A N-propionylsulfonamide prodrug of K-5a2 provided improved aqueous solubility and hERG inhibition

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Abstract

Background Having the potential disadvantages and safety risk of the use of anti-HIV-1 drug candidate **K-5a2** in the longterm treatment of HIV patients in mind, we set out with the goal of finding a second-generation backup compound of **K-5a2** with the appropriate anti-HIV potency, significantly reduced hERG activity, decreased induction of the CYP enzyme, and improved aqueous solubility. Herein, using a N-propionylsulfonamide prodrug strategy, we report the discovery of compound **HM-1**

Methods In vitro assay of anti-HIV activities in TZM-bl and MT-4 cells, metabolic stability in HLM and human plasma, measurements of water solubility and Log P, assay procedures for hERG activity, acute and subacute toxicity experiment and cytochrome P450 inhibition assay were carried out for **HM-1**.

Results **HM-1** can be rapidly hydrolyzed to parent drug **K-5a2** and exhibited high potency against HIV-1_{NL4-3} strain ($EC_{50} = 7.99$ nM) in TZM-bl cells, HIV-1_{IIIB} strain ($EC_{50} = 2.9$ nM) and HIV-1_{Y181C} strain ($EC_{50} = 5.5$ nM) in MT-4 cells. And it also showed a > 70-fold improvement in aqueous solubility and presented a low acute toxicity in mice ($LD_{50} > 2$ g·kg⁻¹); no obvious organ damage was detected in the assessment of subacute toxicity. Meanwhile, **HM-1** also showed 50 times lower hERG inhibition ($IC_{50} = 6.39$ μM) than **K-5a2** ($IC_{50} = 0.13$ μM).

Conclusions It was **HM-1** appeared to be free of most of the drawbacks associated with **K-5a2** and has been selected for further development as an oral anti-HIV-infection agent.

Background

Non-nucleoside reverse transcriptase inhibitors (NNRTIs), which target the binding pocket of reverse transcriptase (RT), are a vital component of highly active antiretroviral therapy (HAART) regimens for the treatment of human immunodeficiency virus type 1 (HIV-1) infection,^[1–3] due to their potent antiviral activity, high selectivity, modest toxicity, and favorable pharmacokinetics.^[4]

K-5a2, a promising anti-HIV-1 drug candidate, was recently discovered by structure-based design in our lab. Compared to etravirine (ETR), it is gratifying that **K-5a2** was 2.8 fold more potent against HIV-1 wild-type (WT) strain, and 5-fold more potent against single mutants Y188L, E138K and double mutant F227L + V106A, and almost equipotent against L100I, K103N, and Y181C, which justifies its further development as a potential clinical candidate for the treatment of HIV-1 infection (Fig. 1).^[5]

Despite these encouraging results, further development of **K-5a2** faces many challenges for its low aqueous solubility (< 1 μg/mL), which is one of the important parameters to achieve a desired drug concentration in systemic circulation to achieve the intended pharmacological response.^[5–7] In addition, high hERG liability ($IC_{50} = 0.13$ μM) is an important problem encountered with **K-5a2**, which may engender the potential risk for cardiotoxicity. Hence, challenges associated with poor aqueous solubility and high hERG inhibition to **K-5a2**, highlight the need for continuous research and development of rational

chemical modification. The application of the prodrug approach proved to be a successful strategy for improving aqueous solubility and other drug-like profiles of the parental drugs result in a positive impact on their biological efficacy.^[8]

Recent studies demonstrated that prodrug modification to improve water solubility of some parent drug containing sulfonamide group has been successfully achieved using N-propionyl moiety.^[9–11] For example, as a potent NNRTI, GW678248 was limited into clinical trials due to its poor water solubility (0.18 µg/ml). With the aim to improve solubility, N-propionyl sulfonamide prodrug of GW678248 was discovered. GW695634 obviously enhanced aqueous solubility (92 mg/ml). And the oral bioavailability of GW695634 in rats and dogs (> 40%) was also increased.^[12] Besides, the marketed parecoxib was also the N-propionyl sulfonamide prodrug of valdecoxib which is relatively insoluble in water at about 10 µg/mL.^[13] Elulfavirine, a new generation NNRTI, is the prodrug of the active compound VM 1500A that obtained its first market authorization in Russia (Fig. 2).^[14]

Herein, inspired by these recent successful examples, with the aim to enhance aqueous solubility and drug-like profiles of our drug candidate **K-5a2**, we looked for a second-generation backup compound for **K-5a2**, with the appropriate anti-HIV potency, significantly reduced hERG activity, decreased induction of the CYP enzyme, and improved aqueous solubility. Consequently, the N-propionylsulfonamide prodrug strategy was employed in a structural modification. The synthesis and characterization of prodrug **HM-1** (Fig. 3), including antiviral activity, acute toxicity, subacute toxicity, hERG inhibition, and CYP enzymatic inhibitory activity, are presented in this paper.

Methods

N-((4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)sulfonyl)propionamide (HM-1)

K-5a2 (0.5 g, 0.9 mmol) was dissolved in propionic acid (4 mL) and propanoic anhydride (1 mL) was then added. The mixture was then stirred at 120 °C for 2 h and checked by TLC until completion. The reaction solution was concentrated under reduced pressure and then extracted with dichloromethane (20 mL), washed with water (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and the residue was purified by silica gel chromatography (CH₂Cl₂: CH₃OH = 30:1) and then recrystallized from ethyl acetate/petroleum ether to give the title compound **HM-1** as white solid in 36% yield, mp 130–133 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.360–10.713 (m, 1H, SO₂NH), 8.13 (d, *J* = 5.4 Hz, 1H, C₆-thienopyrimidine-H), 7.79 (d, *J* = 7.9 Hz, 2H, C₃,C₅-Ph'-H), 7.65 (s, 2H, C₃,C₅-Ph''-H), 7.45 (d, *J* = 8.0 Hz, 2H, C₂,C₆-Ph'-H), 7.245–7.134 (m, 1H, C₇-thienopyrimidine-H), 6.86 (s, 1H, NH), 3.69 (s, 1H), 3.51 (s, 2H, N-CH₂), 2.784–2.622 (m, *J* = 35.5 Hz, 2H), 2.13 (q, *J* = 7.5 Hz, 2H,), 2.04 (s, 6H, CH₃ × 2), 1.894–1.1053 (m, 6H), 0.82 (t, *J* = 7.4 Hz, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 173.21, 162.51, 160.56,

153.45, 139.23, 133.25, 133.01, 129.62, 127.90, 119.03, 109.08, 61.69, 52.64, 31.44, 29.43, 16.25, 8.90.
ESI-MS: m/z 605.43 (M + 1). C₃₀H₃₂N₆O₄S₂ (604.1926).

In vitro anti-HIV assay in TZM-bl cells

Different concentrations of **K-5a2** and **HM-1** were added to TZM-bl cells, which were infected by NL4-3 virus. After two days, remove the culture medium and then add 100 µL of Bright Glo reagent.

Various concentrations of **K-5a2** and **HM-1** were affiliated to TZM-bl cells, and then incubated. Four days later, add XTT solution (1 mg/mL), which involved 0.02 µM PMS. The absorbance was detected at 450 nm four hours later. EC₅₀ (50% effective concentration) was the concentration that caused a 50% decrease in luciferase activity, CC₅₀ (50% cytotoxic concentration) was concentration that caused a 50% reduction in cell viability.

In vitro anti-HIV assay in MT-4 cells

This kind of anti-HIV activity and cytotoxicity assay were conducted in MT-4 cells named MTT method. 10 different concentrations of **K-5a2**, **HM-1** and reference drugs were added in the corresponding volumes to formulate two series of triplicate wells to evaluate anti-HIV activity simultaneously on mock- and HIV-infected cells. Serial 5-fold dilutions of relevant compounds were added in flat-bottomed 96-well microtiter trays. HIV stock at 100–300 CCID₅₀ or culture medium was added to the infected or mock-infected wells, and untreated HIV- and mock-infected cell samples were as controls. After the MT-4 cells being centrifuged, the supernatant was deserted. MT-4 cells were resuspended and transfer 50 µL volumes for infecting. Five days later, MTT assay was conducted on mock-and HIV-infected cells. All data were calculated by the median absorbance value. EC₅₀ was concentration achieving 50% protection against the cytopathic effect of the virus in infected cells, CC₅₀ was the concentration reducing the absorbance (OD₅₄₀) of the mock-infected control sample by 50%.

Metabolic stability in HLM and human plasma

To validate our hypothesis, prodrug **HM-1** was further assessed for its ability to undergo conversion to the parent drug **K-5a2**. HLM stability assay was primarily carried out. **HM-1** incubated with microsome solution for 10 minutes, and then 2 mM MgCl₂ was added. Each bioanalysis plate was sealed after adding into stop solution for LC-MS/MS analysis.

We also tested the stability of **HM-1** in human plasma. Blank plasma spiked with **HM-1** (100 µM) was incubated at 37 °C. Stop solution was added at 0, 10, 30, 60 and 120 min. The supernatant was transferred from each well and mixed with ultra pure water for LC-MS/MS analysis.

Measurements of water solubility and Log P

Water solubility was measured in phosphate buffer at pH 7.4 and 2.0 by using a HPLC-UV method.^[16] **HM-1** or **K-5a2** was dissolved in DMSO, and then spiked into 1 mL purified water. After ultrasonic for 2 h,

the saturated solution was filtrated through a filter membrane and prepared two samples. LC-20AT HPLC-UV (SHIMADZU) system was used to compare the peak area of the saturated solution with a standard curve plotted peak area. Stock solution of **HM-1** or **K-5a2** were prepared for HPLC analysis. The P (partition coefficient) value was obtained by the peak area ratios in n-octanol and in H₂O.

Assay procedures for hERG activity

The hERG channels were stably expressed in Chinese Hamster Ovary (CHO) cells that had no endogenous IKr, so CHO-hERG cells obtained from Sophion Biosciences were used in this assay.^[17] CHO-hERG cells were maintained at 37 °C (± 2 °C) under 5% CO₂ (4%~8%) in a high humidity incubator. The whole-cell patch-clamp technique was used to record hERG current controlled with Patchmaster Pro software at room temperature and output signals from the patch-clamp amplifier was digitized and low-pass filtered at 2.9 KHz. Electrode was recorded by a micropipette filled with ICS, which was drawn out from borosilicate glass to give a tip resistance between 3 ~ 5 MΩ. The initial holding potential was -80 mV, and then the voltage was increased to +60 mV for 850 ms to open the hERG channels. After that, the voltage was decreased to -50 mV for 1275 ms to cause a "rebound" or tail current, before the voltage was decreased to the holding potential (-80 mV), the peak tail current was measured and collected for data analysis. This command voltage protocol was repeated every 15 s continuously.

Acute and subacute toxicity experiment

All animal treatments were performed strictly in accordance with the institutional guidelines of Animal Care and Use Committee at Shandong University, after gaining approval from the Animal Ethical and Welfare Committee (AEWC). Healthy male Kunming mice (17 ~ 22 g) were randomly divided into three groups: blank group, **K-5a2** group, and **HM-1** group. Each group consisted of 5 mice. **K-5a2** and **HM-1** were suspended in dimethyl sulfoxide (DMSO): normal saline: polyethylene glycol (PEG) 400 = 5%: 25%: 70% at concentrations of 200 mg·mL⁻¹, respectively, and all mice had been fasted for 12 h, and then the mice were given by gavage a dose of 2000 mg/kg of blank solvent, **K-5a2** and **HM-1** respectively for the following 7 days. The death, body weight and behavioral abnormalities were monitored.

Another group of healthy male Kunming mice were also randomly divided into three groups: blank group, **K-5a2** group, and **HM-1** group. Each group consisted of 5 mice. Suspension of **K-5a2** and **HM-1** were prepared (DMSO: sodium carboxymethylcellulose: H₂O = 3%: 77%: 20%), respectively, with concentrations of 6 mg·mL⁻¹, and administered intragastrically after the mice had been fasted for 12 h. Before dosages of 60 mg·kg⁻¹ of blank solvent, **K-5a2** and **HM-1** were administered respectively to three groups, and the body weight of the mice was measured. All of the mice were dissected on the last day, and the heart, liver, spleen, lung, and kidney were extracted. These organs were then examined by HE to see if any damage had occurred.

Kunming mice were purchased from the animal experimental center of Shandong University. Animals were maintained during the experiment in standard conditions of 12 hours light–dark cycle, food and water ad libitum, temperature 24 °C, humidity above 55%. When the experiments finished, all animals

were euthanized using the method of spinal dislocation. The details were as follow: grasp the mouse with the right hand and pull it backward hard, while pressing the mouse's head with the thumb and index finger of the left hand to pull the spinal cord and brain marrow to break, and the mouse died immediately.

Cytochrome P450 inhibition assay

Compound **HM-1** and standard inhibitors (α -naphthoflavone, sulfaphenazole, (+)-N-3-benzylirvanol, quinidine, ketoconazole) were prepared. Then, the substrates solutions, potassium phosphate buffer (PB), **HM-1** and positive control working solution were added into homologous wells, respectively. Then, the HLM solutions pre-warmed for 10 min and NADPH cofactor were added. Before centrifuged, the 400 μ L cold stop solution was added and then 200 μ L supernatant was transferred for LC/MS/MS analysis.

Results

Chemistry

The synthetic routes of **HM-1** was shown in Scheme 1, **K-5a2** was prepared according to our previously reported method.^[5] **HM-1** was synthesized from the 2,4-dichlorothiophene[3,2-d]pyrimidine (**1**); nucleophilic substitution reactions with 3,5-dimethyl-4-hydroxybenzonitrile and 4-(tert-butoxycarbonyl)-aminopiperidine in the presence of K_2CO_3 and DMF afforded intermediate **3**. Then intermediate **3** underwent deprotection with trifluoroacetic acid at room temperature in DCM to generate compound **4**, which led to **K-5a2** with 4-bromomethylbenzenesulfonamide, K_2CO_3 and DMF. Then, **K-5a2** (0.5 g, 0.9 mmol) was dissolved in propionic acid (4 mL) and propanoic anhydride (1 mL) was then added. The mixture was then stirred at 120°C for 2 h.

The target compound **HM-1** was completely characterized by the spectral means including 1H NMR, ^{13}C NMR and MS. The purity was > 95% as determined by analytical HPLC.

Anti-HIV activity assay in TZM-bl cells

To examine the antiviral activity of **HM-1**, along with **K-5a2**, reduction in luciferase gene expression was detected in TZM-bl cells. As exhibited in Table 1, the new compound **HM-1** still revealed high potency suppressing HIV-1_{NL4-3} strain at a nanomolar concentration (EC_{50} = 7.99 nM), although it was inferior to that of the parent drug **K-5a2** (EC_{50} = 1.17 nM). The CC_{50} values of **HM-1** and **K-5a2** were greater than 210 nM, which illustrated that both compounds had low cytotoxicity in TZM-bl cells.

Table 1
Antiviral potency against HIV-1 NL4-3 and cytotoxicity in TZM-bl cells.

Compds.	EC_{50} (nM, NL4-3)	CC_{50} (μ M)	SI (NL4-3)
K-5a2	1.17 $\pm$ 0.44	> 0.23	> 196.58
HM-1	7.99 $\pm$ 2.73	> 0.21	> 26.28

Anti-HIV activity assay in MT-4 cells

Furthermore, we also evaluate the anti-HIV-1 potency against wild type (WT) and common mutant strains in MT-4 cells. The results in Table 2 showed that **HM-1** still exhibited high antiviral activity against HIV-1_{IIIB} with EC₅₀ values of 2.9 nM, which was better than the reference drug **ETR** (EC₅₀ = 3.0 nM). Besides, the cytotoxicity of **HM-1** (CC₅₀ = 75.7 µM, SI = 26161) in MT-4 cells was far lower than **ETR** (CC₅₀ > 4.6 µM, SI > 1546). And **HM-1** didn't show inhibitory against HIV-2 strain.

Table 2
Antiviral potency against WT and cytotoxicity in MT-4 cells.

Compds.	EC ₅₀ (nM)		CC ₅₀ (µM)	SI (IIIB)
	IIIB	ROD		
HM-1	2.9 ± 0.4	> 7127.0	75.7 ± 24.1	26161
K-5a2	1.4 ± 0.2	> 227819.5	> 227.9	> 161290
NVP	84.0 ± 10.7	–	> 9.5	> 113
AZT	18.3 ± 4.2	–	> 7.5	> 410
EFV	2.5 ± 0.8	–	> 6.3	> 2572
ETR	3.0 ± 0.6	–	> 4.6	> 1546

As showed in Table 3, **HM-1** exhibited higher anti-HIV-1 activities towards mutant strains than **ETR** except RES056 strain. Notably, the potency against Y181C strain of **HM-1** (EC₅₀ = 5.5 nM) was superior to the parent **K-5a2** (EC₅₀ = 5.9 nM).

Table 3
Antiviral potency against mutant strains in MT-4 cells.

Compds.	EC ₅₀ (nM)						
	L100I	K103N	Y181C	Y188L	E138K	F227L/V106A	RES056
HM-1	4.7 ± 1.4	2.1 ± 0.6	5.5 ± 1.8	5.6 ± 2.2	8.5 ± 4.0	6.9 ± 2.9	101.6 ± 34.4
K-5a2	3.2 ± 0.8	1.2 ± 0.2	5.9 ± 0.9	3.7 ± 0.7	4.6 ± 1.1	3.6 ± 0.8	53.5 ± 20.2
NVP	372.9 ± 154.4	1470.7 ± 457.9	2325.1 ± 1074.2	3388.2 ± 1331.0	72.8 ± 20.6	2730.3 ± 1258.4	6747.7 ± 2322.7
AZT	7.0 ± 2.6	12.6 ± 3.4	8.7 ± 1.7	9.9 ± 7.8	14.5 ± 2.7	4.7 ± 1.7	20.1 ± 4.7
EFV	30.8 ± 18.4	63.7 ± 14.6	5.2 ± 1.7	148.3 ± 40.8	5.7 ± 1.2	168.5 ± 63.9	273.2 ± 153.4
ETR	10.2 ± 10.3	2.3 ± 0.4	14.0 ± 2.6	15.5 ± 6.8	9.8 ± 3.8	8.2 ± 2.2	60.2 ± 24.7

Metabolic stability assays

Since the prodrug Parecoxib with the N-propionyl moiety was rapidly hydrolyzed by the liver to valdecoxib, ^[18] the stability of N-propionylsulfonamide prodrug **HM-1** in the presence of HLM was determined. The results demonstrated that **HM-1** was rapidly converted to the parent drug **K-5a2** in 10 min. In addition, **HM-1** was highly stabilized in human plasma via plasma stability assay (Table 4).

Table 4
The metabolic stability of **HM-1** in HLM and human plasma.

Compd.	Metabolite	Time Point (min)	Analyte/IS	Metabolite formation
HM-1 (HLM)	K-5a2	0	0.004	2.1%
		2	0.093	53.0%
		5	0.146	83.4%
		10	0.179	102.2%
		20	0.192	109.2%
		30	0.179	102.0%

Solubility and lipophilic efficiency

As well known, poor water solubility and hydrophilicity/lipophilicity will influence drug metabolism and distribution, **K-5a2** displayed poor solubility which can limit its further development as a drug. HPLC was

used to test the solubility and logP of **K-5a2** and **HM-1**, and other drug-like properties such as MW (molecular weight) and nON (no. of hydrogen bond acceptors) were predicted (Table 5). Surprisingly, **HM-1** showed a > 70-fold improvement in aqueous solubility compared to **K-5a2**, which supported that the strategy of N-propionylsulfonamide can improve water solubility effectively.

Table 5
Main physicochemical properties of **K-5a2**, **HM-1** and **ETV**.

Compd.	Metabolite	Time Point (min)	% Remaining	Formation of Metabolite (nM)
HM-1 (human plasma)	K-5a2	0	100	2.2281
		10	101.99	2.377
		30	103.56	3.244
		60	100.54	3.817
		120	108.58	2.898
Parameters	Rule of five	K-5a2	HM-1	ETV
MW	< 500	548.68	604.74	435.28
nON	< 10	9	10	7
nOHNH	< 5	3	2	3
nrotb	≤ 10	7	9	4
tPSA	< 140	134.24	137.31	120.65
logP	< 5	3.33 ^a	-0.11	> 5 ^b
Solubility (pH = 7.0)		< 1	69.66	
Solubility (pH = 7.4) ^d		< 0.1	162.64	<< 1 ^b
Solubility (pH = 2.0) ^d		ND	18.09	<< 1 ^b
mp		223–225 °C	130–133 °C	255–257 °C ^c

^aUsing free on-line software (<http://www.molinspiration.com/>); MW = molecular weight; nON = no. of hydrogen bond acceptors; nOHNH = no. of hydrogen bond donors; nrotb = no. of rotatable bonds; tPSA = topological polar surface area; log P = logarithm of partition coefficient; ND = not determined.

^bSee Ref [19]. ^cSee Ref [20]. ^dMeasured in phosphate buffer by HPLC in duplicate

Assessment of hERG activity

Acquired long QT syndrome caused by inhibition of the voltage-dependent ion channel, which is encoded by human ether-a-go-go-related gene (hERG), is a critical adverse effect of noncardiovascular therapeutic

drugs.^[21] As we all know, decreasing alkalinity is an important strategy for structure optimization of lead compounds to reduce the inhibitory activity of hERG.^[22] Through predicting by ChemDraw, the alkalinity of **HM-1** was decreased, which revealed that **HM-1** may have lower hERG activity. Indeed, in vitro manual patch-clamp electrophysiology was used to evaluate the activity against the hERG potassium channel to estimate the potential risk for cardiotoxicity,^[23] and the result verified our prediction exactly.

As shown in Fig. 4, **HM-1** showed an IC_{50} value of 6.39 μM against the potassium channel, which was nearly 50 times higher than that of **K-5a2** ($IC_{50} = 0.13 \mu M$). Although the hERG IC_{50} value of **HM-1** was not greater than 10 μM , it was also 12 times better than rilpivirine (RPV, $IC_{50} = 0.5 \mu M$).^[24] Therefore, the hERG inhibitory activity of **HM-1** was much lower than that of **K-5a2**, suggesting that the potential cardiotoxicity was significantly diminished.

Assessment of acute and subacute toxicity.

Regarding the relatively low cytotoxicity of **K-5a2**, a single-dose acute toxicity trial of **HM-1** in healthy Kunming mice was carried out. One mouse died after intragastric administration of **K5a2** and **HM-1** at a dose of 2000 mg/kg. However, no significant behavior abnormalities (lethargy, clonic convulsion, anorexia, and ruffed fur) were observed and the body weights changes was the same as in the blank group (Fig. 5A). Therefore, **HM-1** can be tolerated at a dose of 2000 mg/kg with low toxicity.

To further evaluate the *in vivo* safety of **HM-1**, the subacute toxicity was investigated in healthy mice. No mice died, and no behavioral abnormalities occurred during the treatment period of mice treated po with 60 mg/kg·day⁻¹ of **HM-1** for 2 weeks. The body weight of mice in the **HM-1** group increased gradually and the mice grew normally as in the **K-5a2** group (Fig. 5B). Additionally, organs toxicity experiments were carried out by hematoxylin – eosin (HE) staining after dissecting all the mice. As depicted in Fig. 5C, the heart, liver, and spleen of the mice were not damaged with either **HM-1** or **K-5a2**, but after treatment with **K-5a2**, it was remarkable that there were alveolar hemorrhage and alveolar interstitial thickening (red arrows) in the lung and glomerular edema (blue circles) in the kidney, which were obviously improved with **HM-1**. The subacute toxicity assessment results indicated that **HM-1** possessed favorable *in vivo* safety profiles.

In vitro effects of HM-1 on CYP enzymatic inhibitory activity.

In the liver, the majority of drugs are metabolized by enzymes like cytochrome P450 enzymes, whose activity can be inhibited or induced by drugs. Therefore, when co-administering different drugs, therapeutic effect may be decreased or increased, this is called metabolism-mediated drug – drug interactions (DDI). Previous studies disclosed that ETR is an inducer of CYP3A4 and inhibitor of CYP2C9 and CYP2C19, and DDI will occur when ETR is co-administrated with other anti-HIV-1 drugs, which limits the selection of concomitant antiretrovirals. Hence, **HM-1** was evaluated for its ability to inhibit CYP drug-metabolizing enzymes in vitro.

As shown in Table 6 and Table 7, the CYP3A4 inhibition of HM-1 ($IC_{50} > 50 \mu M$) was far superior to K-5a2 ($IC_{50} = 18.9 \mu M$), which revealed that HM-1 might decrease toxicity and DDI caused by CYP3A4 inhibition in anti-HIV infection. In addition, HM-1 displayed no CYP enzymatic inhibition of CYP1A2 and CYP2D6. Although the CYP2C9 and CYP2C19 inhibitions ($IC_{50} < 3 \mu M$) of HM-1 were inferior to K-5a2,^[25] which may induce side effects in the liver and probably result in DDI when co-administered with other different drugs, the perfect result of CYP3A4 inhibition was still worthy to emphasized.

Table 6
Inhibitory activity on CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 of **HM-1** and positive controls

CYP isozyme	Standard inhibitor	IC_{50} (μM)	Compd.	IC_{50} (μM)
1A2	α -naphthoflavone	0.216	HM-1	> 50
2C9	sulfaphenazole	0.609	HM-1	1.24
2C19	(+)-N-3-benzylnirvanol	0.227	HM-1	1.83
2D6	quinidine	0.134	HM-1	> 50
3A4	ketoconazole	0.0375	HM-1	> 50

Table 7
Inhibitory activity on CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 of **K-5a2** and positive controls

CYP isozyme	Standard inhibitor	IC_{50} (μM)	Compd.	IC_{50} (μM)
1A2	α -naphthoflavone	0.311	K-5a2	> 50
2C9	sulfaphenazole	0.643	K-5a2	> 50
2C19	(+)-N-3-benzylnirvanol	0.259	K-5a2	> 50
2D6	quinidine	0.158	K-5a2	> 50
3A4	ketoconazole	0.0433	K-5a2	18.9
Tables				

Discussion

A high-quality drug should emerge balanced efficacy against its therapeutic targets, safety, physicochemical properties and ADME properties.^[26] In order to improve key drug-like properties, namely the solubility, an effort to identify prodrugs was undertaken. This has led to the discovery of a N-propionylsulfonamide prodrug **HM-1**. **HM-1** could be rapidly converted to the parent drug **K-5a2** in the liver, and exhibit remarkable potency against HIV-1_{NL4-3} strain with an EC_{50} value of 7.99 nM. Furthermore, **HM-1** also showed excellent potency against HIV-1_{IIIB} ($EC_{50} = 2.9$ nM) and HIV-1_{Y181C} strain

(EC₅₀ = 5.5 nM). The physicochemical evaluation showed that **HM-1** exhibited a 70-fold increase in aqueous solubility (69.66 µg/mL) when compared to that of **K-5a2** (< 1 µg/mL). The hERG liability (IC₅₀ = 6.39 µM) of **HM-1** was significantly reduced by 50 times, compared to **K-5a2** (IC₅₀ = 0.13 µM), which would allow higher doses to be used in the clinic thereby realizing full potential of this compound. In addition, **HM-1** showed a low acute toxicity in mice (LD₅₀ > 2 g·kg⁻¹) as well as no obvious organ damage detected in the assessment of subacute toxicity.

Conclusion

In summary, the N-propionylsulfonamide prodrug approach has wide applicability in sulfonamide-bearing compounds and provides an effective means of augmenting the therapeutic potential of our anti-HIV-1 drug candidate **K-5a2**. What can not be ignored is that **HM-1** may induce some side effects in the liver and DDI probably occur when co-administrated with other drugs with an IC₅₀ value less than 3 µM against CYP2C9 and CYP2C19. Thus, further studies should be carried out to discover potential prodrugs with improved physiochemical and PK properties, as well as decreased induction of CYP enzymes.

Abbreviations

CC₅₀, 50% cytotoxicity concentration; EC₅₀, the concentration causing 50% inhibition of antiviral activity; ETR, etravirine; HAART, highly active antiretroviral therapy; hERG, human ether-a-go-go-related gene; HIV-1, human immunodeficiency virus type 1; HLM, human liver microsomes. NNRTIs, non-nucleoside reverse transcriptase inhibitors; RPV, rilpivirine; SI, selection index; WT, wild-type.

Declarations

Declarations

Author declares that there is no conflict of interests.

Ethics approval and consent to participate

We performed this study with an authorized standard method to detect acute and subacute toxicity using mice and accredited by Laboratory Animal Ethical and Welfare Committee of Shandong University Cheeloo College of Medicine.

Consent to publish

Not applicable.

Availability of data and materials

All data obtained and analyzed during this work are included in this article. Extra information is available upon reasonable request from the corresponding author.

Competing interests

The authors declare no competing financial interest.

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Authors' contributions

PZ and XL provided part of the funding support and revised the manuscript. XZ designed and performed research and wrote the final manuscript. ZH analyzed the data. DK, EDC and CP revised the manuscript. TZ performed the organs toxicity experiments. CHC and KHL performed the anti-HIV activity assay. All authors read and approved the final manuscript.

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Figures

Figure 1. The discovery of K-5a2 and its anti-HIV-1 activities.

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The discovery of K-5a2 and its anti-HIV-1 activities.

Figure 2. Structures of successful N-propionyl sulfonamide prodrugs

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Structures of successful N-propionyl sulfonamide prodrugs

Figure 3. The design concept of **K-5a2** prodrug.

Figure 3

The design concept of K-5a2 prodrug.

Figure 4. Activity of **HM-1** and **K-5a2** against hERG potassium channel in CHO-hERG cells.

Figure 4

Activity of HM-1 and K-5a2 against hERG potassium channel in CHO-hERG cells.

Figure 5. **A.** Body weight of all mice in three groups (g)-time (day) in acute toxicity experiment. **B.** Body weight of all mice in three groups (g)-time (day) in subacute toxicity experiment. **C.** HE of different organs of normal mice with **K-5a2** (60 mg/kg) and **HM-1** (60 mg/kg). Scale bars represent 50 μ m.

Figure 5

A. Body weight of all mice in three groups (g)-time (day) in acute toxicity experiment. B. Body weight of all mice in three groups (g)-time (day) in subacute toxicity experiment. C. HE of different organs of normal mice with K-5a2 (60 mg/kg) and HM-1 (60 mg/kg). Scale bars represent 50 μ m.

Scheme 1. Synthetic route of target compound **HM-1**.

Reagents and conditions: (i) 3,5-dimethyl-4-hydroxybenzonitrile, DMF, K_2CO_3 , rt; (ii) 4-(tert-butoxycarbonyl)aminopiperidine, DMF, K_2CO_3 , $120^\circ C$; (iii) TFA, DCM, rt; (iv) 4-bromomethylbenzenesulfonamide, DMF, K_2CO_3 , rt; (v) Propionic anhydride: Propionic acid = 1:4 (V/V), $120^\circ C$, 2h.

Figure 6

Scheme 1. Synthetic route of target compound **HM-1**.

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