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Solution-Phase, Parallel Synthesis and Pharmacological Evaluation of Acylguanidine Derivatives as Potential Sodium Channel Blockers

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Dedicated to Professor Hitomi Suzuki on the occasion of his retirement from Kyoto University, Japan.

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Abstract—Solution-phase synthesis of various acylguanidine derivatives and the evaluation of a small library of compounds as potential sodium channel blockers are described.

Previously, we have described several classes of guanidines that act as ion-channel blockers, including NMDA-activated cation channel and Type II neuronal sodium channel blockers.[1–3] The guanidine substituents profoundly influence the physicochemical properties and associated biological actions of these compounds. Introduction of an acyl group on one of the guanidine nitrogen atoms markedly reduces the basicity. Thus the pK_a of benzoylguanidine is 6.98,[4] compared with phenylguanidine, pK_a 10.77,[5] and the unsubstituted guanidine molecule, pK_a 12.6.[6] Notable examples (Fig. 1) of acylguanidines of biological interest include the α_2-adrenoceptor agonist guanfacine (**1**)[7] and the multiple ion-channel blocker amiloride (**2**).[8] Amiloride analogues and some corresponding phenyl derivatives,[9] example **3** (HOE 694), Cariporide[10] (**4**, HOE 642) are Na^+/H^+ antiporter inhibitors and multiple ion-channel blockers. SAR studies[11,12] on amiloride analogues indicate that substitution of a guanidine nitrogen atom by hydrophobic groups decreases Na^+/H^+ antiporter activity and increases sodium channel blockade. Voltage-dependent sodium channel blockers[13,14] are of clinical importance due to their use as local anesthetics, antiarrhythmic agents and anticonvulsants, and they are also of potential interest as treatments for stroke and other neurological disorders. In this paper,[15] we describe the solution-phase, parallel synthesis of a small library of acylguanidine derivatives and their evaluation as inhibitors of type II neuronal sodium channels.

Preparation of various acylguanidine derivatives was achieved following the synthetic scheme given in Figure 2. Acylguanidines derived from aromatic amines were synthesized through the condensation of aroylcyanamides[16] and the corresponding aniline hydrogen halides in toluene or xylenes at reflux in good yields and in high HPLC purity[17] without any purification (Method A).[18] No reaction occurred, or a complex reaction mixture was obtained, using sterically hindered aniline derivatives like 2,6-dimethylaniline or less basic heterocyclic amines.

It was found that under similar conditions, cyanamides derived from arylalkyl (phenylacetylcyanamide[19]), alkyl (adamantane-1-carbonylcyanamide[20]), and heterocyclic (furan, thiophene, and pyridine) carboxylic acids gave highly pure products from the reactions with various aniline derivatives (Method A).

Initially, synthesis of *N*-aroyl-*N*′-alkylguanidines, through condensation of aroyl cyanamide with aliphatic amines, posed problems. After many attempts, successful

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condensation of aroyl *S*-methylisothiourea[16] with aliphatic amines in the presence of triethyl amine using toluene or xylenes at reflux led to the synthesis of *N*-aroyl-*N*′-alkyl/arylalkylguanidine derivatives in moderate to high purity without any purification (Method B).[21]

Further investigations established a general procedure[22] for the synthesis of acylguanidines derived from both aliphatic and aromatic amines through condensation of aromatic amines and aliphatic amines with the corresponding acylthiourea derivatives in the presence of triethyl amine using toluene or xylenes as solvent at reflux.

It may be of interest to compare the potencies of acylguanidines from anilines and aliphatic amines. The compounds derived from both methods (A and B) exhibited a range of potencies from weak to potent sodium channel blockade. However, more compounds derived from aliphatic amines showed in vivo activity in the various efficacy models compared to those derived from aromatic amines.

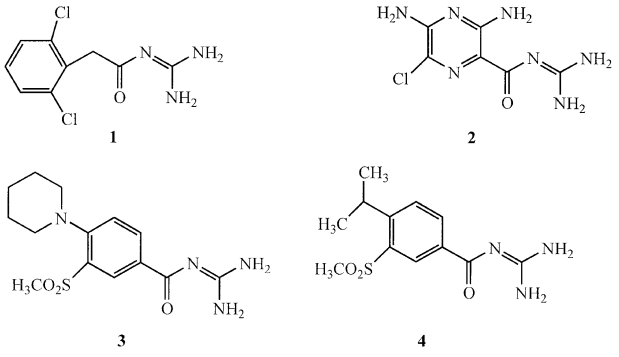

Figure 1. Structures of selected acylguanidines.

Preparation of *N*-aroyl-*N*′,*N*″-dialkylguanidines was achieved through successive replacement[23] of methylthio groups in *S*,*S*-dimethyl *N*-aroylcarbimidodithiolate[24] (Method C)[25] and these compounds showed very weak sodium channel blockade.

Reaction of excess of *N*-alkylguanidine hydrogen halide salts with esters[26] in the presence of sodium ethoxide afforded *N*-alkanoyl-*N*′-alkylguanidine derivatives in good yields and moderate purity (Method D).[27] These acylguanidines showed only moderate activity in the sodium channel assay.

Solution-phase, parallel reactions were carried out using a manifold apparatus[28] capable of running five or 10 reactions at a time or using a Robosynthon multireactor™ (24 vessels) apparatus.[29] Based on the initial optimized conditions, ~200 acylguanidine derivatives were synthesized and isolated products were found to be of moderate to high purity by HPLC and ^{1}H NMR.[30]

Sodium channel blockade of the various acylguanidine derivatives in this study were tested in a functional assay using Chinese hamster ovary (CHO) cell line expressing either the mammalian type IIA neuronal (CNaIIA)[31] or the human cardiac (hH1)[32] sodium channels. The assay[33] measures percent inhibition of [^{14}C]guanidinium flux through veratridine-stimulated, tetrodotoxin-sensitive sodium channels. From dose–response (% inhibition) curves the corresponding IC_{50} values for each compound were determined. These IC_{50} values are given in Tables 1 and 2.

One of the early potent compounds derived from aromatic amines, *N*-(4-methylbenzoyl)-*N*′-(4-isopropylphenyl)guanidine (**7**, Table 1) was evaluated in the audiogenic mouse antiseizure (DBA/2 mice[34]) model for in vivo anticonvulsant activity (see Table 3), and exhibited very weak activity in the DBA/2 model (30% inhibition

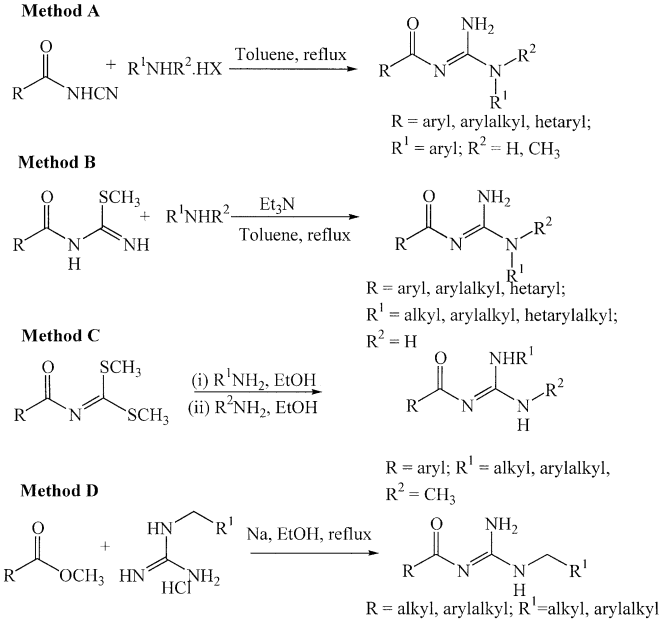

Figure 2. Synthetic scheme of acylguanidines.

@ 20 mg/kg). To probe the role of physicochemical properties, in binding to sodium channels and in transport to the site of action in the CNS as measured in the audiogenic DBA/2 mouse model, we determined the pK_a and $logD_{7.4}$ of **7** as 6.35 ± 0.05 and 4.4, respectively.

The weak activity of **7** in the audiogenic mouse antiseizure test[35] may be related to the low population of the protonated species (<10%) under physiological conditions because of its low pK_a. It was anticipated that acylguanidines derived from arylalkyl amines (i.e., introducing a spacer linker between the aromatic ring and the guanidinium group) would lead to compounds with increased pK_a and therefore an increased population of cationic species. Sodium channel blockade and in vivo activity, in the antiseizure (DBA/2) model of the

Table 1. Acylguanidines derived from 4-methylbenzoic acid and different amines (R^1R^2NH)

Compd	R^1R^2NH	HPLC Purity	IC_{50} (μM)	
			CNaII	hH1
	Method A			
5	1-Naphthylamine	98	3.29±0.85	nt
6	4-Benzyloxyaniline	96	10.09±2.6	nt
7	4-Isopropylaniline	99	1.95±0.24	19.7±1.3
8	4-Isopropoxyaniline	nt	2.02±0.86	5.75±0.95
9	2-Isopropylaniline	nt	4.36±0.72	4.5±0.5
10	4-*t*-Butylaniline	99	>24	nt
11	2,5-Dibromoaniline	nt	>25	nt
12	3,4,5-Trimethoxyaniline	nt	1.92±0.14	24.35±2.75
13	*N*-Methyl-3-iodoaniline	94	9.45±0.11	nt
14	*N*-Methyl-3-methylthioaniline	94	5.44±0.75	nt
15	Indoline	99	8.81±1.5	>100
16	7-Trifluoromethyl-1,2,3,4-tetrahydroquinoline	96	>25	nt
17	1,2,3,4-Tetrahydroquinoline	nt	18.77±4.3	29.7±3.3
	Method B			
18	1-Butylamine	67.7	17.35±1.9	9±0.8
19	Benzylamine	95	3.87±0.13	nt
20	2-Phenethylamine	93	2.66±0.52	5.65±0.65
21	3-Phenylpropylamine	nt	6.03±2.84	5.25±0.35
22	4-Phenylbutylamine	99	1.14±0.41	2.85±0.35
23	5-Phenylpentylamine	97	2.11±0.35	3.6±0.5
24	1-Naphthylmethylamine	nt	2.38±0.03	8.85±0.75
25	2-(4-Chlorophenyl)ethylamine	nt	1.97±0.26	8.5±1.9
26	2-Phenoxyethylamine	72	3.23±0.33	14.2±3.0
27	3-Phenoxypropylamine	94	2.67±0.45	5.6±0.5
28	3-Dimethylaminopropylamine	99	>25	nt
29	2-(Benzylthio)ethylamine	74	3.9±1.28	10.8±4.99
30	2-(5-Methoxyindol-3-yl)ethylamine	92	1.76±0.77	1.76±0.16
31	2-(6-Methoxyindol-3-yl)ethylamine	95	3.35±0.81	3.92±0.62
32	2-(Thien-2-yl)ethylamine	nt	4.95±0.35	10.5±0.4
	Method C			
33	4-Phenylbutylamine and methylamine	93	4.16±0.70	9±1.61

nt, not determined.

Table 2. Acylguanidines derived from different carboxylic derivatives (RCO-) and 4-phenylbutylamine using Method B

Compd	Acyl group (RCO-)	HPLC Purity	IC_{50} (μM)	
			CNaII	hH1
34	Benzoyl	99.9	5.35±0.25	6.9±2.82
22	4-Methylbenzoyl	99	1.14±0.41	2.85±0.25
35	2-Methylbenzoyl	96	1.20±0.17	1.75±0.05
36	4-Chlorobenzoyl	83	4.3±1.1	3.6±0.4
37	2,3-Dichlorobenzoyl	97.2	1.48±0.19	2.71±0.3
38	2,5-Dichlorobenzoyl	87.3	3.25±0.48	7.89±1.84
39	2,6-Dichlorobenzoyl	91.7	5.03±2.49	5.4±0.65
40	3,4-Dichlorobenzoyl	98	7.11±2.57	15.5±0.5
41	4-Methoxybenzoyl	98.9	0.82±0.15	2.1±0.1
42	4-Ethoxybenzoyl	99	0.76±0.01	1.1±0.1
43	4-Butoxybenzoyl	99	0.63±0.13	0.56±0.15
44	3,4,5-Trimethoxybenzoyl	92	1.39±0.22	1.51±0.33
45	1-Naphthoyl	nt	0.86±0.05	4.1±0.7
46	Furan-2-carbonyl	83.4	13.75±3.09	7.9±2.58
47	Thiophene-2-carbonyl	nt	4.31±0.1	2.35±0.25

nt, not determined

Table 3. Percentage inhibition of seizure in DBA/2 mice of acylguanidines

Compd Name (No.)	% Inhibition @ 20 mg/kg (ip)
N-(4-Methylbenzoyl)-*N*′-(4-isopropylphenyl)guanidine (**7**)	30
N-(4-Methylbenzoyl)-1-indolinyl carboxamidine (**15**)	16
N-(4-Methylbenzoyl)-*N*′-(4-phenylbutyl)guanidine (**22**)	75
N-(4-Methylbenzoyl)-*N*′-(5-phenylpentyl)guanidine (**23**)	7
N-(4-Methylbenzoyl)-*N*′-(3-phenoxylpropyl)guanidine (**27**)	54
N-(2-Methylbenzoyl)-*N*′-(4-phenylbutyl)guanidine (**35**)	53
N-(4-Methoxylbenzoyl)-*N*′-(4-phenylbutyl)guanidine (**41**)	42
N-(4-Ethoxybenzoyl)-*N*′-(4-phenylbutyl)guanidine (**42**)	60
N-(4-Butoxybenzoyl)-*N*′-(4-phenylbutyl)guanidine (**43**)	97
N-(1-Naphthoyl)-*N*′-(4-phenylbutyl)guanidine (**45**)	9
N-(2-Furanoyl)-*N*′-(4-phenylbutyl)guanidine (**46**)	5

acylguanidines derived from arylaliphatic amines were studied. The improved activity of *N*-(4-methylbenzoyl)-*N*′- (4-phenylbutyl)guanidine (Table 1, **22**) in the DBA/2 model (75% inhibition @ 20 mg/kg) may be related to its increased pK_a (7.09 ± 0.08).

Tables 1 and 2 show a representative set of various amines and acid derivatives used in the synthesis of acylguanidines, along with the HPLC purity of isolated products and their sodium channel blockade data. The in vivo data, as percent inhibition of seizure in the DBA/2 mouse, of various acylguanidines are given in Table 3.

In summary, syntheses and sodium blocking activities of a series of acylguanidines with diverse substituents on both sides of the guanidine unit are described. From these studies, a number of acylguanidines exhibiting good activities in different animal models for neuroprotection including focal ischemia, glaucoma, and pain, have been identified.

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17. HPLC purity determinations were carried out using a Beckman 126 gradient system with UV detection at 220 nm. Linear 30 min gradient: 2 to 98% CH_3CN in H_2O (0.1% TFA) Column: Ultrasphere ODS (AC-2) 5 mm 4.6×250 μm with C-18 guard column, flow rate 1 mL/min.
18. Method A: *N*-(4-Methylbenzoyl)-*N*′-(4-isopropylphenyl)-guanidine, hydrochloride: A mixture of 4-methylbenzoylcyanamide (160 mg, 1 mmol) and 4-isopropylaniline hydrochloride (185 mg, 1 mmol) in toluene (4 mL) was refluxed for 3 h. The reaction mixture was cooled to room temperature, the white solid separated was filtered, washed with toluene and finally with hexanes to afford the title product (268 mg, 78%); mp 208–210 °C; Purity 99.3% (HPLC); ^{1}H NMR (CD_3OD) δ 1.28 (2s, 6H, CH_3), 2.42 (s, 3H, Ar–CH_3), 3.02 (m, 1H, CH), 7.38 (d, 2H, ArH), 7.42 (m, 4H, ArH), 8.0 (d, 2H, ArH).
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21. Method B: *N*-(4-Methylbenzoyl)-*N*′-(4-phenylbutyl)guanidine, hydrochloride: A suspension of *S*-methyl 4-methylbenzoyl isothiourea derivative (1.04 g, 5 mmol), in touene (10 mL) was added phenylbutyl amine (0.75 mL, 4.75 mmol) and triethyl amine (0.7 mL, 5 mmol). The reaction mixture was heated in an oilbath to reflux and maintained at reflux for 3 h. The free base separated on cooling was filtered, washed with hexanes and dried to afford the solid (1.3 g). The free base (1.3 g) was dissolved in methanol (30 mL) and dichloromethane (25 mL) and cooled in an ice water bath. Hydrogen chloride (1 M in ether, 2 mL) was added, stirred for 30 mins, concentrated under reduced pressure. *N*-(4-methylbenzoyl)-*N*′-(4-phenylbutyl)guanidine, hydrochloride white solid (1.43 g, 84%); mp 166–170 °C; purity: 99% (HPLC); ^{1}H NMR (CD_3OD) δ 1.74 (m, 4H, CH_2), 2.43 (s, 3H, CH_3), 2.69 (t, 2H, CH_2), 3.38 (t, 2H, CH_2), 7.2 (m, 5H, ArH), 7.4 (d, 2H, ArH), 7.9 (d, 2H, ArH).
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25. Method C: *N*-(4-Methylbenzoyl)-*N*′-(4-phenylbutyl)-*N*″-methylguanidine hydrochloride: A mixture of *S*,*S*-dimethyl *N*-(4-methylbenzoyl)carbimidodithiolate (240 mg, 1 mmol) and phenylbutylamine (150 mg, 1 mmol) in ethanol (5 mL) was stirred overnight at room temperature. Concentrated and the oil obtained was repeatedly coevaporated with dichloromethane, when solid separated. This solid was triturated with hexanes, filtered and dried to afford *N*-(4-methylbenzoyl)-*N*′-(4-phenylbutyl)-*S*-methylthiourea as white solid (80 mg); ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 4H, CH_2), 2.38 (s, 3H, ArMe), 2.61 (s and t, 5H, CH_2 and SMe), 3.35 (t, 2H, CH_2) 7.2 (m, 5H, ArH), 7.28 (d, 2H, ArH), 8.12 (d, 2H, ArH).

A solution of *N*-(4-methylbenzoyl)-*N*′-(4-phenylbutyl)-*S*-methylthiourea (80 mg) in 5 mL of methylamine (2 M in methanol) was stirred at room temperature for 48 h. After removal of the solvent the residue was dissolved in methanol (3 mL) an ethereal solution of hydrogen chloride (5 mL) was added. The solid separated was filtered, washed with ether, and dried. Pale-yellow solid (50 mg); purity: 93% (HPLC); ^{1}H NMR (CD_3OD) δ 1.71 (m, 4H, CH_2), 2.40 (s, 3H, $ArCH_3$), 2.65 (t, 2H, CH_2), 3.05 (s, 3H, NMe), 3.4 (t, 2H, CH_2), 7.13 (m, 5H, Ar), 7.35 (d, 2H, ArH), 7.82 (d, 2H, ArH).

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27. Method D: *N*-(2,6-Dichlorophenylacetyl)-*N*′-benzylguanidine, hydrochloride: To sodium ethoxide [prepared by reacting sodium (60 mg, 2.61 mmol) and anhydrous ethanol (5 mL)] benzylguanidine.hydrochloride (580 mg, 3.12 mmol) was added and refluxed in an oil-bath for 1 h. The reaction mixture was cooled to room temperature and filtered all insoluble materials. Methyl 2,6-dichlorophenylacetate (285 mg, 1.3 mmol) was added to the filtrate and refluxed for 2 h. After cooling to room temperature the reaction mixture was concentrated and converted to the hydrochloride salt by the addition of hydrogen chloride (1 M in ether). White solid (310 mg); purity: 89.3% (HPLC); ^{1}H NMR (CD_3OD) δ 4.38 (s, 4H, CH_2), 7.32–7.41 (m, 8H, Ar). Sodium channel blockade potencies (IC_{50}) of *N*-(2,6-dichlorophenylacetyl)-*N*′-benzylguanidine, hydrochloride are 1.19 ± 0.62 μM for neuronal (CNaIIA) and 1.46 ± 0.82 μM for cardiac (hH1) channels, respectively.

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33. Cultures (CNaIIA-1 and hH1 cells seeded in 96-well plates for 3 days) were incubated with 200 μL of preincubation buffer (mM/KCl, 5.4; $MgSO_4$, 0.8; HEPES, 50; choline chloride, 130; guanidine, HCl, 1.0; D-glucose, 5.5; BSA, 0.1 mg/mL; pH 7.4) at 37 °C for 10 min. Aliquots (50 μL) of the different concentrations of the test compounds prepared by serial dilution in uptake buffer (pre-incubation buffer + ~2.5 mCi/mL [^{14}C]-guanidine·HCl and 200 μM veratridine) were added to the 96-well plates and incubated at room temperature for 1 h. Each plate also had the following controls: basal uptake (obtained in the absence of test compound and veratridine), veratridine-only-induced flux, and veratridine-induced fluxes in the presence of 30 μM TTX (a measure of nonspecific flux). The assay was terminated by rinsing the plates with ice cold wash buffer. Scintillation fluid (100 μLs) was then added to each well, the plates were shaken and then counted in a liquid scintillation counter. Net flux is obtained by subtracting the non-specific flux from the total flux (flux in the presence of veratridine only or veratridine and competing ligand).

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35. Audiogenic DBA/2 mouse model: Testing procedure—Mice (Jackson Lkabs, ME, USA; weight range 6.5–12 g, 20–23 days of age) were placed individually in a glass jar (25 cm id) and exposed to pure tone sound of 12 kHz and 120 dB for 45 s. Animals were injected ip with the drug or vehicle (0.3 M, mannitol, in a volume of 10 mL/kg of body weight) 30 min prior to exposure to the sound, unless otherwise noted. All experiments were done in a fully blinded fashion and took place between 11 am and 5 pm, and % response inhibition = (MRS control − MRS treatment)/MRS control × 100. Mean response scores (MRS) were calculated as the average seizure score of a test group of mice on our scale from 0 to 4. The Jonckheere nonparametric trend test, one-sided, was used to determine the lowest effective dose in dose-response studies. Otherwise, the Kruskal–Wallis nonparametric test was used, with Dunn's post-hoc test.