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Synthesis and in vitro evaluation of 2,4-diamino-1,3,5-triazine derivatives as neuronal voltage-gated sodium channel blockers

Xiang Ma [a,b,*], Thong-Yuen Poon [a], Peter Tsun Hon Wong [c], Wai-Keung Chui [a,*]

[a] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore
[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive, Singapore 117576, Singapore
[c] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 10 Medical Drive, Singapore 117597, Singapore

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ABSTRACT

Neuronal sodium channels blockers interfere with ion flux and have been used for managing neuropathic pain, epilepsy, and cerebral ischemic disorders. In the current study, four groups of 2,4-diamino-1,3,5-triazine derivatives were synthesized and investigated for their neuronal sodium channels binding activity. 5-Aryl-1,3,5-triazaspiro[5.5]undeca-1,3-diene-2,4-diamines (**4a**–**4j**) were found to have the best neuronal sodium binding activity among the four groups of triazines evaluated. Derivatives **4a**–**4j** blocked the sodium channels with IC_{50} values ranged from 4.0 to 14.7 μM. The result from this study showed that analogues of 2,4-diamino-1,3,5-triazines could be used as leads for the discovery of neuronal sodium channels blockers for managing central nervous system related disorders.

Voltage-gated sodium channels are large transmembrane proteins which are essential for the generation of action potentials in excitable cells that lead to a rapid transmission of depolarizing impulses throughout cells and cell networks.[1] The neuronal sodium channels have been identified as therapeutic targets, either selectively or in combination with other cellular processes. Interference of the neuronal sodium channels may be useful to treat central nervous system (CNS) related disorders such as epilepsy, neuropathic pain, neurodegeneration associated ischemic stroke, and other conditions.[2,3] Phenytoin (**1**), an antiepileptic drug, was the milestone in recognition of neuronal sodium channel blockers' potential in the treatment of CNS-related disorders.[4] Phenytoin has been shown to be efficacious in treating partial and generalized tonic-clonic seizures in humans.[4] Lamotrigine (**2**), a phenyltriazine derivative, is a novel antiepileptic drug that shares similar mode of action on neuronal sodium channels as phenytoin.[5] It emerged from the screening process of putative antifolates as anticonvulsant agents, prompted by the observation that folates induce seizures in animals.[6–8] Unverferth et al. identified a common pharmacophore model based on some well known voltage-gated sodium channel blockers including phenytoin and lamotrigine (Chart 1).[9] The essential structural features which could be responsible for an interaction with the active site of voltage-gated sodium channels were a hydrophobic unit, an electron donor group, and a hydrogen donor/acceptor unit (Chart 1). 2,4-Diamino-1,3-diaza pharmacophore, indicated as one of the hydrogen donor/acceptor units in the above pharmacophore model, is very common in many types of antifolates (Chart 1).[10] It was once believed that antifolate activity might be related to several sodium channels blockers' anticonvulsant activity.[11] Lamotrigine was then developed as an antifolate drug. However, as it turns out, lamotrigine does not have marked antifolate action and there is no correlation between the antifolate action and antiepileptic effects.

2,4-Diamino-1-(4-chlorophenyl)-1,2-dihydro-1,3,5-triazines (**3**) was known to be a potent antifolate.[12] However, our investigation and other SAR studies of this class of triazines suggested that the spiro triazines **4**, the Dimroth rearranged products **5**, the N^1-benzyl substituted triazines **6** and the aromatic 1,3,5-triazines **7** were either weak antifolates or were totally devoid of antifolate activity.[12–15] The triazine derivatives are composed of a phenyl ring, amino groups, and a 2,4-diamino-1,3-diaza structure, which are also present in voltage-gated sodium channel blockers such as phenytoin and lamotrigine (Chart 1). We therefore hypothesized that the 2,4-diamino-1,3,5-triazine scaffold would exhibit neuronal sodium channels blockade activity; if so, the weak antifolate activity of the scaffold would avoid antifolate side effect. In this letter, four groups of 2,4-diamino-1,3,5-triazines (**4**–**7**) were used to elucidate if 2,4-diamino-1,3,5-triazines would block the neuronal sodium channels (Chart 1).

The synthesis of 5-aryl-1,3,5-triazaspiro[5.5]undeca-1,3-diene-2,4-diamine (**4a**–**4j**) was accomplished using a well known '3-component' method[16] as described in Scheme 1. Three components: aniline, cyanoguanidine, and cyclohexanone were refluxed in the presence of catalytic amount of concentrated hydrochloric acid in

* Corresponding authors. Tel.: +65 6516 2657; fax: +65 6779 1554 (X.M.); tel.: +65 6516 2933; fax: +65 6779 1554 (W.K.C.).
E-mail addresses: chemx@nus.edu.sg (X. Ma), phacwk@nus.edu.sg (W.-K. Chui).

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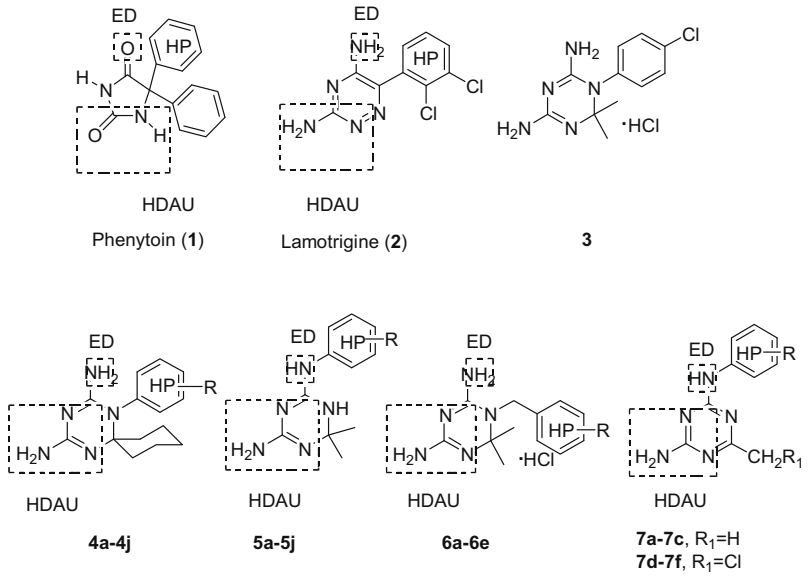
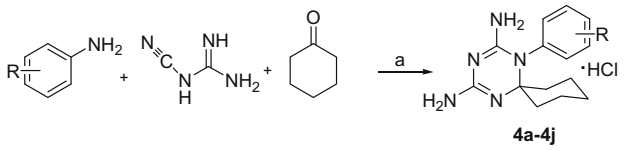


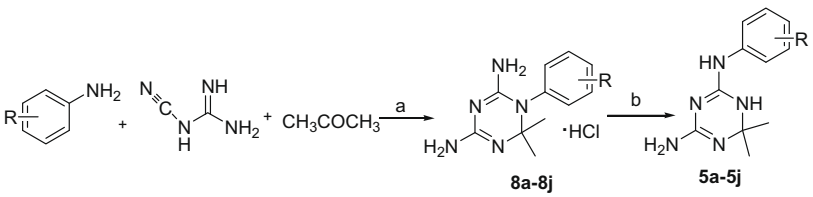
Chart 1. Structures of sodium channel modulators. The essential structure elements for the pharmacophore of Unverferth et al. are indicated by dotted rectangles.[9]



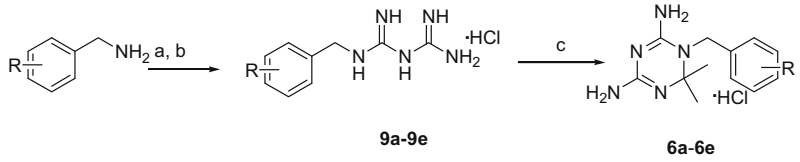
Scheme 1. Synthesis of **4a**–**4j**. Reagents and conditions: (a) concd HCl, abs ethanol, reflux.

alcohol to give **4a**–**4j** in 21–56% yields. Scheme 2 shows the synthesis of 6,6-dimethyl-N^2-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamine (**5a**–**5j**). 2,2-Dimethyl-1,3,5-triazines **8a**–**8j** were prepared according to the '3-component' method, involving the reflux of an appropriate aniline, cyanoguanidine, and hydrochloric acid in acetone. Compounds **8a**–**8j** were subsequently subjected to Dimroth rearrangement which produced target compounds **5a**–**5j** in 60–92% yields. The Dimroth rearrangement is an isomerization process whereby *exo*- and *endo*cyclic heteroatom are translocated on a heterocyclic ring.[16–18] The conversion of compounds **8a**–**8j** to **5a**–**5j** was monitored by UV spectroscopy. In general, compounds **8a**–**8j** exhibited a maximum UV absorption at 240 nm in water. The λ_{max} of the corresponding **5a**–**5j** in each case would appear at a longer wavelength, and thus the rearrangement of **8a**–**8j** to the target **5a**–**5j** could be easily followed. Proton NMR also provided an important tool in differentiating the *ortho*-substituted rearrangement isomers. For example, the proton NMR spectra of 1-(2-methoxyphenyl)-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine (**8k**) and N^2-(2-methoxyphenyl)-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine (**5k**) in deuterated DMSO showed different features of the chemical shifts of 6,6-dimethyl groups.[19] The dimethyl groups in **8k** appeared in the spectrum as two singlet peaks at around 1.2 and 1.4 ppm, respectively; while both methyl groups in **5k** occurred as a singlet peak at about 1.3 ppm.[19] It could be explained that the hindered conformational change of the two methyl groups affected by the *ortho*-substitution on the phenyl ring in **8k**, and the methyl groups are diastereoisotopic and appear as two singlets of equal intensity. However, the hindrance did not exist in structure **5k**. The synthesis of **6a**–**6e** was carried out by two-component synthesis (Scheme 3).[17] Firstly, fusing of the substituted benzylamine hydrochloride and cyanoguanidine at 170–180 °C for 0.5 h provided biguanide hydrochlorides (**9a**–**9e**) in 77–82% yields. Then, the various substituted biguanide hydrochlorides (**9a**–**9e**) were condensed with acetone in catalytic amount of hydrochloric acid to give **6a**–**6e** in 32–48% yields. In the reaction, 2,2-dimethoxypropane was used as a water scavenger, and it could in situ remove water from the condensation reaction to shift the reaction equilibrium to the product side. When reacted with water, it generated acetone which was one of the starting materials. The synthesis of the target compounds **7a**–**7f** was performed according to the procedure as shown in Scheme 4. Phenylbiguanide hydrochlorides **10a**–**10c** were obtained from the reaction of refluxing substituted anilines and cyanoguanidine in the present of catalytic amount of hydrochloric acid. The compounds **7a**–**7f** were then prepared by the alkoxide-catalyzed condensation of biguanide **10a**–**10c** with suitably substituted carboxylic esters in 42–60% yields.

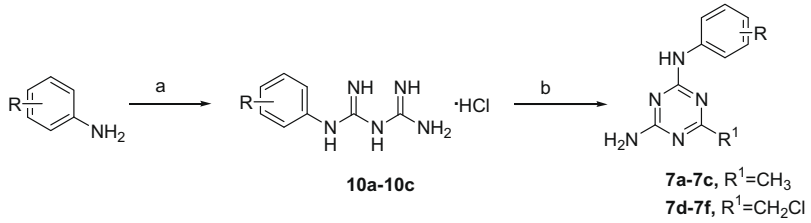
Four groups of triazines prepared for this study were examined in two assays to determine sodium channel binding activity and



Scheme 2. Synthesis of **5a**–**5j**. Reagents and conditions: (a) concd HCl, acetone, reflux; (b) NaOH, 50% ethanol (aq), reflux.



Scheme 3. Synthesis of **6a–6e**. Reagents and conditions: (a) HCl, ether; (b) cyanoguanidine, 170–180 °C; (c) acetone, 2,2-dimethoxypropane, concd HCl, ethanol, reflux.



Scheme 4. Synthesis of **7a–7f**. Reagents and conditions: (a) cyanoguanidine, concd HCl, ethanol, reflux; (b) R^1CH_2COOEt, MeONa/MeOH, rt.

antifolate activity against bovine dihydrofolate reductase (DHFR). In the [^{3}H]BTX binding assay, the competition of all compounds with the binding of [^{3}H]batrachotoxinin-A-20α-benzoate ([^{3}H]BTX-B) to neurotoxin site 2 of the voltage-gated sodium channels from rat brain synaptosomes were studied, and compared with the displacement properties of phenytoin (**1**) tested in the same in vitro model.[20] At the same time, since these structures have the pharmacophore that exhibits antifolate activity, the DHFR inhibition assay was also used to verify whether the antifolate activity was present in the target compounds.[13]

As shown in Table 1, sodium channel binding activities are expressed as IC_{50} values. Compounds **4a–4j** were observed to have the best neuronal sodium channels binding activity among the four groups of triazines evaluated in this study. They were found to block the neuronal sodium channels at IC_{50} values of between 4.0 and 14.7 μM. All the compounds with substitution in phenyl ring demonstrated better activity than unsubstituted compound **4a**. *ortho*-Substitution in the phenyl ring was less favorable than *meta* or *para* substitution. Compound **4c** with a chloro substitution in the *meta* position of the phenyl ring showed the best binding affinity (IC_{50} = 4.0 ± 0.5 μM). In comparison, the standard anticonvulsant phenytoin (**1**) was used as the positive control for the assay and it exhibited an IC_{50} of 126.0 μM. In the series **5a–5j**, *ortho*-substitution was also less favorable, and *meta*-substituted anilino triazines showed better activity than the *para*-substituted anilino triazines. An electron withdrawing group (Br, Cl) located on N^2-phenyl ring, as in **5b–5g**, showed the highest sodium channel binding activity with IC_{50} values ranging from 12.6 to 57.5 μM, with the sole exception of the *ortho* chloro-substituted compound **5b**. Compounds with electron donating groups (CH_3, CH_3O) on the N^2-phenyl ring demonstrated relatively weaker activity ranging from 71.51 to 581.73 μM. Compounds **6a–6e** showed more potent sodium channels binding activity than the positive control, phenytoin. However, aromatic triazines **7a–7f** were found to be weak blockers of the sodium channels with higher IC_{50} values compared to phenytoin. All compounds were tested for antifolate activity against bovine DHFR, and the results obtained as expressed as IC_{50}, are collated in Table 1. Only compounds **6a–6e** retained some inhibitory activity against bovine DHFR with IC_{50} from 12.8 to 58.8 μM. Not surprisingly, the rest of the compounds including phenytoin were found to be devoid of DHFR inhibitory activity (IC_{50} >100 μM). These results suggested that these sodium channel blockers, particularly compound **4c**, showed a promise in further development as it did not possess side-effect liability through DHFR inhibition. The results also confirmed that DHFR inhibitory activity may not be relate to the sodium channel binding activity among triazines.[21] To the best of our knowledge, these four groups of the 1,3,5-triazines are the first examples to show sodium channel binding activity.

Table 1
Sodium channels binding activity and antifolate activity against bovine DHFR of the synthesized compounds

Compound	R	BTX, IC_{50}[a] (μM)	DHFR: IC_{50} (μM)
4a	H	14.7 (1.3)	>100
4b	*o*-Cl	13.0 (0.6)	>100
4c	*m*-Cl	4.0 (0.5)	>100
4d	*p*-Cl	4.4 (0.3)	>100
4e	*o*-CH_3	9.1 (1.2)	>100
4f	*m*-CH_3	4.7 (0.1)	>100
4g	*p*-CH_3	4.2 (0.6)	>100
4h	*o*-OCH_3	12.3 (2.1)	>100
4i	*m*-OCH_3	5.1 (0.3)	>100
4j	*p*-OCH_3	7.8 (0.8)	>100
5a	H	258.6 (15.4)	>100
5b	*o*-Cl	85.5 (6.3)	>100
5c	*m*-Cl	16.5 (0.2)	>100
5d	*p*-Cl	24.7 (0.8)	>100
5e	*o*-Br	57.5 (2.0)	>100
5f	*m*-Br	12.6 (0.8)	>100
5g	*p*-Br	18.4 (1.2)	>100
5h	*o*-CH_3	581.7 (34.7)	>100
5i	*m*-CH_3	185.9 (5.9)	>100
5j	*p*-CH_3	71.5 (1.5)	>100
5k	*o*-OCH_3	343.1 (38.7)	>100
5l	*m*-OCH_3	76.2 (1.6)	>100
5m	*p*-OCH_3	207.2 (2.3)	>100
6a	H	53.2 (2.0)	19.5
6b	4-Cl	35.3 (0.5)	29.9
6c	4-CH_3	32.2 (1.0)	24.6
6d	4-CH_3O	59.8 (2.1)	58.8
6e	3,4-diCl	19.0 (0.8)	12.8
7a	*m*-Cl	245.5 (15.5)	>100
7b	3,4-diCl	273.8 (13.8)	>100
7c	*m*-Br	198.2 (17.2)	>100
7d	*m*-NO_2	189.9 (18.0)	>100
7e	3,4-diCl	168.1 (13.3)	>100
7f	*m*-Br	171.9 (5.1)	>100
Phenytoin		126.4 (7.0)	>100

[a] Values are mean (SEM), n = 3.

In conclusion, this preliminary study demonstrated that four groups of 1,3,5-triazines structures were new versatile scaffolds that exhibited sodium channel binding activity. Among these four structures, structure **4** showed the best activity. The most active compound **4c** prepared herein was about 30-fold more potent than

the control, phenytoin. In addition, most of these compounds including **4c** are devoid of DHFR inhibitory activity. The results from this study suggests that the compound **4c** can be utilized as a lead molecule for further investigation and optimization for neuronal sodium channel binding activity, the therapeutic benefits of which are yet to be established.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.052.

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19. All final products were characterized by UV, ^{1}H NMR, ESI-MS and elemental analysis. Representative examples, N^2-(2-methoxyphenyl)-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine (**5k**). 82.4% yield. Mp: 210–212 °C. ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 6H, $2CH_3$), 3.81 (s, 3H, OCH_3), 6.80 (m, 3H, aromatic C-H), 8.47 (d, 1H, aromatic C–H, J = 6.8 Hz). ESI-MS m/z 248.2 (M+1), λ_{max} (water) = 246.6 nm. Anal. Calcd for $C_{12}H_{17}N_5O{\cdot}0.3H_2O$: C, 57.04; H, 7.02; N, 27.71. Found: C, 57.00; H, 6.83; N, 27.42. Corresponding 1-(2-methoxyphenyl)-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine (**8k**). 67.2% yield; mp: 220–221 °C ^{1}H NMR (DMSO-d_6) δ 1.22 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.05–7.52 (m, 4H, aromatic C–H). ESI-MS m/z 248.2 (M+1), λ_{max} (water) = 240.0 nm.
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