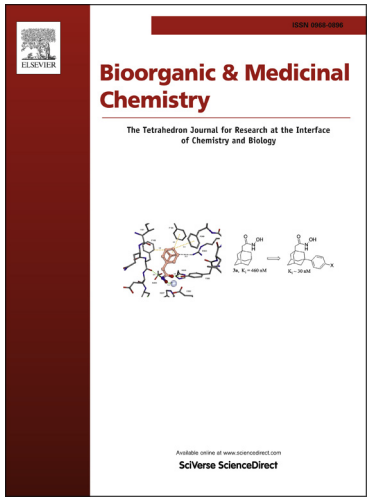

Design and bio-evaluation of indole derivatives as potent Kv1.5 inhibitors

Xiaoke Guo, Qian Yang, Jing Xu, Li Zhang, Hongxi Chu, Peng Yu, Yingying Zhu, Jinglian Wei, Weilin Chen, Yaozhong Zhang, Xiaojin Zhang, Haopeng Sun, Yiqun Tang, Qidong You

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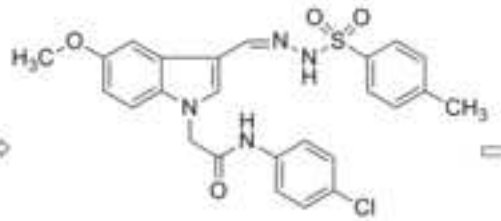
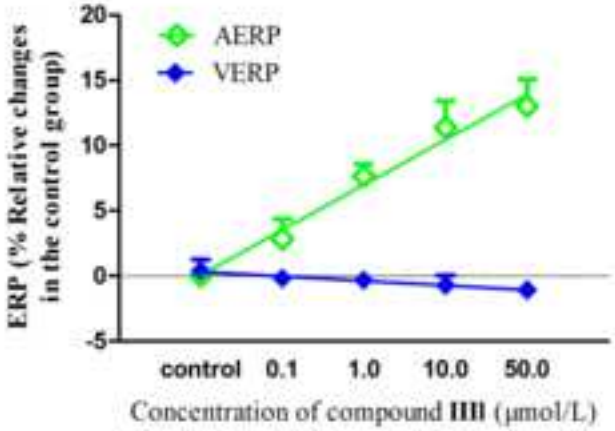
*Graphical Abstract

Mapping of compound **IIII** to the Kv1.5 pharmacophore model



compound **IIII**
Kv1.5 IC_{50} = 0.51 μ M
*h*ERG IC_{50} = 418.35 μ M
I_{Na} IC_{50} = 52.60 μ M

Compound **IIII** with potent Kv1.5 inhibitory activity and ideal selectivity



Selective profile of Compound **IIII** on pharmacodynamic model

Design and bio-evaluation of indole derivatives as potent Kv1.5 inhibitors

Xiaoke Guo [a, b, 1], Qian Yang [a, b, 1], Jing Xu [d], Li Zhang [d], Hongxi Chu [a, c], Peng Yu [d], Yingying Zhu [d], Jinglian Wei [c], Weilin Chen [c], Yaozhong Zhang [c], Xiaojin Zhang [a], Haopeng Sun [a, b, c, *], Yiqun Tang [d, *], Qidong You [a, b, *]

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Abstract: Atrial fibrillation (AF) is one of the common arrhythmias that threaten human health. Kv1.5 potassium channel is reported as an efficacious and safe target for the treatment of AF. In this paper, we designed and synthesized 3 series of compounds through modifying the lead compound RH01617 that was screened out by the pharmacophore model we reported earlier. All of the compounds were evaluated by the whole-patch lamp technology and most of them possessed potent inhibitory activities against Kv1.5. Compounds **IIIi** and **IIIl** were evaluated for the target selectivity as well as the pharmacodynamic effects in an isolated rat model. Due to the promising pharmacological behavior, compound **IIIl** deserves further pharmacodynamic and pharmacokinetic evaluations.

Keywords: Atrial fibrillation (AF); Kv1.5; Indole derivatives; Structure-activity relationship (SAR)

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1. Introduction

As the most common sustained form of arrhythmia, atrial fibrillation (AF) affects a large and growing population, [1-4] some further projections [5] indicate that the morbidity of AF will at least double in the next 5 decades with the ageing population and will lead to substantial mortality of stroke and heart failure. [6, 7]

Antiarrhythmic medications remain a mainstay in the treatment of AF. [8] Currently the antiarrhythmic agents, [9] such as dofetilide and sotalol, are limited in efficacy and considerable risks [2, 10] for their unselective blockade of the potassium currents in both atrial and ventricular myocytes. [11] One interesting potential target is the ultra-rapid potassium channel (I_{Kur}, encoded by Kv1.5 gene) which is functionally expressed in the atrium but not ventricle in humans, [12-14] suggesting Kv1.5 potassium channel as a novel selective target for the treatment of AF. [15]

Our previous studies [16, 17] reported a pharmacophore model of Kv1.5 (Figure 1) that was made up of one hydrogen bond acceptor, one aromatic ring, and two hydrophobic groups. After structure-based virtual screening of the compounds from the Maybridge database, in silico druglike property prediction and electrophysiological evaluation were undertaken, [18, 19] leading to a lead compound RH01617 with outstanding Kv1.5 inhibitory activity (Figure 1). Herein, with the aim of carefully investigating the structure-activity relationship (SAR), 3 series of derivatives containing 30 compounds were designed, synthesized and bioevaluated. Among them, compounds **IIIi** and **IIIl** showed potent activity and were chosen for further pharmacodynamic evaluations. The results showed that compound **IIIl** possessed remarkable selectivity Kv1.5 potassium channel activity over *h*ERG (human ether-a-go-go gene) potassium channel and sodium channel, while the atrial selective profile was also proved in the animal model *in vitro*.

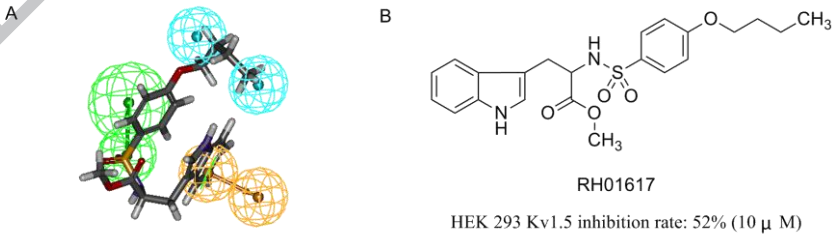


Figure 1. A: Mapping of RH01617 to the pharmacophore model (Green: hydrogen bond acceptor; Orange: aromatic ring; Blue: hydrophobic groups). B: Kv1.5 potassium channel inhibition of RH01617

2. Result and discussion

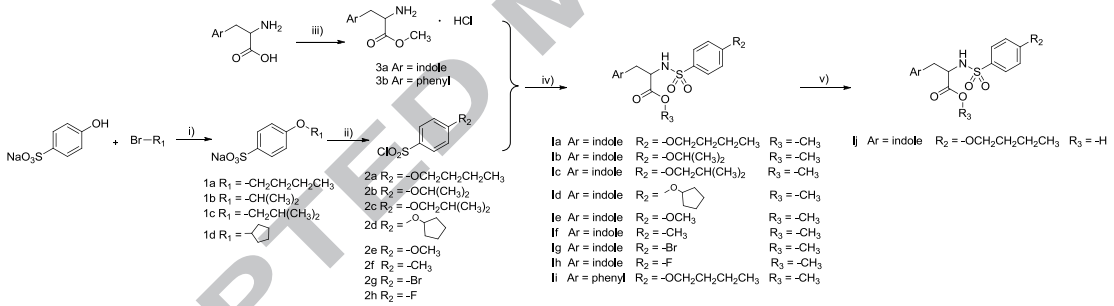
2.1 Chemistry

Compounds **Ia-Ii** were prepared from methyl tryptophan ester hydrochloride and commercially available or synthesized sulfonyl chloride derivatives (Scheme 1). Starting from 4-hydroxybenzenesulfonate and alkyl bromide,

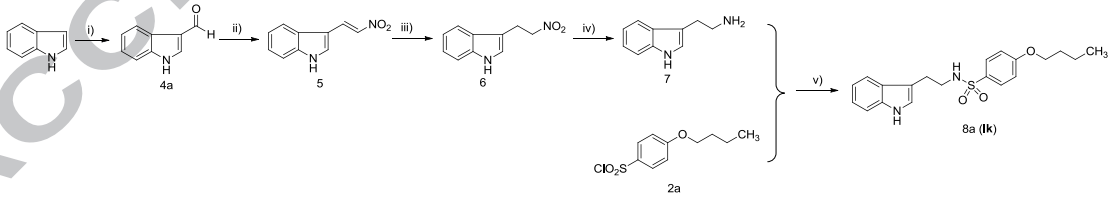
compounds **2a-2e** were obtained via substitution and acylation reaction. Compounds **3a-3b** were obtained from tryptophan or phenylalanine and CH_3OH in the presence of $SOCl_2$. Reaction of **2a-2e** with **3a-3b** in CH_2Cl_2 provided the target compounds **Ia-Ii**. Compound **Ia** was hydrolyzed by LiOH (1mol/L) to give the compound **Ij**.

With the purpose of investigating the SAR, we designed a compound **Ik** (Scheme 2) without the ester group. 1*H*-indole-3-carbaldehyde (**4a**) was synthesized using Vilsmeier Reaction from indole, and then be treated with CH_3NO_2 and NH_4OAc to give compound **5**. 2-(1*H*-indol-3-yl)ethanamine (**7**) was obtained by deoxidized compound **5** with $NaBH_4$ and H_2 (Pd/C) respectively, and then compound **7** reacted with **2a** to get the compound **8a** (**Ik**).

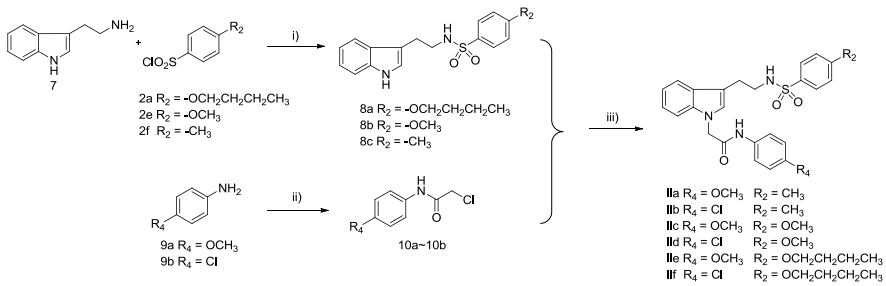
The synthetic routes of derivatives **IIa-IIf** and **IIIa-IIIm** were described in Schemes 3–4. Compound **7** reacted with **2a, 2b** or **2f** respectively to form the compounds **8a-8c**. Reaction of **8a-8c** with **10a-10b** that obtained from substituted aniline (**9a**, **9b**) provided the 2nd series of compounds. In the same way, treated compound **4** with 4-methylbenzenesulfonohydrazide (**11**) to get the compound **12**. Reaction of compound **12** with **10a-10j** provided the 3rd series of compounds.



Scheme 1. Reagents and Conditions: i) DMF (or CH_3OH), NaOH, 75 °C, 20 h; ii) CH_2Cl_2, $SOCl_2$, reflux, 4 h; iii) CH_3OH, $SOCl_2$, r.t., 30 min; iv) Et_3N, CH_2Cl_2, r.t., 4 h; v) LiOH (1mol/L), THF, r.t., 10 h.

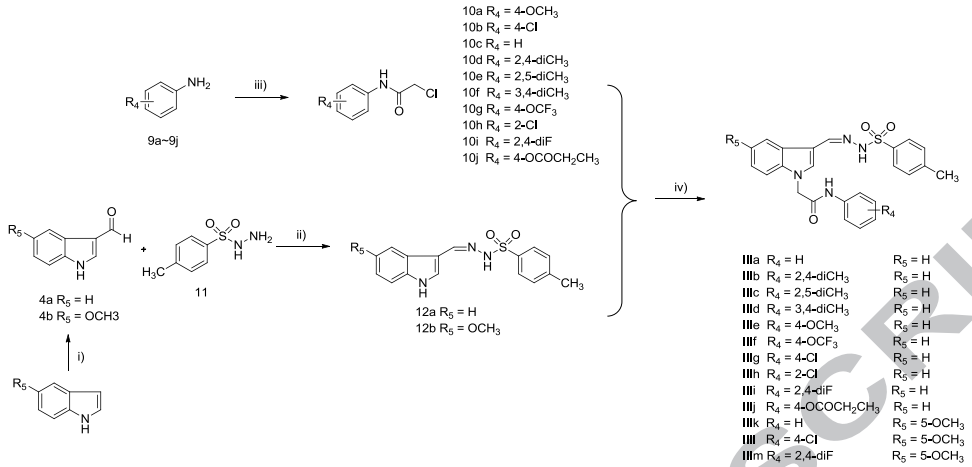


Scheme 2. Reagents and Conditions: i) $POCl_3$, DMF, 5 °C for 20 min and 35 °C for 1 h; ii) CH_3NO_2, NH_4OAc, 105 °C, 4 h; iii) $NaBH_4$, THF, CH_3OH, r.t., 30 min; iv) H_2, Pd/C, CH_3OH, 40 °C, 12 h; v) Et_3N, CH_2Cl_2, r.t., 3 h.



Scheme 3. Reagents and Conditions: i) Et_3N, CH_2Cl_2, r.t., 4 h; ii) $ClCH_2COCl$, K_2CO_3, CH_3CN, 0 °C and r.t., 2 h;

iii) NaH, DMF, N_2 protection, 5 °C for 2 h and r.t. for 4 h.



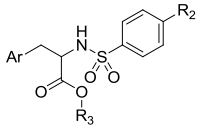
Scheme 4. Reagents and Conditions: i) $POCl_3$, DMF, 5 °C for 20 min and 35 °C for 1 h; ii) EtOH, reflux, 2 h; iii) $ClCH_2COCl$, K_2CO_3, DMF, r.t., 2 h; iv) NaH, DMF, N_2 protection, 5 °C for 2 h and r.t. for 4 h.

2.2 Structure–activity relationship

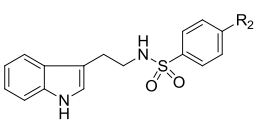
The *in vitro* Kv1.5 inhibitory activity of the 30 target compounds were tested using whole cell patch-clamp technique in a representative HEK 293 cell stably expressing *h*Kv1.5 channels. (Table 1 and 2).

We started our investigation by examining stereo-hindrance effects of substituent groups on phenyl moiety of the lead compound RH01617. Our results (Table 1) demonstrated that the steric hindrance at R_2 (**Ia-Ih**) had no apparent influence on the Kv1.5 activity, indicating a structural tolerance at this position. Removing the ester group to avoid potential instability and the chirality led to compound **Ik** which maintained the potency of Kv1.5 close to **Ia** (**Ik** IC_{50} = 9.95, **Ia** IC_{50} = 8.37). However, a significant loss (~ 13 fold) in Kv1.5 inhibitory potency was observed while hydrolyzing the ester group (**Ia**) to carboxyl group (**Ij**), presumably due to changed physicochemical properties. Exchanging indole ring to phenyl group caused a dramatic drop of activity of Kv1.5 affinity (**Ii** *vs* **Ia**), inferring indole ring was a crucial group to the inhibitory activity.

Table 1. The activity of Kv1.5 inhibition of 1st series of compounds



Ia~Ij



Ik

Comp.	Ar	R_2	R_3	HEK 293 *h*Kv1.5 IC_{50} (μM) [a]	CHO *h*ERG IC_{50} (μM) [a, b]	*h*ERG / Kv1.5
Ia	3-indolyl	$-OCH_2CH_2CH_2CH_3$	$-CH_3$	8.37 ± 0.953	ND	
Ib	3-indolyl	$-OCH(CH_3)_2$	$-CH_3$	3.93 ± 0.428	ND	
Ic	3-indolyl	$-OCH_2CH(CH3)_2$	$-CH_3$	3.56 ± 0.341	ND	
Id	3-indolyl		$-CH_3$	2.38 ± 0.792	9.56 ± 1.207	4.02

Ie	3-indolyl	-OCH_3	-CH_3	5.45 ± 0.688	ND	
If	3-indolyl	-CH_3	-CH_3	4.72 ± 0.340	ND	
Ig	3-indolyl	-Br	-CH_3	2.25 ± 0.191	4.66 ± 0.716	2.07
Ih	3-indolyl	-F	-CH_3	6.45 ± 0.782	ND	
Ii	3-indolyl	-$OCH_2CH_2CH_2CH_3$	H	109.74 ± 9.359	ND	
Ij	phenyl	-$OCH_2CH_2CH_2CH_3$	-CH_3	65.42 ± 7.443	ND	
Ik	-	-$OCH_2CH_2CH_2CH_3$	-	9.95 ± 0.941	3.18 ± 0.588	0.32

[a] Data are expressed as mean ± SD, n = 3. [b] ND for Not Determined

Lessening of *h*ERG channel activity is supposed to mitigate the safety risks associated with current antiarrhythmic agents. [20-26] With the aim to search a selective inhibitor of Kv1.5, 3 compounds (**Id**, **Ig** and **Ik**) that possessed potent Kv1.5 activity were selected to test their *in vitro h*ERG inhibitory activities using whole cell patch-clamp technique in a representative CHO cell stably expressing *h*ERG channels. [27] The results showed that this series of compounds were poor at Kv1.5 selectivity over *h*ERG, especially the compound **Ik** which activity ratio (*h*ERG / Kv1.5) was 0.32. Recently, some researches inferred that discrete structural modification was a satisfactory approach to decrease *h*ERG affinity. [28, 29] Moreover, a comparison between the compound **Ik** with some *h*ERG inhibitors (dofetilide, sotalol and *N*-acetylprocainamide), showed (Figure 2) a similar chemical structure – a long and flexibility chain. In order to reduce the *h*ERG affinity, *N*-phenyl amide group was introduced to compound **Ik** to change the molecular topological characteristics, resulting in 2nd series of compounds. To further increase the rigidity and bulkiness of the molecular, the coupling chain was modified from '-CH_2-CH_2-' to '-C=N-', affording 3rd series of compounds.

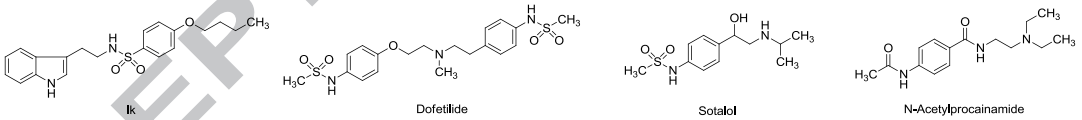


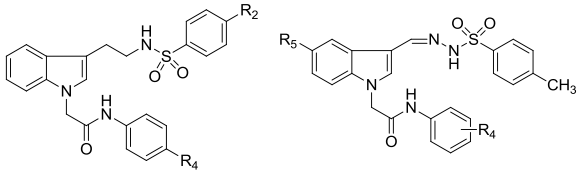
Figure 2. The structure of *h*ERG inhibitors and compound **Ik**

Compound **IIe** manifested similar Kv1.5 inhibitory effect to that of compound **Ik** (Table 2). Compounds with methoxyl substitution at R_2 (**IIc** and **IId)** showed more potent inhibitory activity than with methyl (**IIa** and **IIb)** and with *n*-butoxyl (**IIe** and **IIf)**. No remarkable difference in the activity was found using -Cl to replace -OCH_3 at R_4 except **IIf**. The selectivity of the 2nd series of compounds was slightly ameliorated.

As shown in Table 2, the 3rd series of compounds changing the coupling chain from '-CH_2-CH_2-' to '-C=N-' increased the Kv1.5 activity (**IIIa** *vs* **IIIe**, **IIIb** *vs* **IIIg**). Electron-withdrawing groups such as trifluoromethoxy group (**IIIf**) and 2,3-difluoro group (**IIIi**, **IIIm**) exhibited preferable activities than electron-donating groups such as alkyl groups (**IIIb**, **IIIc**, **IIId**) and methoxy group (**IIIe**). The introduction of a methoxyl group at R_5 (**IIIk**, **IIIl**, **IIIm**) provided similar inhibitory potency to unsubstituted ones (**IIIa**, **IIIg**, **IIIi**). It is noteworthy that 3rd series of

compounds exhibited remarkably increased selectivity. Compound **IIIl** exhibited almost a 2600-fold selective profile compared to compound **Ik** and 300-fold compared to compound **IId**, highlighting these compounds would be safe inhibitors.

Table 2. The activity of Kv1.5 inhibition of 2nd and 3rd series of compounds



IIa-IIf IIIa-IIIm

Comp.	R_2	R_4	R_5	HEK 293 *h*Kv1.5 IC_{50} (μM) [a]	CHO *h*ERG IC_{50} (μM) [a, b]	*h*ERG / Kv1.5
IIa	$-CH_3$	$-OCH_3$	-	38.73 ± 4.505	ND	
IIb	$-CH_3$	-Cl	-	12.92 ± 1.968	ND	
IIc	$-OCH_3$	$-OCH_3$	-	1.72 ± 0.037	10.23 ± 1.230	5.95
IId	$-OCH_3$	-Cl	-	3.14 ± 0.822	11.49 ± 1.931	3.66
IIe	$-OCH_2CH_2CH_2CH_3$	$-OCH_3$	-	6.21 ± 0.501	ND	
IIf	$-OCH_2CH_2CH_2CH_3$	-Cl	-	185.14 ± 12.293	ND	
IIIa	-	H	H	0.81 ± 0.012	43.62 ± 5.725	53.85
IIIb	-	2,4-diCH_3	H	24.01 ± 1.529	ND	
IIIc	-	2,5-diCH_3	H	1.77 ± 0.731	ND	
IIId	-	3,4-diCH_3	H	11.24 ± 0.694	ND	
IIIe	-	4-OCH_3	H	16.91 ± 1.082	ND	
IIIf	-	4-OCF_3	H	1.52 ± 0.079	ND	
IIIg	-	4-Cl	H	1.90 ± 0.136	ND	
IIIh	-	2-Cl	H	0.74 ± 0.057	18.66 ± 2.018	25.22
IIIi	-	2,4-diF	H	0.25 ± 0.041	42.97 ± 4.834	171.88
IIIj	-	4-$OCOCH_2CH_3$	H	2.14 ± 0.758	ND	
IIIk	-	H	5-OCH_3	3.73 ± 0.674	ND	
IIIl	-	4-Cl	5-OCH_3	0.51 ± 0.035	418.35 ± 33.219	820.29
IIIm	-	2,4-diF	5-OCH_3	2.18 ± 0.493	ND	

[a] Data are expressed as mean ± SD, n = 3. [b] ND for Not Determined

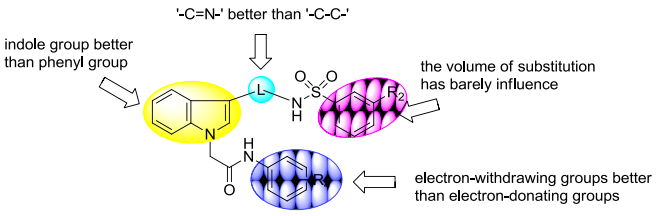


Figure 3. Graphical depiction of the general SAR for Kv1.5 activity based on the IC_{50} results on HEK293 cell lines

Our newly synthesized 29 compounds exerted significant *in vitro* inhibitory effects on Kv1.5 potassium channel and some of them had an obvious selective profile between Kv1.5 and *h*ERG. Taken together (Figure 3), these data indicated that the indole ring is an essential group for the activity against Kv1.5; '-C=N-' double bond as

the coupling chain showed better Kv1.5 affinity than '-C–C-' single bond and possessed improved selectivity; the potency is not correlated with the size of substitution at R_2 directly; electron-withdrawing group at R_4 had a greater contribution for activity than electron-donating group.

Compounds with potent Kv1.5 inhibitory effects and ideal selective profile were selected for further pharmacodynamics studies.

2.3 Selective assay on sodium-channel

After investigating *h*ERG potassium channel selectivity, a sodium channel inhibition assay was undertaken to research the effects of 3 compounds on other ion-channels in heart. Compounds **IIIa**, **IIIi** and **IIIl** were chosen for this test using whole cell patch-clamp technique in the fresh isolated ventricular myocytes from guinea pig (Table 3). Our compounds showed brilliant selectivity over sodium channel, especially compound **IIIi** (activity ratio = 459). This result proved once again our compounds could be safe and selective blockers of Kv1.5. Due to this intriguing selective behavior, further pharmacological studies were taken on compounds **IIIi** and **IIIl**.

Table 3. The activity of sodium channel inhibition of the compounds

Comp.	I_{Na} IC_{50} (μM) [a]	HEK 293 *h*Kv1.5 IC_{50} (μM) [a]	I_{Na} /Kv1.5
IIIa	77.97 ± 8.273	0.81 ± 0.012	96.26
IIIi	114.76 ± 12.314	0.25 ± 0.041	459.06
IIIl	52.60 ± 5.621	0.51 ± 0.035	103.14

[a] Data are expressed as mean ± SD, n = 3.

2.4 Pharmacodynamic studies of IIIi and IIIl

Compounds **IIIi** and **IIIl** had distinguished Kv1.5 activities and acceptable selective profiles and were further evaluated for efficacy in the rat pharmacodynamics model. Effect on atrial effective refractory period (AERP) and ventricular effective refractory period (VERP) of compounds **IIIi** and **IIIl** were tested respectively in isolated rat hearts. Two compounds both demonstrated a dose dependent prolongation in AERP without increasing VERP (Figure 4). Compared these two compounds, **IIIl** was more promising for its better selectivity between *h*ERG and Kv1.5 and the better linearity on rat model *in vitro*. These results indicated that compound **IIIl** could be a safe and potent Kv1.5 inhibitor and it desiring further pharmacodynamic and pharmacokinetic evaluations.

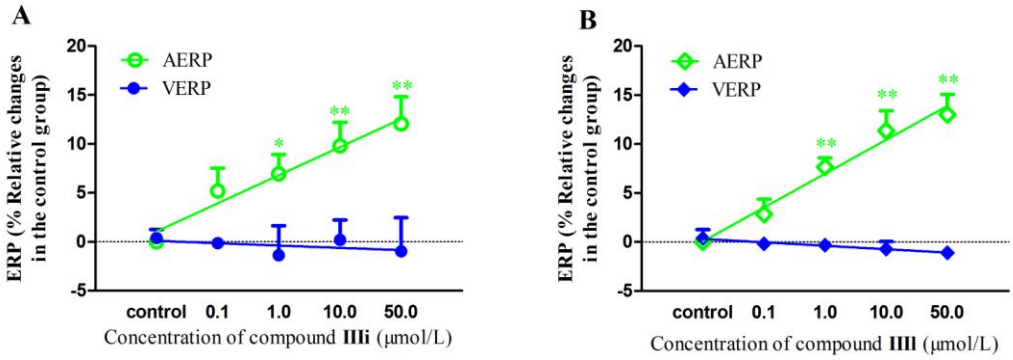


Figure 4. Effect on ERP in rats for compounds **IIIi** (A) and **IIIl** (B). (AERP = Atrial Effective Refractory Period, VERP = ventricular effective refractory period; Data are expressed as mean ± SEM, n = 3; * $P < 0.05$, ** $P < 0.01$)

3. Conclusion

Drug targeted cardiac Kv1.5 channel is supposed to have a safety advantage over current market drugs. In this study, we have designed and synthesized 29 novel compounds modified from the lead compound **Ia** which was identified from virtual screening we previously reported, aiming at discovering potent and selective Kv1.5 inhibitors. Structural modifications mainly addressing the side chain of phenyl, ester group, coupling chain and *N*-substituents at indolyl enabled modulation of Kv1.5 inhibitory potency, and most of the derivatives endowed effective affinity, these compounds produced a concentration-dependent inhibition of *h*Kv1.5 current. After introducing the rigidity and bulkiness moieties, selectivity over *h*ERG potassium channel and sodium channel of our compounds was significantly improved, and the pharmacodynamic effect on VERP reflected the selectivity for Kv1.5 over ventricular ion channels.

We discovered compound **IIIl** to be a very potent and selective blocker of Kv1.5. This compound also showed a significant pharmacodynamic effect in rats. For these reasons it was chosen for further preclinical development.

4. Experimental section

4.1. Chemistry

4.1.1. General methods, materials, and spectroscopic details

Melting points were measured with a Melt-Temp II instruments. IR spectra were recorded on a Nicolet Impact 410 spectrometer. EI-MS was recorded Shimadzu GC-MS 2050 apparatus; ESI-MS was recorded on Agilent 1100 LC/MSD (70 ev) spectrometers. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-300 or AV-500MHz instruments in DMSO-d_6 and $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. Chemical shifts were reported as d values (parts per million) relative to solvent peak. Coupling constants were reported in hertz. The multiplicity was defined by a s (singlet), d (doublet), t (triplet), or m (multiplet).

Column and thin-layer chromatography (CC and TLC, resp.) were performed on silica gel (200-300 mesh) and silica gel GF_{254} resp., supplied by *Qingdao Marine Chemical Factory*.

4.1.2. Sodium 4-butoxybenzenesulfonate (**1a**)

To a stirred solution of sodium 4-hydroxybenzenesulfonate (1.96g, 0.010mol) and NaOH (0.44g, 0.011mol) in DMF (15 mL) was added 1-bromobutane (2.20g, 0.016mol). The mixture was stirred at 75 °C for 20 h, and then concentrated under reduced pressure to 1/3 of its original volume and filtrated to get the title compound **1a** as white solid (0.77g, 33.31%). Mp > 250 °C.

4.1.3. Sodium 4-isopropoxybenzenesulfonate (**1b**)

To a stirred solution of dried sodium 4-hydroxybenzenesulfonate (2.94g, 0.015mol) and NaOH (0.60g, 0.015mol) in CH_3OH (100 mL) was added 2-bromopropane (2.77g, 0.023mol). The mixture was stirred at 80 °C for 48 h, and then concentrated under reduced pressure. The residue was dissolved in acetone and filtrated to remove the insoluble impurities and the filtrate was concentrated under reduced pressure to obtain the compound **Ib** as yellow solid (1.98g, 55.41%). Mp > 250 °C.

4.1.4. Sodium 4-isobutoxybenzenesulfonate (**1c**)

Compound **Ic** was synthesized from 4-hydroxybenzenesulfonate and 1-bromo-2-methylpropane with the procedure described for compound **Ia** (pink solid, 44.44%). Mp > 250 °C.

4.1.5. Sodium 4-(cyclopentyloxy)benzenesulfonate (**1d**)

Compound **Id** was synthesized from 4-hydroxybenzenesulfonate and bromocyclopentane with the procedure described for compound **Ia** (pink solid, 41.62%). Mp > 250 °C.

4.1.6. 4-butoxybenzene-1-sulfonyl chloride (**2a**)

$SOCl_2$ (1.3 mL, 0.018mol) and DMF (1~2 drops) were added to a stirring solution of compound **1a** (0.77g, 0.003mol) in anhydrous CH_2Cl_2 (25 mL) under ice-water bath. After being refluxed for 4 h, the mixture was filtrated to remove the insoluble impurities and the filtrate was washed with NaCl saturated solution (x 3), then concentrated under reduced pressure to give the compound **2a** as a yellow oil (0.46g, 61.33%).

4.1.7. 4-isopropoxybenzene-1-sulfonyl chloride (**2b**)

Compound **2b** was synthesized from $SOCl_2$ and sodium 4-isopropoxybenzenesulfonate (**1b**) with the procedure described for compound **2a** (colorless oil, 52.62%).

4.1.8. 4-isobutoxybenzene-1-sulfonyl chloride (**2c**)

Compound **2c** was synthesized from $SOCl_2$ and sodium 4-isobutoxybenzenesulfonate (**1c**) with the procedure

described for compound **2a** (colorless oil, 40.40%).

4.1.9. 4-(cyclopentyloxy)benzene-1-sulfonyl chloride (**2d**)

Compound **2d** was synthesized from $SOCl_2$ and sodium 4-(cyclopentyloxy)benzenesulfonate (**1d**) with the procedure described for compound **2a** (colorless oil, 54.99%).

4.1.10. Methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**)

To a solution of DL-tryptophane (8.16g, 0.040mol) in methanol (80 mL) was added dropwise $SOCl_2$ (14.28g, 0.120mol), and the mixture was stirred at r.t. for 30 min. The solution was concentrated and filtered, then the residue was washed with acetone and dried under infrared light (IR) to give the compound **3a** as a white solid (8.07g, 92.54%). Mp = 220 °C, Lit. [30] Mp = 221-222 °C.

4.1.11. Methyl 2-amino-3-phenylpropanoate hydrochloride (**3b**)

Compound **3b** was synthesized from phenylalanine with the procedure described for compound **3a** (white solid, 93.61%). Mp = 159 °C, Lit. [30] Mp = 156-157 °C.

4.1.12. 1*H*-indole-3-carbaldehyde (**4a**)

To a stirring solution of $POCl_3$ (18.36g, 0.120mol) in DMF (25 mL), indole (11.7g, 0.100mol) in DMF (15 mL) was added dropwise. The mixture was stirred at 35 °C for 1 h, and then be poured into ice water and neutralized with 20% NaOH a.q. The solution was filtered and the residue was washed with water and dried under IR to give the compound **4a** as yellow needle (13.86g, 95.48%). Mp = 192-194 °C, Lit. [31] Mp = 196-197 °C.

4.1.13. 5-methoxy-1H-indole-3-carbaldehyde (**4b**)

Compound **4b** was synthesized from 5-methoxy-1*H*-indole with the procedure described for compound **4a** (yellow solid, 89.4%). Mp = 57 °C, Lit. [31] Mp = 54-55 °C.

4.1.14. 3-(2-nitrovinyl)-1*H*-indole (**5**)

To a solution of 1*H*-indole-3-carbaldehyde (**4a**, 1.45g, 0.010mol) in nitromethane (15 mL) was added ammonium acetate (0.77g, 0.010mol), the mixture was stirred at 105 °C for 4 h and evaporated until dryness to give the compound **5** as yellow solid (1.09g, 57.98%). Mp = 168-170 °C, Lit. [32] Mp = 169-172 °C.

4.1.15. 3-(2-nitroethyl)-1*H*-indole (**6**)

$NaBH_4$ was added slowly to a stirring solution of 3-(2-nitrovinyl)-1*H*-indole (**5**, 18.80g, 0.100mol) and CH_3OH (20 mL) in THF (100 mL), the mixture was stirred at r.t. for 30 min and concentrated to 1/5 of its original volume and neutralized by HCl (1 mol/L). Then, the solution was extracted by EA (50 mL x 3) and the organic layer was concentrated under reduced pressure. The crude material was purified by column chromatography (CC) (petroleum ether/AcEt = 5:1) to get the compound **6** as yellow solid (9.12g, 47.95%). Mp = 56-58°C, Lit. [33] Mp =

55-57 °C.

4.1.16. 2-(1*H*-indol-3-yl)ethanamine (**7**)

To a solution of 3-(2-nitroethyl)-1*H*-indole (**6**, 9.12g, 0.048mol) in CH_3OH (100 mL) was added Pd/C (10%, 1g), the mixture was stirred at 40 °C with continuous H_2 for 12 h, and then was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in water (100 mL) and extracted by EA (40 mL x 3), the organic layer was evaporated until dryness to give the compound **7** as yellow solid (5.50g, 71.61%). Mp = 110-112 °C, Lit. [34] Mp = 112 °C.

4.1.17. *N*-(2-(1*H*-indol-3-yl)ethyl)-4-butoxybenzenesulfonamide (**8a**, **Ik**)

4-butoxybenzene-1-sulfonyl chloride (**2a**, 4.97g, 0.020mol) was added to a stirring solution of 2-(1*H*-indol-3-yl)ethanamine (**7**, 3.20g, 0.020mol) and Et_3N (4.04g, 0.040mol) in CH_2Cl_2 (100 mL). After being stirred at r.t. for 3 h, the mixture was washed by water (30 mL x 3) and was concentrated under reduced pressure. The crude material was purified by CC (petroleum ether/AcEt = 2:1) to get the compound **8a** (**Ik**) as white solid (4.64g, 85.91%). Mp = 84-87 °C.

^{1}H-NMR(300 MHz, DMSO) δ 10.81 (1H, s), 7.73-7.70 (2H, m), 7.57 (1H, t, J = 5.70 Hz), 7.45-7.31 (2H, m), 7.12-6.92 (5H, m), 4.03 (2H, t, J = 6.30 Hz), 3.01-2.94 (2H, m), 2.81-2.76 (2H, m), 1.75-1.65 (2H, m), 1.49-1.36 (2H, m), 0.92 (3H, t, J = 7.50 Hz). MS (ESI) *m/z* 372 [M^+]. HR-MS ([M + NH_4]$^+$) Calcd for $C_{20}H_{24}N_4O_3S.NH_4$, 390.184; Found 390.1845.

4.1.18. *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methoxybenzenesulfonamide (**8b**)

Compound **8b** was synthesized from 4-methoxybenzene-1-sulfonyl chloride (**2e**) and 2-(1*H*-indol-3-yl)ethanamine (**7**, 3.20g, 0.020mol) with the procedure described for compound **8a** (white solid, 12.64%). Mp = 94-96 °C.

4.1.19. *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methylbenzenesulfonamide (**8c**)

Compound **8c** was synthesized from 4-methylbenzene-1-sulfonyl chloride (**2f**) and 2-(1*H*-indol-3-yl)ethanamine (**7**, 3.20g, 0.020mol) with the procedure described for compound **8a** (white solid, 73.95%). Mp = 107-109 °C.

4.1.20. 2-chloro-*N*-(4-methoxyphenyl)acetamide (**10a**)

To a solution of a4-methoxyaniline (6.16g, 0.050mol), Et_3N (5.05g, 0.050mol) in DMF (40 mL) was added chloroacely chloride (6.78g, 0.06mol) dissolved in DMF (15 mL) under ice bath. The mixture was stirred at r.t. for 2 h and poured into water and then filtered to get the compound compound **10a** as yellow solid (9.61g, 96.25%). Mp = 120-122 °C, Lit. [35] Mp = 124 °C.

4.1.21. 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**)

Compound **10b** was synthesized from 4-chloroaniline with the procedure described for compound **10a** (white solid, 94.82%). Mp = 171-173 °C, Lit. [35] Mp = 170-171 °C.

4.1.22. 2-chloro-*N*-phenylacetamide (**10c**)

Compound **10b** was synthesized from aniline with the procedure described for compound **10a** (white solid, 80.33%). Mp = 133-135 °C. Lit. [35] Mp = 134-137 °C.

4.1.23. 2-chloro-*N*-(2,4-dimethylphenyl)acetamide (**10d**)

Compound **10d** was synthesized from 2,4-dimethylaniline with the procedure described for compound **10a** (white solid, 93.76%). Mp = 152-153 °C.

4.1.24. 2-chloro-*N*-(2,5-dimethylphenyl)acetamide (**10e**)

Compound **10e** was synthesized from 2,5-dimethylaniline with the procedure described for compound **10a** (white solid, 90.83%). Mp = 150 °C.

4.1.25. 2-chloro-*N*-(3,4-dimethylphenyl)acetamide (**10f**)

Compound **10f** was synthesized from 3,4-dimethylaniline with the procedure described for compound **10a** (white solid, 97.82%). Mp = 106-107 °C.

4.1.26. 2-chloro-*N*-(4-(trifluoromethoxy)phenyl)acetamide (**10g**)

Compound **10g** was synthesized from 4-(trifluoromethoxy)aniline with the procedure described for compound **10a** (white solid, 97.79%). Mp = 131-133 °C.

4.1.27. 2-chloro-*N*-(2-chlorophenyl)acetamide (**10h**)

Compound **10h** was synthesized from 2-chloroaniline with the procedure described for compound **10a** (white solid, 90.17%). Mp = 66-67 °C.

4.1.28. 2-chloro-N-(2,4-difluorophenyl)acetamide (**10i**)

Compound **10i** was synthesized from 2,4-difluoroaniline with the procedure described for compound **10a** (white solid, 89.31%). Mp = 76-78 °C.

4.1.29. 4-(2-chloroacetamido)phenyl propionate (**10j**)

Compound **10j** was synthesized from 4-(2-chloroacetamido)aniline with the procedure described for compound **10a** (white solid, 83.66%). Mp = 109-110 °C.

4.1.30. *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**)

A solution of 1*H*-indole-3-carbaldehyde (**4a**, 5.80g, 0.040mol) and 4-methylbenzenesulfonohydrazide (7.44g, 0.040mol) in EtOH (60 mL) was stirred at 80 °C for 2 h, then cooled to r.t. The mixture was concentrated and

purified by CC (CH_2Cl_2:CH_3OH = 5:1) to obtain the compound **12a** as white solid (7.78g, 62.06%). Mp = 142-144 °C (dec), Lit. [36] Mp = 145 °C.

4.1.31. *N*'-((5-methoxy-1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12b**)

Compound **12b** was synthesized from 5-methoxy-1H-indole-3-carbaldehyde (**4b**) with the procedure described for compound **12a** (white solid, 53.88%). Mp = 197-198 °C (dec).

4.1.32. Methyl 2-(4-butoxyphenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Ia**)

To a stirring solution of methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**, 1.91g, 0.008mol) and Et_3N (3.03g, 0.030mol) in CH_2Cl_2 (40 mL) was added dropwise 4-butoxybenzene-1-sulfonyl chloride (**2a**, 1.86g, 0.008mol), the mixture was stirred at r.t. for 4 h and washed with water (x 3) and NaCl saturated solution (x 1). Then, the organic layer was concentrated under reduced pressure to obtain the compound **Ia** as white solid (0.04g, 52.35%). Mp = 120-122 °C.

IR (KBr): 586, 1094, 1153, 1265, 1595, 1623, 1724, 3267, 3407, 3469 cm^{-1}. ^{1}H-NMR(300 MHz, DMSO) δ 8.10 (1H, s), 7.63 (2H, d, *J* = 2.10 Hz), 7.43 (1H, d, *J* = 7.80 Hz), 7.32 (1H, d, *J* = 8.10 Hz), 7.18-7.13 (1H, m), 7.09-7.01 (2H, m), 6.81 (2H, d, *J* = 8.70 Hz), 5.11 (1H, d, *J* = 9.00 Hz), 4.26-4.19 (1H, m), 3.96 (2H, t, *J* = 6.30 Hz), 3.45 (3H, s), 3.22 (2H, d, *J* = 5.70 Hz), 1.81-1.72 (2H, m), 1.55-1.43 (2H, m), 0.98 (3H, t, *J* = 7.20 Hz). ^{13}C-NMR (75MHz, $CDCl_3$) δ 171.3, 162.0, 135.6, 130.2, 128.7, 126.7, 123.0, 121.6, 119.1, 117.9, 113.9, 110.8, 108.5, 67.6, 55.5, 51.9, 30.5, 28.7, 18.6, 13.3. MS (ESI) *m/z* 430 [M^+]. Anal. ($C_{22}H_{26}N_2O_5S$) C, H, N. Calcd for: 61.38, 6.09, 6.51; Found: 61.25, 6.35, 6.38.

4.1.33. Methyl 2-(4-isopropoxyphenylsulfonamido)-3-(1*H*-indol-3-yl) propanoate (**Ib**)

Compound **Ib** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-isopropoxybenzene-1-sulfonyl chloride (**2b**) with the procedure described for compound **Ia** (white solid, 50.10%). Mp = 118-119 °C.

IR (KBr): 592, 745, 833, 1092, 1110, 1151, 1260, 1326, 1596, 1731, 2973, 3244, 3390 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.03 (1H, br s), 7.63 (2H, d, *J* = 8.10 Hz), 7.46-7.04 (5H, m), 6.82 (2H, d, *J* = 8.40 Hz), 5.05 (1H, d, *J* = 8.70 Hz), 4.58 (1H, q, *J* = 6.30 Hz), 4.24 (1H, q, *J* = 3.30 Hz), 3.44 (3H, s), 3.24 (2H, d, *J* = 5.40 Hz), 1.34 (6H, d, *J* = 6.00 Hz). MS (EI) m/z 416 [M^+]. Anal. ($4C_{21}H_{24}N_2O_5S{\cdot}3H_2O$) C, H, N. Calcd for: 60.66, 5.98, 6.51; Found: 58.66, 6.06, 6.42.

4.1.34. Methyl 2-(4-isobutoxyphenylsulfonamido)-3-(1*H*-indol-3-yl) propanoate (**Ic**)

Compound **Ic** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-isobutoxybenzene-1-sulfonyl chloride (**2c**) with the procedure described for compound **Ia** (white solid, 11.53%).

Mp = 157-159 °C.

IR (KBr): 585, 739, 824, 1024, 1096, 1150, 1257, 1587, 1751, 2955, 3292, 3391cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, br s), 7.63 (2H, d, J = 8.70 Hz), 7.45-7.03 (5H, m), 6.82 (2H, d, J = 8.40 Hz), 5.07 (1H, d, J = 8.70 Hz), 4.23 (1H, q, J = 8.40 Hz), 3.72 (2H, d, J = 6.30 Hz), 3.46 (3H, s), 3.22 (2H, d, J = 5.3740 Hz), 2.08 (1H, m, J = 6.30 Hz), 1.03 (6H, d, J = 6.60 Hz). MS (EI) m/z 430 [M^+]. Anal. ($C_{22}H_{26}N_2O_5S$) C, H, N. Calcd for: 61.38, 6.09, 6.51; Found: 61.13, 6.08, 6.13.

4.1.35. Methyl 2-(4-(cyclopentyloxy)phenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Id**)

Compound **Id** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-(cyclopentyloxy)benzene-1-sulfonyl chloride (**2d**) with the procedure described for compound **Ia** (white solid, 23.18%). Mp = 157-158 °C.

IR (KBr): 783, 824, 1092, 1152, 1258, 1325, 1384, 1593, 1724, 2359, 2949, 3274, 3386 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.04 (1H, br s), 7.44 (2H, d, J = 7.80 Hz), 7.34-7.03 (5H, m), 6.80 (2H, d, J = 8.70 Hz), 5.06 (1H, d, J = 8.10 Hz), 4.76-4.74 (1H, m), 4.27-4.20 (1H, m), 3.44 (3H, s), 3.23 (2H, d, J = 5.70 Hz), 1.93-1.53 (8H, m). MS (EI) m/z 442 [M^+]. Anal. ($2C_{23}H_{26}N_2O_5S{\cdot}H_2O$) C, H, N. Calcd for: 61.18, 6.03, 6.20; Found: 61.37, 6.20, 6.02.

4.1.36. Methyl 2-(4-methoxyphenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Ie**)

Compound **Ie** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-methoxybenzene-1-sulfonyl chloride (**2e**) with the procedure described for compound **Ia** (white solid, 13.40%). Mp = 136-137 °C.

IR (KBr): 566, 742, 1091, 1157, 1258, 1335, 1597, 1729, 3282, 3396 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, s), 7.62 (2H, d, J = 2.70 Hz), 7.44 (1H, d, J = 8.10 Hz), 7.33 (2H, d, J = 15.90 Hz), 7.19-7.19 (1H, m), 7.09-7.02 (1H, m), 6.85-6.81 (2H, m), 5.08 (1H, d, J = 9.00 Hz), 4.27-4.20 (1H, m), 3.82 (3H, s), 3.46 (3H, s), 3.23 (2H, d, J = 5.70 Hz). MS (EI) m/z 388 [M^+]. Anal. ($C_{19}H_{20}N_2O_5S$) C, H, N. Calcd for: 58.75, 5.19, 7.21; Found: 58.38, 5.51, 7.12.

4.1.37. Methyl 2-(4-methylphenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**If**)

Compound **If** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-methylbenzene-1-sulfonyl chloride (**2f**) with the procedure described for compound **Ia** (white solid, 60.83%). Mp = 108-109 °C.

IR(KBr): 518, 554, 586, 661, 755, 766, 818, 853, 950, 1091, 1159, 1213, 277, 1321, 1354, 1458, 1759, 2954, 3310, 3392 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.08 (1H, s), 7.61-7.31 (4H, m), 7.18-7.02 (5H, m), 5.12(1H, d, J = 8.70 Hz), 4.28-4.21 (1H, m), 3.43(3H, s), 3.23 (2H, d, J = 5.40 Hz), 2.37 (3H, s). MS (EI) m/z 372 [M^+]. Anal.

($C_{19}H_{20}N_2O_4S$) C, H, N. Calcd for: 61.27, 5.41, 7.52; Found: 61.04, 5.28, 7.26.

4.1.38. Methyl 2-(4-bromophenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Ig**)

Compound **Ig** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-bromobenzene-1-sulfonyl chloride (**2g**) with the procedure described for compound **Ia** (white solid, 49.27%). Mp = 134-135 °C.

IR(KBr):555, 613, 737, 821, 949, 1008, 1068, 1089, 1110, 1158, 1226, 1275, 1334, 1176, 1458, 1573, 1732, 3215, 3379cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.04 (1H, br s), 7.50-7.32 (6H, m), 7.22-6.99 (3H, m), 5.17(1H, d, J = 8.70 Hz), 4.28-4.23 (1H, m), 3.53 (3H, s), 3.28-3.20 (2H, m). MS (EI) m/z 438 [M$^+$]. Anal. ($C_{18}H_{17}BrN_2O_4S$) C, H, N. Calcd for: 49.44, 3.92, 6.41; Found: 49.54, 3.85, 6.20.

4.1.39. Methyl 2-(4-fluorophenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Ih**)

Compound **Ih** was synthesized from methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**3a**) and 4-fluorobenzene-1-sulfonyl chloride (**2h**) with the procedure described for compound **Ia** (white solid, 42.30%). Mp = 100-102 °C.

IR(KBr): 548, 661, 677, 745, 840, 951, 1008, 1091, 1153, 1168, 1244, 1291, 1338, 1432, 1458, 1494, 1591, 1731, 3269, 3353 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, br s), 7.70-7.32 (4H, m), 7.21-6.98 (5H, m), 5.13(1H, d, J = 8.70 Hz), 4.29-4.24 (1H, m), 3.51 (3H, s), 3.30-3.22 (2H, m). MS (EI) m/z 376 [M$^+$]. Anal. ($C_{18}H_{17}FN_2O_4S$) C, H, N. Calcd for: 57.44, 4.55, 7.44; Found: 57.19, 4.36, 7.25.

4.1.40. Methyl 2-(4-butoxyphenylsulfonamido)-3-phenylpropanoate (**Ii**)

Compound **Ih** was synthesized from methyl 2-amino-3-phenylpropanoate hydrochloride (**3b**) and 4-butoxybenzene-1-sulfonyl chloride (**2a**) with the procedure described for compound **Ia** (white solid, 44.26%). Mp = 110-111 °C.

IR(KBr): 699, 805, 1018, 1093, 1157, 1260, 1344, 1597, 1729, 2958, 3286, 3414 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 7.66 (2H, d, J = 8.70 Hz), 7.26-7.23 (3H, m), 7.08-7.06 (2H, m), 6.87 (2H, d, J = 9.00 Hz), 4.98 (1H, d, J = 9.00 Hz), 4.21-4.14 (1H, m), 3.99 (2H, t, J = 6.60 Hz), 3.51 (3H, s), 3.02 (2H, d, J = 6.00 Hz), 1.83-1.73 (2H, m), 1.53-1.43 (2H, m), 0.98 (3H, t, J = 7.50 Hz). MS (ESI) m/z 391 [M$^+$]. Anal. ($C_{20}H_{25}NO_5S$) C, H, N. Calcd for: 61.36, 6.44, 3.58; Found: 61.07, 6.62, 3.38.

4.1.41. 2-(4-butoxyphenylsulfonamido)-3-(1*H*-indol-3-yl)propanoic acid (**Ij**)

LiOH (1mol/L, 2.2 mL) was added to a solution of methyl 2-(4-butoxyphenylsulfonamido)-3-(1*H*-indol-3-yl)propanoate (**Ia**, 0.43g, 0.001mol) in THF (10 mL). After being stirred at r.t. for 10 h, the mixture was concentrated under reduced pressure and diluted acidified by HCl (0.1mol/L)

in water (20 mL). Then, the mixture was filtered to obtain the compound **Ij** as white solid (0.15g, 36.06%). Mp = 109-110 °C.

IR(KBr): 740, 830, 1096, 1150, 1726, 2395, 2954, 3400, 3664 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 12.56 (1H, s), 10.79 (1H, s), 8.04 (1H, d, *J* = 8.40 Hz), 7.48 (2H, d, *J* = 8.70 Hz), 7.30-7.26 (2H, m), 7.06-6.85 (5H, m), 3.98 (2H, d, *J* = 6.60 Hz), 3.88-3.80 (1H, m), 3.06-3.00 (1H, m), 2.86-2.80 (1H, m), 1.76-1.66 (2H, m), 1.51-1.38 (2H, m), 0.98 (3H, t, *J* = 7.50 Hz). MS (ESI) m/z 416 [M^+]. HR-MS ([M + NH_4] $^+$) Calcd for $C_{21}H_{28}N_3O_5S$, 434.1744; Found: 434.1743. HPLC purity: 99.90%; retention time: 2.248 min (mobile phase: methanol/water 98:2, v/v).

4.1.42. *N*-(4-methoxyphenyl)-2-(3-(2-(4-methylphenylsulfonamido)ethyl)-1*H*-indol-1-yl)acetamide (**IIa**)

To a stirring solution of *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methylbenzenesulfonamide (**8c**, 0.63g, 0.002mol) and appropriate NaH in DMF (10 mL) was added 2-chloro-*N*-(4-methoxyphenyl)acetamide (**10a**, 0.80g, 0.004mol) at 5 °C under N_2 flow. After being stirred at 5 °C for 2 h and at r.t. for another 4 h, the mixture was poured into water and extracted with $CHCl_3$ (20 mL x 3). The organic layer was concentrated and purified by CC (CH_2Cl_2/AcEt = 2:1) to obtain the compound **IIa** as white solid (0.42g, 43.75%). Mp = 160-161 °C.

IR(KBr): 698, 743, 1165, 1241, 1324, 1511, 1612, 1663, 3260, 3414 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.15 (1H, s), 7.70-7.64 (3H, m), 7.48(2H, d, *J* = 9.00 Hz), 7.41-7.34 (4H, m), 7.14-7.08 (2H, m), 6.98-6.96 (1H, m), 6.88 (2H, d, *J* = 9.00 Hz), 4.92 (2H, s), 3.71 (3H, s), 3.02-2.95 (2H, m), 2.82-2.72 (2H, m), 2.36 (3H, s). ^{13}C-NMR (75MHz, $CDCl_3$) δ 165.9, 142.7, 137.3, 131.7, 129.4, 128.1, 127.3, 126.8, 121.6, 121.2, 119.0, 118.5, 113.7, 111.8, 109.5, 54.7, 49.4, 43.5, 25.4, 20.4. MS (ESI) m/z 477 [M^+]. Anal. ($C_{26}H_{27}N_3O_4S$) C, H, N. Calcd for: 65.39, 5.70, 8.80; Found: 65.39, 5.68, 8.48.

4.1.43. *N*-(4-chlorophenyl)-2-(3-(2-(4-methylphenylsulfonamido)ethyl)-1*H*-indol-1-yl)acetamide (**IIb**)

Compound **IIb** was synthesized from *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methylbenzenesulfonamide (**8c**) and 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**) with the procedure described for compound **IIa** (white solid, 36.57%). Mp = 192-193 °C.

IR(KBr): 552, 736, 1086, 1093, 1156, 1387, 1534, 1597, 1697, 2296, 3259 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.44 (1H, s), 7.69-7.65 (3H, m), 7.62-7.59 (2H, m), 7.41-7.34 (6H, m), 7.15 (1H, s), 7.10 (1H, t, *J* = 8.10 Hz), 7.00 (1H, t, *J* = 7.20 Hz), 4.97 (2H, s), 3.02-2.95 (2H, m), 2.82-2.72 (2H, m), 2.36 (3H, s). MS (ESI) m/z 481 [M^+]. Anal. ($C_{25}H_{24}ClN_3O_3S$) C, H, N. Calcd for: 62.30, 5.02, 8.72; Found: 62.13, 4.94, 8.38.

4.1.44. *N*-(4-methoxyphenyl)-2-(3-(2-(4-methoxyphenylsulfonamido)ethyl)-1*H*-indol-1-yl)acetamide (**IIc**)

Compound **IIc** was synthesized from *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methoxybenzenesulfonamide (**8b**) and 2-chloro-*N*-(4-methoxyphenyl)acetamide (**10a)** with the procedure described for compound **IIa** (white solid,

28.91%). Mp = 155-156 °C.

IR(KBr): 562, 736, 832, 1153, 1248, 1326, 1514, 1534, 1676, 3287 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.16 (1H, s), 7.72 (2H, d, *J* = 9.00 Hz), 7.59 (1H, t, *J* = 5.70 Hz), 7.50-7.47 (2H, m), 7.41-7.34 (2H, m), 7.15-7.06 (4H, m), 7.02-6.98 (1H, m), 6.90-6.86 (2H, m), 4.92 (2H, s), 3.81 (3H, s), 3.71 (3H, s), 3.02-2.94 (2H, m), 2.82-2.73 (2H, m). MS (ESI) m/z 493 [M^+]. Anal. ($C_{26}H_{27}ClN_3O_5S$) C, H, N. Calcd for: 63.27, 5.51, 8.51; Found: 63.02, 5.55, 8.44.

4.1.45. *N*-(4-chlorophenyl)-2-(3-(2-(4-methoxyphenylsulfonamido)ethyl)-1*H*-indol-1-yl)acetamide (**IId**)

Compound **IId** was synthesized from *N*-(2-(1*H*-indol-3-yl)ethyl)-4-methoxybenzenesulfonamide (**8b**) and 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**) with the procedure described for compound **IIa** (white solid, 88.90%). Mp = 166-167 °C.

IR(KBr): 738, 832, 1153, 1328, 1493, 1537, 1599, 1688, 2922, 3268 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.45 (1H, s), 7.72 (2H, d, *J* = 9.00 Hz), 7.63-7.57 (3H, m), 7.42-7.34 (4H, m), 7.16-7.05 (4H, m), 7.03-6.98 (1H, m), 4.97 (2H, s), 3.81 (3H, s), 3.01-2.94 (2H, m), 2.82-2.73 (2H, m). MS (ESI) m/z 497 [M^+]. HR-MS ([M + H] $^+$) Calcd for $C_{25}H_{24}N_3O_5S$, 498.1249; Found: 498.1260. HPLC purity: 99.64%; retention time: 3.615 min (mobile phase: methanol/water 98:2, v/v)

4.1.46. *N*-(4-methoxyphenyl)-2-(3-(2-(4-butoxyphenylsulfonamido)ethyl)-1*H*-indol-1-yl) acetamide (**IIe**)

Compound **IIe** was synthesized from *N*-(2-(1*H*-indol-3-yl)ethyl)-4-butoxybenzenesulfonamide (**8a**) and 2-chloro-*N*-(4-methoxyphenyl)acetamide (**10a)** with the procedure described for compound **IIa** (white solid, 74.25%). Mp = 160-161 °C.

IR(KBr): 584, 739, 829, 1154, 1319, 1524, 1600, 1673, 2954, 3306, 3414 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.16 (1H, s), 7.70 (2H, d, *J* = 8.70 Hz), 7.58 (1H, t, *J* = 5.70 Hz), 7.49 (2H, d, *J* = 9.00 Hz), 7.41-7.34 (2H, m), 7.15-7.05 (4H, m), 7.02-6.97 (1H, m), 6.879 (2H, d, *J* = 9.00 Hz), 4.92 (2H, s), 4.02 (2H, t, *J* = 6.60 Hz), 3.71 (3H, s), 3.01-2.94 (2H, m), 2.81-2.76 (2H, m), 1.75-1.65 (2H, m), 1.46-1.38 (2H, m), 0.92 (3H, t, *J* = 7.50 Hz). MS (ESI) m/z 535 [M^+]. Anal. ($C_{29}H_{33}N_3O_5S$) C, H, N. Calcd for: 65.03, 6.21, 7.84; Found: 64.98, 6.39, 7.73.

4.1.47. *N*-(4-chlorophenyl)-2-(3-(2-(4-butoxyphenylsulfonamido)ethyl)-1*H*-indol-1-yl) acetamide (**IIf**)

Compound **IIf** was synthesized from *N*-(2-(1*H*-indol-3-yl)ethyl)-4-butoxybenzenesulfonamide (**8a**) and 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**) with the procedure described for compound **IIa** (white solid, 17.30%). Mp = 151-153 °C.

IR(KBr): 588, 743, 830, 1153, 1326, 1526, 1596, 1683, 2956, 3272, 3311 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.45 (1H, s), 7.71 (2H, d, *J* = 8.70 Hz), 7.63-7.56 (3H, m), 7.42-7.34 (4H, m), 7.15-6.97 (5H, m), 4.98 (2H, s),

4.02 (2H, t, *J* = 6.30 Hz), 3.01-2.95 (2H, m), 2.79 (2H, t, *J* = 6.90 Hz), 1.75-1.65 (2H, m), 1.46-1.38 (2H, m), 0.92 (3H, t, *J* = 7.50 Hz). MS (ESI) m/z 539 [M^+]. Anal. ($C_{28}H_{30}ClN_3O_4S$) C, H, N. Calcd for: 62.27, 5.60, 7.78; Found: 62.16, 5.46, 7.45.

4.1.48. *N*-phenyl-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIa**)

Compound **IIIa** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-N-phenylacetamide (**10c**) with the procedure described for compound **IIa** (yellow solid, 23.59%). Mp = 150 °C (dec).

IR(KBr): 550, 691, 742, 1162, 1303, 1444, 1539, 1600, 1671, 3035, 3192, 3328, 3441 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.40 (1H, s), 8.08 (1H, s), 7.99 (1H, d, *J* = 7.20 Hz), 7.80 (2H, d, *J* = 8.10 Hz), 7.74 (1H, s), 7.58-7.55 (2H, m), 7.45-7.28 (5H, m), 7.19-7.16 (2H, m), 7.09-7.00 (1H, m), 5.06 (2H, s), 2.33 (3H, s). ^{13}C-NMR (75MHz, $CDCl_3$) δ 165.7, 144.4, 143.1, 138.6, 137.5, 136.3, 134.3, 129.4, 128.8, 127.3, 124.5, 123.5, 122.8, 121.7, 120.8, 199.1, 110.7, 110.1, 49.1, 20.9. MS (ESI) m/z 445 ([M - H]$^+$), 447 ([M + H]$^+$). Anal. ($C_{24}H_{22}N_4O_3S$) C, H, N. Calcd for: 64.56, 4.97, 12.55; Found: 64.23, 5.05, 12.36.

4.1.49. *N*-(2,4-dimethylphenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIb**)

Compound **IIIb** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(2,4-dimethylphenyl)acetamide (**10d**) with the procedure described for compound **IIa** (white solid, 21.10%). Mp = 213-214 °C (dec).

IR(KBr): 553, 682, 742, 814, 1164, 1311, 1467, 1529, 1616, 1669, 3169, 3304 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.81 (1H, s), 9.62 (1H, s), 8.08 (1H, s), 7.99 (1H, d, *J* = 7.80 Hz), 7.81-7.75 (3H, m), 7.47 (1H, d, *J* = 7.50 Hz), 7.38 (2H, d, *J* = 8.10 Hz), 7.26-7.14 (3H, m), 7.01 (1H, s), 6.95-6.92 (1H, m), 5.07 (2H, s), 2.33 (3H, s), 2.22 (3H, s), 2.13 (3H, s). MS (ESI) m/z 475 ([M + H]$^+$). Anal. ($C_{26}H_{26}N_4O_3S$) C, H, N. Calcd for: 65.80, 5.52, 11.87; Found: 66.17, 5.80, 11.85.

4.1.50. *N*-(2,5-dimethylphenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIc**)

Compound **IIIc** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(2,5-dimethylphenyl)acetamide (**10e**) with the procedure described for compound **IIa** (white solid, 15.85%). Mp = 219-220 °C (dec).

IR(KBr): 583, 743, 813, 948, 1164, 1310, 1467, 1534, 1670, 3182, 3303, 3470 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.81 (1H, s), 9.63 (1H, s), 8.09 (1H, s), 7.99 (1H, d, *J* = 7.50 Hz), 7.82-7.76 (3H, m), 7.47 (1H, d, *J* = 8.10 Hz), 7.38 (2H, d, *J* = 8.10 Hz), 7.25-7.17 (3H, m), 7.09-7.07 (1H, m), 6.90-6.88 (1H, m), 5.09 (2H, s), 2.33 (3H, s), 2.21 (3H, s), 2.14 (3H, s). MS (ESI) m/z 473 ([M - H]$^+$), 475 ([M + H]$^+$). Anal. ($C_{26}H_{26}N_4O_3S$) C, H, N.

Calcd for: 65.80, 5.52, 11.87; Found: 65.64, 5.68, 11.82.

4.1.51. *N*-(3,4-dimethylphenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIId**)

Compound **IIId** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(3,4-dimethylphenyl)acetamide (**10f**) with the procedure described for compound **IIa** (white solid, 25.30%). Mp = 220-221 °C (dec).

IR(KBr): 583, 742, 814, 949, 1092, 1164, 1528, 1669, 2832, 3168, 3305, 3472 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.88 (1H, s), 10.23 (1H, s), 8.08 (1H, s), 7.99 (1H, d, J = 7.50 Hz), 7.82-7.79 (2H, m), 7.74 (1H, s), 7.16-7.40 (7H, m), 7.06-7.03 (1H, m), 5.02 (2H, s), 2.34 (3H, s), 2.15 (6H, s). MS (ESI) m/z 473 ($[M - H]^+$), 497 ($[M + Na]^+$). Anal. ($C_{26}H_{26}N_4O_3S$) C, H, N. Calcd for: 65.80, 5.52, 11.87; Found: 66.00, 5.86, 11.90.

4.1.52. *N*-(4-methoxyphenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIe**)

Compound **IIIe** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(4-methoxyphenyl)acetamide (**10a**) with the procedure described for compound **IIa** (white solid, 18.92%). Mp = 217-219 °C (dec).

IR(KBr): 593, 714, 832, 1034, 1303, 1512, 1555, 1666, 2355, 2960, 3195, 3685, 3849 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.27 (1H, s), 8.08 (1H, s), 7.99 (1H, d, J = 7.50 Hz), 7.82-7.79 (3H, m), 7.49-7.37 (5H, m), 7.22-7.16 (2H, m), 6.89-6.86 (2H, m), 5.03 (2H, s), 3.07 (3H, s), 2.34 (3H, s). MS (ESI) m/z 475 ($[M - H]^+$), 477 ($[M + H]^+$). Anal. ($C_{25}H_{24}N_4O_4S$) C, H, N. Calcd for: 63.01, 5.08, 11.76; Found: 62.85, 5.10, 12.10.

4.1.53. *N*-(4-(trifluoromethoxy)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl) phenyl)acetamide (**IIIf**)

Compound **IIIf** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(4-(trifluoromethoxy)phenyl)acetamide (**10g**) with the procedure described for compound **IIa** (white solid, 21.77%). Mp = 220-222 °C (dec).

IR(KBr): 550, 665, 743, 844, 1397, 1470, 1510, 1694, 2228, 2932, 3068, 3198, 3366 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.60 (1H, s), 8.08 (1H, s), 7.99 (1H, d, J = 7.50 Hz), 7.81 (2H, d, J = 8.10 Hz), 7.75 (1H, s), 7.68 (2H, d, J = 9.00 Hz), 7.45-7.31 (5H, m), 7.24-7.14 (2H, m), 5.08 (2H, s), 2.34 (3H, s). MS (ESI) m/z 531 ($[M + H]^+$). Anal. ($C_{25}H_{21}F_3N_4O_4S$) C, H, N. Calcd for: 56.60, 3.90, 10.56; Found: 56.31, 3.99, 10.43.

4.1.54. *N*-(4-chlorophenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIg**)

Compound **IIIg** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**) with the procedure described for compound **IIa** (white solid, 41.74%). Mp = 229-230 °C (dec).

IR(KBr): 550, 742, 953, 1091, 1396, 1491, 1540, 1600, 1617, 1740, 3053, 3180, 3358 cm^{-1}. ^{1}H-NMR (300

MHz, DMSO) δ 10.89 (1H, s), 10.53 (1H, s), 8.08 (1H, s), 7.99 (1H, d, J = 7.20 Hz), 7.82-7.74 (3H, m), 7.60 (2H, d, J = 8.70 Hz), 7.45-7.35 (5H, m), 7.22-7.17 (2H, m), 5.07 (2H, s), 2.34 (3H, s). MS (ESI) m/z 481 ($[M + H]^+$). Anal. ($C_{24}H_{21}ClN_4O_3S$) C, H, N. Calcd for: 59.93, 4.40, 11.65; Found: 59.80, 4.38, 11.77.

4.1.55. *N*-(2-chlorophenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIh**)

Compound **IIIh** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-*N*-(2-chlorophenyl)acetamide (**10h**) with the procedure described for compound **IIa** (white solid, 22.89%). Mp = 216-217 °C (dec).

IR(KBr): 749, 951, 1159, 1303, 1468, 1537, 1681, 2356, 2925, 3175, 3308, 3849 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 9.91 (1H, s), 8.09 (1H, s), 7.99 (1H, d, J = 7.80 Hz), 7.82-7.69 (4H, m), 7.52-7.45 (2H, m), 7.40-7.37 (2H, m), 7.33-7.15 (4H, m), 5.17 (2H, s), 2.33 (3H, s). MS (ESI) m/z 479 ($[M - H]^+$), 481 ($[M + H]^+$). Anal. ($C_{24}H_{21}ClN_4O_3S$) C, H, N. Calcd for: 59.93, 4.40, 11.65; Found: 59.81, 4.44, 11.68.

4.1.56. *N*-(2,4-difluorophenyl)-2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIi**)

Compound **IIIi** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 2-chloro-N-(2,4-difluorophenyl)acetamide (**10i**) with the procedure described for compound **IIa** (white solid, 14.53%). Mp = 201-202 °C (dec).

IR(KBr): 590, 753, 970, 1181, 1385, 1555, 1617, 1673, 3284, 3409, 3473 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.20 (1H, s), 8.08 (1H, s), 7.99 (1H, d, J = 7.80 Hz), 7.85-7.74 (3H, m), 7.45-7.31 (4H, m), 7.25-7.14 (2H, m), 7.07-7.02 (2H, m), 5.13 (2H, s), 2.33 (3H, s). MS (ESI) m/z 481 ($[M - H]^+$). Anal. ($C_{24}H_{20}F_2N_4O_3S$) C, H, N. Calcd for: 59.74, 4.18, 11.61; Found: 59.59, 4.15, 11.63.

4.1.57. 4-(2-(3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamido)phenyl propionate (**IIIj**)

Compound **IIIj** was synthesized from *N*'-((1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12a**) and 4-(2-chloroacetamido)phenyl propionate (**10j**) with the procedure described for compound **IIa** (white solid, 27.25%). Mp = 209-210 °C (dec).

IR(KBr): 550, 737, 859, 1284, 1549, 1620, 1675, 1714, 2939, 3060, 3125, 3189, 3458 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.74 (1H, s), 8.09 (1H, s), 7.99 (1H, d, J = 6.90 Hz), 7.94-7.91 (2H, m), 7.82-7.69 (5H, m), 7.47-7.38 (3H, m), 7.24-7.15 (2H, m), 5.25 (2H, s), 4.24 (2H, q, J = 6.90 Hz), 2.33 (3H, s), 1.30 (3H, t, J = 7.20 Hz). MS (ESI) m/z 519 ($[M - H]^+$), 517 ($[M + H]^+$). Anal. ($C_{27}H_{26}N_4O_5S$) C, H, N. Calcd for: 62.53, 5.05, 10.80; Found: 62.23, 4.68, 10.63.

4.1.58. *N*-phenyl-2-(5-methoxy-3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIk**)

Compound **IIIk** was synthesized from *N*'-((5-methoxy-1*H*-indol-3-yl)methylene)-4-

methylbenzenesulfonohydrazide (**12b**) and 2-chloro-*N*-phenylacetamide (**10c**) with the procedure described for compound **IIa** (white solid, 30.47%). Mp = 207-208 °C (dec).

IR(KBr): 549, 690, 1054, 1163, 1258, 1309, 1536, 1667, 2826, 2932, 3295, 3306, 3457 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.87 (1H, s), 10.36 (1H, s), 8.08 (1H, s), 7.80 (2H, d, J = 8.10 Hz), 7.70 (1H, s), 7.56 (2H, d, J = 7.80 Hz), 7.48 (1H, d, J = 2.40 Hz), 7.41-7.27 (5H, m), 7.08-7.03 (1H, m), 6.83 (1H, d, J = 2.40 Hz), 5.02 (2H, s), 3.76 (3H, s), 2.39 (3H, s). MS (ESI) m/z 475 ($[M - H]^+$), 477 ($[M + H]^+$). HR-MS ($[M + H]^+$) Calcd for $C_{25}H_{24}N_4O_4S$, 477.1591; Found: 477.1596. HPLC purity: 99.74%, retention time: 2.682min (mobile phase: methanol/water 98:2, v/v).

4.1.59. *N*-(4-chlorophenyl)-2-(5-methoxy-3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIl**)

Compound **IIIl** was synthesized from *N*'-((5-methoxy-1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12b**) and 2-chloro-*N*-(4-chlorophenyl)acetamide (**10b**) with the procedure described for compound **IIa** (white solid, 26.49%). Mp = 226-228 °C (dec).

IR(KBr): 548, 670, 812, 1092, 1163, 1229, 1304, 1490, 1533, 1673, 2927, 3199, 3310 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.87 (1H, s), 10.49 (1H, s), 8.08 (1H, s), 7.82-7.79 (2H, m), 7.69 (1H, s), 7.61-7.58 (2H, m), 7.48-7.47 (1H, m), 7.41-7.32 (5H, m), 6.86-6.82 (1H, m), 5.02 (2H, s), 3.76 (3H, s), 2.35 (3H, s). MS (ESI) m/z 511 ($[M]^+$). Anal. ($C_{25}H_{23}ClN_4O_4S$) C, H, N. Calcd for: 58.76, 4.54, 10.96; Found: 58.54, 4.50, 10.87.

4.1.60. *N*-(2,4-difluorophenyl)-2-(5-methoxy-3-((2-tosylhydrazono)methyl)-1*H*-indol-1-yl)acetamide (**IIIm**)

Compound **IIIm** was synthesized from *N*'-((5-methoxy-1*H*-indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (**12b**) and 2-chloro-N-(2,4-difluorophenyl)acetamide (**10i**) with the procedure described for compound **IIa** (white solid, 14.62%). Mp = 221-222 °C (dec).

IR(KBr): 548, 676, 844, 949, 1163, 1314, 1485, 1543, 1616, 1702, 2809, 2971, 3231, 3380 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO) δ 10.89 (1H, s), 10.18 (1H, s), 8.07 (1H, s), 7.85-7.77 (3H, m), 7.70 (1H, s), 7.48 (1H, s), 7.47-7.31 (4H, m), 7.08-7.02 (1H, m), 6.87-6.84 (1H, m), 5.09 (2H, s), 3.77 (3H, s), 2.34 (3H, s). MS (ESI) m/z 513 ($[M + H]^+$). Anal. ($C_{25}H_{22}F_2N_4O_4S$) C, H, N. Calcd for: 58.59, 4.33, 10.93; Found: 58.64, 4.05, 11.15.

4.2. Biological evaluation

4.2.1. Whole-cell patch lamp technique

The HEK 293 cell line that stably expressed *h*Kv1.5 potassium channel and the CHO cell line that stably expressed *h*ERG (I_{Kr}) potassium channel were kindly gifts from Dr. Gui-Rong Li (Department of Medicine and Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, SAR, China). [37] Transfected HEK 293 cells and transfected CHO cells (stably expressed *h*ERG potassium

channel) were maintained at 37 °C in Minimal Eagle Medium (MEM) or Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, and 0.2 mg/mL geneticin (Invitrogen Corporation, Carlsbad, CA). Cells were passaged weekly and used at ≤ 80% confluence. For electrophysiological recordings, the cells were harvested from the culture dish by trypsinization, and then washed twice with standard MEM or DMEM and maintained in culture medium at room temperature for later use on the same day.

Isolated cells from ventricular muscle were prepared as described previously. [38] Briefly, male guinea pigs (weight about 350 g) were sacrificed by cervical dislocation. The hearts were dissected and perfused retrograde via the aorta at 37 °C with nominally Ca^{2+}-free Tyrode solution (in mM: 117 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.2 NaH_2PO_4, 10 glucose, 20 taurine, pH adjusted to 7.4 with NaOH), then with Tyrode solution containing 50 μmol/L $CaCl_2$, 0.3 mg/mL collagenase (type II; Worthington, Lakewood, NJ), 1 mg/mL bovine serum albumin (Sunshine, Nanjing, China). After 10-15 min of treatment, the digested left ventricular free wall was placed in Kraftbruhe (KB) solution (in mM: L-glutamic acid 50, KCl 40, KOH 70, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 0.5, HEPES 10, pH adjusted to 7.4 with KOH) and pipetted gently to produce a cell suspension. The solution was agitated with 95% O_2 + 5% CO_2. The entire enzyme isolation procedure was performed at 37 °C. Isolated myocytes were kept at room temperature (25 °C) in medium for 1 h. A small aliquot (2 mL) of the solution containing the isolated cells was placed in an open perfusion chamber and mounted on the stage of an inverted microscope. Only quiescent and rod-shaped cells showing clear striations were selected for experiments. [39]

The whole-cell membrane currents were recorded by the patch-clamp technique, using an EPC-10 double patch-clamp amplifier (HEKA, Pfalz, Germany). Recording pipettes, made from borosilicate glass (1.2 mm, o.d.), pulled with a pipette puller (PIP5, HEKA, Germany), had resistances of between 4 and 6MΩ when filled with the pipette solution. Pulse software (HEKA, Pfalz, Germany) was used to generate voltage pulse protocols and to record and analyse data. Standard voltage protocols for I_{Kur}: 500-ms depolarizing pulse steps to +60 mV from a holding potential of -80 mV at 0.1 Hz; [39] I_{Kr}: the holding potential was -70 mV and a family of voltage steps from -60 to +50 mV for 2 sec., in 10-mV increments to evoke step currents, followed by a re-polarisation step to -40 mV for 2 sec., to induce tail currents (stimulation frequency of 0.1 Hz); [40] I_{Na}: 30-ms depolarizing pulse to potentials ranging from -80 mV to +40 mV in 10-mV increments at 0.5 Hz, the holding potential was -90 mV. [41] Compounds were applied at least 5 min after current stabilization. IC_{50} value was determined by using cell-by-cell fits with the Hill equation for three cells at least for all concentrations. The data are presented as the mean and standard deviation (x ± SD), n = 3. All experiments were performed at 25 °C.

4.2.2. The effect of compounds **IIIi** and **IIIl** on ERP in isolated rat hearts

SD rats (male, 230 ± 30 g) were anesthetized with 10% chloral hydrate (intraperitoneal injection). Hearts were rapidly removed through a midline sternal incision, and left atrium and right ventricular were isolated immediately and put into Krebs-Henseleit solution bath (composition in mmol/L: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, Glucose 10 and the pH was adjusted to 7.4) gassed with 95% O_2-5% CO_2.

Compounds **IIIi** and **IIIl** were added to the solution bath in serial concentrations of 0.1, 1.0, 10 and 50 μmol/L, allowing 10 minutes for equilibration. Recording the ERP (BL-420 system of *Tai Meng*, *Cheng Du*) of left atrium and right ventricular before and after compounds added.

Data were analyzed using Student's t test and are presented as the mean (x ± SEM), and n = 3.

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