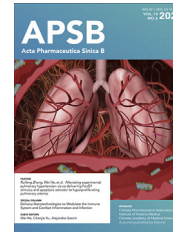




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ORIGINAL ARTICLE

# Discovery of novel sulfonamide substituted indolylarylsulfones as potent HIV-1 inhibitors with better safety profiles



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## KEY WORDS

HIV-1;  
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Cytotoxicity

**Abstract** Indolylarylsulfones (IASs) are classical HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) with a unique scaffold and possess potent antiviral activity. To address the high cytotoxicity and improve safety profiles of IASs, we introduced various sulfonamide groups linked by alkyl diamine chain to explore the entrance channel of non-nucleoside inhibitors binding pocket. 48 compounds were designed and synthesized to evaluate their anti-HIV-1 activities and reverse transcriptase inhibition activities. Especially, compound R<sub>10</sub>L<sub>4</sub> was endowed with significant inhibitory activity towards wild-type HIV-1 (EC<sub>50(WT)</sub> = 0.007 μmol/L, SI = 30,930) as well as a panel of single-mutant strains exemplified by L100I (EC<sub>50</sub> = 0.017 μmol/L, SI = 13,055), E138K (EC<sub>50</sub> = 0.017 μmol/L, SI = 13,123) and Y181C (EC<sub>50</sub> = 0.045 μmol/L, SI = 4753) which were superior to Nevirapine and Etravirine. Notably, R<sub>10</sub>L<sub>4</sub> was characterized with significantly reduced cytotoxicity (CC<sub>50</sub> = 216.51 μmol/L) and showed no

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remarkable *in vivo* toxic effects (acute and subacute toxicity). Moreover, the computer-based docking study was also employed to characterize the binding mode between R<sub>10</sub>L<sub>4</sub> and HIV-1 RT. Additionally, R<sub>10</sub>L<sub>4</sub> presented an acceptable pharmacokinetic profile. Collectively, these results deliver precious insights for next optimization and indicate that the sulfonamide IAS derivatives are promising NNRTIs for further development.

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## 1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a chronic infectious disease with serious threat to human health and global economy<sup>1,2</sup>. Human immunodeficiency virus type 1 (HIV-1), as the pathogen of AIDS, encodes reverse transcriptase (RT) as a key component in its life cycle to conduct the double-stranded DNA synthesis dependent on virus genome RNA<sup>3</sup>. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been widely studied and developed inhibitors targeting RT, taking an important place in highly active anti-retroviral therapy (HAART)<sup>4,5</sup>. In order to control viral load on a long term, RT inhibitors typically require life-long administration. However, in the extensive clinical application of NNRTIs, severe long-term side effects and unfavorable pharmacokinetic properties reduced patient compliance and clinical effects<sup>6,7</sup>. Meanwhile, the rapid mutation of HIV and the wide use of current RT inhibitors has led to a variety of drug-resistant strains<sup>8</sup>. Mutation of certain residues around NNRTIs binding pocket (NNIBP) altered the size, rigidity and electrical property of the binding site, thus impaired ligand binding<sup>9,10</sup>. All these challenges are forcing the earliest generations of NNRTIs out from HIV clinical therapy<sup>4,11</sup>. Developing less toxic HIV-1 NNRTIs with high activity towards all drug-resistant strains continues to be an urgent need.

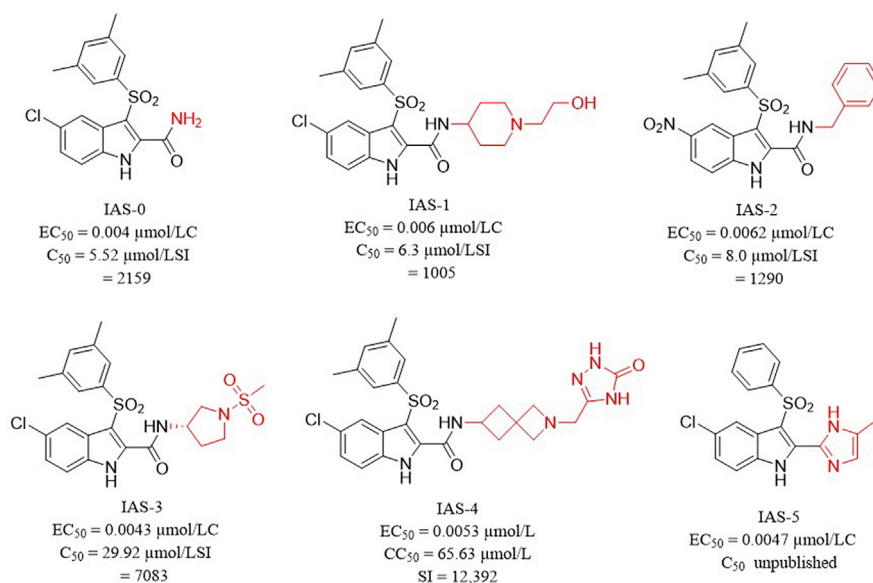
Indolylarylsulfones (IASs) are a class of HIV-1 NNRTIs with unique scaffold and promising antiviral activity<sup>12</sup>. Compound IAS-0 was proved to inhibit HIV-1 wild-type (WT) and several mutant strains at low-nanomolar EC<sub>50</sub> values<sup>13</sup>. However, a series of issues including high cytotoxicity and poor solubility limited its further development into clinical trials. According to previous structure–activity relationship (SAR) study, the 3,5-dimethyl group on phenylsulfonyl moiety of IAS-0 improved its anti-drug resistant profile, but introduced severe cytotoxicity (CC<sub>50</sub> IAS-0 = 5.5 μmol/L)<sup>12</sup>, which is undesirable for drugs to treat long-term viral infectious diseases. How to reduce the toxicity of IAS derivatives has thus been a challenge in further modifications, but has long been ignored. Among those most active IASs with rationally designed modifications (IAS 1–4), the decrease of toxicity was also not satisfying (Fig. 1)<sup>14–17</sup>. Interestingly, our group found that sidearm substitutions containing sulfonamide groups on 2-carboxamide showed a slightly reduced cytotoxicity<sup>12</sup>. And to enhance water solubility, Merck company replaced the 2-carboxamide group with nitrogen-containing aromatic rings, but ended up with compound IAS-5 with no obvious improvements<sup>18</sup>.

An in-depth study revealed the co-crystal structure of another derivative, IAS-6 and WT HIV-1 RT, demonstrating the “four-points” pharmacophore model distinguishing from most known NNRTIs (Fig. 2A and B)<sup>19</sup>. Key interactions from four moieties are observed: i) the pyrrolidinyl-1-sulfonyl group gains

hydrophobic interactions with the surrounding residues, namely Y181, Y188, F227 and Y318; ii) imino NH and carbamoyl oxygen attached to indole ring form dual hydrogen bonding with K101 backbone; iii) halogen-substituted phenyl ring of indole fitted into another hydrophobic sub-pocket formed by F227, Y318, etc.; iv) intramolecular H-bond observed between sulfonyl oxygen and carboxamide NH<sub>2</sub>, thus stabilizing the butterfly-like conformation of NNRTIs. These findings provided an important theoretical foundation for future designing and structural optimization for IAS. To be mentioned, the carboxamide group directs towards the unoccupied entrance of the binding position, a solvent-accessible hydrophilic area surrounded by L100, E138 and V179. This region, referred as the tolerant region II, showed good accommodation for rigid aromatic rings in previous studies<sup>20</sup>. Thus, the quests on modified IAS compounds have been focused on introducing extended groups on 2-carboxamide site of indole scaffold<sup>14–17</sup>. A sidearm with multiple hydrogen bond donors/acceptors could occupy this area to increase ligand affinity and water solubility.

Sulfonamides are frequently-used privileged substituents in drug research and development<sup>21,22</sup>. A large number of structurally novel sulfonamide derivatives have recently been reported to show substantial antiviral activity, as exemplified by the clinically used HIV protease inhibitors, many of the new types of NNRTIs, integrase inhibitors, viral capsid protein allosteric modulators, viral zinc finger protein binders or HIV entry inhibitors<sup>23–29</sup>. Sulfonamide motif offers multiple H-bond donors and acceptors, thus enabling additional interactions of ligands with residues around the binding pocket. Moreover, the presence of sulfonamide in small molecules may adjust acidity, increase solubility, improve oral bioavailability and reduce hERG toxicity, due to its polar nature<sup>30,31</sup>, proving indeed that this versatile moiety may be of great help for modern drug design.

Consequently, with the aim to additionally occupy the second tolerant region of NNIBP, aryl/aliphatic sulfonamide groups were introduced to occupy the entrance region in this work. Terminal substituents were joint with 2-carboxamide by a linear alkyl diamine chain from C2 to C5. Sulfonamides are expected to build up additional interactions with surrounding residues, contributing to improved antiviral activity and reduced cytotoxicity. Highly flexible (CH<sub>2</sub>)<sub>n</sub> linker with different length endows the molecules with better adaption to NNIBP tunnel (Fig. 2C). A total of 14 terminal groups and four kinds of linkers were combined to find out the optimal length and structure of this newly introduced pharmacophore. Herein, we depicted the synthesis, antiviral activity evaluation and structure–activity relationships (SARs) discussion of these newly designed compounds. Additionally, the representative candidate, R<sub>10</sub>L<sub>4</sub> (see below), was further evaluated for its *in vivo* pharmacokinetic profile and toxicity in animal models.



**Figure 1** Structures, anti-HIV-1 activities and cytotoxicities of some representative IAS derivatives.

## 2. Results and discussion

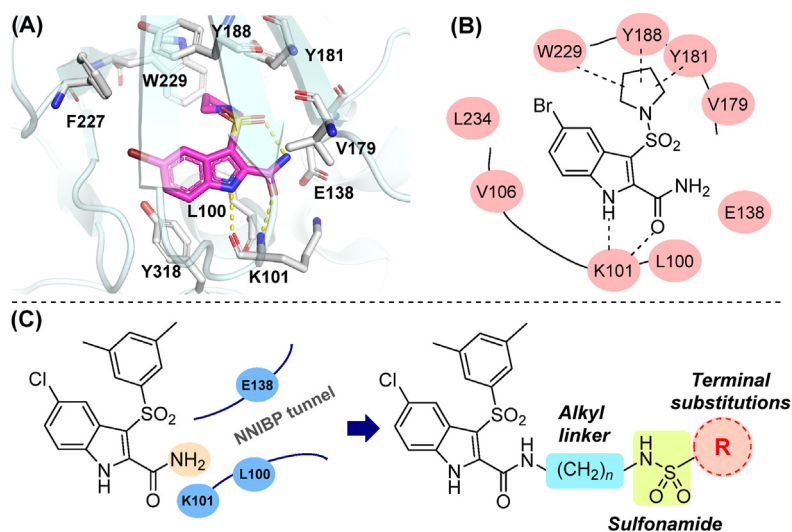
### 2.1. Chemistry

Synthetic routes and protocols of IAS analogues in this study are showed in Scheme 1. In brief, the intermediate **1** was synthesized smoothly starting with the marketable ethyl-5-chloro-1*H*-indole-2-carboxylate and 3,5-dimethylbenzenethiol according to the developed procedures. The afforded intermediate **2** was treated with *m*-chloroperoxybenzoic acid (*m*-CPBA), following the ester hydrolysis under LiOH to give the key intermediate **3**. On the other hand, the sulfonyl chlorides ( $R_m$ ) containing different substituents reacted with diamines ( $L_n$ ) with different carbon chain lengths protected by a single Boc to prepare the intermediates  $4R_mL_n$  with different structures. Subsequently, the Boc-NH groups

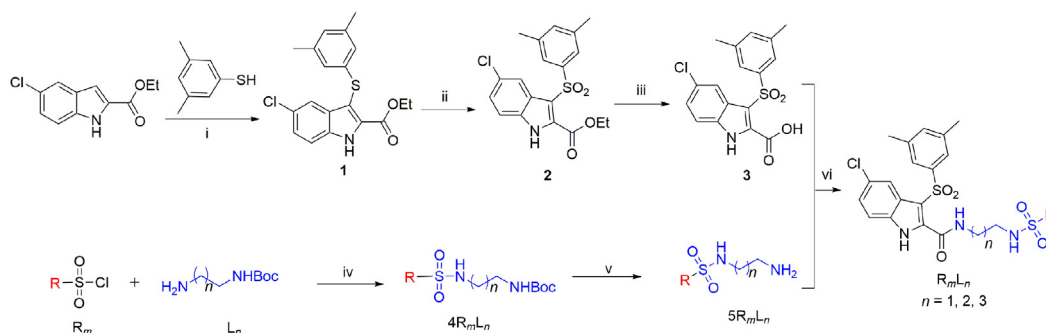
were deprotected by trifluoroacetic acid in dichloromethane to obtain the various intermediates  $5R_mL_n$ . Finally, the different substituted amines  $5R_mL_n$  were condensed with the intermediate **3** to synthesize the corresponding target products.

### 2.2. Anti-HIV activity evaluation

Anti-HIV activity (WT) of the newly synthesized IAS derivatives were evaluated in MT-4 cells. Three drugs: Nevirapine (NVP), Efavirenz (EFV), Etravirine (ETR) and the lead compound IAS-0 were chosen as positive controls. Additionally, several common clinical single mutant strains (L100I, K103N, Y181C, Y188L) as well as double-mutant strains RES056 (K103N/Y181C) and F227L/V106A were assessed. Tables 1 and 2 displayed the measured properties, including  $EC_{50}$ ,  $CC_{50}$  and calculated SI



**Figure 2** Binding mode and design strategy of IAS analogs. (A) 3D view of interaction and surrounding residues of IAS-6 and HIV-1 RT (PDB ID: 2RF2). (B) 2D abstract diagram of general binding pockets and regions of IAS derivatives. (C) Design strategy and general formula of target compounds in this work.



**Scheme 1** Reagents and conditions: (i) 1-(chloromethyl)-4-fluoro-1,4-diazabicyclo [2.2.2]octane-1,4-dium tetrafluoroborate (Selectfluor<sup>TM</sup>), acetonitrile (CH<sub>3</sub>CN), r.t.; (ii) *m*-CPBA, dichloromethane (DCM), 0 °C; (iii) lithium hydroxide, H<sub>2</sub>O, tetrahydrofuran (THF), r.t.; (iv) Et<sub>3</sub>N, DCM, 0 °C–r.t.; (v) CF<sub>3</sub>COOH, DCM, r.t.; (vi) 1*H*-1,2,3-triazolo [4,5-*b*] pyridinium-1-[bis(dimethylamino)-methylene]-3-oxide hexafluorophosphate (HATU), *N,N*-diisopropylethylamine (DIEA), DCM, 0 °C to r.t.

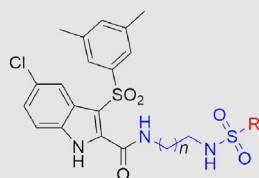
(selectivity index, CC<sub>50</sub>/EC<sub>50</sub> ratio) values. Notably, target compounds synthesized in this study showed remarkable potencies towards WT HIV-1 with low micromolar to nanomolar EC<sub>50</sub> values, meanwhile having low cytotoxicity (Table 1). Among them, compounds R<sub>10</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.0070 μmol/L, SI = 30,930) and R<sub>13</sub>L<sub>2</sub> (EC<sub>50</sub> = 0.0041 μmol/L, SI = 41,951) were proved to be the two most potent inhibitors against HIV-1 (IIIB), showing 18- and 32-fold improvement than that of NVP (EC<sub>50</sub> = 0.13 μmol/L), respectively. Moreover, both compounds maintained similar potency as EFV (EC<sub>50</sub> = 0.0038 μmol/L), ETR (EC<sub>50</sub> = 0.0051 μmol/L) and IAS-0 (EC<sub>50</sub> = 0.0041 μmol/L). It is worth emphasizing that the cytotoxicity of R<sub>10</sub>L<sub>4</sub> (CC<sub>50</sub> = 216.51 μmol/L) and R<sub>13</sub>L<sub>2</sub> (CC<sub>50</sub> = 172.00 μmol/L) are significantly decreased compared to that of IAS-0 (CC<sub>50</sub> = 5.52 μmol/L), which leads to higher selectivity index, nearly 14 times higher than that of IAS-0. This indicates that the newly introduced terminal sulfonamide groups could significantly reduce the cytotoxicity, which is consistent with our design concept. Moreover, compounds R<sub>9</sub>L<sub>4</sub>, R<sub>7</sub>L<sub>4</sub> and R<sub>11</sub>L<sub>4</sub> were also endowed with prominent anti-HIV-1 potencies (EC<sub>50</sub> = 0.0075, 0.0086 and 0.0091 μmol/L, respectively) and low cytotoxicity (CC<sub>50</sub> = 113.34, 89.33 and 189.54 μmol/L, respectively).

Preliminary investigation of the SARs suggested that the nature of sulfonamide moiety and the length of the carbon linker will greatly affect the antiviral potency. This can be concluded from the cellular activity data of each group that the optimal carbon chain length is four carbon atoms, and the general rule is C<sub>4</sub> ≈ C<sub>5</sub> > C<sub>3</sub> > C<sub>2</sub>, exemplified by R<sub>3</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.028 μmol/L) ≈ R<sub>3</sub>L<sub>5</sub> (EC<sub>50</sub> = 0.027 μmol/L) > R<sub>3</sub>L<sub>3</sub> (EC<sub>50</sub> = 0.050 μmol/L) > R<sub>3</sub>L<sub>2</sub> (EC<sub>50</sub> = 0.12 μmol/L) and R<sub>6</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.077 μmol/L) ≈ R<sub>6</sub>L<sub>5</sub> (EC<sub>50</sub> = 0.075 μmol/L) > R<sub>6</sub>L<sub>3</sub> (EC<sub>50</sub> = 0.14 μmol/L) > R<sub>6</sub>L<sub>2</sub> (EC<sub>50</sub> = 0.16 μmol/L). The lengths of carbon chain determine the relative position of sulfonamide substituents. An ethyl group might be inadequate to support terminal sulfonamide to reach the open area, meanwhile causing repulsive contacts. In contrast, longer carbon chains with higher flexibility will enable larger accessibility to different residues at the edge of pocket.

Considering the diverse chemical environment around NNIBP channel, we introduced arylsulfonamides containing lipophilic or hydrophilic groups. Different substitute groups on the *para*-position of benzenesulfonamide have a great impact on the cellular anti-HIV activity. The detailed SARs are as follows: when the *para* substituent of benzenesulfonamide is halogen, smaller atomic structure of

the substituent lead to better antiviral potency (H (R<sub>9</sub>L<sub>4</sub> = 0.0075 μmol/L) > Cl (R<sub>7</sub>L<sub>4</sub> = 0.0086 μmol/L) > F (R<sub>4</sub>L<sub>4</sub> = 0.020 μmol/L) > Br (R<sub>6</sub>L<sub>4</sub> = 0.077 μmol/L)). In addition, it can be seen from the activity data when the substituent on the benzenesulfonamide is a F atom, the *ortho*-position seems most beneficial to increase the activity [R<sub>11</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.0091 μmol/L) > R<sub>4</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.020 μmol/L) ≈ R<sub>12</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.019 μmol/L)]. When the *para*-substituents are strong electron-withdrawing groups (NO<sub>2</sub>/CN) or electron-donating groups (CH<sub>3</sub>/OCH<sub>3</sub>), their activities are no match for most halogen substitutions. Further extending the substituent to benzyl led to compound R<sub>14</sub>L<sub>2</sub> with significantly decreased activity (EC<sub>50</sub> = 0.21 μmol/L), suggesting that large groups are unfavorable for the antiviral potency. In brief, less substituent on the benzenesulfonamide will contribute to a prominent antiviral activity (R<sub>9</sub>L<sub>4</sub>, EC<sub>50</sub> = 0.0075 μmol/L) which demonstrates that the larger groups could prevent inhibitors from entering the NNIBP channel. Moreover, when the benzenesulfonamide is replaced by cyclopropylsulfonamide with a smaller structure, compound R<sub>13</sub>L<sub>2</sub> exhibits the most potency against WT strain (EC<sub>50</sub> = 0.0041 μmol/L) which is comparable to that of EFV (EC<sub>50</sub> = 0.0038 μmol/L), ETR (EC<sub>50</sub> = 0.0051 μmol/L) and IAS-0 (EC<sub>50</sub> = 0.0041 μmol/L). It is worth noting that for the aromatic heterocycles, the pyridine sulfonamide seems more favorable than thiophene sulfonamide when comparing the activity of R<sub>10</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.007 μmol/L) and R<sub>8</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.045 μmol/L). The comprehensive SARs was summarized in Fig. 3. This encouraging improvement in activity might be a consequence of hydrophilic nature of pyridine ring, and additional hydrogen bond between heterocycle N atom and surrounding amino acids. This is confirmed in our molecular docking (see Fig. 4). To our delight, most substituents are conducive to reducing the cytotoxicity, especially the halogen and methyl substituents.

As depicted in Table 2, all the synthesized compounds were further assessed for their inhibitory activities against the common clinical single-mutant strains (L100I, K103N, Y181C and E138K) and double-mutant strains F227L + V106A and RES056 (K103N + Y181C). L100I is selected by EFV, ETR and rilpivirine (RPV), with remarkable resistance towards them. K103N is the most commonly transmitted drug-resistance mutation. Likewise, E138K is mainly seen under RPV treatment. It has moderate resistance to RPV and ETR. Y181C could be selected under a variety of NNRTIs. Y188L is endowed with remarkable resistance towards doravirine (DOR) and EFV. F227L + V106A has 100 folds resistance towards DOR and high-level resistance

**Table 1** Antiviral activity of target compounds against WT HIV-1 strain.

Compd.	n	R	III <sub>B</sub>		
			EC <sub>50</sub> (μmol/L)	CC <sub>50</sub> (μmol/L)	SI
R <sub>1</sub> L <sub>2</sub>	1		0.045 ± 0.007	167.86 ± 17.24	3787
R <sub>1</sub> L <sub>3</sub>	2		0.036 ± 0.012	168.30 ± 23.54	4586
R <sub>1</sub> L <sub>4</sub>	3		0.032 ± 0.012	103.03 ± 59.77	3232
R <sub>1</sub> L <sub>5</sub>	4		0.027 ± 0.005	113.20 ± 3.74	4159
R <sub>2</sub> L <sub>2</sub>	1		0.085 ± 0.043	105.8 ± 23.02	1233
R <sub>2</sub> L <sub>3</sub>	2		0.037 ± 0.0085	162.9 ± 15.3	4354
R <sub>2</sub> L <sub>4</sub>	3		0.036 ± 0.005	22.48 ± 9.14	615
R <sub>2</sub> L <sub>5</sub>	4		0.023 ± 0.0081	109.8 ± 7.89	4772
R <sub>3</sub> L <sub>2</sub>	1		0.12 ± 0.029	>211.84	>1764
R <sub>3</sub> L <sub>3</sub>	2		0.050 ± 0.023	22.99 ± 0.60	464
R <sub>3</sub> L <sub>4</sub>	3		0.028 ± 0.0049	104.1 ± 31.44	3809
R <sub>3</sub> L <sub>5</sub>	4		0.027 ± 0.0079	>197.7	>7548
R <sub>4</sub> L <sub>2</sub>	1		0.028 ± 0.0036	>222.0	>7921
R <sub>4</sub> L <sub>3</sub>	2		0.038 ± 0.0069	34.99 ± 6.97	901
R <sub>4</sub> L <sub>4</sub>	3		0.020 ± 0.0085	>211.47	>10,593
R <sub>4</sub> L <sub>5</sub>	4		0.020 ± 0.0050	143.8 ± 13.20	7527
R <sub>5</sub> L <sub>2</sub>	1		0.065 ± 0.019	23.61 ± 0.58	362
R <sub>5</sub> L <sub>3</sub>	2		0.031 ± 0.0034	22.56 ± 0.89	732
R <sub>5</sub> L <sub>4</sub>	3		0.017 ± 0.010	4.58 ± 3.53	279
R <sub>5</sub> L <sub>5</sub>	4		0.033 ± 0.0082	>204.20	>6158
R <sub>6</sub> L <sub>2</sub>	1		0.16 ± 0.027	150.98 ± 19.26	964
R <sub>6</sub> L <sub>3</sub>	2		0.14 ± 0.013	88.08 ± 18.10	645
R <sub>6</sub> L <sub>4</sub>	3		0.077 ± 0.031	26.91 ± 16.87	347
R <sub>6</sub> L <sub>5</sub>	4		0.075 ± 0.030	37.86 ± 6.32	501
R <sub>7</sub> L <sub>2</sub>	1		0.013 ± 0.0048	174.80 ± 42.78	13,010
R <sub>7</sub> L <sub>3</sub>	2		0.017 ± 0.0067	>210.77	>12,913
R <sub>7</sub> L <sub>4</sub>	3		0.0086 ± 0.00082	89.33 ± 17.84	10,389
R <sub>7</sub> L <sub>5</sub>	4		0.010 ± 0.0014	114.72 ± 8.89	11,132
R <sub>8</sub> L <sub>2</sub>	1		0.10 ± 0.027	161.35 ± 50.34	1558
R <sub>8</sub> L <sub>3</sub>	2		0.057 ± 0.012	120.77 ± 33.45	2105
R <sub>8</sub> L <sub>4</sub>	3		0.045 ± 0.017	86.52 ± 11.78	1953
R <sub>8</sub> L <sub>5</sub>	4		0.067 ± 0.027	35.37 ± 1.05	526
R <sub>9</sub> L <sub>2</sub>	1		0.014 ± 0.0011	185.06 ± 8.31	13,509
R <sub>9</sub> L <sub>3</sub>	2		0.029 ± 0.0036	>223.57	>8047
R <sub>9</sub> L <sub>4</sub>	3		0.0075 ± 0.0010	113.34 ± 26.85	15,106
R <sub>9</sub> L <sub>5</sub>	4		0.015 ± 0.0077	128.61 ± 6.85	8814
R <sub>10</sub> L <sub>2</sub>	1		0.0073 ± 0.0016	23.44 ± 0.75	3205
R <sub>10</sub> L <sub>3</sub>	2		0.0080 ± 0.00089	55.06 ± 8.07	6950
R <sub>10</sub> L <sub>4</sub>	3		0.0070 ± 0.0021	216.51 ± 0.52	30,930
R <sub>10</sub> L <sub>5</sub>	4		0.0076 ± 0.0027	41.90 ± 2.18	5513
R <sub>11</sub> L <sub>2</sub>	1		0.018 ± 0.0036	198.73 ± 8.29	11,116
R <sub>11</sub> L <sub>3</sub>	2		0.015 ± 0.0017	191.69 ± 4.99	12,570
R <sub>11</sub> L <sub>4</sub>	3		0.0091 ± 0.0025	189.54 ± 1.32	20,621
R <sub>12</sub> L <sub>2</sub>	1		0.036 ± 0.0036	186.90 ± 4.81	5151
R <sub>12</sub> L <sub>3</sub>	2		0.035 ± 0.012	204.34 ± 10.80	5799
R <sub>12</sub> L <sub>4</sub>	3		0.019 ± 0.0051	185.57 ± 12.54	10,126
R <sub>13</sub> L <sub>2</sub>	1		0.0041 ± 0.00079	172.00 ± 6.91	41,951
R <sub>14</sub> L <sub>2</sub>	1		0.21 ± 0.048	64.35 ± 11.21	317
NVP	—		0.13 ± 0.041	>15.02	>114
EFV	—		0.0038 ± 0.00063	>6.34	>1622
ETR	—		0.0051 ± 0.0092	>4.59	>900
IAS-4	—		0.0053 ± 0.0014	65.63 ± 18.35	12,392
IAS-0	—		0.0041 ± 0.0014	5.52 ± 0.77	2159

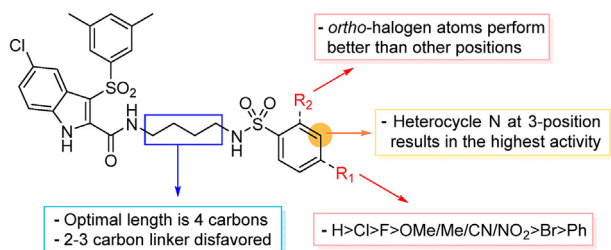
**Table 2** Antiviral activity of the target compounds against HIV-1 mutant strains.

Compd.	EC <sub>50</sub> (μmol/L) <sup>a</sup>						
	L100I	K103N	Y181C	Y188L	E138K	F227L + V106A	RES056
R <sub>1</sub> L <sub>2</sub>	0.68 ± 0.39	0.41 ± 0.11	0.61 ± 0.13	6.42 ± 1.40	0.86 ± 0.36	1.75 ± 0.20	3.38 ± 0.89
R <sub>1</sub> L <sub>3</sub>	0.54 ± 0.42	0.73 ± 0.14	1.19 ± 0.3	7.14 ± 1.4	0.21 ± 0.14	1.26 ± 0.38	5.97 ± 0.65
R <sub>1</sub> L <sub>4</sub>	0.16 ± 0.085	0.31 ± 0.068	0.31 ± 0.068	1.52 ± 0.27	0.13 ± 0.085	0.31 ± 0.034	>103.03
R <sub>1</sub> L <sub>5</sub>	0.17 ± 0.017	0.63 ± 0.017	0.23 ± 0.017	2.53 ± 0.18	0.18 ± 0.017	0.38 ± 0.13	>113.2
R <sub>2</sub> L <sub>2</sub>	0.66 ± 0.23	0.9 ± 0.38	0.85 ± 0.035	>105.8	1.11 ± 0.17	1.58 ± 0.45	>105.8
R <sub>2</sub> L <sub>3</sub>	0.32 ± 0.00	0.66 ± 0.085	0.83 ± 0.068	5.98 ± 0.31	0.25 ± 0.00	0.97 ± 0.051	>162.9
R <sub>2</sub> L <sub>4</sub>	0.18 ± 0.00	0.33 ± 0.033	0.33 ± 0.00	0.80 ± 0.083	0.25 ± 0.05	0.28 ± 0.033	>22.48
R <sub>2</sub> L <sub>5</sub>	0.094 ± 0.047	0.57 ± 0.00	0.19 ± 0.016	1.28 ± 0.44	0.13 ± 0.049	0.28 ± 0.065	>109.8
R <sub>3</sub> L <sub>2</sub>	0.95 ± 0.085	0.59 ± 0.10	1.00 ± 0.17	>211.84	1.15 ± 0.00	6.17 ± 1.14	>211.84
R <sub>3</sub> L <sub>3</sub>	0.35 ± 0.017	0.73 ± 0.12	1.03 ± 0.050	3.29 ± 0.066	0.31 ± 0.050	1.77 ± 0.033	4.92 ± 1.27
R <sub>3</sub> L <sub>4</sub>	0.13 ± 0.018	0.50 ± 0.16	0.40 ± 0.11	0.87 ± 0.065	0.23 ± 0.032	0.36 ± 0.032	1.88 ± 0.68
R <sub>3</sub> L <sub>5</sub>	0.13 ± 0.0063	0.9 ± 0.079	0.30 ± 0.00	2.47 ± 0.16	0.25 ± 0.032	0.54 ± 0.14	25.14 ± 0.047
R <sub>4</sub> L <sub>2</sub>	0.21 ± 0.036	0.36 ± 0.16	0.37 ± 0.14	3.00 ± 0.053	0.37 ± 0.11	0.83 ± 0.12	3.23 ± 0.018
R <sub>4</sub> L <sub>3</sub>	0.24 ± 0.035	0.45 ± 0.10	0.69 ± 0.017	2.84 ± 0.052	0.26 ± 0.017	1.04 ± 0.035	3.55 ± 0.35
R <sub>4</sub> L <sub>4</sub>	0.056 ± 0.036	0.19 ± 0.051	0.20 ± 0.034	1.27 ± 0.20	0.063 ± 0.003	0.29 ± 0.12	>211.47
R <sub>4</sub> L <sub>5</sub>	0.074 ± 0.010	0.51 ± 0.21	0.18 ± 0.017	1.67 ± 0.35	0.073 ± 0.02	0.38 ± 0.017	>31.17
R <sub>5</sub> L <sub>2</sub>	0.77 ± 0.035	0.84 ± 0.035	0.89 ± 0.018	3.07 ± 0.12	1.26 ± 0.00	1.75 ± 0.053	3.37 ± 0.21
R <sub>5</sub> L <sub>3</sub>	0.33 ± 0.034	0.92 ± 0.41	0.98 ± 0.051	3.34 ± 0.017	0.27 ± 0.017	1.22 ± 0.051	4.09 ± 0.00
R <sub>5</sub> L <sub>4</sub>	0.025 ± 0.002	0.067 ± 0.005	0.054 ± 0.002	0.15 ± 0.010	0.032 ± 0.018	0.082 ± 0.035	>4.58
R <sub>5</sub> L <sub>5</sub>	0.077 ± 0.023	0.56 ± 0.13	0.21 ± 0.00	1.70 ± 0.52	0.16 ± 0.016	0.34 ± 0.082	11.88 ± 4.90
R <sub>6</sub> L <sub>2</sub>	0.75 ± 0.064	0.95 ± 0.16	1.01 ± 0.032	≥33.74	1.27 ± 0.11	2.73 ± 0.48	≥12.17
R <sub>6</sub> L <sub>3</sub>	0.78 ± 0.14	1.04 ± 0.19	1.32 ± 0.19	5.26 ± 1.04	0.47 ± 0.20	1.48 ± 0.11	>88.08
R <sub>6</sub> L <sub>4</sub>	0.32 ± 0.15	0.69 ± 0.11	0.60 ± 0.031	1.00 ± 0.15	0.78 ± 0.54	0.51 ± 0.092	>26.91
R <sub>6</sub> L <sub>5</sub>	0.12 ± 0.039	0.74 ± 0.090	0.35 ± 0.00	2.47 ± 0.030	0.32 ± 0.015	0.68 ± 0.075	>37.86
R <sub>7</sub> L <sub>2</sub>	0.17 ± 0.041	0.22 ± 0.00	0.31 ± 0.16	2.66 ± 0.17	0.36 ± 0.017	1.04 ± 0.35	3.09 ± 0.50
R <sub>7</sub> L <sub>3</sub>	0.098 ± 0.052	0.22 ± 0.00	0.57 ± 0.25	0.19 ± 0.19	0.61 ± 0.12	2.61 ± 0.12	9.00 ± 0.51
R <sub>7</sub> L <sub>4</sub>	0.074 ± 0.021	0.13 ± 0.048	0.18 ± 0.016	1.53 ± 0.72	0.051 ± 0.018	0.26 ± 0.00	>89.33
R <sub>7</sub> L <sub>5</sub>	0.061 ± 0.0032	0.43 ± 0.14	0.19 ± 0.00	3.14 ± 0.95	0.052 ± 0.009	0.37 ± 0.11	>114.72
R <sub>8</sub> L <sub>2</sub>	0.80 ± 0.094	1.18 ± 0.091	1.00 ± 0.091	6.24 ± 0.65	1.20 ± 0.018	1.81 ± 0.18	4.63 ± 1.54
R <sub>8</sub> L <sub>3</sub>	0.50 ± 0.14	1.03 ± 0.19	1.06 ± 0.018	3.54 ± 0.34	0.28 ± 0.018	1.65 ± 0.27	5.38 ± 1.29
R <sub>8</sub> L <sub>4</sub>	0.13 ± 0.059	0.47 ± 0.069	0.54 ± 0.00	1.05 ± 0.035	0.19 ± 0.017	0.36 ± 0.035	>86.52
R <sub>8</sub> L <sub>5</sub>	0.17 ± 0.017	1.23 ± 0.22	0.73 ± 0.34	2.71 ± 0.13	0.20 ± 0.051	1.26 ± 0.34	>43.31
R <sub>9</sub> L <sub>2</sub>	0.26 ± 0.11	0.24 ± 0.037	0.26 ± 0.073	2.81 ± 0.29	0.22 ± 0.073	1.12 ± 0.35	3.14 ± 0.11
R <sub>9</sub> L <sub>3</sub>	0.18 ± 0.054	0.32 ± 0.072	0.70 ± 0.27	3.08 ± 0.25	0.055 ± 0.018	0.80 ± 0.25	4.76 ± 0.80
R <sub>9</sub> L <sub>4</sub>	0.028 ± 0.0087	0.15 ± 0.045	0.15 ± 0.033	0.87 ± 0.17	0.031 ± 0.007	0.28 ± 0.052	>114.95
R <sub>9</sub> L <sub>5</sub>	0.044 ± 0.005	0.34 ± 0.051	0.22 ± 0.017	1.46 ± 0.56	0.036 ± 0.007	0.22 ± 0.085	>119.33
R <sub>10</sub> L <sub>2</sub>	0.14 ± 0.046	0.29 ± 0.055	0.29 ± 0.018	2.20 ± 0.40	0.12 ± 0.033	1.48 ± 0.27	4.60 ± 0.31
R <sub>10</sub> L <sub>3</sub>	0.10 ± 0.032	0.20 ± 0.036	0.20 ± 0.018	2.32 ± 0.11	0.043 ± 0.005	0.54 ± 0.27	7.77 ± 2.32
R <sub>10</sub> L <sub>4</sub>	0.017 ± 0.002	0.068 ± 0.012	0.045 ± 0.010	0.54 ± 0.087	0.017 ± 0.007	0.087 ± 0.035	5.26
R <sub>10</sub> L <sub>5</sub>	0.046 ± 0.014	0.19 ± 0.034	0.11 ± 0.022	0.73 ± 0.034	0.029 ± 0.012	0.14 ± 0.075	10.61 ± 6.70
R <sub>11</sub> L <sub>2</sub>	0.25 ± 0.071	0.21 ± 0.053	0.28 ± 0.036	2.68 ± 0.25	0.20 ± 0.036	1.23 ± 0.43	3.04 ± 0.21
R <sub>11</sub> L <sub>3</sub>	0.16 ± 0.078	0.28 ± 0.035	0.61 ± 0.14	7.12 ± 0.76	0.068 ± 0.002	0.88 ± 0.52	12.84 ± 10.12
R <sub>11</sub> L <sub>4</sub>	0.063 ± 0.025	0.17 ± 0.017	0.20 ± 0.017	1.74 ± 0.32	0.036 ± 0.005	0.29 ± 0.034	>189.54
R <sub>12</sub> L <sub>2</sub>	0.46 ± 0.18	0.50 ± 0.089	0.55 ± 0.089	3.30 ± 0.30	0.44 ± 0.11	2.26 ± 0.55	4.94 ± 1.24
R <sub>12</sub> L <sub>3</sub>	0.26 ± 0.087	0.45 ± 0.12	0.75 ± 0.21	3.38 ± 0.45	0.11 ± 0.043	0.99 ± 0.14	4.73 ± 1.04
R <sub>12</sub> L <sub>4</sub>	0.063 ± 0.017	0.24 ± 0.068	0.19 ± 0.034	1.45 ± 0.41	0.049 ± 0.012	0.24 ± 0.034	>173.28
R <sub>13</sub> L <sub>2</sub>	0.053 ± 0.022	0.11 ± 0.020	0.18 ± 0.057	1.65 ± 0.39	0.055 ± 0.016	0.51 ± 0.12	32.57 ± 7.96
R <sub>14</sub> L <sub>2</sub>	0.95 ± 0.097	1.19 ± 0.26	1.42 ± 0.37	>31.20	0.74 ± 0.097	2.99 ± 0.68	>82.35
NVP	1.26 ± 0.60	5.33 ± 1.07	>9.52	>9.52	0.095 ± 0.031	>9.52	>9.52
EFV	0.032 ± 0.006	0.10 ± 0.019	0.007 ± 0.002	0.27 ± 0.016	0.007 ± 0.001	0.27 ± 0.048	0.48 ± 0.19
ETR	0.032 ± 0.028	0.015 ± 0.012	0.058 ± 0.041	0.083 ± 0.053	0.037 ± 0.030	0.088 ± 0.076	0.15 ± 0.12
IAS-4	0.047 ± 0.021	0.056 ± 0.005	0.25 ± 0.018	0.56 ± 0.14	0.04 ± 0.02	0.23 ± 0.070	7.45 ± 2.25
IAS-0	0.015 ± 0.0086	0.017 ± 0.0060	0.039 ± 0.0028	1.1 ± 0.63	0.90 ± 0.065	0.047 ± 0.023	1.8 ± 0.29

<sup>a</sup>EC<sub>50</sub>: Concentration of compound that decreases 50% HIV-1-induced cytopathy of MT-4 cells.

to EFV<sup>32</sup>. Their SI and RF (fold-resistance factor, EC<sub>50</sub> (mutant strain)/EC<sub>50</sub> (WT strain)) were showed in Table 3. Most compounds exhibited moderate to excellent activities to these mutant strains, but slightly reduced compared with that to WT HIV-1 strain. SARs for the inhibition activity of mutant strains are basically in consistent with those of WT strains. Among them, compound

R<sub>10</sub>L<sub>4</sub> displayed the most potent inhibitory activity towards commonly occurring mutant strains, especially for L100I, Y181C and E138K (EC<sub>50</sub> = 0.017, 0.045 and 0.017 μmol/L, respectively), superior to that of NVP and ETR, similar with EFV. However, these compounds are more sensitive to Y188L change than other mutations. When tyrosine 188 alters to



**Figure 3** Schematic overview of SARs of IAS derivatives in this work.

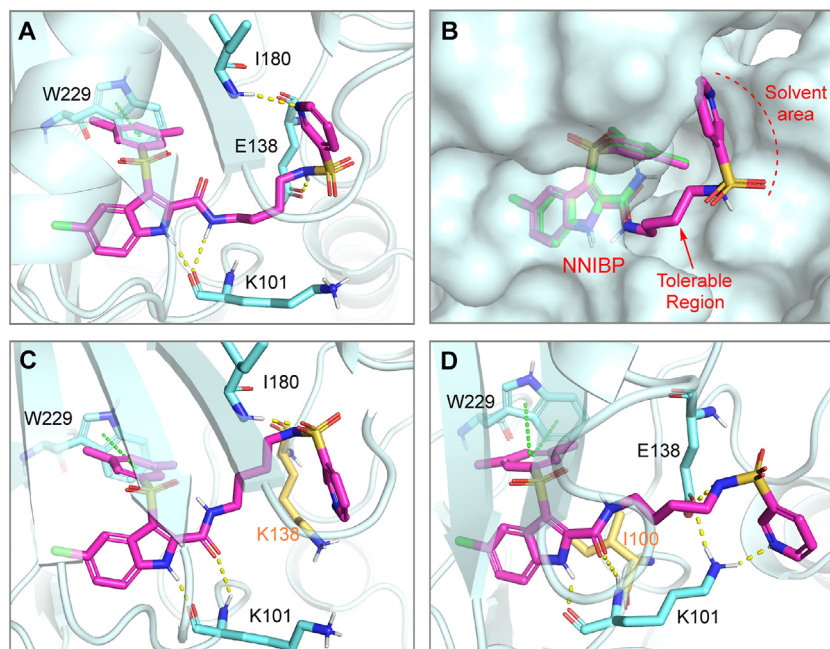
leucine, the loss of key hydrophobic or  $\pi$ - $\pi$  stacking interaction made most of the compounds inactive. Apparently, for the important NNRTIs-resistant mutant strain E138K,  $R_{10}L_4$  exhibited an enhanced inhibitory activity and potent drug resistance ( $EC_{50} = 0.017 \mu\text{mol/L}$ , SI = 13,123, RF = 2.4). Additionally, considering the cytotoxicity,  $R_{10}L_4$  possessed a higher selectivity index and safety profiles than the IAS-0, NVP, EFV, and ETR. It is a pity that  $R_{13}L_2$ , the compound with the best activity against WT strain ( $EC_{50} = 0.0041 \mu\text{mol/L}$ , SI = 41,951), did not show good drug resistance, such as the two most common mutants ( $EC_{50} (K103N) = 0.11 \mu\text{mol/L}$ , RF = 26.8;  $EC_{50} (Y181C) = 0.18 \mu\text{mol/L}$ , RF = 43.9). Moreover, the potency towards double mutant strains RES056 and F227L + V106 were also evaluated. In general, a large part of compounds are rather sensitive to RES056 strain than F227L + V106 mutation. The two most potential compounds  $R_5L_4$  and  $R_{10}L_4$  also exhibited the best inhibitory activities against F227L + V106A, with  $EC_{50}$  values of 0.082 and  $0.087 \mu\text{mol/L}$ , respectively, possessing significantly improved potency than NVP ( $EC_{50} > 9.52 \mu\text{mol/L}$ ) and EFV ( $EC_{50} = 0.27 \mu\text{mol/L}$ ), and comparable to ETR

( $EC_{50} = 0.088 \mu\text{mol/L}$ ). When compared with IAS-4 with similar activity against WT strain ( $EC_{50(WT)} = 0.0053 \mu\text{mol/L}$ ) in our previous studies<sup>15</sup>,  $R_{10}L_4$  possesses lower toxicity feature and is much less influenced by the mutant strains, exemplified in Tables 2 and 3. From the perspective of anti-drug resistance and cytotoxicity, an apparent advantage of sulfonamide substitution over other groups is thus clearly showed. Meanwhile, further experiments on more multi-mutant strains should also be considered.

In summary, the most suitable carbon chain length appeared to be four carbon atoms, and pyridine sulfonamide with hydrogen-bonding receptor was the privileged terminal substituent. It is worth emphasizing that a general decrease in cytotoxicity and improvement of the selectivity index is attained by the introduction of sulfonamide groups, which achieved the primary goal of our design concept.

### 2.3. HIV-1 RT inhibition assay

For the purpose of directly evaluating target affinity of target compounds, all target compounds along with IAS-0 and EFV as control drugs were selected to test their inhibitory effects against WT HIV-1 RT (Supporting Information Table S1). As shown in Table 4, the representative compounds maintained prominent activity ( $IC_{50} = 0.057$ – $0.070 \mu\text{mol/L}$ ) against HIV-1 RT. Excellent enzymatic inhibitory ( $IC_{50} = 0.057 \mu\text{mol/L}$ ) was seen in  $R_{13}L_2$ , one with the best cellular activity. It was comparable to EFV ( $IC_{50} = 0.030 \mu\text{mol/L}$ ) but weaker than the lead IAS-0 ( $IC_{50} = 0.018 \mu\text{mol/L}$ ).  $R_{10}L_2$  ( $EC_{50} = 0.0073 \mu\text{mol/L}$ ,  $IC_{50} = 0.060 \mu\text{mol/L}$ ) exhibited an inferior cellular activity but with a higher enzymatic inhibitory activity than  $R_{10}L_4$  ( $EC_{50} = 0.0070 \mu\text{mol/L}$ ,  $IC_{50} = 0.070 \mu\text{mol/L}$ ). In comparison, IAS-4 showed an  $IC_{50}$  of  $0.17 \mu\text{mol/L}$  in previous studies under



**Figure 4** Graphical representation of docking poses and interactions of  $R_{10}L_4$  with WT HIV-1 RTs (PDB ID: 6C0N) (A and B), E138K (PDB ID: 6C0L) (C) and L100I (PDB ID: 2OPQ) (D).  $R_{10}L_4$  and IAS-0 were shown as sticks with carbon atoms colored in magenta/green. Protein surface and residues were shown in pale cyan. Mutated residues were highlighted by yellow. H-bonds and  $\pi$ - $\pi$  stackings were represented by yellow/green dashed lines.

**Table 3** SI values of the target compounds.

Compd.	SI (RF) <sup>a</sup>						
	L100I	K103N	Y181C	Y188L	E138K	F227L+V106A	RES056
R <sub>1</sub> L <sub>2</sub>	247 (15.3)	404 (9.4)	274 (13.8)	26 (144.8)	197 (19.2)	96 (39.6)	50 (76.4)
R <sub>1</sub> L <sub>3</sub>	308 (14.9)	231 (19.8)	142 (32.2)	24 (194.6)	833 (5.5)	134 (34.3)	28 (162.5)
R <sub>1</sub> L <sub>4</sub>	637 (5.1)	332 (9.7)	327 (9.9)	68 (47.4)	770 (4.2)	333 (9.7)	<1 (>3232)
R <sub>1</sub> L <sub>5</sub>	679 (6.1)	177 (23.5)	491 (8.5)	45 (93.1)	593 (7.0)	301 (13.8)	<1 (>4159.3)
R <sub>2</sub> L <sub>2</sub>	159 (7.8)	118 (10.5)	125 (9.8)	1 (1232.5)	95 (13.0)	67 (18.5)	<1 (>1232.5)
R <sub>2</sub> L <sub>3</sub>	494 (8.8)	246 (17.7)	195 (22.3)	27 (159.7)	636 (6.9)	169 (25.8)	<1 (>4354.1)
R <sub>2</sub> L <sub>4</sub>	126 (4.9)	66 (9.3)	67 (9.1)	28 (21.9)	88 (7.0)	79 (7.8)	<1 (>615.2)
R <sub>2</sub> L <sub>5</sub>	1172 (4.1)	196 (24.4)	546 (8.7)	86 (55.7)	846 (5.6)	391 (12.2)	<1 (>4772.2)
R <sub>3</sub> L <sub>2</sub>	>224 (7.9)	>358 (4.9)	>212 (8.3)	1 (1764.0)	>184 (9.6)	>34 (51.3)	<1 (>1764.0)
R <sub>3</sub> L <sub>3</sub>	65 (7.1)	32 (14.6)	22 (20.8)	7 (66.5)	74 (6.3)	13 (35.9)	5 (99.0)
R <sub>3</sub> L <sub>4</sub>	771 (4.9)	211 (18.1)	255 (15.0)	120 (31.8)	471 (8.1)	299 (12.7)	56 (68.4)
R <sub>3</sub> L <sub>5</sub>	1487 (5.1)	220 (34.3)	652 (11.6)	80 (94.2)	779 (9.7)	366 (20.6)	>8 (959.4)
R <sub>4</sub> L <sub>2</sub>	1043 (7.6)	627 (12.6)	603 (13.1)	74 (107.4)	592 (13.4)	267 (29.6)	69 (115.4)
R <sub>4</sub> L <sub>3</sub>	143 (6.3)	77 (11.7)	50 (18.0)	12 (73.4)	136 (6.6)	34 (26.8)	10 (91.7)
R <sub>4</sub> L <sub>4</sub>	3765 (2.8)	1100 (9.6)	1038 (10.2)	167 (63.5)	3420 (3.1)	732 (14.5)	<1 (>10593.2)
R <sub>4</sub> L <sub>5</sub>	1929 (3.9)	278 (27.1)	776 (9.7)	86 (87.1)	1996 (3.8)	371 (20.3)	≤5 (>1631.1)
R <sub>5</sub> L <sub>2</sub>	30 (11.9)	28 (13.0)	26 (13.7)	8 (47.0)	19 (19.2)	13 (27.0)	7 (51.5)
R <sub>5</sub> L <sub>3</sub>	70 (10.5)	24 (30.2)	23 (31.7)	7 (108.2)	81 (9.0)	19 (39.2)	6 (132.8)
R <sub>5</sub> L <sub>4</sub>	181 (1.5)	68 (4.1)	87 (3.2)	30 (9.2)	147 (1.9)	56 (4.9)	<1 (>278.8)
R <sub>5</sub> L <sub>5</sub>	2657 (2.3)	365 (16.9)	955 (6.5)	121 (51.0)	1315 (4.7)	585 (10.5)	17 (358.4)
R <sub>6</sub> L <sub>2</sub>	198 (4.9)	159 (6.1)	150 (6.4)	4 (≥215.6)	119 (8.1)	55 (55.5)	≤12 (>77.7)
R <sub>6</sub> L <sub>3</sub>	112 (5.7)	85 (7.6)	67 (9.6)	17 (38.5)	188 (3.4)	60 (10.8)	<1 (>644.7)
R <sub>6</sub> L <sub>4</sub>	85 (4.1)	39 (8.9)	45 (7.7)	27 (12.9)	35 (10.0)	54 (6.5)	<1 (>347.4)
R <sub>6</sub> L <sub>5</sub>	316 (1.6)	51 (9.8)	108 (4.7)	15 (32.7)	119 (4.2)	55 (9.0)	<1 (>501.4)
R <sub>7</sub> L <sub>2</sub>	1028 (12.7)	754 (17.3)	575 (22.6)	66 (198.1)	482 (27.0)	169 (77.0)	56 (230.4)
R <sub>7</sub> L <sub>3</sub>	2144 (6.0)	942 (13.7)	363 (35.5)	1097 (11.8)	352 (36.7)	80 (160.6)	23 (552.1)
R <sub>7</sub> L <sub>4</sub>	1215 (8.6)	676 (15.4)	482 (21.6)	58 (177.6)	1730 (6.0)	333 (31.2)	<1 (10389.1)
R <sub>7</sub> L <sub>5</sub>	1885 (5.9)	260 (42.9)	599 (18.6)	36 (305.4)	2255 (4.9)	311 (35.8)	<1 (11132.1)
R <sub>8</sub> L <sub>2</sub>	201 (7.8)	136 (11.5)	161 (9.7)	26 (60.2)	135 (11.5)	89 (17.6)	35 (44.6)
R <sub>8</sub> L <sub>3</sub>	242 (8.7)	117 (18.0)	113 (18.6)	34 (61.7)	427 (4.9)	73 (28.7)	22 (93.9)
R <sub>8</sub> L <sub>4</sub>	658 (3.0)	186 (10.5)	161 (12.1)	82 (23.8)	437 (4.5)	243 (8.0)	<1 (>1952.5)
R <sub>8</sub> L <sub>5</sub>	273 (3.0)	39 (21.3)	65 (12.7)	17 (47.4)	225 (3.7)	37 (22.1)	<1 (>823.9)
R <sub>9</sub> L <sub>2</sub>	596 (18.7)	651 (17.1)	576 (19.3)	54 (207.7)	670 (16.6)	134 (83.2)	48 (232.8)
R <sub>9</sub> L <sub>3</sub>	1289 (7.9)	699 (14.6)	318 (32.2)	73 (140.7)	4026 (2.5)	275 (37.2)	47 (217.6)
R <sub>9</sub> L <sub>4</sub>	4137 (3.9)	777 (20.9)	754 (21.5)	131 (124.1)	3660 (4.4)	408 (39.8)	<1 (16236.9)
R <sub>9</sub> L <sub>5</sub>	2684 (3.6)	347 (27.8)	533 (18.1)	81 (118.3)	3417 (2.8)	554 (17.4)	<1 (>9634.3)
R <sub>10</sub> L <sub>2</sub>	171 (14.0)	81 (29.4)	81 (29.5)	11 (216.8)	200 (11.9)	16 (145.9)	5 (453.8)
R <sub>10</sub> L <sub>3</sub>	551 (11.1)	289 (21.2)	268 (22.9)	24 (258.2)	1284 (4.8)	102 (59.9)	7 (864.4)
R <sub>10</sub> L <sub>4</sub>	13,055 (2.4)	3189 (9.7)	4753 (6.6)	408 (76.9)	13,123 (2.4)	2493 (12.6)	41 (757.2)
R <sub>10</sub> L <sub>5</sub>	1480 (6.8)	353 (28.7)	638 (15.9)	93 (108.8)	2274 (4.5)	466 (21.7)	6 (1594.6)
R <sub>11</sub> L <sub>2</sub>	786 (12.1)	927 (10.3)	698 (13.6)	74 (128.3)	1051 (9.1)	162 (58.7)	65 (145.7)
R <sub>11</sub> L <sub>3</sub>	1188 (9.5)	704 (16.0)	320 (35.1)	27 (411.2)	2908 (3.9)	218 (51.5)	15 (741.0)
R <sub>11</sub> L <sub>4</sub>	3022 (5.3)	1147 (13.8)	914 (17.4)	108 (146.5)	5223 (3.0)	678 (23.4)	<1 (15876.6)
R <sub>12</sub> L <sub>2</sub>	403 (13.2)	374 (14.3)	333 (16.0)	56 (95.6)	420 (12.7)	82 (65.1)	37 (142.9)
R <sub>12</sub> L <sub>3</sub>	>853 (7.9)	>480 (14.0)	>291 (23.1)	>64 (105.3)	>2002 (3.4)	>220 (30.6)	>46 (147.3)
R <sub>12</sub> L <sub>4</sub>	2735 (4.2)	734 (15.7)	910 (12.6)	119 (97.1)	3553 (3.2)	712 (16.2)	<1 (>11508.5)
R <sub>13</sub> L <sub>2</sub>	3205 (12.9)	1503 (26.8)	972 (43.9)	104 (347.1)	3155 (13.4)	343 (105.6)	5 (6867.5)
R <sub>14</sub> L <sub>2</sub>	87 (4.9)	69 (6.2)	58 (7.3)	3 (≥161.2)	112 (3.8)	28 (15.5)	<1 (>425.6)
NVP	>7 (15.2)	>2 (64.0)	1 (>114.2)	<1 (>114.2)	>100 (1.1)	1 (>114.2)	<1 (>114.2)
EFV	>191 (8.5)	>61 (26.6)	>860 (1.9)	>23 (70.0)	>876 (1.9)	>24 (69.0)	>13 (124.9)
ETR	>140 (2.9)	>320 (1.3)	>81 (5.1)	>56 (7.4)	>127 (3.3)	>52 (7.9)	>30 (13.8)
IAS-4	1396 (8.9)	1172 (10.6)	263 (47.2)	117 (105.7)	1640 (7.54)	285 (43.4)	9 (1405.7)
IAS-0	279 (7.3)	131 (15.7)	97 (21.1)	3 (593.9)	377 (5.4)	54 (≥38.5)	≤3 (≥832.6)

<sup>a</sup>RF is the ratio of EC<sub>50</sub> (resistant viral strain)/EC<sub>50</sub> (wild-type viral strain).

the same method<sup>13</sup>. Generally, their measured inhibition to RT maintained good consistence with antivirus activity. According to the analysis of above results, we conclude that sulfonamide substituted indolylarylsulfones are typical and effective HIV-1 NNRTIs.

#### 2.4. Molecular docking study

Molecular docking simulation was conducted to identify the binding pattern between the most potent compound R<sub>10</sub>L<sub>4</sub> (EC<sub>50(MB)</sub> = 0.0070 μmol/L; EC<sub>50(L100I)</sub> = 0.017 μmol/L;



**Table 4** RT inhibitory activity of some selected compounds.

Compd.	R <sub>10</sub> L <sub>2</sub>	R <sub>10</sub> L <sub>4</sub>	R <sub>13</sub> L <sub>2</sub>	IAS-0	EFV <sup>b</sup>
IC <sub>50</sub> (μmol/L) <sup>a</sup>	0.060 ± 0.009	0.070 ± 0.009	0.057 ± 0.012	0.018 ± 0.002	0.030 ± 0.000

<sup>a</sup>IC<sub>50</sub>: Compound concentration required to inhibit 50% incorporation of biotin-labeled UTP by HIV-1 (WT) RT.

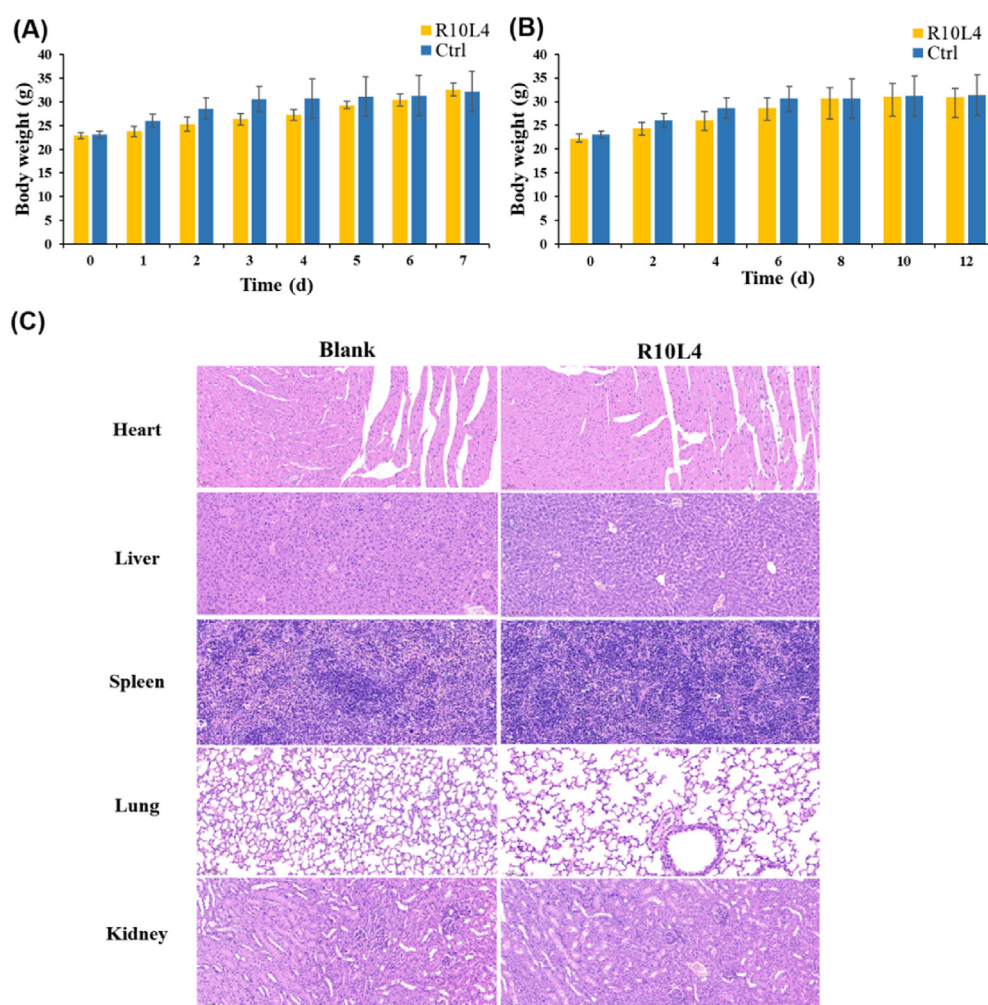
<sup>b</sup>All data were obtained from one batch with the same method.

EC<sub>50(K103N)</sub> = 0.068 μmol/L; EC<sub>50(E138K)</sub> = 0.017 μmol/L and HIV-1 RT (wild type, PDB ID: 6C0N; E138K, PDB ID: 6C0L and L100I, PDB ID: 2OPQ). For this purpose, R<sub>10</sub>L<sub>4</sub> and IAS-0 were both chosen as the ligands and the glide module in Schrödinger suite was employed to conduct dockings. Docking results and 3D interactions are visualized in Pymol ([www.pymol.org](http://www.pymol.org)).

As showed in Fig. 4a, R<sub>10</sub>L<sub>4</sub> was well fitted to NNIBP of WT HIV-1 RT with a high docking score up to −11.645, adapting a double-winged conformation without obvious torsions. Newly introduced 4-(pyridine-3-sulfonamido)butyl moiety is well accommodated by a tunnel at the opening of binding site, which is consistent with our anticipation in compound designing. To be noted, the four-carbon direct chain is slightly twisted, thus

enabling terminal pyridine-3-sulfonamyl group to build up two hydrogen bonds with E138 and I180. The residual part of R<sub>10</sub>L<sub>4</sub> is well aligned with IAS-0, as the hydrophilic interaction and π–π stacking with surrounding residues are conserved (Fig. 4b). Double hydrogen bonds with the backbone of K101 are also seen in the case of R<sub>10</sub>L<sub>4</sub>, but was formed by indole heterocycle NH and 2-carboxamide NH.

Binding mode between R<sub>10</sub>L<sub>4</sub> with E138K and L100I RT mutants was characterized to explain its anti-drug resistance property (Fig. 4c and d). The two mutations exerted no obvious disruptions to the position of indole scaffold, for the interactions towards W229 and K101 remain unchanged. On the other hand, as these mutations reshaped the entry tunnel of NNIBP, significant



**Figure 5** Visualized results from *in vivo* toxicity experiment of R<sub>10</sub>L<sub>4</sub>. (A) Time courses of body weight in 7-days acute toxicity experiment. (B) Time courses of body weight in 12-days subacute toxicity experiment. (C) Images of organ slices from treated mice in subacute toxicity study. Heart, liver, spleen, lung and kidney were sectioned and stained with hematoxylin and eosin.

conformation changes are thus seen in side wing 4-(pyridine-3-sulfonamido)butyl moiety. For E138K model, sulfamide NH no longer interacts with K138, but a close contact with I180 is still viable. Analogously, docking results based on L100I revealed a stable hydrogen bond network among pyridinyl-3-sulfonyl group and sidechains of K101/E138. From a comparison of the results above, the potent inhibition of R<sub>10</sub>L<sub>4</sub> towards mutant strains is mainly contributed by the high flexibility of C4 alkyne linker, and stable interaction of pyridinyl-3-sulfonyl with multiple hydrophilic residues.

### 2.5. *In vivo acute/subacute toxicity assessment*

The safety profiles of R<sub>10</sub>L<sub>4</sub> were conducted in SPF Kunming mice. All relative animal experiments in this study were approved by the ethics committee of Cheeloo College of Medicine, Shandong University (Jinan, China). Procedures performed in the studies were guided by the ethical standards of the institution. In both acute toxicity (800 mg/kg, 7 days) and subacute toxicity (50 mg/kg, 12 days) experiments, R<sub>10</sub>L<sub>4</sub> did not lead to any mortality or observable behavior changes. Records of average body weight are presented in Fig. 5a and b. Compared with blank group, only insignificant body weight loss was seen, and results of experiment groups remain identical with that of control groups at the end of 7 or 12 days of administration. At the end of subacute toxicity test, the hematoxylin and eosin-stained slices from vital organs were prepared. Appearance of cells and structures in all organs showed no marked pathological abnormalities after the administration of R<sub>10</sub>L<sub>4</sub>, compared with blank vehicle group. All these results are consistent with the low cytotoxicity of R<sub>10</sub>L<sub>4</sub> (CC<sub>50</sub> = 216.5 μmol/L), which could be an apparent advantage in its further evaluation and clinical development.

### 2.6. *In vivo pharmacokinetics (PK) study*

The pharmacokinetic profiles of R<sub>10</sub>L<sub>4</sub> were examined in a Sprague–Dawley rat to evaluate its potential for therapeutic application (Table 5). Six rats were equally divided into two groups, 2 mg/kg i.v. dose and 10 mg/kg oral dose was given. After the i.v. dosing, a modest half-life of 1.61 h, a high clearance rate of 4209 mL/h/kg and an area under the curve (AUC) value of 481 h·ng/mL was observed. Absorption of R<sub>10</sub>L<sub>4</sub> was quick when dosed orally at 10 mg/kg ( $T_{max}$  = 0.83 h), indicating its high solubility and permeability. R<sub>10</sub>L<sub>4</sub> also showed a favorable oral half-life ( $t_{1/2}$  = 1.78 h), and a maximum concentration ( $C_{max}$ ) up to 80 ng/mL, far beyond its EC<sub>50</sub> value (approximately 4.0 ng/mL). The oral bioavailability of R<sub>10</sub>L<sub>4</sub> was 8.5%, which may be

inadequate for developing an oral drug. The moderate pharmacokinetic data may also be due to its poor solubility in water, and other efforts should be made on improving the pharmacokinetic features in our laboratory.

## 3. Conclusions

To shed light on the uncharted space of NNIBP tolerant region and reduce the cytotoxicity of IAS-0, alkyne chain linker with different lengths and a variety of sulfonamides containing multiple hydrogen-bonding donors/acceptors were introduced to the 2-carboxamide site of indole scaffold. 48 novel sulfonamide substituted indolylarylsulfones were designed, synthesized and evaluated for antiviral activity. The antiviral results indicated that most of the IASs exhibited potent activity against WT HIV-1, especially R<sub>10</sub>L<sub>4</sub> (EC<sub>50</sub> = 0.007 μmol/L, SI = 30,930) and R<sub>13</sub>L<sub>2</sub> (EC<sub>50</sub> = 0.004 μmol/L, SI = 41,951), the two most promising inhibitors, were superior to NVP and comparable to ETV and ETR. Additionally, R<sub>10</sub>L<sub>4</sub> maintained the effective potency towards mutant strains with EC<sub>50</sub> values of 0.017, 0.068, 0.045, 0.017 and 0.087 μmol/L against L100I, K103N, Y181C, E138K and F227L + V106A strains. The enzyme inhibitory activity assay showed that R<sub>10</sub>L<sub>4</sub> possessed a strong binding affinity for HIV-1 RT (IC<sub>50</sub> = 0.07 μmol/L). To be emphasized, the newly designed sulfonamide inhibitor R<sub>10</sub>L<sub>4</sub> exhibited an almost 40-fold decrease in cytotoxicity (CC<sub>50</sub> = 216.51 μmol/L) compared to IAS-0 (CC<sub>50</sub> = 5.52 μmol/L), which was also confirmed by the acute and subacute toxicity experiments in mice. Furthermore, R<sub>10</sub>L<sub>4</sub> possessed acceptable PK profiles, with moderate clearance and bioavailability ( $F$  = 8.5%). Taken altogether, these results confirm our design concept and the sulfonamide IAS derivatives lay a foundation for the development of novel and potent NNRTIs.

## 4. Experimental

### 4.1. Chemistry

Solvents, reagents and starting materials of synthesis were provided by commercial suppliers. All reactions were monitored *via* thin layer chromatography (TLC) and the purifications of compounds were conducted on silica gel columns (200–300 mesh, Qingdao) through column chromatography. A micro melting point apparatus (YRT-3) was used to determine the melting points (m.p.). A G1313A instrument (Agilent, USA) was used to acquire the mass spectra. The NMR spectra were obtained on 400 or 600 MHz spectrometers (Bruker, Switzerland) in DMSO-*d*<sub>6</sub> with TMS as the internal standard. A LTQ Orbitrap XL instrument (Thermo Fisher) was used to record the HRMS spectra. A rapid and simple HPLC method for the purities of representative compounds were established on a Shimadzu HPLC system. HPLC conditions: Agilent ZORBAX, SB-C18 column (250 mm × 4.6 mm × 5 μm). Isocratic elution method: A: methanol (60%); B: water (40%); velocity: 1.0 mL/min; wavelength: 254 nm, temperature, 30 °C; injection volume, 20 μL. The purities of tested compounds are >95%.

**4.1.1. Synthetic sequence for the ethyl 5-chloro-3-((3,5-dimethylphenyl)thio)-1H-indole-2-carboxylate (intermediate I)**  
Ethyl 5-chloro-1H-indole-2-carboxylate (4.60 g, 20.57 mmol), 3,5-dimethylbenzenethiol (3.06 g, 22.14 mmol) and Selectfluor™ (7.29 g, 20.58 mmol) were dissolved in 100 mL ACN. Stir the

**Table 5** Pharmacokinetic parameters of R<sub>10</sub>L<sub>4</sub><sup>a</sup>.

Parameter	Unit	i.v. <sup>b</sup>	p.o. <sup>c</sup>
$t_{1/2}$	h	1.61 ± 0.04	1.78 ± 0.25
$T_{max}$	h	0.083 ± 0.00	0.83 ± 0.15
$C_{max}$	ng/mL	657 ± 99.2	79.9 ± 14.8
AUC <sub>0–t</sub>	h·ng/mL	472 ± 63.4	199 ± 38.7
AUC <sub>0–∞</sub>	h·ng/mL	481 ± 63.3	203 ± 38.4
MRT <sub>0–∞</sub>	h	1.45 ± 0.09	2.27 ± 0.05
CL	mL/h/kg	4209 ± 552	—
$F$	%	—	8.5

<sup>a</sup>PK parameters (mean ± SD,  $n$  = 3).

<sup>b</sup>Dosed intravenously at 2 mg/kg.

<sup>c</sup>Dosed orally at 10 mg/kg.

reaction at room temperature for 8 h. Upon the completion of reaction, ACN was removed under reduced pressure. To the remaining solid, 60 mL water and 60 mL of DCM were added. Organic phase was collected by a separating funnel, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then evaporated under vacuum concentration. The obtained light-brown crude product was recrystallized with 35 mL of methanol and heated to 60 °C under continuous stirring. Precipitants were collected by filtration to yield an off-white solid as purified intermediate **1** (4.6 g). Yield 62%. m.p.: 135–136 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.56 (s, 1H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 7.35 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.78 (s, 1H), 6.73 (s, 2H), 4.34 (q, *J* = 7.0 Hz), 2.15 (s, 6H), 1.25 (t, *J* = 7.0 Hz, 3H). ESI-MS: *m/z* 358.12 [M–1]<sup>–</sup>, C<sub>19</sub>H<sub>18</sub>ClNO<sub>2</sub>S (359.07).

**4.1.2. Synthetic sequence for ethyl 5-chloro-3-((3,5-dimethylphenyl)sulfonyl)-1H-indole-2-carboxylate (intermediate 2)**  
Intermediate **1** (4.6 g) was added to 160 mL of dichloromethane at room temperature. Then add 3-chlorobenzoperoxoic acid (*m*-CPBA) (6.6 g, 38.25 mmol) to the system. Warm the reaction in a 30 °C water bath until *m*-CPBA is completely dissolved. After 3 h, the mixture was gradually added to ice water and then washed with saturated Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and NaHCO<sub>3</sub> solution sequentially. Dichloromethane was separated, and then concentrated under vacuum concentration. 20 mL ethyl acetate was used to recrystallize the remaining solid. The mixture was heated to 70 °C accompanied with stirring for 30 min. The suspension was cooled down to room temperature. The precipitant was filtered out and vacuum dried to get the target compound **2** (4.7 g). Yield 94%, m.p.: 210–211 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 13.28 (s, 1H), 8.25 (s, 1H), 7.68–7.58 (m, 3H), 7.45 (d, *J* = 8.9 Hz, 1H), 7.28 (s, 1H), 4.36 (q, *J* = 6.6 Hz, 2H), 2.34 (s, 6H), 1.31 (t, *J* = 6.7 Hz, 3H). ESI-MS: *m/z* 390.34 [M–1]<sup>–</sup>. C<sub>19</sub>H<sub>18</sub>ClNO<sub>4</sub>S (391.06).

**4.1.3. Synthetic sequence for 5-chloro-3-((3,5-dimethylphenyl)sulfonyl)-1H-indole-2-carboxylic acid (intermediate 3)**  
Intermediate **2** (4.7 g, mmol) was dissolved in 80 mL of THF under a 0 °C ice bath. Another solution was meanwhile prepared by dissolving LiOH (4.9 g, mmol) into 80 mL of water. The LiOH solution was added into THF solution of intermediate **2** dropwise under a 0 °C ice bath. After 3 h, THF was removed by distillation. 1 mol/L HCl was slowly dropped into the resulting aqueous solution until pH < 3. Gradually precipitated solids were filtered and washed to obtain the intermediate **3** (4.26 g) following the vacuum drying. White solid, Yield 93%, m.p.: 270 °C (decompose). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 14.24 (s, 1H), 13.11 (s, 1H), 8.24 (s, 1H), 7.62 (s, 2H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.25 (s, 1H), 2.32 (s, 6H). ESI-MS: *m/z* 362.14 [M–1]<sup>–</sup>. C<sub>17</sub>H<sub>14</sub>ClNO<sub>4</sub>S (363.03).

**4.1.4. General procedure for the preparation of 4R<sub>m</sub>L<sub>n</sub>**  
Amines (L2, L3, L4, L5) (1.0 eq.) were dissolved in dichloromethane, then added with triethylamine (3.0 eq.). Under a 0 °C ice bath, sulfonyl chlorides (1.2 eq.) were dissolved in dichloromethane and dropped into the system and then the mixture was stirred at 0–30 °C for 4–10 h. After the reaction was finished (monitored by TLC), saturated NaHCO<sub>3</sub> was added. The separated dichloromethane was washed with saturated NaCl, dried with

anhydrous Na<sub>2</sub>SO<sub>4</sub>, and removed solvent under vacuum concentration to acquire colorless to light-yellow oils, which were used without further purification.

#### 4.1.5. General procedure for the preparation of intermediates 5R<sub>m</sub>L<sub>n</sub>

A solution of 4R<sub>m</sub>L<sub>n</sub> series of compounds (1.0 eq.) in dichloromethane was stirred under an ice bath. Trifluoroacetic acid (TFA) (6.0 eq.) was dropped into the mixture and stirred the reaction at 20–30 °C for 5 h. Then remove the organic solvent from the system under reduced pressure. The resulting oils were conducted next step as TFA salts, without further purification.

#### 4.1.6. General procedure for the preparation of final compounds R<sub>m</sub>L<sub>n</sub>

Intermediate **3** (0.15 g, 0.41 mmol) and HATU (0.236 g, 0.62 mmol) were suspended in dichloromethane (8 mL) and DMF (2 mL) under a 0 °C ice bath and vigorous stirring. After 30 min, 4R<sub>m</sub>L<sub>n</sub> (0.45 mmol) and DIEA (0.16 g, 1.24 mmol) were dumped into the round bottom flask, which was then stirred for 5–20 h at 25–30 °C (monitored by TLC). After the reaction mixture was evaporated, 20 mL of 0.5 mol/L HCl solution was added dropwise to the remaining DMF solution. The resulting precipitant was collected by filtration and recrystallized in a mixture of 4 mL dichloromethane and 1.5 mL methanol. Then filter the suspension to afford the purified target compounds R<sub>m</sub>L<sub>n</sub>.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-methylphenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide (R<sub>1</sub>L<sub>2</sub>)**. White solid, Yield 57%, m.p.: 208–210 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.95 (s, 1H), 9.00 (t, *J* = 5.8 Hz, 1H), 7.93 (d, *J* = 2.0 Hz, 1H), 7.76–7.67 (m, 3H), 7.60 (d, *J* = 1.5 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.42–7.31 (m, 3H), 7.25 (s, 1H), 3.40 (q, *J* = 6.6 Hz, 2H), 2.97 (p, *J* = 6.7, 5.3 Hz, 2H), 2.34 (s, 3H), 2.29 (s, 6H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 159.90, 143.21, 142.94, 139.43, 137.89, 137.26, 135.15, 133.26, 130.12, 127.76, 127.01, 125.66, 125.22, 124.16, 119.42, 115.33, 112.11, 41.96, 39.71, 21.37, 21.18. HRMS: *m/z* C<sub>26</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>5</sub>S<sub>2</sub>: Calcd. 559.1002, Found 560.1075 [M+H]<sup>+</sup>.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-methylphenyl)sulfonamido)propyl)-1H-indole-2-carboxamide (R<sub>1</sub>L<sub>3</sub>)**. White solid, Yield 62%, m.p.: 194–196 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 13.00 (s, 3H), 8.94 (t, *J* = 5.6 Hz, 3H), 7.92 (d, *J* = 2.1 Hz, 3H), 7.77–7.58 (m, 15H), 7.53 (d, *J* = 8.8 Hz, 3H), 7.45–7.30 (m, 9H), 7.26 (s, 3H), 3.34 (d, *J* = 5.0 Hz, 15H), 2.87 (q, *J* = 7.0 Hz, 9H), 2.69 (s, 2H), 2.33 (d, *J* = 17.2 Hz, 27H), 1.72 (p, *J* = 7.1 Hz, 6H). <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 159.79, 143.04, 142.95, 139.40, 138.16, 137.75, 135.09, 133.28, 130.03, 127.68, 126.97, 125.67, 125.09, 124.15, 119.36, 115.28, 111.89, 40.91, 37.57, 29.52, 21.38, 21.21. HRMS: *m/z* C<sub>27</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>5</sub>S<sub>2</sub>: Calcd. 573.1159, Found 574.1232 [M+H]<sup>+</sup>.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((4-methylphenyl)sulfonamido)butyl)-1H-indole-2-carboxamide (R<sub>1</sub>L<sub>4</sub>)**. White solid, Yield 45%, m.p.: 194–196 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 13.01 (s, 1H), 8.95 (t, *J* = 5.6 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.72–7.60 (m, 4H), 7.54 (dd, *J* = 7.3, 4.9 Hz, 2H), 7.42–7.31 (m, 3H), 7.25 (s, 1H), 3.33–3.27 (m, 2H), 2.77 (p, *J* = 6.5 Hz, 2H), 2.36 (s, 3H), 2.30 (s, 6H), 1.55 (ddt, *J* = 21.0, 14.5, 7.5 Hz, 4H). <sup>13</sup>C NMR (150 MHz, DMSO-

$d_6$ )  $\delta$  159.59, 143.05, 142.91, 139.42, 138.20, 137.71, 135.12, 133.26, 130.02, 127.70, 126.96, 125.77, 125.11, 124.11, 119.40, 115.29, 111.82, 42.68, 39.38, 26.90, 26.44, 21.38, 21.21. HRMS:  $m/z$   $C_{28}H_{30}ClN_3O_5S_2$ : Calcd. 587.1315, Found 588.1388  $[M+H]^+$ .

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-((4-methylphenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide ( $R_1L_5$ ).** White solid, Yield 58%, m.p.: 144–146 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.91 (t,  $J$  = 5.6 Hz, 1H), 7.94 (d,  $J$  = 2.1 Hz, 1H), 7.67 (d,  $J$  = 8.2 Hz, 2H), 7.62 (d,  $J$  = 1.5 Hz, 2H), 7.56–7.46 (m, 2H), 7.41–7.31 (m, 3H), 7.26 (s, 1H), 3.29 (d,  $J$  = 6.3 Hz, 2H), 2.73 (q,  $J$  = 6.6 Hz, 2H), 2.37 (s, 3H), 2.31 (s, 6H), 1.58–1.29 (m, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.57, 143.08, 142.90, 139.40, 138.26, 137.81, 135.10, 133.26, 130.02, 127.69, 126.95, 125.77, 125.09, 124.09, 119.40, 115.28, 111.75, 42.93, 39.72, 29.16, 28.77, 23.95, 21.38, 21.21. HRMS:  $m/z$   $C_{29}H_{32}ClN_3O_5S_2$ : Calcd. 601.1472, Found 602.1545  $[M+H]^+$ .

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-methoxyphenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide ( $R_2L_2$ ).** White solid, Yield 70%, m.p.: 218–220 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.96 (s, 1H), 9.01 (t,  $J$  = 5.8 Hz, 1H), 7.94 (d,  $J$  = 2.1 Hz, 1H), 7.85–7.70 (m, 2H), 7.61 (d,  $J$  = 2.6 Hz, 3H), 7.54 (d,  $J$  = 8.7 Hz, 1H), 7.35 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.26 (s, 1H), 7.15–7.04 (m, 2H), 3.81 (s, 3H), 3.40 (q,  $J$  = 6.6 Hz, 2H), 2.95 (q,  $J$  = 6.6 Hz, 2H), 2.30 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.67, 159.89, 142.94, 139.43, 137.27, 135.15, 133.26, 132.39, 129.15, 127.75, 125.65, 125.21, 124.16, 119.42, 115.33, 114.85, 112.11, 56.06, 41.96, 39.70, 21.19. HRMS:  $m/z$   $C_{26}H_{26}ClN_3O_6S_2$ : Calcd. 575.0952, Found 576.1024  $[M+H]^+$ . HPLC purity: 95.69%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-methoxyphenyl)sulfonamido)propyl)-1H-indole-2-carboxamide ( $R_2L_3$ ).** White solid, Yield 60%, m.p.: 186–188 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.92 (t,  $J$  = 5.6 Hz, 1H), 7.92 (d,  $J$  = 2.0 Hz, 1H), 7.78–7.70 (m, 2H), 7.61 (s, 2H), 7.52 (dd,  $J$  = 7.3, 3.4 Hz, 2H), 7.34 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.26 (s, 1H), 7.12–6.99 (m, 2H), 3.80 (s, 3H), 3.38–3.32 (m, 3H), 2.86 (q,  $J$  = 6.7 Hz, 2H), 2.30 (s, 6H), 1.71 (p,  $J$  = 7.1 Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.53, 159.78, 143.03, 139.40, 137.75, 135.10, 133.27, 132.69, 129.08, 127.68, 125.66, 125.09, 124.15, 119.36, 115.28, 114.76, 111.90, 56.03, 40.89, 37.59, 29.48, 21.22. HRMS:  $m/z$   $C_{27}H_{28}ClN_3O_6S_2$ : Calcd. 589.1108, Found 590.1181  $[M+H]^+$ .

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((4-methoxyphenyl)sulfonamido)butyl)-1H-indole-2-carboxamide ( $R_2L_4$ ).** White solid, Yield 55%, m.p.: 182–185 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.97 (s, 1H), 8.91 (t,  $J$  = 5.6 Hz, 1H), 7.93 (d,  $J$  = 2.0 Hz, 1H), 7.76–7.68 (m, 2H), 7.61 (s, 2H), 7.53 (d,  $J$  = 8.7 Hz, 1H), 7.43 (t,  $J$  = 5.9 Hz, 1H), 7.34 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.26 (s, 1H), 7.15–7.03 (m, 2H), 3.81 (s, 3H), 2.76 (q,  $J$  = 6.5 Hz, 2H), 2.30 (s, 6H), 1.66–1.44 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.51, 159.60, 143.06, 139.42, 137.72, 135.12, 133.26, 132.75, 129.07, 127.70, 125.76, 125.11, 124.10, 119.40, 115.29, 114.75, 111.81, 56.05, 42.67, 39.40, 26.87, 26.47, 21.21. HRMS:  $m/z$   $C_{28}H_{30}ClN_3O_6S_2$ : Calcd. 603.1265, Found 604.1337  $[M+H]^+$ . HPLC purity: 98.56%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-((4-methoxyphenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide ( $R_2L_5$ ).** White solid, Yield 65%, m.p.: 162–166 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.95 (s, 1H), 8.89 (t,  $J$  = 5.6 Hz, 1H), 7.93 (d,  $J$  = 2.0 Hz, 1H), 7.75–7.67 (m, 2H), 7.60 (s, 2H), 7.53

(d,  $J$  = 8.8 Hz, 1H), 7.39 (t,  $J$  = 5.8 Hz, 1H), 7.34 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.26 (s, 1H), 7.13–7.04 (m, 2H), 3.82 (s, 3H), 2.71 (q,  $J$  = 6.6 Hz, 2H), 1.57–1.25 (m, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.50, 159.58, 143.08, 139.40, 137.83, 135.10, 133.26, 132.80, 129.06, 127.69, 125.76, 125.08, 124.09, 119.39, 115.28, 114.75, 111.75, 56.06, 42.92, 39.72, 29.14, 28.78, 23.98, 21.21. HRMS:  $m/z$   $C_{29}H_{32}ClN_3O_6S_2$ : Calcd. 617.1421, Found 618.1494  $[M+H]^+$ . HPLC purity: 96.89%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-nitrophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide ( $R_3L_2$ ).** White solid, Yield 65%, m.p.: 235–238 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.95 (s, 1H), 9.03 (t,  $J$  = 5.8 Hz, 1H), 8.46–8.34 (m, 2H), 8.19 (t,  $J$  = 5.8 Hz, 1H), 8.11–8.03 (m, 2H), 7.93 (d,  $J$  = 2.1 Hz, 1H), 7.60 (s, 2H), 7.54 (d,  $J$  = 8.8 Hz, 1H), 7.35 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.25 (s, 1H), 3.43 (q,  $J$  = 6.5 Hz, 2H), 3.08 (q,  $J$  = 6.5 Hz, 2H), 2.29 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.88, 150.03, 146.42, 142.89, 139.45, 136.97, 135.18, 133.25, 128.54, 127.81, 125.67, 125.30, 125.05, 124.12, 119.44, 115.36, 112.13, 92.55, 84.00, 42.03, 39.70, 21.18. HRMS:  $m/z$   $C_{25}H_{23}ClN_4O_7S_2$ : Calcd. 590.0697, Found 591.0769  $[M+H]^+$ . HPLC purity: 98.15%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-nitrophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide ( $R_3L_3$ ).** White solid, Yield 80%, m.p.: 174–176 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.90 (s, 1H), 8.86 (t,  $J$  = 5.6 Hz, 1H), 8.34–8.25 (m, 2H), 8.04–7.94 (m, 3H), 7.83 (d,  $J$  = 2.1 Hz, 1H), 7.52 (d,  $J$  = 1.5 Hz, 2H), 7.45 (d,  $J$  = 8.7 Hz, 1H), 7.26 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.17 (s, 1H), 3.28 (q,  $J$  = 6.6 Hz, 2H), 2.92 (q,  $J$  = 6.9 Hz, 2H), 2.23 (s, 6H), 1.67 (p,  $J$  = 7.1 Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.89, 149.95, 146.64, 142.99, 139.38, 137.83, 135.07, 133.29, 128.51, 127.67, 125.59, 125.07, 124.97, 124.14, 119.32, 115.25, 111.86, 41.00, 37.46, 29.52, 21.21. HRMS:  $m/z$   $C_{26}H_{25}ClN_4O_7S_2$ : Calcd. 604.0853, Found 605.0926  $[M+H]^+$ .

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((4-nitrophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide ( $R_3L_4$ ).** White solid, Yield 60%, m.p.: 185–188 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.93 (t,  $J$  = 5.6 Hz, 1H), 8.44–8.35 (m, 2H), 8.09–8.03 (m, 2H), 8.02 (t,  $J$  = 5.8 Hz, 1H), 7.94 (d,  $J$  = 2.1 Hz, 1H), 7.61 (d,  $J$  = 1.5 Hz, 2H), 7.53 (d,  $J$  = 8.7 Hz, 1H), 7.34 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.25 (s, 1H), 3.31 (d,  $J$  = 5.6 Hz, 4H), 2.88 (q,  $J$  = 6.5 Hz, 2H), 2.30 (s, 6H), 1.64–1.50 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.64, 149.98, 146.73, 143.04, 139.41, 137.72, 135.11, 133.27, 128.48, 127.70, 125.74, 125.11, 125.01, 124.10, 119.39, 115.29, 111.81, 42.76, 39.33, 26.97, 26.38, 21.21. HRMS:  $m/z$   $C_{27}H_{27}ClN_4O_7S_2$ : Calcd. 618.1010, Found 619.1082  $[M+H]^+$ . HPLC purity: 97.12%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-((4-nitrophenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide ( $R_3L_5$ ).** White solid, Yield 67%, m.p.: 154–158 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 8.84 (t,  $J$  = 5.6 Hz, 1H), 8.37–8.28 (m, 2H), 8.01–7.94 (m, 2H), 7.91 (t,  $J$  = 5.7 Hz, 1H), 7.86 (d,  $J$  = 2.1 Hz, 1H), 7.54 (d,  $J$  = 1.6 Hz, 2H), 7.46 (d,  $J$  = 8.7 Hz, 1H), 7.26 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.18 (s, 1H), 3.24–3.20 (m, 2H), 2.76 (q,  $J$  = 7.0 Hz, 2H), 2.23 (s, 6H), 1.54–1.19 (m, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.60, 149.98, 146.76, 143.07, 139.40, 137.82, 135.09, 133.26, 128.48, 127.69, 125.75, 125.08, 125.01, 124.08, 119.38, 115.28, 111.74, 42.99, 29.22, 28.71, 23.86, 21.21. HRMS:  $m/z$   $C_{28}H_{29}ClN_4O_7S_2$ : Calcd. 632.1166, Found 633.1239  $[M+H]^+$ . HPLC purity: 98.43%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-fluorophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_4L_2$ ). White solid, Yield 65%, m.p.: 200–203 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.96 (s, 1H), 9.02 (t,  $J$  = 5.8 Hz, 1H), 7.94 (d,  $J$  = 2.1 Hz, 1H), 7.92–7.87 (m, 2H), 7.83 (t,  $J$  = 6.0 Hz, 1H), 7.61 (d,  $J$  = 1.6 Hz, 2H), 7.54 (d,  $J$  = 8.8 Hz, 1H), 7.47–7.40 (m, 2H), 7.35 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.25 (s, 1H), 3.48–3.36 (m, 2H), 3.05–2.95 (m, 2H), 2.30 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  165.46, 163.79, 159.92, 142.94, 139.43, 137.21, 137.17, 137.15, 135.16, 133.26, 130.02, 129.96, 127.77, 125.66, 125.24, 124.15, 119.43, 116.94, 116.79, 115.34, 112.13, 41.97, 21.18. HRMS:  $m/z$   $\text{C}_{25}\text{H}_{23}\text{ClFN}_3\text{O}_5\text{S}_2$ : Calcd. 563.0752, Found 564.0824  $[\text{M}+\text{H}]^+$ . HPLC purity: 95.64%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-fluorophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_4L_3$ ). White solid, Yield 68%, m.p.: 160–162 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.94 (t,  $J$  = 5.6 Hz, 1H), 7.92 (d,  $J$  = 2.1 Hz, 1H), 7.89–7.84 (m, 2H), 7.73 (t,  $J$  = 5.9 Hz, 1H), 7.62 (d,  $J$  = 1.5 Hz, 2H), 7.53 (d,  $J$  = 8.7 Hz, 1H), 7.45–7.37 (m, 2H), 7.34 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.25 (s, 1H), 3.45–3.19 (m, 4H), 2.91 (q,  $J$  = 6.7 Hz, 2H), 2.31 (s, 6H), 1.73 (p,  $J$  = 7.1 Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  165.35, 163.69, 159.83, 143.04, 139.39, 137.80, 137.43, 135.09, 133.28, 129.94, 129.88, 127.67, 125.64, 125.08, 124.15, 119.35, 116.82, 116.67, 115.27, 111.88, 40.93, 37.54, 29.50, 21.21. HRMS:  $m/z$   $\text{C}_{26}\text{H}_{25}\text{ClFN}_3\text{O}_5\text{S}_2$ : Calcd. 577.0908, Found 578.0981  $[\text{M}+\text{H}]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((4-fluorophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_4L_4$ ). White solid, Yield 45%, m.p.: 179–180 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.86 (t,  $J$  = 5.6 Hz, 1H), 7.87 (d,  $J$  = 2.1 Hz, 1H), 7.83–7.72 (m, 2H), 7.59 (t,  $J$  = 5.9 Hz, 1H), 7.55 (d,  $J$  = 1.6 Hz, 2H), 7.46 (d,  $J$  = 8.7 Hz, 1H), 7.38–7.32 (m, 2H), 7.27 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.18 (s, 1H), 3.23 (t,  $J$  = 6.4 Hz, 2H), 2.73 (q,  $J$  = 6.5 Hz, 2H), 2.23 (s, 6H), 1.58–1.34 (m, 4H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  165.33, 163.66, 159.62, 143.06, 139.41, 137.73, 137.48, 137.46, 135.12, 133.27, 129.93, 129.87, 127.70, 125.76, 125.10, 124.11, 119.40, 116.81, 116.66, 115.29, 111.81, 42.69, 39.36, 26.90, 26.42, 21.21. HRMS:  $m/z$   $\text{C}_{27}\text{H}_{27}\text{ClFN}_3\text{O}_5\text{S}_2$ : Calcd. 591.1065, Found 592.1137  $[\text{M}+\text{H}]^+$ . HPLC purity: 97.17%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-((4-fluorophenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_4L_5$ ). White solid, Yield 56%, m.p.: 159–160 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.92 (t,  $J$  = 5.6 Hz, 1H), 7.94 (d,  $J$  = 2.1 Hz, 1H), 7.89–7.80 (m, 2H), 7.68–7.58 (m, 3H), 7.53 (d,  $J$  = 8.8 Hz, 1H), 7.47–7.38 (m, 2H), 7.34 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.25 (s, 1H), 3.30 (q,  $J$  = 6.7 Hz, 2H), 2.82–2.70 (m, 2H), 2.30 (s, 6H), 1.56–1.30 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  165.32, 163.65, 159.59, 143.08, 139.40, 137.84, 137.54, 137.52, 135.10, 133.26, 129.92, 129.86, 127.68, 125.75, 125.08, 124.09, 119.38, 116.81, 116.66, 115.28, 111.74, 42.93, 29.15, 28.76, 23.92, 21.21. HRMS:  $m/z$   $\text{C}_{28}\text{H}_{29}\text{ClFN}_3\text{O}_5\text{S}_2$ : Calcd. 605.1221, Found 606.1294  $[\text{M}+\text{H}]^+$ . HPLC purity: 95.32%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-cyanophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_5L_2$ ). White solid, Yield 69%, m.p.: 197–200 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.88 (s, 1H), 8.96 (t,  $J$  = 5.8 Hz, 1H), 8.04 (t,  $J$  = 5.9 Hz, 1H), 8.01–7.96 (m, 2H), 7.94–7.89 (m, 2H), 7.87 (d,  $J$  = 2.1 Hz, 1H), 7.53 (d,  $J$  = 1.6 Hz, 2H), 7.47 (d,  $J$  = 8.8 Hz, 1H), 7.28 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.18 (s, 1H), 3.39–3.30 (m, 2H), 2.97 (q,  $J$  = 6.5 Hz, 2H), 2.22 (s, 6H).  $^{13}\text{C}$

NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.90, 144.93, 142.90, 139.45, 137.06, 135.19, 133.92, 133.25, 127.81, 127.73, 125.67, 125.29, 124.14, 119.44, 118.08, 115.46, 115.36, 112.13, 41.98, 39.69, 21.19. HRMS:  $m/z$   $\text{C}_{26}\text{H}_{23}\text{ClN}_4\text{O}_5\text{S}_2$ : Calcd. 571.0630, Found 571.0871  $[\text{M}+\text{H}]^+$ . HPLC purity: 97.69%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-cyanophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_5L_3$ ). White solid, Yield 65%, m.p.: 207–210 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.99 (s, 1H), 8.94 (t,  $J$  = 5.6 Hz, 1H), 8.06–7.99 (m, 3H), 7.99–7.95 (m, 2H), 7.91 (d,  $J$  = 2.1 Hz, 1H), 7.61 (d,  $J$  = 1.6 Hz, 2H), 7.53 (d,  $J$  = 8.8 Hz, 1H), 7.34 (dd,  $J$  = 8.7, 2.1 Hz, 1H), 7.26 (s, 1H), 3.35 (q,  $J$  = 6.7 Hz, 2H), 2.96 (q,  $J$  = 6.9 Hz, 2H), 2.31 (s, 6H), 1.73 (p,  $J$  = 7.1 Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.88, 145.18, 143.02, 139.38, 137.86, 135.09, 133.83, 133.29, 127.69, 127.66, 125.60, 125.07, 124.16, 119.32, 118.16, 115.29, 115.27, 111.87, 40.97, 37.47, 29.55, 21.23. HRMS:  $m/z$   $\text{C}_{27}\text{H}_{25}\text{ClN}_4\text{O}_5\text{S}_2$ : Calcd. 584.0955, Found 585.1028  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.56%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((4-cyanophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_5L_4$ ). White solid, Yield 51%, m.p.: 175–178 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.99 (s, 2H), 8.94 (t,  $J$  = 5.6 Hz, 2H), 8.07 (d,  $J$  = 8.4 Hz, 4H), 7.99–7.91 (m, 8H), 7.62 (s, 4H), 7.54 (d,  $J$  = 8.7 Hz, 2H), 7.34 (dd,  $J$  = 8.7, 2.1 Hz, 2H), 7.26 (s, 2H), 5.76 (s, 1H), 3.33–3.29 (m, 4H), 2.85 (q,  $J$  = 6.4 Hz, 4H), 2.51 (t,  $J$  = 1.9 Hz, 3H), 2.31 (s, 12H), 1.65–1.44 (m, 9H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.64, 145.25, 143.04, 139.41, 137.74, 135.12, 133.85, 133.26, 127.70, 127.68, 125.73, 125.11, 124.11, 119.39, 118.18, 115.29, 115.27, 111.81, 55.34, 42.72, 39.33, 26.97, 26.38, 21.22. HRMS:  $m/z$   $\text{C}_{28}\text{H}_{27}\text{ClN}_4\text{O}_5\text{S}_2$ : Calcd. 598.1111, Found 599.1184  $[\text{M}+\text{H}]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-((4-cyanophenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_5L_5$ ). White solid, Yield 65%, m.p.: 165–170 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.91 (s, 1H), 8.85 (t,  $J$  = 5.6 Hz, 1H), 8.00 (d,  $J$  = 8.5 Hz, 2H), 7.91–7.79 (m, 4H), 7.54 (s, 2H), 7.46 (d,  $J$  = 8.7 Hz, 1H), 7.26 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.18 (s, 1H), 3.25–3.19 (m, 2H), 2.73 (q,  $J$  = 6.8, 6.3 Hz, 2H), 2.45–2.42 (m, 2H), 2.23 (s, 6H), 1.49–1.24 (m, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.61, 145.30, 143.07, 139.40, 137.84, 135.10, 133.85, 133.26, 127.67, 125.74, 125.08, 124.10, 119.38, 118.19, 115.28, 115.26, 111.74, 42.96, 29.22, 28.72, 23.85, 21.22. HRMS:  $m/z$   $\text{C}_{29}\text{H}_{29}\text{ClN}_4\text{O}_5\text{S}_2$ : Calcd. 612.1268, Found 613.1341  $[\text{M}+\text{H}]^+$ . HPLC purity: 99.16%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((4-bromophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_6L_2$ ). White solid, Yield 64%, m.p.: 220–224 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.95 (s, 1H), 9.02 (tt,  $J$  = 5.5, 2.4 Hz, 1H), 7.95 (q,  $J$  = 2.2 Hz, 1H), 7.89 (td,  $J$  = 6.0, 2.0 Hz, 1H), 7.81 (d,  $J$  = 8.5 Hz, 2H), 7.76 (dt,  $J$  = 8.5, 1.7 Hz, 2H), 7.61 (s, 2H), 7.55 (dd,  $J$  = 8.8, 1.5 Hz, 1H), 7.35 (dd,  $J$  = 8.8, 2.1 Hz, 1H), 7.24 (d,  $J$  = 3.6 Hz, 1H), 3.46–3.39 (m, 2H), 3.01 (qd,  $J$  = 5.9, 3.0 Hz, 2H), 2.29 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.92, 142.96, 140.10, 139.44, 137.17, 135.17, 133.29, 132.82, 129.04, 127.80, 126.77, 125.71, 125.26, 124.15, 119.47, 115.36, 112.16, 41.99, 21.19. HRMS:  $m/z$   $\text{C}_{25}\text{H}_{23}\text{BrClN}_3\text{O}_5\text{S}_2$ : Calcd. 622.9951, Found 624.0024  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.34%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((4-bromophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_6L_3$ ). White solid, Yield 80%, m.p.: 162–164 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.94 (t,  $J$  = 5.6 Hz, 1H),

7.93 (d,  $J = 2.1$  Hz, 1H), 7.82–7.76 (m, 3H), 7.76–7.71 (m, 2H), 7.62 (d,  $J = 1.6$  Hz, 2H), 7.53 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.25 (s, 1H), 3.36 (q,  $J = 6.7$  Hz, 2H), 2.93 (q,  $J = 6.7$  Hz, 2H), 2.31 (s, 6H), 1.74 (p,  $J = 7.1$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.85, 143.05, 140.36, 139.40, 137.79, 135.10, 133.30, 132.72, 128.99, 127.70, 126.56, 125.67, 125.10, 124.16, 119.37, 115.28, 111.91, 40.95, 37.53, 29.53, 21.22. HRMS:  $m/z$   $\text{C}_{26}\text{H}_{25}\text{BrClN}_3\text{O}_5\text{S}_2$ : Calcd. 637.0108, Found 638.0180  $[\text{M}+\text{H}]^+$ . HPLC purity: 96.51%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-(4-bromophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_7L_4$ ). White solid, Yield 82%, m.p.: 186–190 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.93 (t,  $J = 5.7$  Hz, 1H), 7.95 (d,  $J = 2.1$  Hz, 1H), 7.83–7.78 (m, 2H), 7.74 (d,  $J = 8.6$  Hz, 3H), 7.62 (d,  $J = 1.6$  Hz, 2H), 7.54 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.26 (s, 1H), 2.82 (q,  $J = 6.4$  Hz, 2H), 2.31 (s, 6H), 1.63–1.49 (m, 4H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.63, 143.07, 140.41, 139.43, 137.72, 135.13, 133.28, 132.72, 128.99, 127.72, 126.51, 125.78, 125.12, 124.11, 119.42, 115.30, 111.83, 42.70, 39.36, 26.93, 26.41, 21.22. HRMS:  $m/z$   $\text{C}_{27}\text{H}_{27}\text{BrClN}_3\text{O}_5\text{S}_2$ : Calcd. 651.0264, Found 652.0337  $[\text{M}+\text{H}]^+$ . HPLC purity: 96.29%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-(4-bromophenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_6L_5$ ). White solid, Yield 78%, m.p.: 145–148 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.97 (s, 1H), 8.92 (t,  $J = 5.6$  Hz, 1H), 7.94 (d,  $J = 2.1$  Hz, 1H), 7.82–7.78 (m, 2H), 7.73 (d,  $J = 2.0$  Hz, 1H), 7.72–7.67 (m, 2H), 7.62 (d,  $J = 1.5$  Hz, 2H), 7.54 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.25 (s, 1H), 3.30 (t,  $J = 6.6$  Hz, 5H), 2.80–2.74 (m, 2H), 2.30 (s, 6H), 1.53 (p,  $J = 7.3$  Hz, 2H), 1.44 (h,  $J = 8.5, 7.8$  Hz, 2H), 1.36 (qd,  $J = 8.5, 7.2, 2.2$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.76, 159.60, 143.10, 140.46, 139.41, 137.81, 135.11, 133.28, 132.72, 128.98, 127.71, 126.50, 125.79, 125.10, 124.10, 119.41, 115.29, 111.76, 42.94, 39.70, 36.23, 31.26, 29.19, 28.75, 23.91, 21.22. HRMS:  $m/z$   $\text{C}_{28}\text{H}_{29}\text{BrClN}_3\text{O}_5\text{S}_2$ : Calcd. 665.0421, Found 666.0493  $[\text{M}+\text{H}]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-(4-chlorophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_7L_2$ ). White solid, Yield 77%, m.p.: 217–220 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 2H), 8.96 (t,  $J = 5.8$  Hz, 2H), 7.87 (d,  $J = 2.1$  Hz, 2H), 7.83 (t,  $J = 5.9$  Hz, 2H), 7.76 (d,  $J = 8.4$  Hz, 4H), 7.59 (d,  $J = 8.6$  Hz, 3H), 7.53 (s, 4H), 7.47 (d,  $J = 8.8$  Hz, 2H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 2H), 7.17 (s, 2H), 3.34 (q,  $J = 6.6$  Hz, 4H), 2.93 (q,  $J = 6.7$  Hz, 4H), 2.22 (s, 12H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.93, 142.94, 139.65, 139.45, 137.88, 137.18, 135.17, 133.28, 129.88, 128.94, 127.79, 125.68, 125.26, 124.78, 124.16, 119.45, 115.36, 112.14, 41.97, 21.18. HRMS:  $m/z$   $\text{C}_{25}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ : Calcd. 579.0456, Found 580.0529  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.60%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-(4-chlorophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_7L_3$ ). White solid, Yield 79%, m.p.: 183–186 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.91 (s, 1H), 8.87 (t,  $J = 5.7$  Hz, 1H), 7.85 (d,  $J = 2.1$  Hz, 1H), 7.74 (dd,  $J = 8.6, 6.6$  Hz, 3H), 7.60–7.53 (m, 4H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.26 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.29 (q,  $J = 6.8$  Hz, 2H), 2.85 (q,  $J = 6.7$  Hz, 2H), 2.23 (s, 6H), 1.66 (p,  $J = 7.1$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.86, 143.04, 139.92, 139.40, 137.81, 137.67, 135.10, 133.30, 129.78, 128.89, 127.69, 125.65, 125.09, 124.17, 119.36, 115.28, 111.90, 40.94, 37.53, 29.52, 21.22. HRMS:  $m/z$   $\text{C}_{26}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ : Calcd. 593.0613, Found 594.0685  $[\text{M}+\text{H}]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-(4-chlorophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_7L_4$ ). White solid, Yield 75%, m.p.: 196–198 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.99 (s, 1H), 8.94 (t,  $J = 5.7$  Hz, 1H), 7.95 (t,  $J = 1.8$  Hz, 1H), 7.84–7.79 (m, 2H), 7.74 (t,  $J = 5.8$  Hz, 1H), 7.69–7.64 (m, 2H), 7.64–7.61 (m, 2H), 7.54 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.25 (s, 1H), 3.31 (t,  $J = 6.3$  Hz, 2H), 2.30 (s, 6H), 1.63–1.49 (m, 4H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.64, 143.07, 139.98, 139.43, 137.73, 137.63, 135.13, 133.28, 129.78, 128.88, 127.72, 125.77, 125.12, 124.12, 119.41, 115.30, 111.83, 42.70, 39.36, 26.92, 26.41, 21.21. HRMS:  $m/z$   $\text{C}_{27}\text{H}_{27}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ : Calcd. 607.0769, Found 608.0842  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.95%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-(4-chlorophenyl)sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_7L_5$ ). White solid, Yield 82%, m.p.: 143–146 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.98 (s, 1H), 8.92 (t,  $J = 5.6$  Hz, 1H), 7.95 (d,  $J = 2.1$  Hz, 1H), 7.83–7.78 (m, 2H), 7.73–7.60 (m, 5H), 7.54 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.25 (s, 1H), 3.31 (q,  $J = 6.7$  Hz, 4H), 2.76 (dq,  $J = 13.4, 6.6$  Hz, 2H), 2.30 (s, 5H), 1.53 (p,  $J = 7.2$  Hz, 2H), 1.48–1.31 (m, 5H), 1.26–1.18 (m, 1H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.61, 143.08, 140.03, 139.41, 137.83, 137.61, 135.11, 133.27, 129.78, 128.88, 128.15, 127.70, 125.77, 125.10, 124.10, 119.40, 115.29, 111.75, 42.94, 42.85, 39.75, 39.69, 39.49, 29.17, 29.02, 28.75, 28.18, 23.90, 23.69, 21.21. HRMS:  $m/z$   $\text{C}_{28}\text{H}_{29}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ : Calcd. 621.0926, Found 622.0998  $[\text{M}+\text{H}]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-(thiophene-2-sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_8L_2$ ). White solid, Yield 80%, m.p.: 216–218 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.90 (s, 1H), 8.98 (t,  $J = 5.8$  Hz, 1H), 7.91 (t,  $J = 5.9$  Hz, 1H), 7.87 (dd,  $J = 5.2, 1.5$  Hz, 2H), 7.58–7.54 (m, 3H), 7.47 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.17 (s, 1H), 7.13 (dd,  $J = 5.0, 3.7$  Hz, 1H), 3.37 (q,  $J = 6.6$  Hz, 2H), 3.00 (q,  $J = 6.6$  Hz, 2H), 2.23 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.99, 142.96, 141.60, 139.44, 137.34, 135.16, 133.28, 133.08, 132.10, 128.21, 127.77, 125.65, 125.23, 124.19, 119.43, 115.34, 112.16, 42.19, 39.61, 21.21. HRMS:  $m/z$   $\text{C}_{23}\text{H}_{22}\text{ClN}_3\text{O}_5\text{S}_3$ : Calcd. 551.0410, Found 552.0483  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.02%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-(thiophene-2-sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_8L_3$ ). White solid, Yield 70%, m.p.: 136–139 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.93 (s, 1H), 8.89 (t,  $J = 5.7$  Hz, 1H), 7.90–7.79 (m, 3H), 7.56 (d,  $J = 1.6$  Hz, 2H), 7.53 (dd,  $J = 3.7, 1.4$  Hz, 1H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.18 (s, 1H), 7.10 (dd,  $J = 5.0, 3.7$  Hz, 1H), 3.33–3.26 (m, 2H), 2.92 (q,  $J = 6.7$  Hz, 2H), 2.82 (s, 1H), 2.23 (s, 6H), 1.69 (p,  $J = 7.1$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  162.76, 159.85, 143.05, 141.97, 139.41, 137.82, 135.11, 133.29, 132.74, 131.86, 128.10, 127.69, 125.66, 125.10, 124.18, 119.36, 115.29, 111.90, 41.17, 37.58, 36.23, 31.25, 29.42, 21.22. HRMS:  $m/z$   $\text{C}_{24}\text{H}_{24}\text{ClN}_3\text{O}_5\text{S}_3$ : Calcd. 565.0567, Found 566.0639  $[\text{M}+\text{H}]^+$ . HPLC purity: 98.00%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-(thiophene-2-sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_8L_4$ ). White solid, Yield 76%, m.p.: 188–190 °C.  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.93 (s, 1H), 8.88 (t,  $J = 5.6$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.84 (dd,  $J = 5.0, 1.4$  Hz, 1H), 7.75 (t,  $J = 5.8$  Hz, 1H), 7.55 (d,  $J = 1.6$  Hz, 2H), 7.52 (dd,  $J = 3.7, 1.4$  Hz, 1H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.18 (s, 1H), 7.10 (dd,  $J = 5.0, 3.7$  Hz, 1H), 3.25 (t,  $J = 6.4$  Hz, 2H), 2.82 (q,  $J = 6.4$  Hz, 2H), 2.23 (s, 6H), 1.57–1.44 (m, 4H).  $^{13}\text{C}$  NMR

(150 MHz, DMSO- $d_6$ )  $\delta$  159.65, 143.06, 142.02, 139.43, 137.77, 135.13, 133.27, 132.70, 132.66, 131.80, 128.09, 127.71, 125.76, 125.12, 124.13, 119.41, 115.30, 111.82, 42.92, 39.38, 26.78, 26.45, 21.22. HRMS:  $m/z$   $C_{25}H_{26}ClN_3O_5S_3$ : Calcd. 579.0723, Found 580.0796  $[M+H]^+$ . HPLC purity: 95.61%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-(thiophene-2-sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_8L_5$ ). White solid, Yield 77%, m.p.: 168–170 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  13.00 (s, 1H), 8.94 (t,  $J = 5.7$  Hz, 1H), 7.97–7.89 (m, 2H), 7.79 (t,  $J = 5.8$  Hz, 1H), 7.63 (d,  $J = 1.6$  Hz, 2H), 7.58 (dd,  $J = 3.7, 1.4$  Hz, 1H), 7.54 (d,  $J = 8.7$  Hz, 1H), 7.34 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.25 (s, 1H), 7.18 (dd,  $J = 5.0, 3.7$  Hz, 1H), 3.32 (d,  $J = 6.4$  Hz, 2H), 2.91–2.82 (m, 2H), 2.31 (s, 6H), 1.51 (dp,  $J = 40.7, 7.2$  Hz, 4H), 1.38 (td,  $J = 8.4, 3.9$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.62, 143.09, 142.10, 139.41, 138.77, 137.86, 135.11, 134.72, 133.27, 132.65, 131.77, 128.08, 127.70, 125.76, 125.09, 124.80, 124.11, 120.34, 119.40, 115.29, 111.76, 43.17, 39.72, 29.04, 28.78, 23.96, 21.22. HRMS:  $m/z$   $C_{26}H_{28}ClN_3O_5S_3$ : Calcd. 593.0880, Found 594.0952  $[M+H]^+$ . HPLC purity: 97.81%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-(phenylsulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_9L_2$ ). White solid, Yield 76%, m.p.: 228–231 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 8.95 (t,  $J = 5.8$  Hz, 1H), 7.86 (d,  $J = 2.1$  Hz, 1H), 7.79–7.75 (m, 2H), 7.72 (t,  $J = 6.0$  Hz, 1H), 7.60–7.50 (m, 5H), 7.47 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.34 (q,  $J = 6.6$  Hz, 2H), 2.91 (q,  $J = 6.7$  Hz, 2H), 2.22 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.95, 142.95, 140.75, 139.44, 138.80, 137.31, 135.16, 133.26, 132.96, 129.75, 127.76, 126.95, 125.64, 125.23, 124.79, 124.18, 119.42, 115.34, 112.13, 41.96, 39.72, 21.20. HRMS:  $m/z$   $C_{25}H_{24}ClN_3O_5S_2$ : Calcd. 545.0846, Found 546.0919  $[M+H]^+$ . HPLC purity: 99.03%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-(phenylsulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_9L_3$ ). White solid, Yield 79%, m.p.: 200–202 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.87 (t,  $J = 5.6$  Hz, 1H), 7.86 (d,  $J = 2.0$  Hz, 1H), 7.77–7.72 (m, 2H), 7.62 (t,  $J = 5.9$  Hz, 1H), 7.57–7.53 (m, 3H), 7.50 (dd,  $J = 8.2, 6.6$  Hz, 2H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.26 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.16 (s, 1H), 3.30–3.26 (m, 3H), 2.83 (q,  $J = 6.7$  Hz, 2H), 2.22 (s, 6H), 1.66 (p,  $J = 7.1$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.83, 143.05, 141.05, 139.40, 137.79, 135.10, 133.29, 132.76, 129.64, 127.70, 126.90, 125.67, 125.10, 124.17, 119.37, 115.29, 111.90, 40.94, 37.57, 29.57, 21.21. HRMS:  $m/z$   $C_{26}H_{26}ClN_3O_5S_2$ : Calcd. 559.1002, Found 560.1075  $[M+H]^+$ . HPLC purity: 98.49%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-(phenylsulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_9L_4$ ). White solid, Yield 83%, m.p.: 186–188 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.86 (t,  $J = 5.6$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.77–7.71 (m, 2H), 7.58–7.49 (m, 6H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.8, 2.1$  Hz, 1H), 3.23 (d,  $J = 6.3$  Hz, 2H), 2.73 (q,  $J = 6.5$  Hz, 2H), 2.23 (s, 6H), 1.54–1.41 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.63, 143.06, 141.06, 139.43, 137.75, 135.13, 133.27, 132.73, 129.63, 127.71, 126.90, 125.76, 125.12, 124.12, 119.40, 115.30, 111.81, 42.69, 39.37, 26.92, 26.42, 21.22. HRMS:  $m/z$   $C_{27}H_{28}ClN_3O_5S_2$ : Calcd. 573.1159, Found 574.1232  $[M+H]^+$ .

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-(phenylsulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_9L_5$ ). White solid, Yield 77%, m.p.: 197–200 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.91 (s, 1H), 8.84 (t,  $J = 5.7$  Hz, 1H), 7.86 (d,  $J = 2.1$  Hz, 1H), 7.72 (dt,  $J = 7.0, 1.4$  Hz, 2H), 7.59–7.48 (m, 6H), 7.46 (d,

$J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.18 (s, 1H), 3.25–3.19 (m, 2H), 2.71–2.65 (m, 2H), 2.23 (s, 6H), 1.43 (q,  $J = 7.4$  Hz, 2H), 1.35 (h,  $J = 7.3$  Hz, 2H), 1.27 (qd,  $J = 8.5, 7.2, 2.0$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.60, 143.09, 141.14, 139.41, 137.85, 135.11, 133.27, 132.72, 129.62, 127.69, 126.89, 125.76, 125.09, 124.10, 119.39, 115.29, 111.75, 42.94, 39.70, 29.19, 28.76, 23.92, 21.22. HRMS:  $m/z$   $C_{28}H_{30}ClN_3O_5S_2$ : Calcd. 587.1315, Found 588.1388  $[M+H]^+$ . HPLC purity: 97.04%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-(pyridine-3-sulfonamido)ethyl)-1H-indole-2-carboxamide* ( $R_{10}L_2$ ). White solid, Yield 76%, m.p.: 216–218 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.97 (s, 1H), 9.06 (t,  $J = 5.8$  Hz, 1H), 9.02–8.98 (m, 1H), 8.82 (dd,  $J = 4.8, 1.6$  Hz, 1H), 8.22 (dt,  $J = 8.0, 2.0$  Hz, 1H), 8.06 (t,  $J = 5.9$  Hz, 1H), 7.94 (d,  $J = 2.1$  Hz, 1H), 7.66 (ddd,  $J = 8.1, 4.8, 0.9$  Hz, 1H), 7.62 (d,  $J = 1.5$  Hz, 2H), 7.55 (d,  $J = 8.8$  Hz, 1H), 7.35 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.25 (s, 1H), 3.47–3.40 (m, 2H), 3.05 (q,  $J = 6.6$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.97, 153.61, 147.54, 142.93, 139.45, 137.21, 137.14, 135.17, 135.02, 133.27, 127.78, 125.66, 125.25, 124.81, 124.18, 119.43, 115.36, 112.15, 41.95, 39.70, 21.19. HRMS:  $m/z$   $C_{24}H_{23}ClN_4O_5S_2$ : Calcd. 546.0798, Found 547.0871  $[M+H]^+$ . HPLC purity: 98.64%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-(pyridine-3-sulfonamido)propyl)-1H-indole-2-carboxamide* ( $R_{10}L_3$ ). White solid, Yield 82%, m.p.: 178–180 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.92–8.85 (m, 2H), 8.73 (dd,  $J = 4.8, 1.6$  Hz, 1H), 8.12 (dt,  $J = 8.1, 2.0$  Hz, 1H), 7.89 (t,  $J = 5.8$  Hz, 1H), 7.85 (d,  $J = 2.1$  Hz, 1H), 7.58–7.52 (m, 3H), 7.45 (d,  $J = 8.7$  Hz, 1H), 7.26 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.29 (q,  $J = 6.6$  Hz, 2H), 2.90 (q,  $J = 6.7$  Hz, 2H), 2.23 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.88, 153.42, 147.49, 143.05, 139.39, 137.87, 137.41, 135.09, 134.93, 133.29, 127.68, 125.63, 125.08, 124.72, 124.18, 119.35, 115.27, 111.90, 40.93, 37.50, 31.39, 29.59, 21.21, 14.54, 14.38. HRMS:  $m/z$   $C_{25}H_{25}ClN_4O_5S_2$ : Calcd. 560.0955, Found 561.1028  $[M+H]^+$ . HPLC purity: 99.51%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-(pyridine-3-sulfonamido)butyl)-1H-indole-2-carboxamide* ( $R_{10}L_4$ ). White solid, Yield 71%, m.p.: 198–200 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.91–8.85 (m, 2H), 8.74 (dd,  $J = 4.8, 1.6$  Hz, 1H), 8.11 (dt,  $J = 8.1, 2.0$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.81 (t,  $J = 5.8$  Hz, 1H), 7.59–7.54 (m, 3H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.26–3.22 (m, 2H), 2.79 (q,  $J = 6.4$  Hz, 2H), 2.23 (s, 6H), 1.56–1.42 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.66, 153.40, 147.49, 143.06, 139.43, 138.81, 137.76, 137.43, 135.13, 134.93, 133.27, 127.71, 125.75, 125.11, 124.72, 124.13, 119.40, 115.30, 111.82, 42.69, 39.35, 26.96, 26.39, 21.22. HRMS:  $m/z$   $C_{26}H_{27}ClN_4O_5S_2$ : Calcd. 574.1111, Found 575.1184  $[M+H]^+$ . HPLC purity: 99.43%.

*5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(5-(pyridine-3-sulfonamido)pentyl)-1H-indole-2-carboxamide* ( $R_{10}L_5$ ). White solid, Yield 74%, m.p.: 178–180 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.90–8.83 (m, 2H), 8.74 (dd,  $J = 4.8, 1.6$  Hz, 1H), 8.10 (dt,  $J = 8.0, 2.0$  Hz, 1H), 7.86 (d,  $J = 2.1$  Hz, 1H), 7.79 (t,  $J = 5.8$  Hz, 1H), 7.57 (ddd,  $J = 8.1, 4.8, 0.8$  Hz, 2H), 7.55 (d,  $J = 1.6$  Hz, 2H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.27 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.19–7.16 (m, 1H), 3.23 (q,  $J = 6.6$  Hz, 2H), 2.74 (q,  $J = 7.0$  Hz, 2H), 2.23 (s, 6H), 1.45 (p,  $J = 7.3$  Hz, 2H), 1.37 (p,  $J = 7.2$  Hz, 2H), 1.28 (qd,  $J = 8.6, 7.3, 2.2$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.61, 153.39, 147.48, 143.08, 139.41, 137.85, 137.50, 135.11, 134.92, 133.27, 127.69, 125.75, 125.09, 124.72, 124.11, 119.39, 115.29, 111.75, 42.92, 39.68,

29.21, 28.74, 23.87, 21.21. HRMS:  $m/z$   $C_{27}H_{29}ClN_4O_5S_2$ : Calcd. 588.1268, Found 589.1341  $[M+H]^+$ . HPLC purity: 98.19%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((3-fluorophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide ( $R_{11}L_2$ )**. White solid, Yield 82%, m.p.: 210–212 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 8.96 (t,  $J = 5.8$  Hz, 1H), 8.01 (t,  $J = 5.9$  Hz, 1H), 7.86 (d,  $J = 2.1$  Hz, 1H), 7.76 (td,  $J = 7.6, 1.8$  Hz, 1H), 7.65–7.58 (m, 1H), 7.54 (d,  $J = 1.6$  Hz, 2H), 7.47 (d,  $J = 8.8$  Hz, 1H), 7.37 (ddd,  $J = 10.6, 8.3, 1.1$  Hz, 1H), 7.32 (td,  $J = 7.6, 1.1$  Hz, 1H), 7.27 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.16 (s, 1H), 3.36 (q,  $J = 6.7$  Hz, 2H), 3.04 (q,  $J = 6.7$  Hz, 2H), 2.22 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.96, 159.51, 157.83, 142.96, 139.43, 138.83, 137.30, 135.77, 135.71, 135.15, 134.79, 133.26, 130.10, 128.79, 128.69, 127.77, 125.65, 125.39, 125.37, 125.23, 124.18, 119.43, 117.84, 117.70, 115.33, 112.15, 41.81, 39.86, 21.18. HRMS:  $m/z$   $C_{25}H_{23}ClFN_3O_5S_2$ : Calcd. 563.0752, Found 564.0824  $[M+H]^+$ . HPLC purity: 99.04%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((3-fluorophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide ( $R_{11}L_3$ )**. White solid, Yield 70%, m.p.: 218–220 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.87 (t,  $J = 5.6$  Hz, 1H), 7.92 (t,  $J = 5.8$  Hz, 1H), 7.86 (t,  $J = 1.6$  Hz, 1H), 7.74 (td,  $J = 7.6, 1.8$  Hz, 1H), 7.60 (dddt,  $J = 11.2, 9.2, 6.1, 3.0$  Hz, 1H), 7.55 (d,  $J = 1.6$  Hz, 2H), 7.46 (d,  $J = 8.8$  Hz, 1H), 7.35 (ddd,  $J = 10.6, 8.3, 1.1$  Hz, 1H), 7.32–7.23 (m, 2H), 7.16 (s, 1H), 3.29 (t,  $J = 6.6$  Hz, 2H), 2.95 (q,  $J = 6.6$  Hz, 2H), 2.22 (s, 6H), 1.68 (p,  $J = 7.1$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.84, 159.51, 157.83, 143.04, 139.40, 137.76, 135.58, 135.52, 135.10, 133.29, 130.11, 129.05, 128.95, 127.70, 125.67, 125.29, 125.27, 125.10, 124.16, 119.37, 117.75, 117.62, 115.28, 111.91, 55.34, 40.81, 37.51, 36.23, 31.25, 29.69, 21.20. HRMS:  $m/z$   $C_{26}H_{25}ClFN_3O_5S_2$ : Calcd. 577.0908, Found 578.0981  $[M+H]^+$ . HPLC purity: 98.44%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((3-fluorophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide ( $R_{11}L_4$ )**. Faint yellow solid, Yield 77%, m.p.: 210–212 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.92 (s, 1H), 8.86 (t,  $J = 5.6$  Hz, 1H), 7.89–7.83 (m, 2H), 7.73 (td,  $J = 7.6, 1.8$  Hz, 1H), 7.60 (dddd,  $J = 8.2, 7.0, 5.0, 1.8$  Hz, 1H), 7.55 (d,  $J = 1.7$  Hz, 2H), 7.46 (d,  $J = 8.7$  Hz, 1H), 7.36 (ddd,  $J = 10.5, 8.3, 1.1$  Hz, 1H), 7.32–7.24 (m, 2H), 7.17 (s, 1H), 3.24 (t,  $J = 6.4$  Hz, 2H), 2.85 (q,  $J = 6.4$  Hz, 2H), 2.23 (s, 6H), 1.55–1.42 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.62, 159.51, 157.83, 143.06, 139.43, 137.72, 135.53, 135.48, 135.13, 133.27, 130.11, 129.09, 129.00, 127.72, 125.77, 125.30, 125.28, 125.12, 124.12, 119.41, 117.74, 117.60, 115.30, 111.82, 42.55, 39.34, 27.01, 26.33, 21.21. HRMS:  $m/z$   $C_{27}H_{27}ClFN_3O_5S_2$ : Calcd. 591.1065, Found 592.1137  $[M+H]^+$ . HPLC purity: 97.24%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(2-((3-fluorophenyl)sulfonamido)ethyl)-1H-indole-2-carboxamide ( $R_{12}L_2$ )**. White solid, Yield 82%, m.p.: 205–208 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 8.96 (t,  $J = 5.8$  Hz, 1H), 7.86 (t,  $J = 4.5$  Hz, 2H), 7.61 (td,  $J = 5.2, 4.4, 2.1$  Hz, 1H), 7.59 (dd,  $J = 7.8, 5.1$  Hz, 1H), 7.56–7.52 (m, 3H), 7.47 (d,  $J = 8.8$  Hz, 1H), 7.44 (ddt,  $J = 9.2, 6.3, 1.7$  Hz, 1H), 7.28 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.38–3.32 (m, 2H), 2.95 (dt,  $J = 7.6, 6.1$  Hz, 2H), 2.22 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  163.11, 161.46, 159.95, 142.94, 142.91, 142.87, 139.44, 137.20, 135.17, 133.27, 132.21, 132.16, 127.78, 125.67, 125.25, 124.17, 123.24, 123.22, 120.22, 120.08, 119.43, 115.35, 114.10, 113.94, 112.15, 41.99, 21.18. HRMS:  $m/z$   $C_{25}H_{23}ClFN_3O_5S_2$ :

Calcd. 563.0752, Found 564.0824  $[M+H]^+$ . HPLC purity: 99.58%.

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(3-((3-fluorophenyl)sulfonamido)propyl)-1H-indole-2-carboxamide ( $R_{12}L_3$ )**. White solid, Yield 65%, m.p.: 185–188 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.91 (s, 1H), 8.86 (t,  $J = 5.7$  Hz, 1H), 7.85 (d,  $J = 2.1$  Hz, 1H), 7.76 (t,  $J = 5.9$  Hz, 1H), 7.60 (dt,  $J = 7.8, 1.5$  Hz, 1H), 7.58–7.54 (m, 2H), 7.54–7.51 (m, 2H), 7.45 (d,  $J = 8.8$  Hz, 1H), 7.42 (dddd,  $J = 9.0, 8.0, 2.7, 1.4$  Hz, 1H), 7.26 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.17 (s, 1H), 3.28 (q,  $J = 6.7$  Hz, 2H), 2.87 (q,  $J = 6.7$  Hz, 2H), 2.23 (s, 6H), 1.67 (p,  $J = 7.1$  Hz, 2H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  163.08, 161.44, 159.85, 143.24, 143.20, 143.06, 139.39, 137.81, 135.09, 133.29, 132.08, 132.03, 127.68, 125.66, 125.09, 124.16, 123.17, 123.15, 120.01, 119.87, 119.36, 115.28, 114.02, 113.86, 111.91, 40.97, 37.52, 29.57, 21.21. HRMS:  $m/z$   $C_{26}H_{25}ClFN_3O_5S_2$ : Calcd. 577.0908, Found 578.0981  $[M+H]^+$ .

**5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-N-(4-((3-fluorophenyl)sulfonamido)butyl)-1H-indole-2-carboxamide ( $R_{12}L_4$ )**. White solid, Yield 72%, m.p.: 196–198 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.93 (s, 1H), 8.88 (t,  $J = 5.7$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.71 (t,  $J = 5.9$  Hz, 1H), 7.61–7.52 (m, 5H), 7.52–7.48 (m, 1H), 7.48–7.39 (m, 3H), 7.27 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.20–7.16 (m, 1H), 3.24 (q,  $J = 6.5$  Hz, 2H), 2.76 (q,  $J = 6.4$  Hz, 2H), 2.23 (s, 6H), 1.55–1.41 (m, 4H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  163.08, 161.44, 159.65, 143.23, 143.19, 143.05, 139.43, 137.76, 135.13, 133.27, 132.09, 132.04, 127.70, 125.74, 125.11, 124.13, 123.18, 123.16, 123.13, 119.99, 119.85, 119.39, 115.30, 114.00, 113.96, 113.84, 113.80, 111.81, 42.72, 42.53, 39.34, 26.93, 26.64, 26.39, 21.21. HRMS:  $m/z$   $C_{27}H_{27}ClFN_3O_5S_2$ : Calcd. 591.1065, Found 592.1137  $[M+H]^+$ . HPLC purity: 96.41%.

**5-Chloro-N-(2-(cyclopropanesulfonamido)ethyl)-3-((3,5-dimethylphenyl)sulfonyl)-1H-indole-2-carboxamide ( $R_{13}L_2$ )**. White solid, Yield 66%, m.p.: 210–212 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  9.02 (t,  $J = 5.8$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.58 (d,  $J = 1.6$  Hz, 2H), 7.48 (d,  $J = 8.8$  Hz, 1H), 7.28 (dd,  $J = 8.8, 2.1$  Hz, 1H), 7.18 (s, 1H), 7.12 (t,  $J = 6.1$  Hz, 1H), 3.46–3.40 (m, 2H), 3.20–3.13 (m, 2H), 2.43 (p,  $J = 1.9$  Hz, 1H), 2.24 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  160.07, 142.99, 139.44, 137.53, 135.16, 133.30, 127.76, 125.64, 125.21, 124.23, 119.40, 115.35, 112.12, 42.01, 40.59, 40.27, 29.85, 21.21, 5.20. HRMS:  $m/z$   $C_{22}H_{24}ClN_3O_5S_2$ : Calcd. 509.0846, Found 510.0919  $[M+H]^+$ . HPLC purity: 98.60%.

**N-(2-([1,1'-Biphenyl]-4-sulfonamido)ethyl)-5-chloro-3-((3,5-dimethylphenyl)sulfonyl)-1H-indole-2-carboxamide ( $R_{14}L_2$ )**. White solid, Yield 74%, m.p.: 250–254 °C.  $^1H$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 8.97 (t,  $J = 5.8$  Hz, 1H), 7.87 (d,  $J = 2.1$  Hz, 1H), 7.86–7.82 (m, 2H), 7.82–7.75 (m, 3H), 7.64–7.59 (m, 2H), 7.54 (d,  $J = 1.7$  Hz, 2H), 7.45 (d,  $J = 8.8$  Hz, 1H), 7.44–7.39 (m, 2H), 7.38–7.33 (m, 1H), 7.26 (dd,  $J = 8.7, 2.1$  Hz, 1H), 7.14 (s, 1H), 3.42–3.35 (m, 2H), 2.98 (q,  $J = 6.6$  Hz, 2H), 2.19 (s, 6H).  $^{13}C$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  159.93, 144.51, 142.94, 139.52, 139.44, 138.94, 137.19, 135.16, 133.26, 129.54, 128.92, 127.90, 127.80, 127.68, 127.48, 125.69, 125.25, 124.16, 119.45, 115.34, 112.14, 42.05, 21.17. HRMS:  $m/z$   $C_{31}H_{28}ClN_3O_5S_2$ : Calcd. 621.1159, Found 622.1232  $[M+H]^+$ .

#### 4.2. In vitro anti-HIV assays

Our previously established assay was conducted to evaluate the cellular activity and cytotoxicity of these target compounds<sup>13</sup>. The



compounds to be measured and drugs as positive references were weighted and prepared into stock solutions of specific concentrations in DMSO. Before testing, the stock solutions were diluted into 5 concentration gradients by purified water, meanwhile three duplicates were set for each concentration. Each sample included the untreated HIV-1 infection and mock-infected cells. HIV-1 WT strain, mutant strains L100I, K103N, Y181C, Y188L, E138K, F227L + V106A and RES056 stock solution (50  $\mu$ L) at 100–300 CCID<sub>50</sub> (50% cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells. Mock-infected groups were established to measure the cytotoxicity target compounds. To each well of the cell culture plate, 100  $\mu$ L medium, 50  $\mu$ L MT-4 cell culture medium, and 20  $\mu$ L HIV-infected MT-4 cell suspension or blank medium were added in sequence. The processed cells were incubated in constant environment for 5 days. MTT solution (20 mL, 5 mg/mL) was added to each well, and the cell culture was centrifuged at 1000 rpm to remove the supernatant after 2 h. The cell viability in mock- and HIV-infected cultures was examined spectrophotometrically with MTT method. The absorbance values were used to calculate the EC<sub>50</sub> and CC<sub>50</sub> values. SI values were acquired as a ratio between CC<sub>50</sub> and EC<sub>50</sub>.

#### 4.3. HIV-1 RT inhibition assays

The enzymic inhibition assay was ran by a RT kit purchased from Roche. The relevant ELISA method in the process was performed under the guidance of kit protocol. The template/primer complex, nucleotide (dNTPs) and HIV-1 RT was pre-diluted and mixed under the guidance of assay kit. Assay buffers containing desired concentration of each compound were added as well. The mixtures were allowed to incubate at 37 °C, meanwhile gently shaken for 1 h. Then they were transferred to streptavidin-coated microplate and further incubated at 37 °C for another 2 h. Each well was washed 5 times using the assay buffer. After digoxin antibody-peroxidase solution was added, the whole plate was further incubated for 1 h. Subsequently, the washing procedure above was repeated, and followed by adding the ABST peroxide substrate solution. A microplate reader was applied to measure the absorbance value promptly. In the process, the negative control and the blank group were set up. The percentage inhibitory activity of the tested compounds was calculated by Eq. (1):

$$\text{Inhibition (\%)} = [\text{OD}_{\text{negative}} - \text{OD}_{\text{test}}] / [\text{OD}_{\text{test}} - \text{OD}_{\text{blank}}] \quad (1)$$

The IC<sub>50</sub> values represents the concentrations of the inhibitors required to inhibit biotin-dUTP incorporation by 50%.

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#### Author contributions

Xinyong Liu, Peng Zhan, and Yajie Tang as the supervisors conceived the project and supplied the financial support. Shenghua Gao and Yusen Cheng conducted the synthesis of compounds. Letian Song, Mianling Yang and Bing Ye complete the structure confirmation. Shenghua Gao and Letian Song completed the manuscript. Erik De Clercq and Christophe Pannecouque conducted and supervised the activity assay. The molecular docking was conducted by Fabao Zhao and Letian Song. Shenghua Gao designed the pharmacokinetics, acute toxicity and subacute toxicity experiment. Dongwei Kang, Shu Song, and Wei Zhao done the data analysis.

#### Conflicts of interest

There are no conflicts of interest to declare.

#### Appendix A. Supporting information

Supporting data to this article can be found online at <https://doi.org/10.1016/j.apsb.2023.01.003>.

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