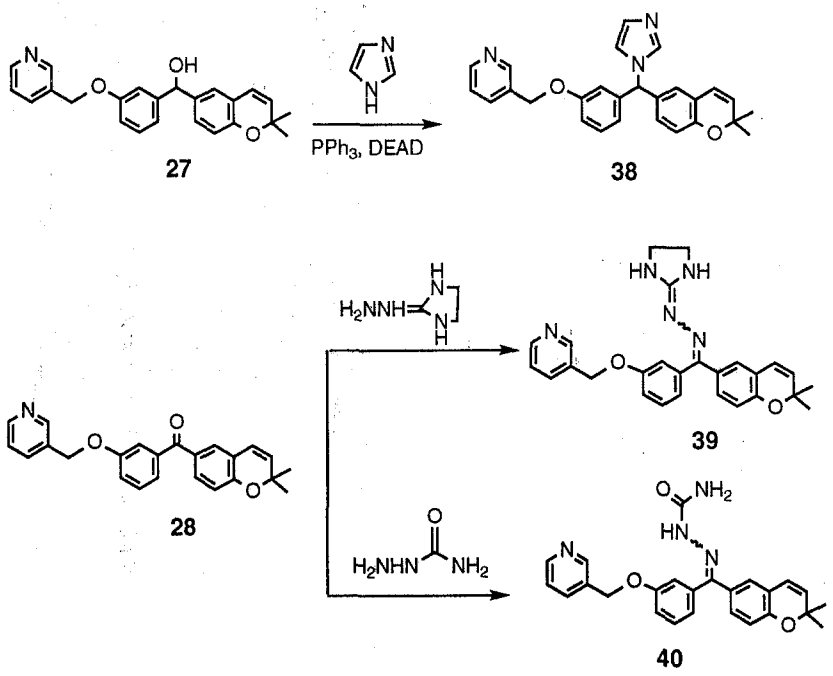


Fig 6. Methylenehydrazinecarboximidamide replacement of **29** and **30**.

aldosterone biosynthesis was stimulated by angiotensin-II in the presence or absence of test compounds. Aldosterone production was then quantified using a commercial aldosterone radioimmunoassay kit. Aldosterone inhibition was expressed as a percentage of the control (angiotensin-II stimulated aldosterone biosynthesis) and IC_{50} values were determined from the dose–response curve.

As shown in table II, guanabenz **7** inhibited aldosterone secretion (IC_{50} 6.2 μM). This property appears to be independent of α_2-adrenergic activity since guanfacine **8** was considerably less potent than **7** and clonidine **9** exhibited little inhibitory activity at 5 μM.

It was quickly determined that the nuclear substitution about the aryl ring influenced potency. Thus, removal of both lipophilic chloro groups and replacement with one cyano produced **13**, a markedly less potent compound (16% inhibition at 5 μM) than guanabenz. It was further established that the aromatic group was not a requirement for aldosterone inhibition since the dichlorophenyl group could be substituted by a nonaryl, cyclododecanone template (*ie* **14**) to provide significant enhancement of potency (IC_{50} 1.9 μM).

Replacement of the aryl group with a chromene nucleus gave **16**, which was less potent (30% inhibition at 5 μM) than guanabenz; however increasing lipophilicity of **16** to **17** (IC_{50} 1.3 μM) produced a compound that was clearly more potent than guana-

Table II. Inhibition of aldosterone biosynthesis in isolated rat adrenal glomerulosa cells.

Compound	*IC_{50} (μM) or (% inhibition at 5 μM)*	*Compound*	*IC_{50} (μM) or (% inhibition at 5 μM)*
Guanabenz **7**	6.2	**24**	0.63
Guanfacine **8**	36	**25**	1.0
Clonidine **9**	(0)	**29**	0.016
Spironolactone **10**	1.9	**30**	0.10
Etomidate **11**	0.165	**32**	0.27
Ketoconazole **12**	0.84	**34**	0.15
13	(16)	**35**	0.56
14	1.9	**37**	0.50
16	(30)	**38**	0.08
17	1.3	**39**	0.050
21	0.84	**40**	0.15
22	1.5		

benz. Additional replacement of the chloro substituent with the more lipophilic trifluoromethyl group (**21** and **22**) and benzyloxy moiety **24** had only modest effects. Small, but notable, differences in potencies were seen with the individual trifluoromethyl isomers **21** (IC_{50} 0.84 μM) and **22** (IC_{50} 1.5 μM).

Since little enhancement of potency was observed with **21**, **22**, and **24** *vs* **17**, we explored more hydrophilic appendages disposed about the periphery of the hydrophobic core. Replacement of the benzyloxy group of **24** with pyridinyl (**29**, **30**, **32**, **34**, **35**) and quinolinyl heterocycles (**37**) resulted in further enhancement of aldosterone biosynthesis inhibitory potency. In particular, the 3-pyridinyl nucleus produced a dramatic increase in potency. Furthermore the potency of the individual isomers **29** (IC_{50} 0.016 μM) and **30** (IC_{50} 0.10 μM) was clearly dependent on hydrazone stereochemistry. The profound effect of the combined lipophilic core and pyridinyl appendage is clearly seen by comparison of the potencies of the individual components **16** (30% inhibition at 5 μM) and **25** (IC_{50} 1.0 μM).

Compounds **38–40** were designed to examine the role of the guanidino head piece on the inhibition of aldosterone biosynthesis in 3-pyridinyl series. Replacement of the guanidino head with either imidazole **38** (IC_{50} 0.08 μM) or semicarbazone **40** (mixture; IC_{50} 0.15 μM) produced potent inhibitors. Imidazoline **39** (mixture of isomers) was approximately equipotent to the mixture of **29** and **30**.

In many respects the compounds of this study bear structural similarities to many P-450-dependent steroid biosynthesis inhibitors that have emerged for the treatment of a number of steroid-dependent diseases (prostatic carcinoma, breast cancer, hypercortisolism, and benign prostatic hyperplasia) [10]. For example, agents such as CGS 18320 **41**, econazole **42**, liarozole **43**, fadrozole **44**, and vorozole **45** [10] possess a basic group coupled to a hydrophobic core (fig 7), analogous to the compounds of the present study. Many of these known agents inhibit aldosterone biosynthesis. As shown in table II, ketoconazole **12** inhibited aldosterone biosynthesis (IC_{50} 0.84 μM), consistent with its reported inhibitory action on 11β-hydroxylase [11]. Fadrozole and CGS 18320 have been reported to inhibit aldosterone secretion (IC_{50}s 1 μM and 6.1 μM, respectively) in a rat adrenal preparation [10]. Etomidate **11**, another known 11β-hydroxylase inhibitor 12], potently inhibited aldosterone biosynthesis as shown in table II.

The compounds of our study are distinguished from known aldosterone biosynthesis inhibitors in 2 respects. Firstly, they lack a steroidal nucleus found in the aldosterone receptor antagonist/biosynthesis inhibitor spironolactone **10** [13] and in other steroidal inhibitors of aldosterone biosynthesis inhibitors [14, 15]. Secondly, the methylenehydrazinecarboximidamide grouping appears to be a novel imidazole replacement in our series.

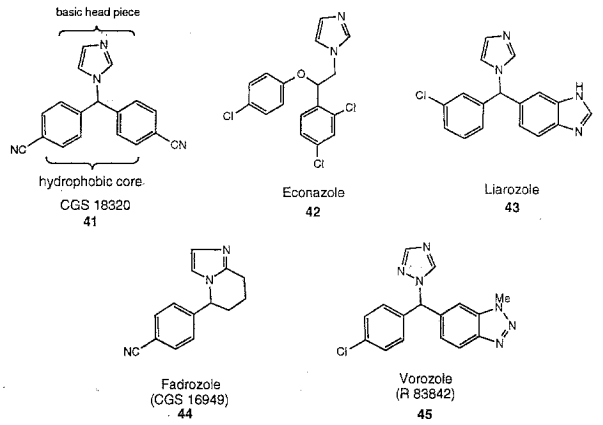


Fig 7. Nonsteroidal inhibitors of P-450-dependent steroid biosynthesis.

In summary, we have described a new series of potent, guanabenz-derived aldosterone biosynthesis inhibitors. Salient features of the structure–activity relationship indicates the requirement of a hydrophobic core, presence of a hydrophilic (or basic) peripheral appendage, and, in some cases dependence on hydrazone stereochemistry. Further studies are needed to elucidate the mechanism of action of this intriguing series.

Experimental protocols

Chemistry

2-[(2-Cyanophenyl)methylene]hydrazinecarboximidamide ***13*** *hydrochloride*

A solution of 1.99 g (15.2 mmol) 2-cyanobenzaldehyde and 6.24 g (45.6 mmol) aminoguanidine nitrate in 50 ml EtOH was refluxed for 2 h. The reaction mixture was cooled to room temperature, quenched with 1 N NaOH (to pH 13), and extracted into THF/CH_2C1_2. The combined organic extracts were washed with brine (3 x), dried (K_2CO_3), and concentrated. The crude product was crystallized from $MeOH/Et_2O$/petroleum ether to give 1.82 g (64%) **13** as the free base. This was dissolved in MeOH, treated with 4 ml 4 N ethanolic HCl, and concentrated to dryness. The solid was recrystallized from MeOH/petroleum ether to give 1.95 g (90%) of the title compound, mp 196–198°C. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.32 (s, 1 H), 8.46 (s, 1 H), 8.37 (d, 1 H), 7.92 (d, 1 H), 7.78 (t, 1 H), and 7.62 ppm (t, 1 H); IR (KBr) 2220 cm^{-1}; mass spectrum (EI), *m/e* 187, 145. Anal $C_9H_{10}ClN_5$ (H, N); C: Calc 48.33; Found 47.60.

2-Cyclododecylidenehydrazinecarboximidamide ***14*** *hydrochloride*

A mixture of 2.0 g (11.0 mmol) cyclododecanone, 1.7 g (12.4 mmol) aminoguanidine nitrate and 20 μl HNO_3 in 30 ml of EtOH was stirred at 70°C for 24 h. The reaction mixture was

cooled to room temperature and was triturated with approximately 30 ml Et_2O/petroleum ether (1:1) to give 3.20 g of a colorless powder. This was added to excess 1 N NaOH and extracted into 10% THF/Et_2O. The organic phase was washed with water, dried (K_2CO_3), and concentrated. The crude material was dissolved in 30 ml THF/CH_2Cl_2 and treated with 4 ml 2.5 M ethanolic HCl. The slurry was diluted further with ether/petroleum ether to give 2.3 g (77%) of the title compound as a colorless powder, mp 220–221°C. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 11.0 (s, 1 H), 7.44 (bs, 4 H), 2.38 (t, 2 H), 2.31 (t, 2 H), 1.70 (m, 2 H), 1.55 (m, 2 H), and 1.2–1.3 ppm; mass spectrum (EI), *m/e*, 238; Anal $C_{13}H_{27}ClN_4$ (C, H, N).

*2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)methylene]hydrazinecarboximidamide **16** hydrochloride*

A mixture of 1.09 g (5.8 mmol) 2,2-dimethyl-2*H*-1-benzopyran-6-carboxaldehyde **15** and 1.1 g (6.2. mmol) aminoguanidine nitrate in 50 ml EtOH was refluxed for 1 h. The reaction was cooled to room temperature, diluted with 200 ml 10% THF/CH_2Cl_2 and washed with brine (3 x 70 ml). Drying and concentration gave 1.15 g (81%) of a tan powder, which was then dissolved in MeOH/CH_2Cl_2 and treated with excess 4 N ethanolic HCl. Removal of solvent *in vacuo* gave the title compound, mp 102–105°C. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 11.75 (s, 1 H), 8.04 (s, 1 H), 7.60 (d, 1 H), 7.58 (s, 1 H), 6.79 (d, 1 H), 6.40 (d, 1 H), 5.82 (d, 1 H), and 1.38 ppm (s, 6 H); mass spectrum (EI, free base), *m/e* 244, 229, 170; Anal $C_{13}H_{17}ClN_4O$ (H), C: Calc 55.61; Found 55.12; N, Calc 19.96; Found 18.29.

*2-[(4-Chlorophenyl)(2,2-dimethyl-2H-1-benzopyran-6-yl)-methylene]hydrazinecarboximidamide **17** hydrochloride*

At 0°C to 640 mg (3.4 mmol) 2,2-dimethyl-2*H*-1-benzopyran-6-carboxaldehyde **15** in 25 ml Et_2O was added 4.4 ml (4.4 mmol) *p*-chlorophenylmagnesium bromide (1.0 M in Et_2O). The cold bath was removed and stirring was continued for 15 min. The reaction mixture was quenched with water and 20 ml of 1 N HCl. The reaction mixture was extracted into Et_2O (100 ml) and the organic phase was washed with saturated sodium bicarbonate, dried (K_2CO_3), and then concentrated to give 1.16 g of crude product which was then used directly in the next reaction.

The crude material was dissolved in CH_2Cl_2 (30 ml), and was then treated with 1.1 g (5 mmol) of pyridinium chlorochromate. After stirring at 35°C for 1 h, the reaction mixture was diluted with Et_2O and then filtered through a thick pad of silica gel (Et_2O elution). Concentration and flash chromatography CH_2Cl_2/petroleum ether (1:2, then 1:1, then 100:0) gave 836 mg (82% yield) of product which was used in the next reaction. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 7.71 (d, 2 H), 7.58 (dd, 1 H), 7.49 (d, 1 H), 7.45 (d, 2 H), 6.82 (d, 1 H), 6.34 (d, 1 H), 5.68 (d, 1 H), and 1.48 ppm (s, 6 H); IR (KBr) 1635 cm^{-1}; mass spectrum (EI) *m/e* 298 and 300, 283 and 285.

A solution of 810 mg (2.7 mmol) of ketone in EtOH containing 418 mg (3.1 mmol) aminoguanidine nitrate and 10 µl concentrated HNO_3 was stirred at 50°C overnight, then at 90°C for 8 h. The reaction mixture was then stirred at 60°C for 2 d to effect completion. The reaction mixture was basified with 2.5 N NaOH, extracted into 20% THF/CH_2Cl_2, dried (K_2CO_3), and flash chromatographed (CH_2Cl_2/MeOH/NH_4OH) (90:7:3, then 85:10:5) to give 898 mg of product as an orange powder. The product (885 mg) was dissolved in CH_2Cl_2 and then treated with 1.1 µl of 2.5 N ethanolic HCl. Trituration into Et_2O/petroleum ether gave a total of 879 mg of a broad melting colorless powder (mixture of isomers by NMR), mp 163–192°C (dec); ^{1}H-NMR (DMSO-d_6; 400 MHz; partial) δ 9.94 and 9.76 (s, 1 H), 6.44 and 6.38 (d, 1 H), 5.83 and 5.79 (d, 1 H), 1.44 and 1.37 ppm (s, 6 H); IR (KBr) 3120, 1665, 1610, and 1580 cm^{-1}; mass spectrum (EI, free base), *m/e* 355 and 357. Anal $C_{19}H_{20}Cl_2N_4O$ (C, H, N).

*4-Trifluoromethylbenzaldehyde **19***

To 25.8 g (0.147 mol) 4-trifluoromethylbenzyl alcohol in 250 ml CH_2Cl_2 was added 45 g (0.21 mol) pyridinium chlorochromate. After stirring for 90 min, the reaction mixture was diluted with Et_2O (1000 ml) and was passed through a thick pad of Celite 545. The filtrate was washed with 2 N HCl. The organic phase was dried ($MgSO_4$) and concentrated to give 23.1 g (90% yield) of the title compound, which was used immediately due to its sensitivity to air oxidation. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 10.1 (s, 1 H), 7.87 (d, 2 H), and 7.28 ppm (d, 2 H).

*2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[4-(trifluoromethyl)-phenyl]methylene]hydrazinecarboximidamide **21** and **22** hydrochloride salts*

At –78°C to 19.2 g (67 mmol) of 2,2-dimethyl-6-iodo-2*H*-benzopyran [5] in 600 ml of anhydrous THF was added slowly 130 ml (0.221 mol) of *t*-butyllithium (1.7 M in pentane) over 20 min. After stirring at –78°C for 35 min, 23 g (0.13 mol) of **19** in 100 ml of THF was added. The cold bath was removed and stirring was continued for 1 h. The reaction mixture was quenched with saturated NaCl, extracted into Et_2O, dried ($MgSO_4$), and flash chromatographed (CH_2Cl_2, then 3% ether/CH_2Cl_2, then 5% Et_2O/CH_2Cl_2) to give 30.1 g **20** which was used directly in the next reaction.

An analytical sample of **20**, was obtained as a colorless gum. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 7.58 (d, 2 H), 7.50 (d, 2 H), 7.05 (dd, 1 H), 6.92 (d, 1 H,), 6.72 (d, 1 H), 6.26 (d, 2 H), 5.79 (d, 1 H), 5.61 (d, 1 H), 2.18 (d, 1 H), and 1.41 ppm (s, 6 H); IR ($CHCl_3$) 3590 cm^{-1}; mass spectrum (EI) *m/e* 334, 319, 302. Anal $C_{19}H_{17}F_3O_2$ (C, H).

A mixture of the 30.1 g **20** and 31 g pyridinium chlorochromate in CH_2Cl_2 (250 ml) was stirred at room temperature for 40 min. Another 5 g pyridinium chlorochromate was added and the reaction mixture was stirred for another 20 min to effect total oxidation. The reaction mixture was passed through a thick pad of solka floc (Et_2O rinse) and the filtrate was concentrated. The residue was flash chromatographed (CH_2Cl_2/petroleum ether (3: 2)) to give 17.8 g of ketone. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 7.84 (d, 2 H), 7.73 (d, 2 H), 7.59 (dd, 1 H), 7.51 (d, 1 H; J = 2.6 Hz), 6.82 (d, 1 H), 6.34 (d, 1 H), 5.69 (d, 1 H), 1.49 (s, 3 H), and 1.47 ppm (s, 3 H).

A mixture of 17.8 g (53.6 mmol) of ketone and 26 g aminoguanidine nitrate in ethanol (400 ml) containing 2 ml concentrated HNO_3 was stirred at 70°C for 6 d, then at room temperature for 5 d. The reaction mixture was basified with 1 N NaOH, and extracted into Et_2O (2 x). The combined organic phases were washed with water (2 x 300 ml) and then brine (2 x 300 ml), dried (K_2CO_3), and concentrated to give 19.9 g of a mixture of hydrazone isomers (96% yield). The material was dissolved in CH_2Cl_2 (100 ml), treated with 41 ml 2 M ethanolic HCl, and then diluted with a mixture of Et_2O (400 ml) and petroleum ether (500 ml). The solvent was removed *in vacuo*, the oily residue was dissolved in MeOH (150 ml), diluted with Et_2O (300 ml) and then petroleum ether (700 ml). After stirring for 20 min, the less polar of the 2 isomers (as determined by tlc using Et_2O/CH_2Cl_2/MeOH/NH_4OH (50:43:5:2)) deposited selectively (9.9 g of nearly pure isomer). Pure isomer **21** was obtained by dissolving the salt into a small amount of a mixture of CH_2Cl_2 and MeOH and then triturating into a solution of Et_2O and petroleum ether. This procedure was repeated several

times to afford 7.74 g of pure **21** as a coloress powder, mp > 250°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.83 (s, 1 H), 7.97 (d, 2 H), 7.58 (d, 2 H), 7.43 (s, 1 H,), 7.24 (dd, 1 H), 6.74 (d, 1 H), 6.39 (d, 1 H), 5.79 (d, 1 H), and 1.37 ppm (s, 6 H); IR (KBr) 3200 (br), 1670, 1610, and 1580 cm^{-1}; mass spectrum (FAB), *m/e* 389. Anal $C_{20}H_{20}ClF_3N_4O$ (C, H, N).

The more polar of the 2 isomers began to crystallize after the mother liquors became enriched in this material (9.6 g isolated). A final purification was achieved by dissolving the material in a little MeOH/CH_2Cl_2 mixture and then triturating with Et_2O to provide pure isomer **22** as a colorless powder, mp 208–211°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 10.1 (s, 1 H), 7.86 (d, 2 H), 7.75 (d, 2 H), 7.09 (dd, 1 H), 7.08 (d, 1 H,), 6.95 (d, 1 H), 6.45 (d, 1 H), 5.84 (d, 1 H), and 1.44 ppm (s, 6 H); IR (KBr) 3200 (br), 1670, 1605, and 1580 cm^{-1}; mass spectrum (FAB), *m/e* 389. Anal $C_{20}H_{20}ClF_3N_4O$ (C, H, N).

2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[4-(phenylmethoxy)-phenyl]methylene]hydrazinecarboximidamide ***24*** *hydrochloride*

At –78°C to 1.51 g (5.28 mmol) 2,2-dimethyl-6-iodo-2*H*-benzopyran **18** in THF (15 ml) was added slowly 7 ml (11.9 mmol) *t*-butyllithium (1.7 M in pentane)). After stirring for 5 min at –78°C, a solution of 1.3 g (6.1 mmol) of **23** in THF (10 ml) was added. The cold bath was removed and stirring was continued for 45 min. The reaction mixture was extracted into Et_2O, and the combined organic phases were dried (K_2CO_3) and concentrated. The residue was then dissolved in CH_2Cl_2 (50 ml) and treated with 2 g of pyridinium chlorochromate. After stirring for 1 h at ambient temperature, the reaction mixture was diluted with Et_2O and passed through a thick pad of silica gel (Et_2O elution). Concentration and purification of the residue by flash chromatography (CH_2Cl_2/petroleum ether (3:1), then Et_2O) gave 418 mg of ketone as an orange oil, which was used in the next reaction. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 7.78 (d, 2 H), 7.2–7.6 (m, 9 H), 7.03 (d, 2 H), 6.81 (d, 1 H), 6.35 (d, 1 H), 5.67 (d, 21 H), S.15 (s, 2 H), and 1.50 ppm (s, 6 H).

A mixture of ketone (418 mg (1.13 mmol) and 400 mg of aminoguanidine nitrate in EtOH (10 ml) containing 20 µl of concentrated HNO_3 was heated at 70°C for 16 h. The reaction mixture was basified with 2.5 N NaOH, diluted with water and then extracted into 10% THF/CH_2C_2. The organic extract was dried (K_2CO_3) and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH (85:10:5) to give 323 mg of an orange foam. The product was dissolved in CH_2Cl_2 and treated with 1 ml of 2.5 M ethanolic HCl. Repeated concentration from Et_2O/petroleum ether gave the title compound as a tan powder (mixture of isomers as determined by NMR), mp 118°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz, partial) δ 9.7 and 9.66 (s, 1 H), 6.93 and 6.74 (d, 1 H), 6.45 and 6.37 (d, 1 H), 5.83 and 5.81 (d, 1 H), 5.18 and 5.14 (s, 2 H), and 1.44 and 1.37 ppm (s, 6 H); IR (KBr) 310 (br), 1660, 1605, and 1575 cm^{-1}; mass spectrum (EI) *m/e* 426, 335, and 91. Anal $C_{26}H_{27}ClN_4O_2$·0.5 H_2O (C, H, N).

2-[[3-(3-Pyridinylmethoxy)phenyl]methylene]hydrazine-carboximidamide ***25*** *dihydrochloride*

A solution of 1.3 g (6.1 mmol) **26** in 40 ml EtOH containing 2.51 g (18.3 mmol) of aminoguanidine nitrate and 5.45 ml (6.1 mmol) 1.12 M ethanolic HBr was refluxed for 1.5 h. The reaction mixture was cooled to room temperature, quenched with 100 ml 1 N NaOH, and extracted into CH_2Cl_2. The combined organic extracts were dried (K_2CO_3) and concentrated to give 1.48 g crude product, which was combined with the crude product (0.269 g) from another experiment using 201 mg (0.944 mmol) of **26**. The material was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH (86.5:9:4.5) to give 1.12 g (59%) of **25**. The free base was converted into the title compound by dissolving 1.02 g of **25** (3.78 mmol) in CH_2Cl_2 (50 ml) and treating with 2.52 ml (7.56 mmol) 3 M ethereal HCl. Removal of solvent *in vacuo* gave the title compound (1.19 g), mp 91–105°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 12.16 (s, 1 H), 8.98 (s, 1 H), 8.84 (d, 1 H), 8.50 (d, 1 H). 8.15 (s, 1 H), 7.96 (dd, 1 H), 7.66 (s, 1 H), 7.42 (d, 1 H), 7.38 (t, 1 H), and 7.13 ppm (brd, 1 H); mass spectrum (CI), *m/e* 270, 228. Anal $C_{14}H_{17}Cl_2N_5O$·0.5 H_2O (C, H, N).

3-(3-Pyridinylmethoxy)benzaldehyde ***26***

At 0°C to a solution of 20.1 g (0.165 mol) 3-hydroxybenzaldehyde, 19.8 g (0.181 mol) 3-pyridylcarbinol, and 47.5 g (0.181 mol) triphenylphosphine in THF (700 ml) was added slowly 28.7 ml (0.181 mol) diethyl azodicarboxylate. The reaction mixture was stirred overnight (6 h) to room temperature. Since tlc analysis indicated incomplete consumption of phenol, the reaction mixture was cooled to 0°C, and treated sequentially with 3-pyridylcarbinol (16.0 ml), triphenylphosphine (43.2 g), and diethyl azodicarboxylate (26.1 ml). The reaction mixture was then allowed to stir to room temperature over 16 h. The reaction mixture was quenched with 2.5 N NaOH, and extracted into Et_2O. The organic extracts were washed with water (3 x), and then was extracted into 1 N HCl. The aqueous extracts were washed with Et_2O (3 x), basified with 50% NaOH, and extracted into Et_2O. The organic extracts were dried (K_2CO_3) and concentrated to give 28.6 g crude product, which was purified *via* preparative HPLC using a gradient of EtOAc/hexane (20:80, to 100:0). This provided 7.0 g of pure 3-(3-pyridinylmethoxy)benzaldehyde. An analytical sample was prepared as its HCl salt, mp 144–148°C, by dissolving a 1.1 g of the aldehyde in CH_2Cl_2, treating with 1.9 ml 3 M ethereal HCl, and trituration into Et_2O. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.99 (s, 1 H), 9.00 (s, 1 H), 8.85 (s, 1 H), 8.55 (d, 1 H), 7.99 (dd, 1 H), 7.54–7.60 (m, 3 H), 7.39–7.44 (m, 1 H), and 5.41 ppm (s, 2 H); IR (KBr) 2370, and 1680 cm^{-1}; mass spectrum, *m/e* 213. Anal $C_{13}H_{12}ClNO_2$ (C, H, N).

2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(3-pyridinylmethoxy)-phenyl]methanone ***28***

At –78°C to 31.5 g (0.110 mol) **18** in THF (300 ml) was added slowly 112 ml (0.190 mol) *t*-butyllithium (1.7 M in pentane). After stirring at –78°C for 1 h, another 12 ml *t*-butyllithium (1.7 M in pentane) was added. After stirring at –78°C for 15 min, the reaction mixture was transferred *via* cannula to a solution of 21.4 g (0.100 mol) **26** in THF (200 ml) at –78°C. The cold bath was removed and stirring was continued for 1 h. The reaction mixture was quenched with water (1 l) and extracted into CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated. Purification was achieved by flash chromatography CH_2Cl_2/MeOH/NH_4OH (95.5:3:1.5) to afford 19.9 g of pure **27** as a foam, as well as 6.32 g of impure material. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 8.68 (d, 1 H, J = 2 Hz), 8.59 (dd, 1 H, J = 2.5 Hz), 7.83 (dd, 1 H), 7.2–7.5 (m, 3 H), 6.9–7.1 (m, 5 H), 6.88 (dd, 1 H), 6.72 (d, 1 H), 6.27 (d, 1 H), 7.73 (s, 1 H), 5.59 (d, 1 H), 5.07 (s, 2 H), and 1.41 ppm (s, 6 H).

To a solution of 19.9 g (53 mmol) **27** in CH_2Cl_2 (500 ml) was added 46 g (0.53 mol) of MnO_2. After stirring at reflux for 16 h, the reaction mixture was filtered through a thick pad of silica gel (5% THF/CH_2Cl_2 elution) and concentrated to give 15.2 g (77% yield) of crude **28**, which was used without further purification. An analytical sample was characterized as its HCl salt, mp 171–174°C, by dissolving 1.6 g **28** in CH_2Cl_2, and

treating with 3.3 ml of 1.44 M ethereal HCl. The solvent was removed *in vacuo*. The residue was dissolved in a MeOH/CH_2Cl_2 mixture and then triturated into ether at 0°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 8.96 (s, 1 H), 8.83 (d, 1 H), 8.49 (d, 1 H), 7.96 (dd, 1 H), 7.4–7.6 (m, 3H), 7.2–7.6 (m, 3H), 6.86 (d, 1H), 6.51 (d, 1 H), 5.84 (d, 1 H), 5.37 (s, 2 H), and 1.12 ppm (s, 6 H); IR (KBr) 3540 (br), 2510 (br), 1645, 1630, and 1595 cm^{-1}. Anal $C_{24}H_{22}ClNO_3 \cdot 0.25\ H_2O$ (C, H, N).

*2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(3-pyridinylmethoxy)-phenyl]methylene]hydrazinecarboximidamide **29** and **30** dihydrochloride*

A solution of 10.2 g (27.6 mmol) **28** in EtOH (200 ml) containing 9.44 g (69 mmol) aminoguanidine nitrate and 2.2 ml concentrated HNO_3 was stirred at reflux for 8 h. Another 1 ml of concentrated HNO_3 was added and stirring was continued at reflux for 16 h. The reaction mixture was quenched with 750 ml of 2.5 N NaOH at room temperature and then extracted into 10% THF/CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated. Flash chromatography (CH_2Cl_2/MeOH/NH_4OH (91:6:3)) gave 4.21 g of the less polar isomer **29** (as determined by tlc (CH_2Cl_2/MeOH/ammonium hydroxide (85:10:5), 4.12 g of the more polar isomer, **30**, and 5.61 g of mixed fractions. The isomers were then obtained as salts by treating a CH_2Cl_2 solution of the free bases with 2 equivalents of HCl (3 M ethereal HCl) and then removing solvent *in vacuo*. Analytical data for **29**, mp 180–185°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.91 (s, 1 H), 8.92 (s, 1 H), 8.81 (dd, 1 H), 8.41 (d, 1 H), 7.90 (dd, 1 H), 7.43 (t, 1 H, J = 2 Hz), 7.33 (t, 1 H), 7.14 (dd, 1 H), 7.08 (d, 1 H), 7.0–7.05 (m, 2 H), 6.92 (d, 1 H), 6.45 (d, 1 H), 5.82 (d, 1 H), 5.33 (s, 2 H), and 1.44 ppm (s, 6 H); IR (KBr) 3179, 3250, 3150, 1665, 1615, and 1585 cm^{-1}; mass spectrum, *m/e* 428. Anal $C_{25}H_{27}Cl_2N_5O_2 \cdot H_2O$ (C, H, N). Analytical data for **30**, mp 148–153°C. ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.79 (s, 1 H), 8.93 (s, 1 H), 8.81 (d, 1 H), 8.47 (d, 1 H), 7.93 (dd, 1 H), 7.56 (t, 1 H), 7.45 (d, 1 H, J = 2 Hz), 7.2–7.3 (m, 2 H), 7.02 (s, 1 H), 6.89 (d, 1 H), 6.73 (d, 1 H), 6.36 (d, 1 H), 5.79 (d, 1 H), 5.37 (s, 12 H), and 1.37 ppm (s, 6 H); IR (KBr) 3360, 1675, 1610, and 1575 cm^{-1}; mass spectrum (CI), *m/e* 428. Anal $C_{25}H_{27}Cl_2N_5O_2 \cdot H_2O$ (C, H, N).

*3-(2-Pyridinylmethoxy)benzaldehyde **31***

At 0°C to 3.0 g (24.6 mmol) 3-hydroxybenzaldehyde, 2.4 ml (24.7 mmol) 2-pyridylcarbinol, and 6.44 g (24.6 mmol) triphenylphosphine in 50 ml THF was added slowly 4.1 ml (26 mmol) diethyl azodicarboxylate. The cold bath was removed and the reaction mixture was stirred for 15 min. To the reaction mixture was added 200 ml of 2 N NaOH. After stirring at room temperature for 20 min, the reaction was extracted into 10% THF/CH_2Cl_2, dried (K_2CO_3) and concentrated. The residue was diluted with Et_2O, and extracted into 2 N HCl. The aqueous phase was basified with 2 N NaOH and extracted into CH_2Cl_2. The organic phase was dried (K_2CO_3) and concentrated. The residue was purified by flash chromatography (Et_2O/petroleum ether, 1:1, the 3:1, then 100:0) to give 3.18 g (61% yield) **31**. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 9.97 (s, 1 H), 8.62 (d, 1 H), 7.73 (dt, 1 H), 7.2–7.6 (m, 7 H), and 5.27 ppm; IR (KBr) 1705 cm^{-1}; mass spectrum, *m/e* 213.

*2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(2-pyridinylmethoxy)-phenyl]methylene]hydrazinecarboximidamide **32** dihydrochloride*

At –78°C, to 1.48 g (5.17 mmol) **18** in THF (25 ml) was added slowly 6.8 ml (11.6 mmol) *t*-butyllithium (1.7 M in pentane). After stirring at –78°C for 20 min, the yellow solution was transferred slowly *via* cannula to a flask containing 1.32 g (6.2 mmol) **31** in THF (10 ml). The cold bath was removed, and the reaction mixture was quenched with saturated NaCl solution, diluted with water, and extracted into Et_2O. The organic phase was dried (K_2CO_3) and concentrated. The crude product was diluted with CH_2Cl_2 and treated with 1.5 equiv of pyridinium chlorochromate. The reaction mixture was stirred at room temperature until complete as judged by tlc (CH_2Cl_2/Et_2O, 3:1). The reaction mixture was diluted with Et_2O, passed through a thick pad of silica gel (Et_2O elution). The filtrate was concentrated and the residue purified by flash chromatography (CH_2Cl_2/Et_2O, 9:1, then 4:1) to give 512 mg of ketone product as an oil, which was used directly in the next reaction.

A mixture of 512 mg (1.38 mmol) of ketone in 20 ml EtOH containing 1.0 g (7.3 mmol) aminoguanidine nitrate and 30 μl of concentrated HNO_3 was heated at 70°C overnight. Another 30 μl of concentrated HNO_3 was added. After stirring overnight, at 90°C, the reaction mixture was diluted with water, dried (K_2CO_3), and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 85:10:5) to give 444 mg of product as a pale yellow powder. The product was dissolved in CH_2Cl_2, treated with 1.7 ml 2.5 N ethanolic HCl, and concentrated from a mixture of Et_2O/petroleum ether/CH_2Cl_2 to give the title compound (mixture of isomers as determined by NMR) as a colorless powder, mp 117°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz, partial) δ 9.89 and 9.70 (s, 1 H), 8.63–8.64 (m, 1 H), 6.45 and 6.37 (d, 1 H), 5.83 and 5.79 (d, 1 H), 5.30 and 5.28 (s, 2 H), and 1.45 and 1.27 ppm (s, 6 H); IR (KBr) 3120 (br), 1670, 1620, and 1585 cm^{-1}; mass spectrum, *m/e* 428. Anal $C_{25}H_{27}Cl_2N_5O_2$ (C, H, N).

*3-(4-Pyridinylmethoxy)benzaldehyde **33***

At 0°C to 9.0 g (74 mmol) 3-hydroxybenzaldehyde in CH_2Cl_2 (100 ml) containing 8.07 g (74 mmol) 4-pyridylcarbinol and 19.5 g (74.4 mmol) triphenylphosphine was added dropwise 12.3 ml (78 mmol) of diethylazodicarboxylate. The cold bath was removed and stirring was continued for 20 min. The reaction mixture was quenched with 200 ml of 2 N NaOH and was stirred at ambient temperature for 20 min. The organic phase was separated, diluted with 3 vol of Et_2O, and extracted into 2 N HCl. The aqueous extracts were then basified and extracted into Et_2O. The ethereal phase was dried (K_2CO_3), concentrated, and crystallized from CH_2Cl_2/petroleum ether/Et_2O to give 5.23 g **33** as a brown powder which was used as such in the next reaction. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 9.99 (s, 1 H), 8.64 (d, 2 H), 7.2–7.6 (m, 6 H), and 5.7 ppm (s, 2 H).

*2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(4-pyridinylmethoxy)-phenyl]methylene]hydrazinecarboxamidamide **34** and **35** dihydrochlorides*

At –78°C to 6.5 g (22.9 mmol) **18** in 70 ml THF was added over 5 min 23 ml (39 mmol) *t*-butyllithium (1.7 M in pentane). After 45 min at –78°C, the reaction mixture was transferred slowly *via* cannula to a solution of 4.52 g (2.12 mmol) of **33** in THF (100 ml) at –78°C. The reaction mixture was stirred at –78°C for 30 min, quenched with water (500 ml) and extracted into Et_2O. Drying (K_2CO_3) and concentration gave 8.54 g of crude alcohol, which was used directly in the next reaction. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 8.57 (d, 2 H), 7.33 (d, 2 H), 6.9–7.1 (m, 5 H), 6.84 (dd, 1 H), 6.72 (d, 1 H), 6.27 (d, 1 H), 5.72 (s, 1 H), 5.60 (d, 1 H), 5.07 (s, 2 H), 2.47 (bs, 1 H), and 1.41 ppm (s, 6 H).

To a solution of alcohol in CH_2Cl_2 (100 ml) was added 8.0 g MnO_2. The reaction mixture was stirred at 40°C throughout. Over the course of 4 h, another 32 g MnO_2 was added in 8-g

portions. After a total of 5 h reaction, the reaction mixture was cooled to room temperature, passed through a pad of silica gel (Et_2O/CH_2Cl_2, 9:1 rinse), and concentrated to give 7.83 g of crude ketone which was used directly in the next reaction. An analytical sample was purified by flash chromatography (CH_3CN/CH_2Cl_2, 5:95, then 10:90, then 50:50). ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 8.63 (d, 2 H), 7.59 (dd, 1 H), 7.51 (d, 1 H, J = 2.6 Hz), 7.1–7.5 (m, 6 H), 6.80 (d, 1 H), 6.36 (d, 1 H), 5.15 (s, 2 H), and 1.48 ppm (s, 6 H); IR (CHC_3), 1640 cm^{-1}; mass spectrum (HRMS). Anal calc for $C_{24}H_{21}NO_3$ 371.15213, found 371.15192.

A mixture of 6.82 g crude ketone and 10 g aminoguanidine nitrate in EtOH (200 ml) containing 1 ml concentrated HNO_3 was stirred at 70°C for 2 d. The reaction mixture was quenched with excess 2.5 M NaOH and extracted into a mixture of THF/CH_2Cl_2 (1:10). The organic extract was washed with water, dried (K_2CO_3), and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 88:8:4) to provide 3.09 g of the free base of the less polar isomer **35** (as determined by tlc (CH_2Cl_2/MeOH/NH_4OH, 88:84) and 2.78 g of the more polar isomer **34**. The salts of the these compounds were prepared by dissolving the free base in CH_2Cl_2, treating with 2 ml of 2 N ethanolic HCl, and then trituration into a mixture of Et_2O/petroleum ether. Analytical data for **34**, mp 157°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.95 (s, 1 H), 8.82 (bs, 2 H), 7.88 (d, 12 H), 7.44 (t, 1 H, J = 2.3 Hz), 7.32 (t, 1 H), 7.11 (dd, 1 H), 7.08 (d, 1 H), 7.0–7.1 (m, 2 H), 6.92 (d, 1 H), 6.44 (d, 1 H), 5,83 (d, 1 H), 5.45 (s, 2 H), and 1.44 ppm (s, 6 H); IR (KBr) 3120 (br), 1675, 1615, and 1590 cm^{-1}. Anal $C_{25}H_{27}Cl_2N_5O_2$•0.25 H_2O (C, H, N). Analytical data for **35**, mp 196°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.87 (s, 1 H), 8.89 (bs, 2 H), 8.03 (d, 2 H), 7.57 (t, 1 H), 7.45 (d, 1 H), 7.25 (dt, 2 H), 7.02 (q, 1 H), 6.91 (d, 1 H), 6.72 (d, 1 H), 6.36 (d, 1 H), 5.79 (d, 1 H), 5.52 (s, 2 H), and 1.37 ppm (s, 6 H); IR (KBr) 3150 (br), 1675,1610, and 1585 cm^{-1}. Anal $C_{25}H_{25}N_5O_2$•2 HCl•0.1 H_2O (C, H, N).

3-(2-Quinolinylmethoxy)benzaldehyde ***36***

A mixture of 3.2 g (26 mmol) 3-hydroxybenzaldehyde and 4.0 g (22.4 mmol) α-chloroquinaldine and 17 g (0.123 mol) K_2CO_3 in CH_3CN (150 ml) was mechanically stirred at 75°C for 3.5 h. The reaction mixture was quenched with water (500 ml) and extracted into Et_2O (200 ml). The organic phase was then extracted into 2 N HCl, the aqueous layer basified with 2.5 N NaOH, and then extracted into Et_2O. The organic extract was dried over K_2CO_3 and flash chromatographed (CH_2Cl_2, the 5% Et_2O/CH_2Cl_2) to give 4.84 g (82% yield) of 3-(2-quinolinylmethoxy)benzaldehyde. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 9.96 (s, 1 H), 8.20 (d, 1 H), 8.15 (s, 1 H), 7.2–7.9 (m, 8 H), and 5.43 ppm (s, 2 H).

2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(2-quinolinylmethoxy)-phenyl]methylene]hydrazinecarboximidamide ***37*** *dihydrochloride*

At –78°C to 1.44 g (5.03 mmol) **18** in 50 ml of Et_2O was added slowly 6.5 ml (11.1 mmol) of *t*-butyllithium (1.7 M in pentane). After stirring for –78°C for 20 min, the reaction mixture was transferred *via* cannula to a solution of 1.85 g (7.0 mmol) of **36** in a mixture of THF (*ca* 10 ml) and Et_2O (50 ml). The cold bath was removed and the red reaction mixture was stirred for 20 min. The reaction mixture was quenched with water and extracted into CH_2Cl_2. The organic phase was dried (K_2CO_3), concentrated, and flash chromatographed (10 and 20% Et_2O/CH_2Cl_2) to give 474 mg of alcohol product as an oil. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 8.18 (d, 1 H), 8.07 (d, 1 H), 7.5–7.9 (m, 4 H), 6.8–7.3 (m, 7 H), 6.68 (d, 1 H), 6.24 (d, 1 H), 5.72 (d, 1 H), 5.58 (d, 1 H), 5.37 (s, 2 H), 2.17 (d, 1 H), and 1.41 ppm (s, 6 H).

To a solution of the above product in CH_2Cl_2 (25 ml) was added 1 g (4.64 mmol) of pyridinium chlorochromate. After stirring at 40°C for 30 min, the reaction mixture was diluted with 10 ml of THF, passed through a thick pad of silica gel (20% Et_2O/CH_2Cl_2) and concentrated. The residue was further purified by flash chromatography (5 and 10% Et_2O/CH_2Cl_2) to give 313 mg of ketone product as a colorless foam. ^{1}H-NMR ($CDCl_3$; 200 MHz) δ 8.21 (d, 1 H), 8.07 (d, 1 H), 7.2–8.9 (m, 9 H), 6.71 (d, 1 H), 7.30 (d, 1 H), 5.65 (d, 1 H), 5.42 (s, 2 H), and 1.47 ppm (s, 6 H).

A mixture of 312 mg (0.74 mmol) of ketone, 650 mg (4.74 mmol) aminoguanidine nitrate in EtOH (10 ml) containing 20 μl concentrated HNO_3 was stirred at 70°C for 2 d. The reaction mixture was cooled to room temperature, quenched with 2 N NaOH, and extracted into 10% THF/CH_2Cl_2. The organic extract was dried (K_2CO_3) and flash chromatographed (CH_2Cl_2/MeOH/NH_4OH, 95:3:2, then 93:5:2) to give 305 mg of the product as a yellow foam. The product was dissolved in a little CH_2Cl_2 and treated with 1 ml 2 N ethanolic HCl. Repeated concentrations from Et_2O/MeOH/petroleum ether/CH_2Cl_2 mixture gave the title compound as colorless powder (mixture of isomers by NMR), mp 170–183°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz, partial) δ 9.86 and 9.67 (s, 1 H), 8.46–8.50 (m, 1 H), 8.01–8.02 (m, 1 H), 6.44 and 6.32 (d, 1 H), 5.82 and 5.77 (d, 1 H), 5.45 and 5.43 (s, 2 H), 1.43 and 1.36 ppm (s, 6 H); IR (KBr) 3150 (br), 1670, 1620, and 1585 cm^{-1}; mass spectrum (FAB), *m/e* 478. Anal $C_{29}H_{29}Cl_2N_5O_2$ (C, H, N).

3-[[3-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)(1H-imidazol-1-yl)methyl]phenoxy]methyl]pyridine ***38*** *dihydrochloride*

At 0°C to a solution of 2.52 g (6.76 mmol) **27** in CH_2Cl_2 (50 ml) containing 2.20 g (8.40 mmol) triphenylphosphine, and 1.40 g (21 mmol) imidazole was added slowly 1.3 ml (8.26 mmol) of diethylazodicarboxylate. After stirring for 30 min at room temperature, tlc suggested incomplete reaction. The reaction mixture was recooled to 0°C, and treated sequentially with 1.4 g imidazole, 2.2 g of triphenylphosphine, and 0.80 ml diethylazodicarboxylate. After stirring overnight, the reaction mixture was quenched with 2.5 N NaOH and extracted into CH_2Cl_2. The organic extract was diluted with 1 vol petroleum ether and then extracted into 1 N HCl (3 x 100 ml). The aqueous phase was then basified with 2 N NaOH, dried (K_2CO_3), and flash chromatographed (Et_2O/CH_2Cl_2/MeOH/NH_4OH, 33:57:7:3) to give 2.28 g of impure product as an orange oil. Reflash chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:2:1) gave 1.28 g of pure free base as an orange oil. The product was dissolved in a little CH_2Cl_2, treated with 5 ml 2 N ethanolic HCl, and concentrated from a mixture of petroleum ether/Et_2O/CH_2Cl_2 to provide the title compound, mp 108°C (dec). ^{1}H-NMR (DMSO-d_6; 400 MHz) δ 9.12 (s, 1 H), 8.83 (s, 1 H), 8.73 (d, 1 H), 8.26 (d, 1 H), 7.7–7.8 (m, 3 H), 7.40 (t, 1 H), 7.11 (dd, 1 H), 6.95–7.05 (m, 3 H), 6.90 (bs, 1 H), 6.84 (d, 1 H), 6.78 (d, 1 H), 6.36 (d, 1 H), 5.78 (d, 1 H), 5.24 (s, 2 H), and 1.36 ppm (s, 6 H); IR (KBr) 2910 (br) cm^{-1}. Anal $C_{27}H_{27}Cl_2N_3O_2$•H_2O (C, H, N).

2-Imidazolidinone 2-[(2,2-dimethyl-2H-1-benzopyran-6-yl[3-(3-pyridinylmethoxy)phenyl]methylene]hydrazone ***39*** *dihydrochloride*

A solution of 1.89 g (5.09 mmol) **28** in EtOH (50 ml) containing 2.77 g (15.3 mmol) 2-hydrazinoimidazoline hydrobromide and 4.6 ml (5.09 mmol) 1 M methanolic HBr was refluxed for 4 h and then stirred at room temperature for 3 d. The reaction mixture was quenched with 1 N NaOH (200 ml) and then extracted into 20% THF/CH_2Cl_2. The combined orga-

nic extracts were dried over Na_2SO_4, concentrated and flash chromatographed twice (CH_2Cl_2/MeOH/NH_4OH, 95.5:3:1.5) to give 1.06 g of pure product and 0.87 g of slightly impure material. The pure free base was dissolved in CH_2Cl_2 and was treated with 1.6 ml of 3 M ethereal HCl. The reaction mixture was stirred for 30 min and concentrated. The residue was dissolved in a little MeOH and triturated with Et_2O to provide 1.03 g of the title compound as an off-white solid, mp 248–256°C (1:3 mixture of isomers by NMR). ^{1}H-NMR (DMSO-d_6; 400 MHz, partial) δ 10.62 and 10.46 (s, 1 H), 8.92 (d, 1 H), 8.80 (dd, 1 H), 6.44 and 6.37 (d, 1 H), 5.83 and 5.80 (d, 1 H), 5.35 and 5.36 (s, 2 H), and 1.44 and 1.38 ppm (s, 6 H); IR (KBr) 3420, 2450, 1650, and 1605 cm^{-1}. Anal $C_{27}H_{29}Cl_2N_5O_2$ (C, H, N).

2-[(2,2-Dimethyl-2H-1-benzopyran-6-yl)[3-(3-pyridinylmethoxy)-phenyl]methylene]hydrazinecarboxamide ***40*** *hydrochloride*

A solution of 1.40 g (3.78 mmol) **28** in MeOH (30 ml) containing 3.38 ml 1.1 M ethanolic HBr and 1.26 g (11.4 mmol) semicarbazide hydrochloride was refluxed for 16 h. An additional 0.42 g semicarbazide hydrochloride and 3.38 m 1.1 M ethanolic HBr was added to the reaction mixture and the reaction mixture was refluxed for another 8 h. The reaction mixture was basified with 1 N NaOH and extracted into CH_2Cl_2. The organic extracts were dried over K_2CO_3 and concentrated. Flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 95.5:3:1.5) gave 1.19 g (75% yield) of pure product. This material was converted to its hydrochloride salt by dissolving the free base in CH_2Cl_2, treating with 1 equivalent of 3 M ethereal HCl and triturating with Et_2O to provide 1.35 g of the title compound as a colorless powder, mp 207–214°C (1:1 mixture of isomers by NMR). ^{1}H-NMR (DMSO-d_6; 400 MHz, partial) δ 8.93 (s, 1 H) 8.80 (d, 1 H), 8.45 (t, 1 H), 8.14 and 7.7 (bs, 1 H), 7.89–7.94 (m, 1 H), 6.44 and 6.37 (d, 1 H), 5.81 and 5.77 (d, 1 H), 5.34 and 5.31 (s, 2 H), 1.42 and 1.36 ppm (s, 6 H); IR (KBr) 3360, 2350, and 1690 cm^{-1}. Anal $C_{25}H_{25}ClN_4O_3$ (C, H, N).

Pharmacology

Aldosterone biosynthesis was evaluated in an isolated rat adrenal glomerulosa cell preparation in which aldosterone biosynthesis was stimulated by angiotensin-II. Briefly, isolated rat (Sprague-Dawley) adrenal glomerulosa cells, prepared by collagenase dispersion method, were diluted to 5 x 10^5 cells/ml in incubation media (media 199 with Hanks' salts (120 ml; Gibco), 80 ml media 199 with Hanks' salts minus potassium (80 ml; VWR), 2.0 ml penicillin/streptomycin solution, 0.4 g BSA, and pH adjusted to 7.2–7.4). To 1.0 ml aliquots of cell suspension was added 10 µl of angiotensin-II (10^{-9} final concentration) to stimulate aldosterone. Typical stimulation by angiotensin-II was 10–30 times basal activity. Test compounds were then added (10 µl of test solutions prepared in 50% DMSO and m199 media) such that final concentration of 0.1 µM, 0.5 µM, 1.0 µM and 5.0 µM) were achieved. The tubes were vortexed, incubated at 37°C for 2 h, and then centrifuged at 3000 RPM for 15 min. The supernatant was removed from the pellet and the supernatant assayed for aldosterone levels using a conventional, commercial aldosterone radioimmunoassay kit (^{125}I-Aldosterone Coat-a-Count by Diagnostics Products Corporation, Los Angeles, CA). Aldosterone values for each drug concentration were averaged and expressed as a percentage of the control value (angiotensin-II-stimulated aldosterone production in absence of drug). Dose–response curves were plotted for each compound and the IC_{50} determined.

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