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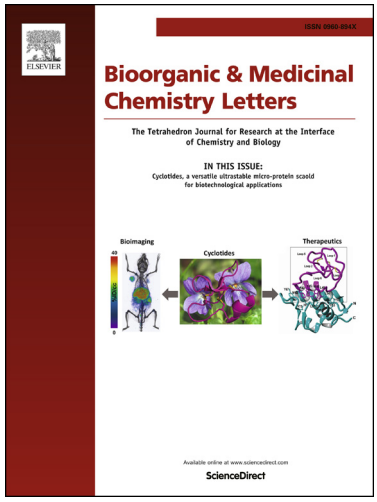

Design, Synthesis, Biological Evaluation, Homology Modeling and Docking Studies of (E)-3-(benzo[d][1,3]dioxol-5-ylmethylene) Pyrrolidin-2-one Derivatives as Potent Anticonvulsant Agents

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Design, Synthesis, Biological Evaluation, Homology Modeling and Docking Studies of (E)-3-(benzo[d][1,3]dioxol-5-ylmethylene) Pyrrolidin-2-one Derivatives as Potent Anticonvulsant Agents

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Abstract:

A series of (E)-3-(benzo[d][1,3]dioxol-5-ylmethylene)pyrrolidin-2-one derivatives were designed, synthesized, and evaluated for their anticonvulsant activities. In the preliminary screening, compounds **5**, **6a-6f** and **6h-6i** showed promising anticonvulsant activities in MES model, while **6f** and **6g** represented protection against seizures at doses of 100 mg/kg and 0.5 h in scPTZ model. The most active compound **6d** had a high-degree protection against the MES-induced seizures with ED_{50} value of 4.3 mg/kg and TD_{50} value of 160.9 mg/kg after intraperitoneal (*i.p.*) injection in mice, which provided **6d** in a high protective index (TD_{50}/ED_{50}) of 37.4 comparable to the

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reference drugs. Beyond that, **6d** has been selected and evaluated *in vitro* experiment to estimate the activation impact. Apparently, **6d** clearly inhibits the $Na_v1.1$ channel. Our preliminary results provide new insights for the development of small-molecule activators targeting specifically Nav1.1 channels to design potential drugs for treating epilepsy. The computational parameters, such as homology modeling, docking study, and ADME prediction, were made to exploit the results.

Keywords: (E)-3-(benzo[d][1,3]dioxol-5-ylmethylene)pyrrolidin-2-one, design; synthesis, anticonvulsant; homology modeling; docking study

Nowadays, it is urgent to discover new chemical entities as antiepileptic drugs (AEDs), since about one-third of patients undergoing epilepsy is still insufficiently treated[1]. Besides, currently available AEDs tend to cause multiple serious side-effects, such as drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism, and megaloblastic anaemia[2-4].

Due to incomplete information on the pathogenesis of human epilepsy and the complex mechanisms of actions of AEDs, it is challenging to use rational methodologies to discover new AEDs[5]. Therefore, the ligand-based pharmacophore approach is an important strategy for designing novel anticonvulsant agents[6-8]. For example, Malik S. *et. al.* reported a series of 3-(benzo[d]isoxazol-3-yl)-N-substituted pyrrolidine-2, 5-diones, which were designed dependent upon the existing biological data from old drugs, new drugs, and some other anticonvulsant active components. In this regard, we examined the structural characteristics of typical anticonvulsant agents, such as ethosuximide[9], levetiracetam[10], brivaracetam[11], and seletracetam[12] (Figure 1). The inspection showed that these drugs shared a common pyrrolidinone moiety in their molecules. Inspired by this observation, we assumed that the compound containing pyrrolidinone moiety in a single molecule could be favorable to

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anticonvulsant activity.

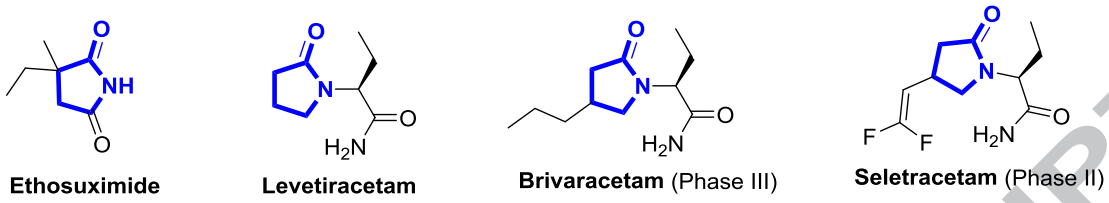

Figure 1. Anticonvulsant agents bearing pyrrolidinone fragments

An earlier structure-activity relationship studies of stiripentol[13] revealed the remarkable anticonvulsant activity for the skeleton of benzo[d][1,3]dioxole. Aboul-enein *et. al.*[13] attached semicarbazone moiety to the backbone of stiripentol (strategy **a)** and cyclization of the semicarbazone (strategy **b**) to afford the semicarbazone **S_1** and racemic pyrazoline **S_2** (Figure 2), respectively. **S_1** showed an excellent ED_{50} value of 87 mg/kg in the MES model, while **S_2** had a good activity against scPTZ-induced seizures (ED_{50} =110 mg/kg).

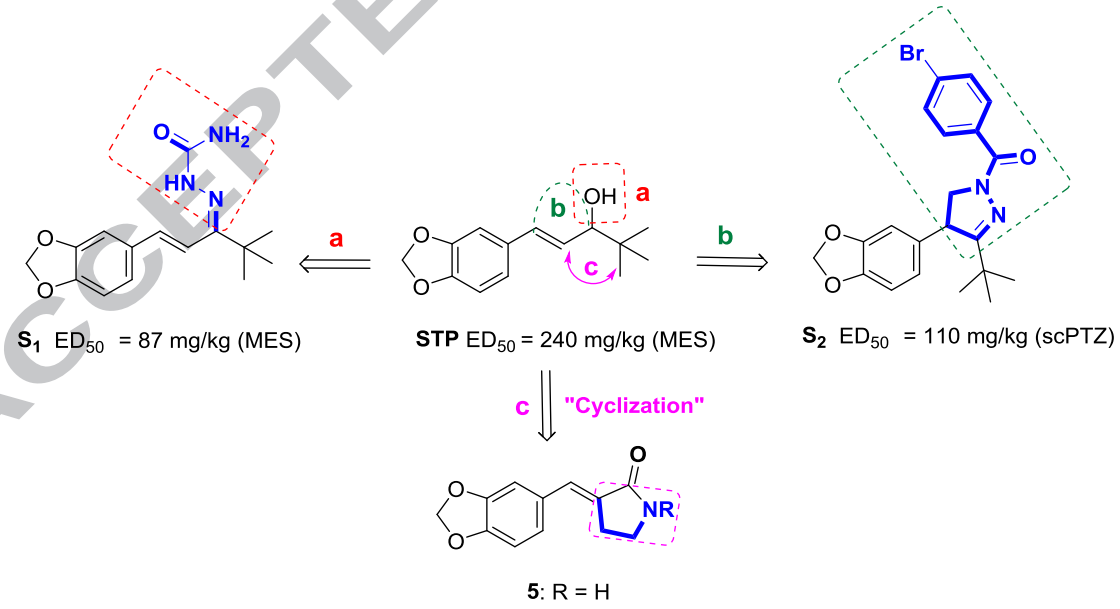

Figure 2. Stiripentol and designed anticonvulsant agents

In this study, we implemented the strategy **c**. To be specific, a five-membered pyrrolidinone ring was used to modify neopentyl alcohol, which aimed to generate a

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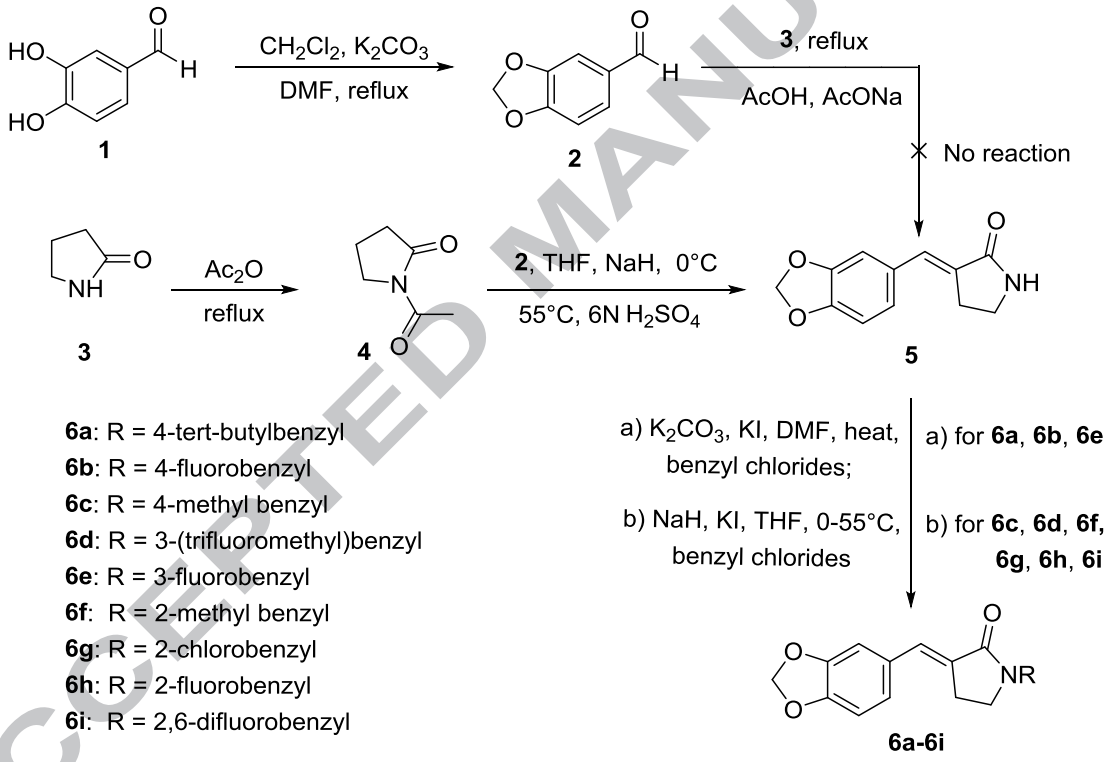
synergetic effect in the treatment of epilepsy. The structures of designed targeting compounds along with stiripentol were presented in Figure 2.

Generally speaking, the benzyl-based derivatives were beneficial and important for anticonvulsant activity, which had been depicted in numerous studies[14-16] including the marketed drugs Lacosamide, Rufinamide, Safinamide, and Retigabine. Herein, the influences of N-terminal benzyl substitutions on the anticonvulsant profiles of **5** were further explored *via* synthesis and pharmacological evaluation of **6a-6i**.



Scheme 1. The synthesis route of targeting compounds **6a-6i**.

The synthetic route of targeting compounds was illustrated in Scheme 1. 3, 4-Dihydroxy benzaldehyde **1** was firstly reacted with CH_2Cl_2 in presence of DMF according to the published method[17]. Besides, the resulting **2** was used as an active scaffold for the synthesis of *E*-configuration **5** carrying Knoevenagel condensation reaction. However, due to the weak acidity of pyrrolidone **3** α-hydrogens, benzo[*d*][1,3]dioxole-5-carbaldehyde **2** could not be directly condensed with **3**.

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Instead, compound **3** was initially reacted with acetic anhydride to produce **4**. Herein, the N-acetyl group is an electron-withdrawing group which facilitates the condensation reaction through enhancing the acidity of the α-hydrogens[18]. Then, an excess of base was used during the condensation and the activating group was easily removed from the product **5**. Furthermore, the resulting **5** was modified with different substituted benzyl chlorides and **6a-6i** was obtained.

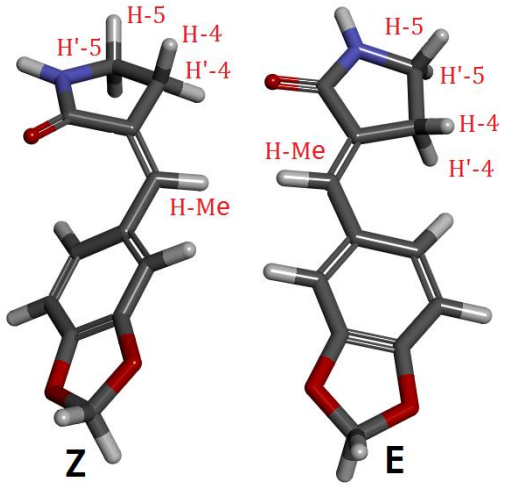

Figure 4. Computational geometries of the hypothetical Z- and E- configurations of **5** obtained by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10 (Discovery Studio 4.5 version).

Compound **5** did not form crystals suitable for X-ray diffraction (XRD) analysis. As a result, the further structural elucidations were limited to the ^{1}H-NMR experiments. From the two hypothetical conformations of **5** (Figure 4), the *E*-configuration was proposed as the more feasible one. Besides, a long-range spin-spin coupling of H-4, H′-4 and H-Me was observed in the NMR spectrum of **5**. Additionally, the signal of protons H-4 and H′-4 appeared as a triplet of doublets with two vicinal coupling constants of $J_{H4,\,H'4\text{-}\,H5,\,H'5}$ = 6.6 and $J_{H4,\,H'4\text{-}\,\text{-}HMe}$ = 3.0 (in Hz). It is worth mentioning that the NOE experiment showed no correlations between the H-Me and H-4, H′-4 signals, suggesting that H-Me and H-4 H′-4 were on the opposite

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sides of the double bond, which was only possible for the *E*-configuration **5** (See Supplementary Figure S2 ^{1}H-NMR and NOE spectrums in Supplementary data).

The preclinical discovery and development of novel chemical entities to treat epilepsy heavily rely on using animal models of seizures. The maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screening are two important and routine animal models for the anticonvulsant studies.[19, 20] Almost all AEDs are protective in at least one of these two models.[21] Therefore, these two kinds of anticonvulsant tests are significant for the clinical prediction of the anticonvulsant drug candidates. Besides, the acute neurological toxicity (NT) was required and determined by the rotarod test.[22] The screening results had been summarized in Table 1.

Table 1 The primary anticonvulsant data in mice of compounds **5** and **6a**–**6i** (*i.p.*).

Compounds	Intraperitoneal injection in mice[a]					
	MES screening[b]		scPTZ screening[c]		NT screening[d]	
	0.5h	4h	0.5h	4h	0.5h	4h
5	30	100	-	-	300	300
6a	100	100	-	-	300	300
6b	30	-	-	-	300	300
6c	30	30	-	-	300	300
6d	30	30	-	-	300	300
6e	-	30	-	-	300	300
6f	-	30	100	-	300	300
6g	-	-	100	-	300	300
6h	30	30	-	-	300	300
6i	100	100	-	-	300	300
Phenytoin	30	30	-	-	100	100

[a] Number of animal used = 3, Doses of 30, 100 and 300 mg/kg were administered. The figure in the table indicates the minimum dose whereby bioactivity was demonstrated in two or three mice. After injection was made, the animals were examined 0.5 and 4.0 h. The dash (-) indicates the absence of activity at maximum dose administered (300 mg/kg). [b] Maximal electroshock test. [c] Subcutaneous pentylenetetrazole test. [d] Neurotoxocity screening (rotarod test).

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As shown in Table 1, compounds **5**, **6a-6f** and **6h-6i** exhibited considerable anticonvulsant activities in the MES test. An instilling into these outcomes showed that **6c, 6d** and **6h** were more effective in a dose of 30 mg/kg at the both reported time intervals (0.5 and 4.0 h), which depicted a quick onset and prolonged anticonvulsant potential of these derivatives at the minimum dose. Among the entire compounds in scPTZ test, **6f** and **6g** were found to be significantly active at a dose of 100 mg/kg after 0.5 h, but not continue to show activity after 4.0 h. The rotarod test indicated that all compounds were less toxic than phenytoin and displayed motor impairment at the dose of 300 mg/kg at 0.5 and 4.0 h.

As a result of the primary screening, the selected highly active compounds (**5**, **6c**, **6d** and **6h**) were further subjected to secondary trials to quantify their preliminary anticonvulsant activity in mice. As displayed in Table 2, **6d** was the most active and promising compound in this study. With an ED_{50} of 4.3 mg/kg and TD_{50} value of 160.9 mg/kg, it can cause a PI value of 37.4. The order of anticonvulsant activities in the MES test as judged from ED_{50} values is **6d** > **6h** > **6c** > **5**. As shown by the pharmacological results, these compounds were active against the MES test, which could be recommended to develop as anticonvulsant candidates in treating grand mal seizures.

Table 2 The secondary quantitative anticonvulsant evaluation in MES model in mice (*i.p.*).

Compounds	TPE (h)[a]	ED_{50}[b]	TD_{50}[c]	PI[d]
5	1	17.3(12.1-27.7)[e]	179.4 (142.1-223.1)	10.4
6c	1	6.3(1.4-13.3)	167.2 (123.7-195.3)	26.5
6d	1	4.3(2.3-7.3)	160.9 (115.3-224.4)	37.4
6h	1	5.6(1.0-11.3)	238.5(220.0--272.0)	42.6
Phenytoin	2	9.5 (8.1-10.4)	65.5(52.5-72.9)	6.9
Phenobarbital	1	21.8(21.825.5)	69(62.8-72.9)	3.2

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[a] Time to peak effect; [b] ED_{50}: median effective dose affording anticonvulsant protection in 50% of animals, the dose is measured in mg/kg; [c] TD_{50}: median toxic dose eliciting minimal neurological toxicity in 50% of animals, the dose is measured in mg/kg; [d] PI: protective index (TD_{50}/ED_{50}); [e] 95% confidence intervals given in parentheses.

A wealth of evidence[23-26] shows that compounds effective in the MES model are thought to be sodium channel blockers and they can block seizure spread (e.g., phenytoin, carbamazepine), while those effective in the scPTZ model are often $GABA_A$ agonists. To explain the possible mechanism of anticonvulsant action, for the chosen active **6d**, its influence on voltage-dependent Na^+ channel was tested *in vitro*. The selected Na_V 1.1 (encoded by the SCN1A gene), as a test channel, is the most frequent target of mutations, which is responsible for genetic epilepsy syndromes with a wide range of severity[27, 28].

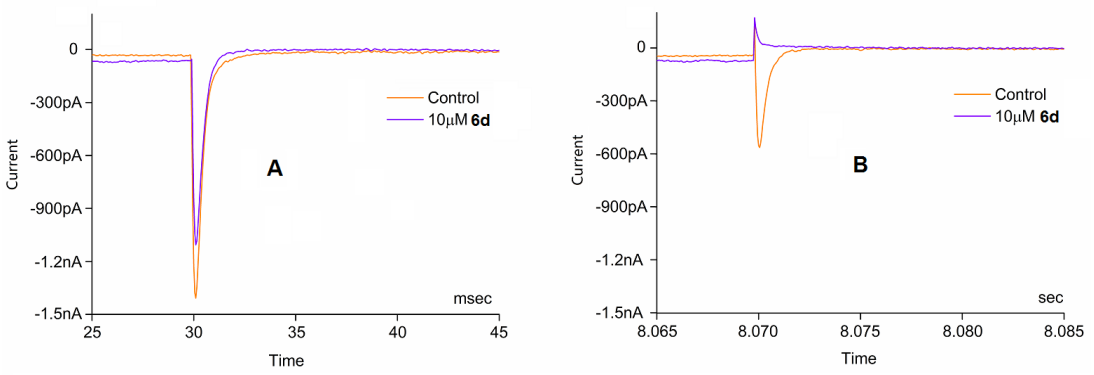

Figure 3. State- and use-dependent block of Na_V 1.1 voltage-gated Na^+ channel by **6d**. (A): state-dependency (TP1); (B): use-dependency (TP2).

Small molecule sodium channel modulators frequently possess state- and use-dependency. An ideal sodium channel blocker has much higher affinity to the inactivated (use-dependency) than the resting conformation of the sodium channel protein (state-dependency) [29-30]. In the present study, we compared the state- and use-dependent **6d** block of Na^+ currents carried by Na_V 1.1 isoform expressed in CHO-K1 cells, and the result is summarized in Table 3 and illustrated in Figure 3.

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The state-dependent block of Na_V 1.1 channel by **6d** was weak, with a 19.5% inhibition ratio. By contrast, the use-dependent block of Na_V 1.1 channel isoform by **6d** during repetitive pulses was strong at 10 μM, up to 99.9% inhibition ratio for Nav1.1. As indicated by this result, **6d** was highly selective for use-dependent block of Na_v 1.1 voltage-gated Na^+ channel, which would have little toxic and side-effects on the sodium channel of normal resting state.

Table 3. Electrophysiology studies for **6d**. Anticonvulsant drugs on voltage dependent sodium currents in CHO-K1 cells.

Dependency	Current (pA)		Inhibition (%)
	Control	**6d** (10μM)	
state-dependency	-1205.8 ± 117.0	-969.6 ± 78.4	19.5 ± 1.3
use-dependency	-702.7 ± 196.5	3.1 ± 6.6	99.9 ± 0.1

In order to illustrate the possible binding mode of the title compound **6d** towards the Na_v1.1 channel, we have planned to perform molecular docking simulations. However, because of the unavailability of the crystal structure of Na_v1.1, the 3D structure of Na_v1.1 was constructed based on the homology modeling using the crystal structure of the open-pore Electric eel Nav1.4 as the template (Supporting Information, Figure S3, S4 and S5). The homology modeling algorithm generated ten Na_v1.1 models, and the quality of generated Na_v1.1 models were assessed using "Verify Protein (Profiles-3D)" protocol in Discovery Studio 4.5 version (DS). Beyond that, the best model regarding the least Discrete Optimized Protein Energy (DOPE) score was chosen. Validation of the model was performed by analyzing Ramachandran plot (Supporting Information, Figure S6.). The resulting Ramachandran plot showed more than 98% residues distributed in the allowed regions, indicating that the generated model is reasonable.

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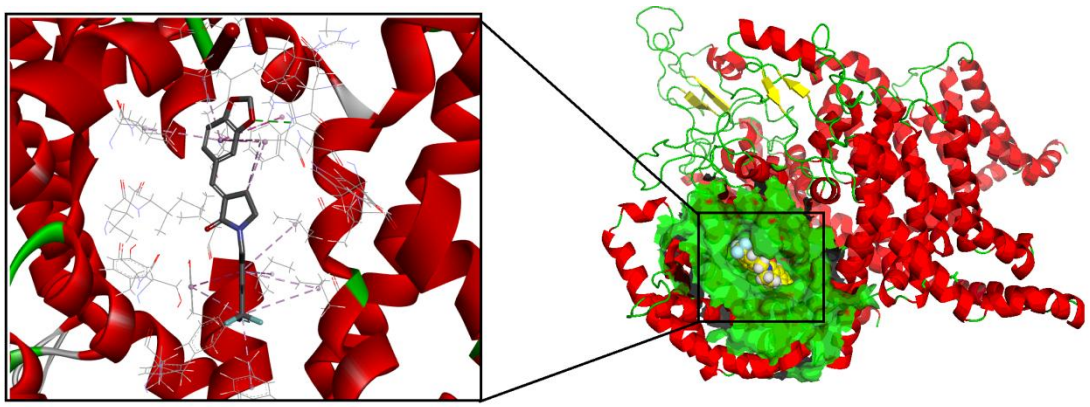

Figure 5. The 3D binding mode of **6d** in our human $Na_v1.1$ open-pore conformation homology model was shown in the cartoon form. The helix was shown in red, and the sheet strands were yellow. The green region was a space filling representation of atoms lining the active site.

The 2D/3D binding model of **6d** in our human $Na_v1.1$ open-pore homology model was shown in Figure 5 and 6. Besides, the sodium channel-blocking antiepileptic drug phenytoin was performed by a molecular docking to compare the binding modes with **6d** in Nav1.1 (Figure 6). The most important residues in the binding mode of $Na_v1.1$ include TRP668, ILE1067, TYR1068, PHE1071, IEU705, LEU708, ALA667, LEU724, VAL1160 and LEU1159. The benzo[d][1,3]dioxole fragment of compound **6d** occupies the large hydrophobic pocket directed toward TRP668, ALA667, LEU724, and LEU1159. Specifically, one of the ether oxygen groups at the benzo[d][1,3]dioxole ring interacts with TRP668 of the $Na_v1.1$ active domain via the formation of a medium-strong hydrogen bond (3.33Å). This would help to stabilize the open form and generate tighter binding to the catalytic sites of $Na_v1.1$. Furthermore, the interactions were also stabilized by a π-π stacked interactions with TRP668 (3.64Å), and three alkyl interactions with ALA667 (4.21Å), LEU724 (5.39Å) and LEU1159 (6.56Å). In addition to the above interactions, two alkyl interactions with TRP668 (5.03Å) and ALA667 (4.84Å) are observed in the pyrrolidinone ring. It

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is worth mentioning that the benzyl moiety plays an important role in the binding, as the aromatic moiety is involved in a π-π stacked interaction with TYR1068 (5.83Å) as well as six alkyl interactions with ILE1067 (6.57Å), TYR1068 (4.27Å), PHE1071 (4.75Å), IEU705 (5.52, 6.08Å) and LEU708 (6.18Å). More importantly, the fluorine atom of benzyl group presented in **6d** showed a halogen bonding interaction with ILE1067 (4.71Å) as well as a C-H bonding interaction with TYR1068 (3.50Å).

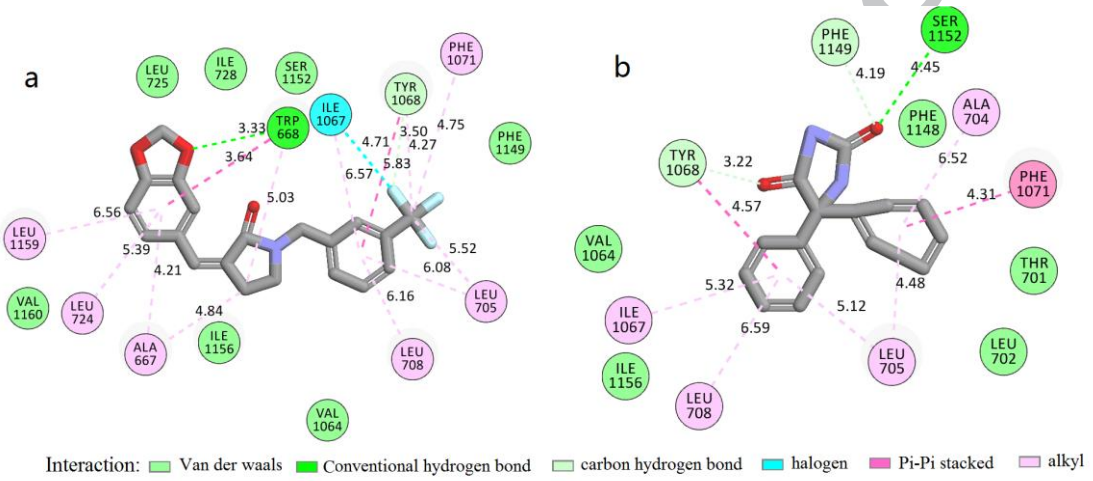

Figure 6. The 2D binding mode of **6d** and Phenytoin in our $Na_v1.1$ open-pore conformation homology model

For comparison, phenytoin not only entirely occupied the hydrophobic pocket but also formed an H-bond between the carbonyl oxygen atom and the residue SER1152 (4.45Å). Apparently, two benzene rings of phenytoin are responsible for two π-π stacked interactions as well as five alkyl interactions with the hydrophobic residues of the inner cavity, such as TYR1068, ALA704, PHE1071, LEU705, LEU708 and ILE1067 as the active pocket consisted of 13 amino acid residues in Figure 6b. These docking study results demonstrated a minimal difference between the binding modes of **6d** and phenytoin. However, they still share four critical residues (PHE1071, LEU705, LEU708 and ILE1067) in the active site of $Na_v1.1$ receptor.

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A computational study to predict the absorption, distribution, metabolism and excretion (ADME) properties of the compounds was performed. Topological polar surface area (TPSA) is a descriptor that is strongly correlated with passive molecular transport through membranes, and accordingly predicts the transport properties of drugs in the intestines and blood brain barrier crossing[31]. Other included parameters are the number of rotatable bonds, molecular volume, topological polar surface area, percentage absorption, and *in vitro* plasma protein binding. These parameters allow ascertaining oral absorption, or membrane permeability that occurs when evaluated molecules obey Lipinski's rule-of-five. The results were calculated for **5**, **6a-6i** and were shown in Table 4. From the data, it could be observed that the title compounds **5**, and **6b-6i** obeyed Lipinski's rule of five with good drug-like properties and membrane permeability (log P≤5, MW<500, HBD<5 and HBA<10). *In vitro* plasma protein binding (%) for the compounds **5**, **6a-6i** was given in Table 4. Compounds **5**, **6a-6i** presented less than 92% *in vitro* plasma protein binding, thus suggesting weak plasma protein binding of these compounds[32].

Table 4. Structural and pharmacokinetic properties of compounds **5** and **6a–6i**[a]

Comp.	MW	cLog *P*	HBA	HBD	nVio	n-ROTB	Volume	TPSA	% ABS	iPPB
Rule	<500	≤5	<10	<5	≤1	-	-	-	-	-
5	217.07	1.715	4	1	0	1	189.54	47.57	93.28	51.3
6a	363.18	5.325	4	0	1	4	344.32	38.78	95.62	91.0
6b	325.11	3.642	4	0	0	3	283.06	38.78	95.62	89.5
6c	321.14	3.998	4	0	0	3	294.69	38.78	95.62	88.0
6d	375.11	4.382	4	0	0	4	309.43	38.78	95.62	92.0
6e	325.11	3.642	4	0	0	3	283.06	38.78	95.62	89.5
6f	321.14	3.948	4	0	0	3	294.69	38.78	95.62	88.1
6g	341.08	4.212	4	0	0	3	291.67	38.78	95.62	90.3
6h	325.11	3.642	4	0	0	3	283.06	38.78	95.62	88.9
6i	343.10	3.785	4	0	0	3	287.99	38.78	95.62	89.7

[a] cLog P, calculated partition co-efficient; MW, molecular weight; HBA, the number of hydrogen bond acceptors; HBD, the number of hydrogen bond donors; nVio, the number of violations from Lipinski's rule-of-five; n-ROTB, the number of rotatable bonds; volume, molecular volume; iPPB,

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in vitro plasma protein binding (%); TPSA, topological polar surface area; %ABS, absorption percentage.

In conclusion, by using both the MES and PTZ tests, a series of 5-substituted benzo[*d*][1,3]dioxole derivatives were designed, synthesized, and evaluated for their anticonvulsant activities. Among them, **6d** was found to be the most potent with ED_{50} value of 4.3 mg/kg and protective index ($PI = TD_{50}/ED_{50}$) value of 37.4. The *in vivo* experiments demonstrated that it could block the Nav1.1 channel. Moreover, the study results may promote the rational design of additional novel anticonvulsants.

Acknowledgments

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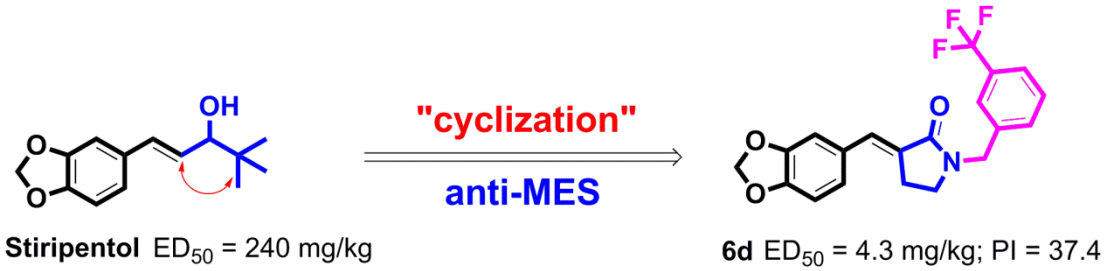
Graphical Abstract



Stiripentol ED_{50} = 240 mg/kg

6d ED_{50} = 4.3 mg/kg; PI = 37.4

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