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Synthesis and biological evaluation of 3-phenyl-3-aryl carboxamido propanoic acid derivatives as small molecule inhibitors of retinoic acid 4-hydroxylase (CYP26A1)

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ABSTRACT

All-*trans*-retinoic acid (ATRA), the biologically active metabolite of vitamin A, is used medicinally for the treatment of hyperproliferative diseases and cancers. However, it is easily metabolized. In this study, the leading compound **S8** was found based on virtual screening. To improve the activity of the leading compound **S8**, a series of novel **S8** derivatives were designed, synthesized and evaluated for their in vitro biological activities.

All of the prepared compounds showed that substituting the 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole group for the 2-*tert*butyl-5-methylfuran scaffold led to a clear increase in the biological activity. The most promising compound **32**, with a CYP26A1 IC_{50} value of 1.36 μM (compared to liarozole (IC_{50} = 2.45 μM) and **S8** (IC_{50} = 3.21 μM)) displayed strong inhibitory and differentiation activity against *HL60* cells. In addition, the study focused on the effect of β-phenylalanine, which forms the coordination bond with the heme of CYP26A1. These studies suggest that the compound **32** can be used as an appropriate candidate for future development.

1. Introduction

All-*trans*-retinoic acid (ATRA), a natural metabolite of vitamin A, is present in mammalian tissues and plays a crucial role in the regulation of cellular differentiation, proliferation and gene expression.[1–3] A large number of clinical studies have proven that ATRA can be used as a valuable drug in treating skin related diseases as well as in tumor therapy; which is especially true in against promyelocytic leukemia (APL), changing the prognosis of APL from a fatal leukemia to a highly curable disease.[4–7] Although ATRA and retinoid compounds can be useful in treating tumors and skin-related diseases, clinical uses of ATRA have been significantly hampered by the emergence of drug resistance; since ATRA is very easily metabolized into 4-hydroxyl-RA by CYP26A1, an inducible cytochrome P450 enzyme, and eventually into more polar metabolites.[8–12] CYP26A1 is usually not expressed in non-ATRA treated cells.[13] Using *HL60* or breast cancer cells to observe ATRA-induced differentiation and growth inhibition, CYP26A1 is rapidly induced by ATRA at pharmacological concentrations.[14–16] Therefore, the highly inducible enzyme CYP26A1 has been considered as a primary reason for ATRA resistance and disease relapse of APL; and it is a feasible strategy to find an efficient inhibitor of CYP26A1 to cure cancer, skin, neurodegenerative and autoimmune diseases. In the past few years, there has been significant success in the inhibition of the ATRA metabolism.[17–21] For example, there has been an emergence of ATRA metabolism blocking agents, such as liarozole, talarozole, ketoconazole and tetralone1 (Fig. 1). Some of these blocking agents have been evaluated in clinical trials and have shown some encouraging preclinical and clinical results. However, those aromatase inhibitors have been discontinued because of adverse side effects attributed to their high toxicity, lacks of specificity, poor solubility and drug resistance.[22–24] Research on CYP26A1 inhibition is slow, with only a few inhibitors currently available; thus, it is necessary to perform other studies on CYP26A1 inhibitors from design to synthesis.

Recently, we have constructed a 3D structure of CYP26A1 through a threading method using Sybyl/GeneFold with P450BM-3 (PDB code 1BU7) as a template.[25,26] Based on this constructed model, the leading compound 3-phenyl-2-(5-*tert*butyl-2-methylfuran-3-carboxamido) propanoic acid (**S8**) was found as a potential CYP26A1 inhibitor by virtual screening of a 3D database based on

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0968-0896/

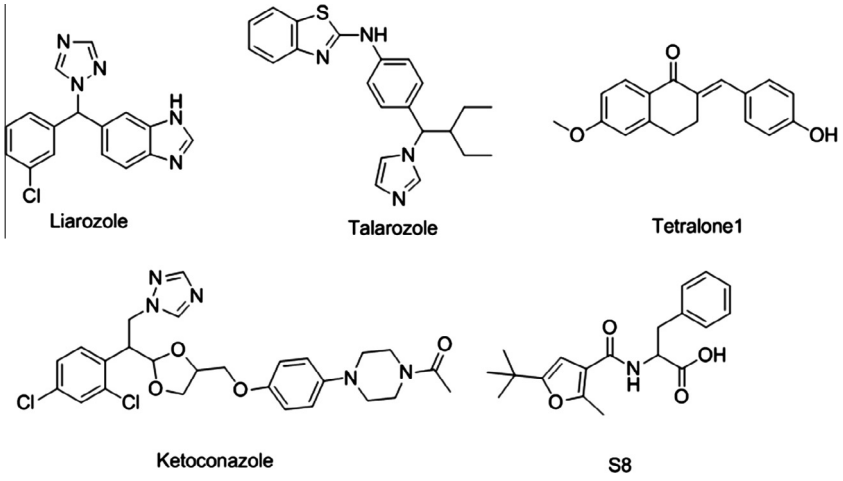


Figure 1. Structures of **S8** and other CYP26A1 inhibitors.

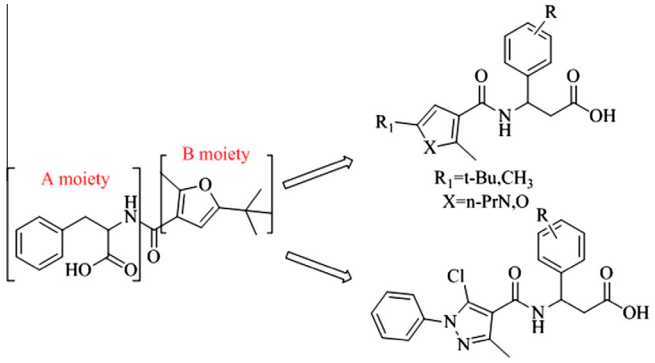


Figure 2. General structure of the target compounds **S8** derivatives.

the complex structure of CYP26A1 (Fig. 1).[27] The leading compound **S8** has two clear structural characteristics in its linker between moiety A and moiety B. Part A is phenylalanine, containing a carboxyl group that could coordinate to the iron atom of heme in the active site of CYP26A1 and play a major role in the binding affinity. Part B is a 2,5-substituted furan scaffold that occupies the hydrophobic cavity of the active site. In this study, we attempted to modify the structure of **S8** to improve the potency and selectivity, including a furan scaffold modification and a variation of the spacer linking core. Additionally, various R_1 substituted phenyl rings were introduced to the 3-position of the phenylalanine moiety to investigate their effects on activity, while optimizing the drug-like properties of these compounds (Fig. 2).

2. Chemistry

The synthetic routes of the key intermediates 3 or 5 are illustrated in Scheme 1. Starting from the commercially available starting material, ethyl acetoacetate was treated with Na in the presence of anhydrous ethanol to provide the ethyl 3-oxobutanoate sodium salt; the salt was converted to compound **1** using 1-chloropinacolone or chloroacetone as the nucleophilic reagent and sodium iodide catalyzed conditions for 11 h (75% yield).[28] Next, a ring closure under Paal–Knorr conditions using 5 M H_2SO_4 or *n*-propylamine provided compounds **2** or **4**; then, the key intermediate acids **3** or **5** were produced in 2 M sodium hydroxide solution at 60 °C for 2 h, respectively.[29]

Scheme 2 shows the synthesis of the key intermediate 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid. The starting material, phenylhydrazine was treated with ethyl acetoacetate in the presence of anhydrous ethanol to obtain compound **6** (5-methyl-2-phenyl-2, 4-dihydro-pyrazol-3-one); compound **6** was refluxed with excess phosphorus oxychloride in *N*,*N*-dimethylformamide and provided compound **7**. Compound **7** was oxidized with potassium permanganate to obtain the purified intermediate (compound **8**).[30]

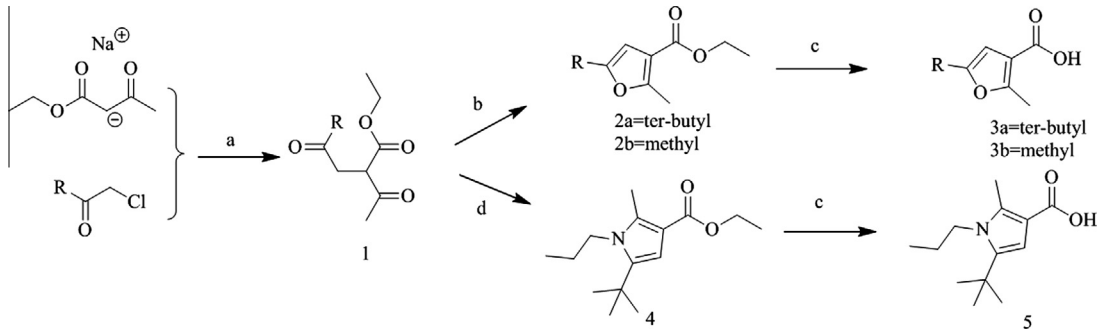
The synthesis of target compounds **15**–**34** are summarized in Scheme 3. Various benzaldehydes were separately treated with acetic acid ammonium salt and malonic acid in refluxing ethanol to produce the white solid (compound **9**);[31] chlorination of compound **9** with dichlorosulfoxide ($SOCl_2$) in methyl alcohol at room temperature for 6 h produced compound **10**, which was converted to compounds **11**, **12**, **13** or **14** with catalytic amounts of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and *N*-hydroxybenzotriazole (HOBt) under basic conditions.[32] Next, the substitution of compounds in the presence of 2 M sodium hydroxide in an ethanol/water solution provided acid **14** in good yield (73%), after purification by recrystallization with a methanol/water system to obtain the target compounds.[33]

3. Biology

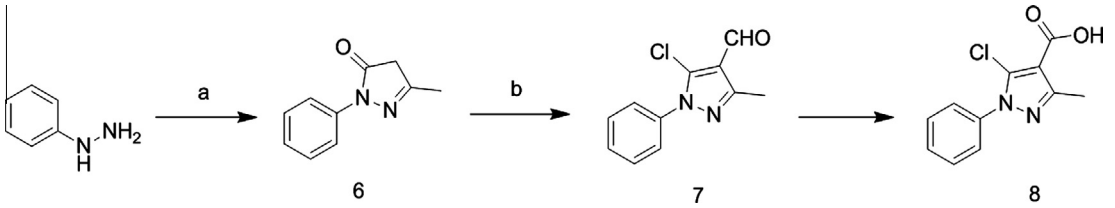
3.1. CYP26A1 enzyme inhibition assay

The CYP26A1 enzyme inhibitory activity was evaluated using a cell-free microsomal assay as previously described.[34] *HL-60* cells were cultured in RPMI 1640 medium, ATRA was added to the culture medium, then *HL-60* cells were incubated for 12 h before cell harvest. Cells were washed twice with PBS and scraped from plates. Cells were pelleted and resuspended in homogenization buffer (0.1 M Tris–Cl, pH 7.4, 0.1 M DTT, 0.2 mM EDTA, 1.15% w/v KCl, 0.1 mM PMSF and 20% v/v glycerol). Microsomes were prepared by differential centrifugation of homogenized cells. Homogenate was spun at 17,000×*g* and the supernatant spun again at 100,000×*g*. The pellet was resuspended in 25 mM potassium phosphate, pH 7.4, 20% v/v glycerol and stored at −80 °C.

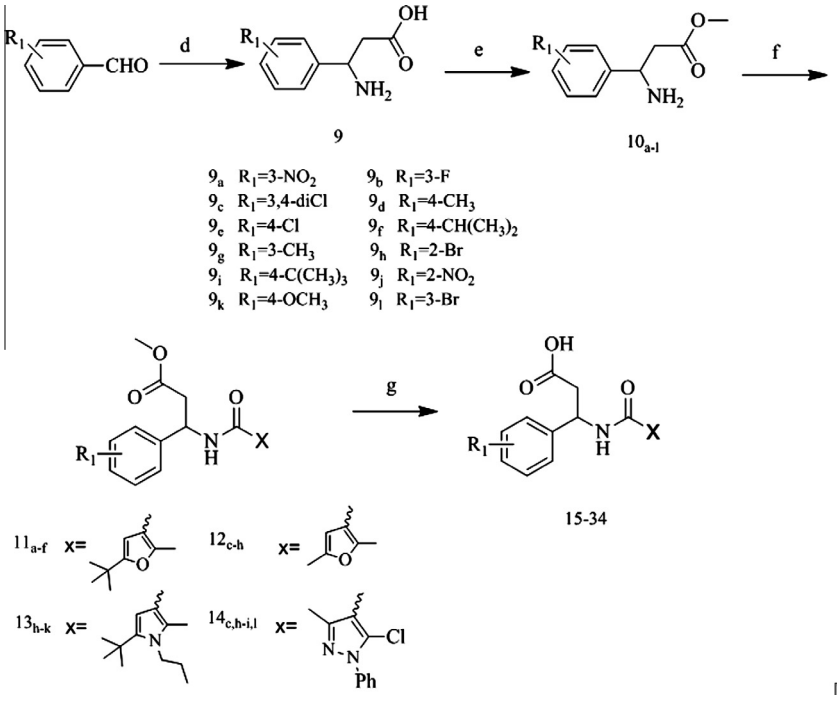
Enzymatic assays (HPLC biochemical CYP26A1 assay) were performed in a total volume of 100 μL in a reaction mixture composed of 100 mM Tris pH 7.4, 150 mM KCl, 10 mM $MgCl_2$, 2 mM NADPH, 50 nM ATRA, and varying concentrations of novel compound dissolved in ethanol. The final concentration of ethanol in all



Scheme 1. Reagents and conditions: (a) 1-chloropinacolone or chloroacetone, NaI, CH_3COCH_3, reflux, 11 h; (b) 5 M H_2SO_4 or *n*-propylamine, CH_3OH, reflux, 2 h; (c) NaOH, CH_3CH_2OH, reflux, 2 h.



Scheme 2. Reagents and conditions: (a) ethyl acetoacetate, anhydrous ethanol, reflux, 7 h; (b) $POCl_3$, DMF, reflux, 2 h; (c) $KMnO_4$, H_2O, 90-95 °C, 4 h.



Scheme 3. Reagents and conditions: (d) $CH_2(COOH)_2$, CH_3COONH_4, CH_3CH_2OH, reflux, 2 d; (e) $SOCl_2$, CH_3OH, reflux, 6 h; (f) EDCI, HOBt, DIEA, DMF, 2 h; (g) NaOH, MeOH, 6 h.

experiments never exceeded 0.8%. The reactions were incubated at 37 °C for 30 min in the dark. The reaction was quenched with acetonitrile, mixed and spun at 10,000×*g* for 10 min. The supernatant was removed and ATRA and metabolites were separated on a C18 Waters Spherisorb column with an in-line radiometric detector with a flow rate of 1 mL/min and detected at 350 nM. The gradient used was the mixture of 60 mM ammonium acetate, pH 5.2/30% CH_3OH, solvent A and solvent B (CH_3OH). An increasing CH_3OH gradient was run for the separation of products by increasing solvent B.

3.2. Cell growth inhibition assay

Growth inhibitory activities were evaluated on the CYP26A1 high expression *HL-60* (acute myeloid leukemia) cell line. The effects of the compounds on cell viability were evaluated using the MTT assay. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1–104 cells/well. The cells were incubated for 24 h at 37 °C. The cells in the wells were treated with target compounds at the concentration (10 μM) for 48 h. Then, 50 μL MTT (2 mg/mL) was added to each

well and incubated for 4 h at 37 °C. After the supernatant was discarded, 150 μL DMSO was added to each well, and the absorbance values were determined using a microplate reader (Bio-Rad Instruments) at 570 nm.

3.3. Cell induced differentiation assay

The induced differentiation activities of the compounds were evaluated using the CYP26A1 high expression *HL60* cell line in vitro with liarozole and **S8** as the positive control. The cancer cell lines were cultured in standard RPMI-1640 media supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere. Approximately 5×10^5 cells were plated in 24-well plates and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds were added to the culture medium at the indicated final concentrations, and the cell cultures were continued for 72 h. Next, the chromogenic agent (300 μL NBT and 15 μL PMC) was added to each well, and the percent cellular differentiation was calculated as [Np/(Np + Nn)] × 100. Np represents the cell number with the NBT positive results, and Nn represents the cell number with the NBT negative results. All experiments were repeated at least three times.

4. Results and discussion

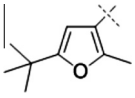
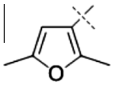
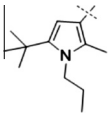
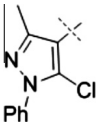
4.1. CYP26A1 enzyme inhibition assay

All the newly synthesized 3-phenyl-2-(5-*tert*butyl-2-methylfuran-3-carboxamido) propanoic acid derivatives were evaluated for their inhibitory activity toward CYP26A1 enzyme using a cell-free microsomal assay as previously described,[34] with all-*trans* retinoic acid as the substrate. The liarozole and compound **S8** were included in all experiments as comparative standards. The results expressed as IC_{50} were summarized in Table 1.

As illustrated in Table 1, all the tested compounds displayed CYP26A1 enzymatic activity with IC_{50} values ranging from 1.36 to 5.19 μM. Compared with liarozole (IC_{50} = 2.45 μM) and compound **S8** (IC_{50} = 3.21 μM), six of them (**17**, **21**, **26**, **31**, **32**, **34**) exhibited equivalent or higher potency with IC_{50} values, which indicated that replacement of the 5-*tert*butyl-2-methylfuran fragment with 2,5-dimethylfuran, 5-*tert*butyl-2-methyl-1-propyl-1*H*-pyrrole or 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole fragment maintained the CYP26A1 inhibitory efficacy. In addition, the activity of compounds **31**, **32**, **34** have the appropriate improvement than compounds **15**–**30**, suggesting that the introduction of a 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole group led to an obvious increase in efficiency.

Further analysis revealed that the introduction of different R_1 groups also had some influence on activity. Introduction of electron-withdrawing groups (EWGs) in phenyl increased inhibitory activity compared with the electron-donating groups (EDGs) derivatives. For example, compare the **S8**, the introduction of double double-EWGs R_1 = 3, 4-diCl (**17**, IC_{50} = 2.62 μM; **21**, IC_{50} = 2.83 - μM; **31**, IC_{50} = 2.07 μM) or mono-EWGs R_1 = 4-Cl (**19**, IC_{50}=3.02 μM; **23**, IC_{50} = 2.73 μM) led to an moderate improvement in activity. However, the introduction of EDGs (**16**, R_1 = 4-CH_3, IC_{50} = 3.26 μM; **20**, R_1 = 4-$CH(CH_3)_2$, IC_{50} = 5.07 μM; **24**, R_1 = 4-$CH(CH_3)_2$, IC_{50} = 4.02 μM; **28**, R_1 = 4-$CH(CH_3)_2$, IC_{50} = 5.19 μM; **30**, R_1 = 4-OCH_3, IC_{50} = 3.48 μM; **33**, R_1 = 4-$CH(CH_3)_2$, IC_{50} = 3.22 μM) caused the potency reduce. Moreover, it was worth noting that the compounds **32** (IC_{50} = 1.36 μM) with 2-Br substituted phenyl group derivatives displayed higher potency than those compounds.

Table 1
CYP26A1 enzymatic activity of the **S8** derivatives **15**–**34**

Compd	X	R_1	IC_{50} (μM) CYP26A1
15		3-NO_2	3.04
16		4-CH_3	3.26
17		3,4-DiCl	2.62
18		3-F	2.81
19		4-Cl	3.02
20		4-$CH(CH_3)_2$	5.07
21		3,4-DiCl	2.83
22		4-CH_3	3.09
23		4-Cl	2.73
24		4-$CH(CH_3)_2$	4.02
25		3-CH_3	3.14
26		2-Br	2.34
27		2-Br	3.13
28		4-$C(CH_3)_3$	5.19
29		2-NO_2	3.35
30		4-OCH_3	3.48
31		3,4-DiCl	2.07
32		2-Br	1.36
33		4-$C(CH_3)_3$	3.22
34		3-Br	1.87
S8[a]			3.21
Liarozole[a]			2.45

[a] Used as positive control.

4.2. In vitro cell growth inhibition assay

All the newly synthesized 3-phenyl-2-(5-*tert*butyl-2-methylfuran-3-carboxamido) propanoic acid derivatives were evaluated for their inhibitory activity toward the CYP26A1 high expression *HL60* cells line using a thiazolyl blue tetrazolium bromide (MTT) assay. Liarozole and leading compound **S8** were used as positive controls, and the all-*trans*-retinoic acid was used as the substrate. The results expressed as percent growth inhibition are summarized in Table 2. The percent growth inhibition is the average of at least three independent experiments.

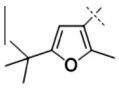
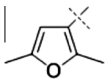
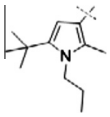
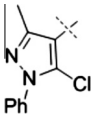
The data listed in Table 2 revealed that compounds **15**–**34** possessed moderate abilities enhance the cytotoxicity of ATRA against the tested cell line *HL60*. A number of tested compounds showed remarkable effects on the anticancer activity with percent growth inhibition ranging from 31.20 ± 3.29 to 59.72 ± 7.19. Among these compounds, **15**–**30** did not significantly increase the growth inhibition of *HL60* cells. Whereas the activity of compounds **31**, **32**, **34** have the appropriate improvement than the leading compound **S8**, and they have similar trends with CYP26A1 enzyme inhibition results, especially the compound **32** (IC_{50} = 1.36 μM) with percent growth inhibition 59.72 ± 7.19.

4.3. In vitro cell induced differentiation assay

The differentiation induction abilities of the compounds combined with ATRA were determined in human *HL60* cells. It has been reported that ATRA-induced differentiation of *HL60* cells is dose-dependent. We selected a low dose of ATRA (0.1 μM), which has less differentiation induction ability, but did induce the expression of CYP26A1. Liarozole and leading compound **S8** were used as the positive control group. Furthermore, some potent compounds were further evaluated for their differentiation induction abilities. The results are summarized in Table 3. The values are the average of at least three independent experiments.

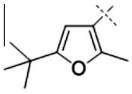
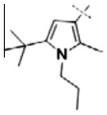
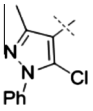
The data listed in Table 3 revealed the same trend with the inhibitory activity study. Compounds **27**–**30** had a slight influence on differentiation activity, and compounds **15**, **18**, **19**, **21**, **22**, **23**, **26** only moderate increased to induce differentiation of *HL60* cells. The promising compounds **31**, **32**, and **34** were more active than liarozole and leading compound **S8** with percent cellular differen-

Table 2
Growth inhibitory effects of **S8** derivatives in combination with ATRA in *HL60* cells

Compd	X	R_1	0.1 μM ATRA	10 μM inhibitor	10 μM inhibitor +0.1 μM ATRA
ATRA			23.76 ± 0.49		
15		3-NO_2		6.47 ± 3.19	45.52 ± 3.87
16		4-CH_3		7.15 ± 3.16	38.63 ± 4.16
17		3,4-DiCl		7.26 ± 2.61	46.28 ± 3.68
18		3-F		8.15 ± 3.10	44.83 ± 4.84
19		4-Cl		5.62 ± 1.83	41.12 ± 1.36
20		4-$CH(CH_3)_2$		8.15 ± 2.40	34.56 ± 4.50
21		3,4-DiCl		6.69 ± 1.31	47.27 ± 5.96
22		4-CH_3		5.85 ± 1.49	44.85 ± 7.17
23		4-Cl		10.35 ± 3.82	49.53 ± 2.60
24		4-$CH(CH_3)_2$		6.19 ± 1.68	37.41 ± 1.61
25		3-CH_3		8.82 ± 1.37	44.25 ± 3.83
26		2-Br		11.45 ± 2.51	51.14 ± 3.78
27		2-Br		9.15 ± 1.40	46.63 ± 7.33
28		4-$C(CH_3)_3$		8.56 ± 2.78	31.20 ± 3.29
29		2-NO_2		5.51 ± 1.32	47.60 ± 5.36
30		4-OCH_3		7.21 ± 1.19	41.39 ± 2.55
31		3,4-DiCl		10.54 ± 2.41	54.38 ± 3.30
32		2-Br		7.16 ± 1.39	59.72 ± 7.19
33		4-$C(CH_3)_3$		9.56 ± 2.42	39.92 ± 2.11
34		3-Br		6.16 ± 2.19	56.15 ± 8.68
S8[a]				6.26 ± 1.48	35.58 ± 5.22
Liarozole[a]				15.41 ± 3.53	48.26 ± 3.36

[a] Used as positive control.

Table 3
Differentiation induction of **S8** derivatives in combination with ATRA in *HL60* cells

Compd	X	R_1	0.1 μM ATRA	10 μM inhibitor	10 μM inhibitor +0.1 μM ATRA
ATRA			26.07 ± 0.59		
15		3-NO_2		0	43.40 ± 4.61
16		4-CH_3		0	37.41 ± 3.60
17		3,4-diCl		0	39.19 ± 2.71
18		3-F		0	42.16 ± 5.37
19		4-Cl		0	45.31 ± 1.70
20		4-$CH(CH_3)_2$		0	35.12 ± 4.32
21		3,4-diCl		0	45.57 ± 2.14
22		4-CH_3		0	42.62 ± 1.61
23		4-Cl		0	46.61 ± 3.64
24		4-$CH(CH_3)_2$		0	36.42 ± 0.65
25		3-CH_3		0	41.47 ± 6.26
26		2-Br		0	48.69 ± 1.54
27		2-Br		0	41.36 ± 2.57
28		4-$C(CH_3)_3$		0	35.47 ± 4.30
29		2-NO_2		0	38.51 ± 6.18
30		4-OCH_3		0	40.73 ± 5.26
31		3,4-diCl		0	49.31 ± 3.39
32		2-Br		0	51.61 ± 2.43
33		4-$C(CH_3)_3$		0	42.57 ± 4.72
34		3-Br		0	48.51 ± 2.93
S8[a]				0	41.27 ± 1.73
Liarozole[a]				0	46.71 ± 2.58

[a] Used as positive control.

tiation of 49.31 ± 3.39, 51.61 ± 2.43 and 48.51 ± 2.93, respectively. The study of structure–activity relationships (SARs) indicated that these analogs showed similar SARs; the analyzed data showed the following: (A) compounds with the same R_1 but different X groups showed marked differences in activity, while compounds with the same X but different R_1 exhibited equivalent activity; (B) compounds bearing EWGs were generally more active than those with EDGs; (C) compounds large volume EDGs were lower than those with substituted groups.

4.4. The study of prediction pharmacokinetics characteristics

Promising compound **32** were further examined to determine if they had appropriate pharmacokinetic characteristics. Molecular modeling was performed on liarozole, **S8** and **32** to predict their pharmacokinetics (ADMET) activities (Table 4). Five characteristics, including the aqueous solubility, blood brain barrier penetration (BBB), cytochrome P450 2D6 inhibition, hepatotoxic, and intestinal absorption were measured. The data listed in Table 4 revealed **32**

Table 4
The pharmacokinetics (ADMET) of liarozole, **S8** and **32**

ADMET	Solubility	BBB	EXT-CYP2D6	EXT-hepatotoxic	Absorption-level
Liarozole	−4.991	0.186	0.752	0.927	−4.991
S8	−4.053	−0.35	0.376	0.589	−4.053
32	−5.026	−0.23	0.356	0.688	−5.078

1. Log(Sw) < −8.0, extremely low; 2. −8.0 < log(Sw) < −6.0, very low; 3. −6.0 < log(Sw) < −4.0, low; 4. −4.0 < log(Sw) < −2.0, moderate; 5. −2.0 < log(Sw) < 0, optimal; 6.0 < log(Sw), too high.

(BBB = −0.23; EXT-CYP2D6 = 0.356; EXT-Hepatotoxic = 0.688) demonstrated stable BBB level, less Hepatotoxic and CYP2D6 inhibition. However, it was worth noting the compound **32** (solubility = −5.026; absorption-level = −5.078) with low solubility and absorption-level, which suggest that we further introduce hydrophilic group to improve bioavailability.

5. Molecular docking model analysis of compound 32 in CYP26A1

In previous studies, the docking result of **S8** revealed that the phenylalanine group is an essential moiety for their potent inhibition of CYP26A1. To further elucidate the underlying structural reasons for the high affinity of promising compound **32** with CYP26A1, a detail docking analysis was performed within the active site of the CYP26A1 homology model, following the previously reported methodology.[25] The conformations were conducted using Auto Dock 4.2 because of its powerful conformational search capabilities. The image files were generated using the Accelrys DS visualizer 4.0 systems. The docking model was exemplified by the interaction of compound **32** with CYP26A1. As shown in Figure 3A and B, compound **32** occupied the substrate binding site of CYP26A1, in which the oxygen atoms of the phenyl carboxylic moiety formed a coordination bond interaction with the Fe^{3+} of the heme. Furthermore, the chlorine atoms of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole moiety in compound **32** generated one hydrogen-bond with the important residue Pro332 of CYP26A1, and the 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole scaffold of compound **32** was surrounded by hydrophobic residues (Trp73, Leu81, Phe183, Pro332, Phe260, Pro332 and Pro439), and the aromatic ring moieties formed pi–pi contacts stacking interaction with residues Phe183, Trp73 and Phe260, which may have accounted for the higher inhibitory potency for CYP26A1 compared to other derivatives. Furthermore, the 2-bromine atoms of phenyl formed two additional hydrogen-bond interaction with the residue Trp73 and Leu81.

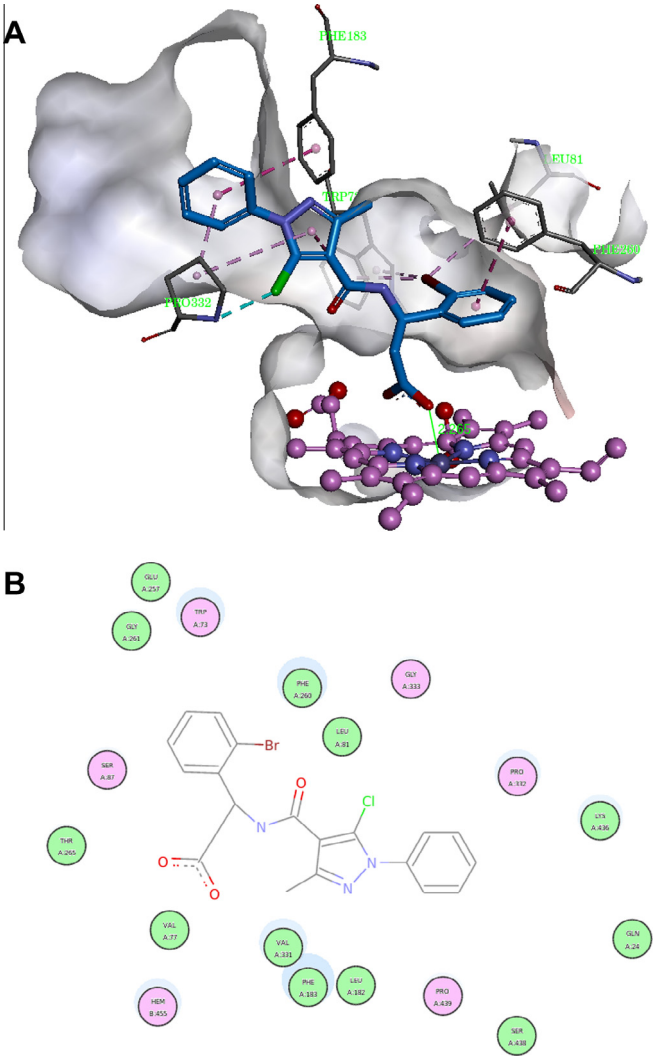


Figure 3. The proposed binding mode of compound **32** and CYP26A1. (A) Binding positions of compound **32** with CYP26A1. (B) LIGPLOT diagram depicting interactions between compound **32** and the binding site residues of CYP26A1.

6. Conclusions

In this study, a series of 3-phenyl-2-(5-*tert*butyl-2-methylfuran-3-carboxamido) propanoic acid derivatives (**15**–**34**) were designed, synthesized and evaluated for their in vitro biological activities with CYP26A1 high expression *HL60* cells. The preliminary investigation showed that most compounds displayed good to excellent potency against the *HL60* cell line. The most promising compound **32**, with a CYP26A1 IC_{50} of 1.36 μM (compared to liarozole (IC_{50} = 2.45 μM) and **S8** (IC_{50} = 3.21 μM)) was further confirmed as a CYP26A1 inhibitor using CYP26A1 inhibition assays. The analysis of SARs indicated that compounds with the 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole group on the B moiety were more active than those with other substituents. Conversely, the introduction of a bromine atom to the 2-position of the phenyl ring on the A moiety led to a sharp increase in activity. In addition, the pharmacokinetics (ADMET) activity of compound **32** was examined and showed appropriate pharmacokinetic characteristics. Thus, the compounds containing 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole group moieties, especially compound **32**, can be considered novel leading compounds to further optimize.

7. Experimental

Commercial reagents and solvents were used without additional purification. The reaction progression was determined by thin layer chromatography (TLC) on Silica Gel 60 F254 plates (Jiangyou, Yantai). Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong,

China). The mass spectra (MS) were taken in ESI mode on an Agilent 1200 LC–MS (Agilent, Palo Alto, CA, USA). All melting points were determined on a Büchi Melting Point B-540 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DPX 300, 300 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. The chemical shifts (d) were reported in ppm relative to internal tetramethylsilane. Peak multiplicities were expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; dt, doublet of triplet; td, triplet of doublet; ddd, doublet of doublet of doublet; m, multiplet; br s, broad.

7.1. Ethyl 2-acetyl-5, 5-dimethyl-4-oxohexanoate (1a) and ethyl 2-acetyl-4-oxopentanoate (1b)

Ethyl 3-oxobutanoate sodium salt (2.38 g, 15.6 mmol) and sodium iodide were dissolved in acetone. Subsequently, 1-chloropinacolone (2.04 ml, 15.6 mmol) or chloroacetone (1.24 ml, 15.6 mmol) was added dropwise at a rate to maintain the reaction temperature below 25 °C. Upon the completion of addition, the reaction mixture was allowed to warm up to 56 °C and stirred for 11 h, after which the solvent was removed under reduced pressure, the residue was poured slowly into the mixed solution of water (200 mL) and dichloromethane (100 mL). The organic layer was separated and washed with brine (50 mL × 3), dried with anhydrous sodium sulfate and evaporated. The residue was cooled and dried at room temperature to give light yellow oil, yield: 86.3%.

7.2. Ethyl 5-(*tert*Butyl)-2-methylfuran-3-carboxylate (2a) and ethyl 2,5-dimethylfuran-3-carboxylate (2b)

A stirred solution of ethyl 2-acetyl-5,5-dimethyl-4-oxohexanoate **1a** (5 g, 21.93 mmol) or ethyl 2-acetyl-4-oxopentanoate **1b** (5 g, 26.88 mmol) in anhydrous methanol (50 mL) was added dropwise with 5 M sulfuric acid solution (8 mL, 20 mmol), and the resulting reaction mixture was heated to 75 °C for 2 h, after the anhydrous methanol was removed under reduced pressure, the residue was poured slowly into the mixed solution of water (200 mL) and dichloromethane (100 mL). The organic layer was separated and washed with brine (50 mL × 3), dried with anhydrous sodium sulfate and evaporated. The residue was cooled and dried at room temperature to give light yellow oil, yield: 73.4%.

7.3. 5-(*tert*Butyl)-2-methylfuran-3-carboxylic acid (3a) and 2,5-dimethylfuran-3-carboxylic acid (3b)

A stirred solution of ethyl 5-*tert*butyl-2-methylfuran-3-carboxylate **2a** (2.16 g, 10.27 mmol) or ethyl 2,5-dimethylfuran-3-carboxylate **2b** (1.73 g, 10.27 mmol) in anhydrous methanol (30 mL) was added with 2 M sodium hydroxide solution (10.27 mL, 20.55 mmol) at 60 °C for 2 h, after the anhydrous methanol was removed under reduced pressure, adjusted to pH 1. A lot of precipitated white solid was filtered and dried in vacuo at 40 °C for 24 h to give a light white solid. **3a** (1.57 g, yield: 83.9%), mp: 88.2–91.5 °C; MS (ESI) *m*/*z* (%): 180.8 $[M-H]^-$; **3b** (1.32 g, yield: 92.0%); mp: 136.0–138.4 °C; MS (ESI) *m*/*z* (%): 138.8 $[M-H]^-$. ^{1}H NMR (DMSO-d_6, 300 MH): δ 2.18 (s, 3H), 2.43 (s, 3H), 6.18 (s, 1H), 12.34 (s, 1H).

7.4. Ethyl 5-*tert*Butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carboxylate (4)

A stirred solution of ethyl 2-acetyl-5,5-dimethyl-4-oxohexanoate **1a** (4.56 g, 20 mmol) in anhydrous methanol (50 mL) was added dropwise with propylamine solution (1.65 mL, 20 mmol), and the resulting reaction mixture was heated to 75 °C for 3 h, after the anhydrous methanol was removed under reduced pressure, the residue was poured slowly into the mixed solution of water (30 mL) and ethyl acetate (30 mL). The organic layer was separated and washed with brine (30 mL × 3), dried with anhydrous sodium sulfate and evaporated. The residue was cooled and dried at room temperature to give a brown viscous material (3.26 g, yield: 65.0%).

7.5. 5-*tert*Butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carboxylic acid (5)

A stirred solution of ethyl 5-*tert*butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carboxylate **4** (1.71 g, 6.8 mmol) in anhydrous methanol (20 mL) was added with 2 M sodium hydroxide solution (13.60 ml, 27.30 mmol) at 60 °C for 2 h, after the anhydrous methanol was removed under reduced pressure, adjusted to pH 1. A lot of precipitated white solid was filtered and dried in vacuo at 40 °C for 24 h to give a light white solid (1.35 g, yield: 89.0%). Mp: 185.0–187.8 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90–0.95 (t, 3H), 1.27 (s, 9H), 1.52–1.60 (m, 2H), 2.41 (s, 3H), 3.82–3.87 (m, 3H), 6.40 (s, 1H), 11.41 (s, 1H); ESI-MS *m*/*z*: 224.2 $[M+H]^+$.

7.6. 3-Methyl-1-phenyl-1*H*-pyrazol-5(*4H*)-one (6)

A stirred solution of phenylhydrazine (21.6 g, 200 mmol) in anhydrous ethanol (30 mL) was added with ethyl acetoacetate (26.0 g, 200 mmol), and the resulting reaction mixture was heated to 75 °C for 5 h, then the anhydrous ethanol was removed under reduced pressure, final yellow solid was obtained (29.3 g, yield 85.0%). Mp: 128.0–130.3 °C; ESI-MS *m*/*z*: 175.1 $[M+H]^+$; 197.1 $[M+Na]^+$; 172.9 $[M-H]^-$.

7.7. 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (7)

Phosphorus oxychloride (64 mL, 700 mmol) was added dropwise at a rate to a cold solution (0–5 °C) of *N*,*N*-dimethylformamide (23 ml, 300 mmol). After 30 min, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*) one **6** (17.48 g, 100 mmol) was added. The resulting mixture was allowed to heat to 120 °C for 1 h. The reaction mixture was poured slowly into the mixed solution of cold water (600 mL), a lot of precipitated solid was filtered and dried in vacuo at 40 °C for 24 h to give a light yellow solid (19.0 g, yield: 86.4%); mp: 139.0–140.8 °C; ESI-MS *m*/*z*: 221.1 $[M+H]^+$; 243.1 $[M+Na]^+$.

7.8. 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (8)

5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **7** (16.85 g, 76.4 mmol) and potassium permanganate was added to the solution of water (300 mL) and heated to 90–95 °C for 4 h. The reaction mixture was filtered, adjusted to pH 1. A lot of precipitated white solid was filtered and dried in vacuo at 40 °C for 24 h to give a white solid (16.6 g, yield: 92.2%); mp: 232.1–234.7 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.41 (s, 3H), 7.56 (s, 5H), 12.88 (s, 1H); ESI-MS *m*/*z*: 237.1 $[M+H]^+$; 259.1 $[M+Na]^+$.

7.9. General procedure for preparation of β phenylalanine (9a–9l)

Benzaldehyde (1 equiv), propanedioic acid (2 equiv) and ammonium acetate (2 equiv) were added to the solution of anhydrous ethanol (30 mL) and heated to 78 °C for 17 h. A lot of precipitated solid was filtered and dried in vacuo at 40 °C for 24 h to give a light white solid.

7.10. General procedure for preparation of β phenylalanine methyl ester hydrochloride (10_a–10_l)

Thionylchloride (1 equiv) was added dropwise to the solution of anhydrous methanol at a rate to maintain the temperature below 5 °C. After 30 min, β phenylalanine $\mathbf{9_a}$–$\mathbf{9_l}$ (3 equiv) was added in the reaction mixture at 65 °C for 6 h, respectively. Then the anhydrous methanol was removed under reduced pressure, final white solid was obtained.

7.11. General procedure for preparation of compounds (11_a–14_l)

EDCI (1.1 equiv) and HOBt (1.1 equiv) were added to a solution of appropriate organic acids **3a**, **3b**, **5 and 8** (1 equiv) in anhydrous DMF, respectively. The mixture was stirred at room temperature for 2 h, then β phenylalanine methyl ester hydrochloride ($\mathbf{10_a}$–$\mathbf{10_l}$) (1.1 equiv) and DIEA (4 equiv) were added, and the mixture was heated at 30 °C for 2 h. The reaction mixture was poured into ice water, filtered and produced a white solid.

7.12. General procedure for preparation of compounds (15–34)

A stirred solution of compounds $\mathbf{11_a}$–$\mathbf{14_l}$ (1 equiv) in anhydrous methanol was added with 2 M sodium hydroxide solution (5 equiv) at 60 °C for 2 h, after the anhydrous methanol was removed under reduced pressure, adjusted to pH 1. A lot of precipitated white solid was filtered and dried in vacuo at 40 °C for 24 h to give a light white solid. The white solid was purified using $MeOH/H_2O$ recrystallization.

7.12.1. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-(3-nitrophenyl) propionic acid (15)

Light white solid; yield: 53.2%; mp: 223.9–226.7 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.23 (s, 9H), 2.42 (s, 3H), 2.75–2.91 (m, 2H), 5.39–5.43 (q, 1H), 6.48 (s, 1H), 7.60–7.65 (t, 1H), 7.83 (d, 1H, J = 7.56 Hz), 8.10 (d, 1H, J = 7.47 Hz), 8.23 (s, 1H), 8.49 (d, 1H, J = 7.95 Hz); ^{13}C NMR (DMSO): 13.68 (CH_3), 29.13 ($3 \times CH_3$), 32.45 (C), 40.76 (CH_2), 49.57 (CH), 101.91 (ArCH), 115.91 (ArC), 121.72 (ArCH), 122.46 (ArCH), 130.33 (ArCH), 134.18 (ArCH), 145.89 (ArC), 148.29 (ArC), 154.85 (ArC), 161.36 (ArC), 162.91 (C, C=O), 172.21 (C, C=O). ESI-MS m/z: 373.0 $[M-H]^-$.

7.12.2. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-*p*-tolyl propionic acid (16)

Light white solid; yield: 56.3%; mp: 261.9–263.0 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.22 (s, 9H), 2.25 (s, 3H), 2.41 (s, 3H), 2.64–2.83 (m, 2H), 5.27–5.35 (q, 1H), 6.49 (s, 1H), 7.10 (d, 2H, J = 7.77 Hz), 7.22 (d, 2H, J = 7.77 Hz), 8.22 (d, 1H, J = 8.31 Hz), 12.25 (s,1H); ^{13}C NMR (DMSO): 13.68 (CH_3), 21.09 (CH_3), 29.09 ($3 \times CH_3$), 32.43 (C), 41.18 (CH_2), 49.45 (CH), 102.01 (ArCH), 116.19 ($ArCH_2$), 126.94 ($2 \times$ ArCH), 129.21 ($2 \times$ ArCH), 136.37 (ArC), 140.48 (ArC), 154.54 (ArC), 161.18 (ArC), 162.62 (C, C=O), 172.47 (C, C=O). ESI-MS m/z: 342.0 $[M-H]^-$.

7.12.3. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-(3,4-dichlorophenyl) propionic acid (17)

Light white solid; yield: 53.6%; mp: 207.8–211.7 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.21 (s, 9H), 2.42 (s, 3H), 2.63–2.80 (m, 2H), 5.23–5.30 (q, 1H), 6.46 (s, 1H), 7.31–7.35 (m, 1H), 7.54–7.58 (m, 2H), 7.30–7.37 (q, 1H), 8.59 (d, 1H, J = 7.53 Hz); ^{13}C NMR (DMSO): 13.68 (CH_3), 29.11 ($3 \times CH_3$), 32.43 (C), 41.26 (CH_2), 49.39 (CH), 101.91 (ArCH), 115.99 (ArC), 127.55 (ArCH), 129.09 (ArCH), 129.79 (ArCH), 130.89 (ArC), 131.23 (ArC), 145.05 (ArC), 154.72 (ArC), 161.32 (ArC), 162.79 (C, C=O), 172.60 (C, C=O). ESI-MS m/z: 396.0$[M-H]^-$.

7.12.4. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-(3-fluorophenyl) propionic acid (18)

Light white solid; yield: 57.2%; mp: 236.5–238.7 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.21 (s, 9H,), 2.42 (s, 3H), 2.62–2.80 (m, 2H), 5.33–5.35 (q, 1H), 6.49 (s, 1H), 7.01–7.06 (m, 1H), 7.14–7.20 (m, 2H), 7.30–7.37 (m, 1H), 8.48 (d, 1H, J = 7.95 Hz); ^{13}C NMR (DMSO): 13.69 (CH_3), 29.14 ($3 \times CH_3$), 32.44 (C), 41.43 (CH_2), 49.62 (CH), 101.94 (ArCH), 113.62 (ArCH), 113.84 (ArCH), 123.15 (ArC), 130.57 (ArCH), 130.57 (ArCH), 130.65 (ArCH),146.73 (ArC), 154.66 (ArC), 161.29 (ArC), 162.74 (C, C=O), 170.56 (C, C=O). ESI-MS m/z: 346.0$[M-H]^-$.

7.12.5. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-(4-chlorophenyl) propionic acid (19)

Light white solid; yield: 57.3%; mp: 264.7–266.8 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.22 (s, 9H), 2.42 (s, 3H), 2.66–2.84 (m, 2H), 5.28–5.33 (q, 1H), 6.49 (s,1H), 7.37 (s, 4H), 8.35 (d, 1H, J = 8.82 Hz); ^{13}C NMR (DMSO): 13.51 (CH_3), 29.24 ($3 \times CH_3$), 32.14 (C), 41.11 (CH_2), 49.37 (CH), 102.23 (ArCH), 114.25 (ArC), 126.37 ($2 \times$ ArCH), 127.87 ($2 \times$ ArCH), 133.32 (ArC), 146.43 (ArC), 155.15 (ArC), 161.47 (ArC), 162.57 (C, C=O), 172.18 (C, C=O). ESI-MS m/z: 362.0$[M-H]^-$.

7.12.6. 3-[(5-*tert*Butyl-2-methylfuran-3-carbonyl)amino]-3-(4-isopropylphenyl) propionic acid (20)

Light white solid; yield: 54.7%; mp: 215.4–217.0 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.17 (d, 6H, J = 6.9 Hz), 1.22 (s, 9H), 2.42 (s, 3H), 2.67–2.88 (m, 2H), 5.29–5.37 (q, 1H), 6.50 (s, 1H), 7.17 (d, 2H, J = 8.13 Hz), 8.20 (d, 1H, J = 8.34 Hz), 12.21 (s, 1H); ^{13}C NMR (DMSO): 13.69 (CH_3), 24.37 ($2 \times CH_3$), 29.16 ($3 \times CH_3$), 32.44 (C), 33.55 (CH), 40.99 (CH_2), 49.40 (CH), 102.01 (ArCH), 116.17 (ArC), 126.59 ($2 \times$ ArCH), 127.02 ($2 \times$ ArCH), 140.81 (ArC), 147.41 (ArC), 154.58 (ArC), 161.19 (ArC), 162.62 (C, C=O), 172.37 (C, C=O). ESI-MS m/z: 370.1$[M-H]^-$.

7.12.7. 3-(3,4-Dichlorophenyl)-3-[(2,5-dimethylfuran-3-carbonyl)amino] propionic acid (21)

Light white solid; yield: 54.9%; mp: 163.4–164.0 °C. ^{1}H NMR (DMSO, 300 MHz): δ 2.21 (s, 3H), 2.40 (s, 3H), 2.40 (s, 3H), 2.70–2.87 (m, 2H), 5.25–5.33 (q, 1H), 6.47 (s, 1H), 7.33–7.36 (m,1H), 7.67–7.60 (m, 2H), 8.28 (d, 1H, J = 8.1 Hz); ^{13}C NMR (DMSO): 13.42 (CH_3), 13.53 (CH_3), 40.62 (CH_2), 49.07 (CH), 105.39 (ArCH), 116.35 (ArC), 127.62 (ArCH), 129.13 (ArCH), 129.97 (ArCH), 130.98 (ArC), 131.31 (ArC), 144.63 (ArC), 149.55 (ArC), 154.91 (ArC), 162.77 (C, C=O), 171.99 (C, C=O). ESI-MS m/z: 353.7$[M-H]^-$.

7.12.8. 3-[(2,5-Dimethylfuran-3-carbonyl)amino]-3-*p*-tolyl propionic acid (22)

Light white solid; yield: 63.9%; mp: 132.1–132.9 °C. ^{1}H NMR (DMSO, 300 MHz): δ 2.21 (s, 3H), 2.25 (s, 3H), 2.40 (s, 3H), 2.65–2.84 (m, 2H), 5.30–5.35 (q, 1H), 6.48 (s, 1H), 7.10 (d, 2H, J = 7.95 Hz), 7.23 (d, 2H, J = 7.98 Hz), 8.17 (d, 1H, J = 8.4 Hz), 12.20 (s, 1H); ^{13}C NMR (DMSO): 13.43 (CH_3), 13.52 (CH_3), 21.08 (CH_3), 41.14 (CH_2), 49.43 (CH), 105.49 (ArCH), 116.68 (ArC), 126.93 ($2 \times$ ArCH), 129.22 ($2 \times$ ArCH), 136.39 (ArC), 140.43 (ArC), 149.36 (ArC), 154.60 (ArC), 162.54 (C, C=O), 172.35 (C, C=O). ESI-MS m/z: 299.8 $[M-H]^-$.

7.12.9. 3-(4-Chlorophenyl)-3-[(2,5-dimethylfuran-3-carbonyl)amino] propionic acid (23)

Light white solid; yield: 52.7%; mp: 196.6–197.2 °C. ^{1}H NMR (DMSO, 300 MHz): δ 2.21 (s, 3H), 2.40 (s, 3H), 2.40 (s, 3H), 2.70–2.86 (m, 2H), 5.28–5.35 (q,1H), 6.48 (s, 1H), 7.37 (s, 4H), 8.25 (d, 1H, J = 8.25 Hz), 12.28 (s, 1H); ^{13}C NMR (DMSO): 13.43 (CH_3), 13.52 (CH_3), 40.81 (CH_2), 49.24 (CH), 105.43 (ArCH), 116.50 (ArC), 128.67 ($2 \times$ ArCH), 128.97 ($2 \times$ ArCH), 131.92 (ArC), 142.44

(ArC), 149.45 (ArC), 154.77 (ArC),162.67 (C, C=O), 172.16 (C, C=O). ESI-MS *m*/*z*: 319.7[M−H]$^-$.

7.12.10. 3-[(2,5-Dimethylfuran-3-carbonyl)amino]-3-(4-isopropylphenyl) propionic acid (24)

Light white solid; yield: 58.6%; mp: 149.3–150.2 °C. ^{1}H NMR (DMSO, 300 MHz): δ 1.17 (d, 6H, *J* = 8.4 Hz), 2.21 (s, 3H), 2.40 (s, 3H), 2.68–2.86 (m, 2H), 5.29–5.36 (q,1H), 6.49 (s, 1H), 7.17 (d, 2H, *J* = 8.13 Hz), 7.26 (d, 2H, *J* = 8.13 Hz), 8.19 (d, 1H, *J* = 8.4 Hz), 12.21 (s, 1H); ^{13}C NMR (DMSO): 13.44 (CH_3), 13.53 (CH_3), 24.36 (2 × CH_3), 33.55 (CH), 41.07 (CH_2), 49.42 (CH), 105.48 (ArCH), 116.64 (ArC), 126.59 (2 × ArCH), 126.98 (2 × ArCH), 140.83 (ArC), 147.40 (ArC), 149.36 (ArC), 154.64 (ArC), 162.55 (C, C=O), 172.39 (C, C=O). ESI-MS *m*/*z*: 327.7 [M−H]$^-$.

7.12.11. 3-[(2,5-Dimethylfuran-3-carbonyl)amino]-3-*m*-tolyl propionic acid (25)

Light white solid; yield: 57.3%; mp: 132.1–132.9 °C. ^{1}H NMR (DMSO, 300 MHz): δ 2.21 (s, 3H), 2.28 (s, 3H), 2.40 (s, 3H), 2.65–2.84 (m, 2H), 5.30–5.32 (q, 1H), 6.50 (s, 1H), 7.02–7.19 (m, 4H), 8.19 (d, 1H, *J* = 8.34 Hz), 12.21 (s, 1H); ^{13}C NMR (DMSO): 13.44 (CH_3), 13.53 (CH_3), 21.58 (CH_3), 41.17 (CH_2), 49.68 (CH), 105.49 (ArCH), 116.64 (ArCH), 124.09 (ArC), 127.64 (ArCH), 127.99 (ArCH), 128.63 (ArCH), 137.70 (ArC), 143.41 (ArC), 149.38 (ArC), 154.64 (ArC), 162.55 (C, C=O), 172.34 (C, C=O). ESI-MS *m*/*z*: 299.8[M−H]$^-$.

7.12.12. 3-(2-Bromophenyl)-3-[(2,5-dimethylfuran-3-carbonyl)amino] propionic acid (26)

Light white solid; yield: 57.8%; mp: 188.7–190.0 °C. ^{1}H NMR (DMSO, 300 MHz): δ 2.23 (s, 3H), 2.39 (s,3H), 2.65–2.84 (m,2H),5.60–5.65 (q,1H), 6.56 (s,1H), 7.15–7.21 (m,1H), 7.33–7.38 (t, 1H), 7.47 (d, 1H, *J* = 7.68 Hz), 7.58 (d, 1H, *J* = 7.83 Hz), 8.37 (d, 1H, *J* = 7.77 Hz); ^{13}C NMR (DMSO): 13.44 (CH_3), 13.51 (CH_3), 40.62 (CH_2), 49.82 (CH), 105.51 (ArCH), 116.63 (ArC), 122.58 (ArC), 128.09 (ArCH), 128.40 (ArCH), 129.39 (ArCH), 133.05 (ArCH), 142.51 (ArC), 149.46 (ArC), 154.86 (ArC), 162.77 (C, C=O), 171.94 (C, C=O). ESI-MS *m*/*z*: 363.7 [M−H]$^-$.

7.12.13. 3-(2-Bromophenyl)-3-[(5-*tert*butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carbonyl)amino] propionic acid (27)

Light white solid; yield: 51.4%; mp: 171.4–172.1 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90–0.95 (t, 3H), 1.33 (s, 9H), 1.54–1.57 (q, 2H), 2.38 (s, 3H), 2.54–2.77 (m, 2H), 3.80–3.86 (t, 2H), 5.59–5.65 (q, 1H), 6.38 (s, 1H), 7.14–7.18 (t, 1H), 7.32–7.37 (t, 1H), 7.48 (d, 1H, *J* = 7.68 Hz), 7.56 (d, 1H, *J* = 7.38 Hz), 8.03 (d, 1H, *J* = 7.74 Hz); ^{13}C NMR (DMSO):11.21 (CH_3), 11.42 (CH_3), 24.41 (CH_2), 31.15 (3 × CH_3), 32.09 (C), 40.59 (CH_2), 46.55 (CH), 49.63 (CH_2), 103.97 (ArCH), 113.17 (ArC), 122.58 (ArC), 128.23 (ArC), 128.26 (ArCH), 129.14 (ArCH), 132.91 (ArCH), 134.11 (ArC), 139.17 (ArCH), 143.27 (ArC), 164.88 (C, C=O), 172.25 (C, C=O). ESI-MS *m*/*z*: 447.1 [M−H]$^-$.

7.12.14. 3-[(5-*tert*Butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carbonyl)amino]-3-(4-*tert*butyl phenyl) propionic acid (28)

Light white solid; yield: 61.9%; mp: 171.4–172.5 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90–0.95 (t, 3H), 1.25 (s, 9H), 1.30 (s, 9H), 1.53–1.56 (q, 2H), 2.40 (s, 3H), 2.66–2.84 (m, 2H), 3.79–3.85 (t, 2H), 5.30–5.38 (q, 1H), 6.29 (s, 1H), 7.25–7.32 (m, 4H), 7.84 (d, 1H, *J* = 8.52 Hz), 12.16 (s, 1H); ^{13}C NMR (DMSO):11.22 (CH_3), 11.41 (CH_3), 24.38 (2 × CH_3), 24.41 (CH_2), 31.13 (3 × CH_3), 32.07 (C), 33.56 (CH), 41.14 (CH_2), 46.52 (CH), 49.06 (CH_2), 103.90 (ArCH), 113.37 (ArC), 126.45 (2 × ArCH),127.05 (2 × ArCH), 133.90 (ArC), 141.50 (ArC), 147.14 (ArC), 164.71 (C, C=O), 172.65 (C, C=O). ESI-MS *m*/*z*: 425.1 [M−H]$^-$.

7.12.15. 3-[(5-*tert*Butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carbonyl)amino]-3-(2-nitrophenyl) propionic acid (29)

Light white solid; yield: 55.6%; mp 179.2–180.0 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.89–0.94 (t, 3H), 1.32 (s, 9H), 1.49–1.57 (q, 2H), 2.33 (s, 3H), 2.66–2.96 (m, 2H), 3.78–3.84 (t, 2H), 5.65–5.70 (q, 1H), 6.34 (s, 1H), 7.44–7.49 (t, 1H), 7.67–7.76 (m, 2H), 7.87–7.90 (m, 1H), 8.12 (d, 1H, *J* = 7.38 Hz), 12.34 (s, 1H). ^{13}C NMR (DMSO): 11.18 (CH_3), 11.39 (CH_3), 24.36 (CH_2), 31.11 (3 × CH_3), 32.08 (C), 40.60 (CH_2), 45.67 (CH), 46.53 (CH_2), 103.87 (ArCH), 112.83 (ArC), 124.25 (ArCH), 128.46 (ArCH), 128.94 (ArCH), 133.99 (ArC), 134.25 (ArC), 139.24 (ArCH), 139.51 (ArC), 148.60 (ArC), 165.05 (C, C=O), 172.01 (C, C=O). ESI-MS *m*/*z*: 413.9 [M−H]$^-$.

7.12.16. 3-[(5-*tert*Butyl-2-methyl-1-propyl-1*H*-pyrrole-3-carbonyl)amino]-3-(4-methoxyphenyl)propionic acid (30)

Light white solid; yield: 54.5%; mp: 205.4–206.2 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90–0.95 (t, 3H), 1.30 (s, 9H), 2.40 (s, 3H), 2.64–2.80 (m, 2H), 3.71 (s, 3H), 3.79–3.85 (t, 2H), 5.28–5.35 (q, 1H), 6.27 (s, 1H), 6.85 (d, 2H, *J* = 8.55 Hz), 7.26 (d, 2H, *J* = 8.61 Hz), 7.79 (d, 1H, *J* = 8.55 Hz), 12.13 (s, 1H). ^{13}C NMR (DMSO): 11.21 (CH_3), 11.41 (CH_3), 24.39 (CH_2), 31.13 (3 × CH_3), 32.07 (C), 41.29 (CH_2), 46.52 (CH_2), 48.74 (CH), 55.48 (CH_3), 103.87 (ArCH), 113.41 (ArC), 113.93 (2 × ArCH), 128.22 (2 × ArCH), 133.86 (ArC), 136.07 (ArC), 139.08 (ArC), 158.49 (ArC), 164.68 (C, C=O),, 172.63 (C, C=O). ESI-MS *m*/*z*: 399.0 [M−H]$^-$.

7.12.17. 3-[(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl)amino]-3-(3,4-dichloro-phenyl) propionic acid (31)

Light white solid; yield: 52.7%; mp: 187.7–189.3 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.30 (s, 3H), 2.80–2.82 (m, 2H), 5.30–5.37 (q, 1H), 7.40–7.43 (q, 1H), 7.50–7.68 (m, 7H), 8.61 (d, 1H, *J* = 8.07 Hz), 12.48 (s, 1H); ^{13}C NMR (DMSO): 13.70 (CH_3), 40.68 (CH_2), 49.58 (CH), 115.40 (ArC), 125.77 (2 × ArCH), 126.63 (ArCH),127.59 (ArCH), 129.17 (ArCH), 129.36 (ArCH), 129.81 (2 × ArCH), 130.08 (ArC), 131.03 (ArC), 131.39 (ArC), 137.70 (ArC), 144.06 (ArC),149.08 (ArC), 161.20 (C, C=O),172.02 (C, C=O). ESI-MS *m*/*z*: 449.9 [M−H]$^-$.

7.12.18. 3-(2-Bromophenyl)-3-[(5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl)amino] propionic acid (32)

Light white solid; yield: 55.8%; mp: 196.0–197.0 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.30 (s, 3H), 2.68 (d, 2H, *J* = 7.56 Hz), 5.64–5.66 (q, 1H), 7.19–7.24 (t, 1H), 7.37–7.42 (t, 1H), 7.57–7.63 (m, 7H), 8.68 (s, 1H), 12.46 (s, 1H). ^{13}C NMR (DMSO): 13.69 (CH_3), 40.56 (CH_2), 50.31 (CH), 115.59 (ArC), 122.62 (ArC), 125.74 (2 × ArCH), 126.55 (ArCH), 128.18 (ArCH), 128.48 (ArCH), 129.34 (ArCH), 129.59 (ArCH), 129.81 (2 × ArCH), 133.16 (ArC), 137.72 (ArC), 141.79 (ArC), 149.07 (ArC), 161.17 (C, C=O), 171.98 (C, C=O). ESI-MS *m*/*z*: 461.9 [M−H]$^-$.

7.12.19. 3-(4-*tert*Butylphenyl)-3-[(5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl)amino] propionic acid (33)

Light white solid; yield: 53.9%; mp: 152.4–153.3 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.27 (s, 9H), 2.31 (s, 3H), 2.72–2.79 (m, 2H), 5.33–5.40 (q, 1H), 7.31–7.38 (m, 4H), 7.48–7.60 (m, 5H), 8.49 (d, 1H, *J* = 8.31 Hz), 12.35 (s, 1H); ^{13}C NMR (DMSO): 13.76 (CH_3), 31.62 (3 × CH_3), 34.64 (C), 41.27 (CH_2), 49.89 (CH), 115.74 (ArC), 125.54 (2 × ArCH), 125.73 (2 × ArCH), 126.45 (ArCH), 126.64 (2 × ArCH), 129.29 (ArC), 129.79 (2 × ArCH), 137.76 (ArC), 139.75 (ArC), 149.05 (ArC),149.74 (ArC), 160.99 (C, C=O), 172.42 (C, C=O). ESI-MS *m*/*z*: 438.1 [M−H]$^-$.

7.12.20. 3-(4-Bromophenyl)-3-[(5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl)amino] propionic acid (34)

Light white solid; yield: 54.6%; mp: 155.6–157.1 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.30 (s, 3H), 2.73–2.86 (m, 2H), 5.30–5.37 (q, 1H), 7.28–7.61 (m, 9H), 8.58 (d, 1H, J = 8.16 Hz), 12.40 (s, 1H); ^{13}C NMR (DMSO): 13.69 (CH_3), 40.96 (CH_2), 49.94 (CH), 115.54 (ArC), 122.12 (ArC), 125.75 (2 × ArCH), 126.24 (2 × ArCH), 126.56 (ArCH), 129.34 (ArCH), 129.80 (2 × ArCH), 130.40 (ArCH), 131.04 (ArC), 137.71 (ArC), 145.65 (ArC), 149.04 (ArC), 161.14 (C, C=O), 172.13 (C, C=O). ESI-MS m/z: 462.1 $[M-H]^-$.

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Supplementary data

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