Accepted Manuscript

Discovery of tetrahydrocarbazoles with potent hypoglycemic and hypolipemic activities

Ji-Quan Zhang, Shu-Min Li, Xiao Ma, Gang Zhong, Rui Chen, Xiao-Shuang Li, Gao-Feng Zhu, Bo Zhou, Bing Guo, Hao-Shu Wu, Lei Tang

PII: S0223-5234(18)30206-X

DOI: 10.1016/j.ejmech.2018.02.069

Reference: EJMECH 10246

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 19 October 2017

Revised Date: 19 February 2018

Accepted Date: 21 February 2018

Please cite this article as: J.-Q. Zhang, S.-M. Li, X. Ma, G. Zhong, R. Chen, X.-S. Li, G.-F. Zhu, B. Zhou, B. Guo, H.-S. Wu, L. Tang, Discovery of tetrahydrocarbazoles with potent hypoglycemic and hypolipemic activities, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.02.069.

Discovery of Tetrahydrocarbazoles with Potent Hypoglycemic and Hypolipemic Activities

Ji-Quan Zhang,[a,b,c] Shu-Min Li,[a] Xiao Ma,[a] Gang Zhong,[a] Rui Chen,[a] Xiao-Shuang Li,[a] Gao-Feng Zhu,[a] Bo Zhou,[c] Bing Guo,[b] Hao-Shu Wu,[d] and Lei Tang*[,a,b,c]

[a] College of Pharmacy, Guizhou Medical University, Guiyang, 550025, PR China

[b] State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, PR China

[c] Guizhou Provincial Engineering Technology Research Center for Chemical Drug R&D, Guizhou Medical University, Guiyang, 550004, PR China

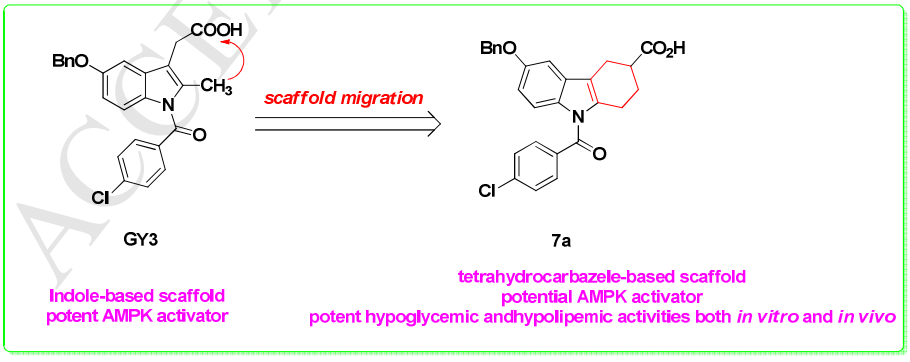
[d] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, PR China

Fax: (+86)-851-86908318; *tlei1974@hotmail.com*.

Abstract

A series of tetrahydrocarbazole derivatives was designed and synthesized on the basis of the AMP-activated protein kinase activator GY3. All the synthesized compounds were screened in HepG2 cell lines for glucose consumption activity and several of them showed potent glucose decreasing activity. *In vivo* evaluation of the hypoglycemic and hypolipemic effects indicated that 7a exhibited comparable activity with pioglitazone, but with a weaker body-weight increasing effect. The pharmacokinetic profiles of 7a were also investigated.

Colour graphic



Discovery of Tetrahydrocarbazoles with Potent Hypoglycemic and Hypolipemic Activities

Ji-Quan Zhang,[a,b,c] Shu-Min Li,[a] Xiao Ma,[a] Gang Zhong,[a] Rui Chen,[a] Xiao-Shuang Li,[a] Gao-Feng Zhu,[a] Bo Zhou,[c] Bing Guo,[b] Hao-Shu Wu,[d] and Lei Tang*[,a,b,c]

[a] College of Pharmacy, Guizhou Medical University, Guiyang, 550025, PR China
[b] State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, PR China
[c] Guizhou Provincial Engineering Technology Research Center for Chemical Drug R&D, Guizhou Medical University, Guiyang, 550004, PR China
[d] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, PR China

KEYWORDS: Tetrahydrocarbazole, AMPK, hypoglycemic and hypolipemic activities, T2DM

ABSTRACT: A series of tetrahydrocarbazole derivatives was designed and synthesized on the basis of the AMP-activated protein kinase activator GY3. All the synthesized compounds were screened in HepG2 cell lines for glucose consumption activity and several of them showed potent glucose decreasing activity. *In vivo* evaluation of the hypoglycemic and hypolipemic effects indicated that **7a** exhibited comparable activity with pioglitazone, but with a weaker body-weight increasing effect. The pharmacokinetic profiles of **7a** were also investigated.

1. Introduction

The global incidence of type 2 diabetes (T2DM) is increasing rapidly, with a morbidity of 415 million in 2015 (projected to rise to 642 million adults by 2040), resulting in a serious health problem worldwide [1-3]. T2DM is characterized by insulin resistance (IR) and hyperglycemia, which cause a series of chronic complications, such as lipidic abnormality, cardiovascular disease, hepatic and renal failure. Therefore, maintaining blood glucose at a normal level is of great importance for diabetic patients. Recent studies have proved that impaired handling of cellular energy homeostasis is closely associated with IR, lipid metabolism and other metabolic syndromes [1,4]. On this basis, molecules that regulate cellular energy metabolism will be a promising therapeutic choice for diabetes and hyperlipemia [5].

AMP-activated protein kinase (AMPK), which acts as a sensor for cellular energy status, plays a central role in maintaining the energy and metabolic landscape of cells [6]. AMPK is a heterotrimeric complex comprised of highly conserved catalytic α and regulatory β/γ subunits. The α and β subunits exist as two isoforms (α1, α2 and β1, β2) and the γ subunit exists as three isoforms (γ1, γ2, γ3). Seven subunits can theoretically combine to create 12 AMPK isoforms, which show differential tissue distribution and function [7-9]. Phosphorylation of Thr-172 located at the activation loop of the kinase domain in the α subunit leads to a 2-3 order of magnitude increase in AMPK activity [10]. Once AMPK is activated, it stimulates glucose uptake in skeletal muscle through independent pathways in IR conditions [1]. Many studies have demonstrated that AMPK mediates a number of pharmacological and physiological factors that exert beneficial effects on T2DM [11]. In addition, the clinical agents metformin and rosiglitazone functionalize their anti-diabetic activities *via* the indirect activation of AMPK. Thus, targeting AMPK is an attractive strategy for the therapy of T2DM and related metabolic syndromes.

Our previous work has revealed that the novel indole-based derivative GY3 possessed potent insulin-sensitizing activity [12] and excellent *in vivo* hypoglycemic and hypolipemic efficacies. In addition, an anti-hyperglycemic mechanism indicated that GY3 exerted its improvement on glucose and lipid metabolism *via* activation of the AMPK pathway [13]. Promoted by our continuing interest in exploring novel agents for the treatment of T2DM, we envisaged that tetrahydrocarbazoles derived from GY3 *via* scaffold migration may also be used as AMPK activators and thus, display desirable hypoglycemic and hypolipemic activities. Herein, we report our recent findings on the design, synthesis and biological evaluation of novel tetrahydrocarbazoles with potent hypoglycemic and hypolipemic activities both *in vitro* and *in vivo*.

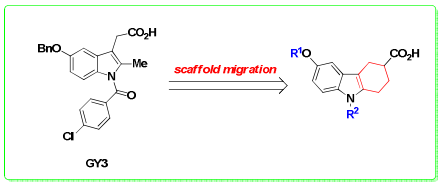


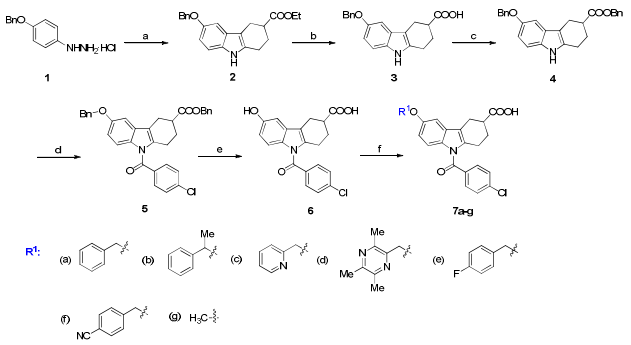
Fig. 1. Design of the tetrahydrocarbazole derivatives.

2. Results and Discussion

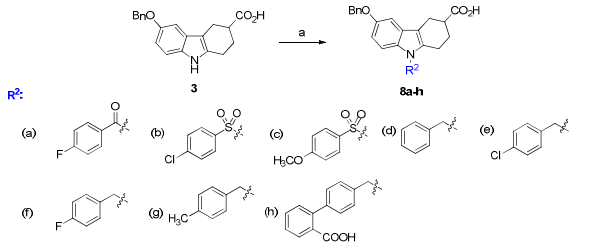
2.1 Chemistry

Using scaffold migration and structure-based design, a series of tetrahydrocarbazole-3-carboxylic acids was derived from GY3. As outlined in Scheme 1, the desired compounds **7a-g** were prepared from (4-(benzyloxy)phenyl)hydrazine hydrochloride (**1**) over 5 steps, which included the Fischer indole synthesis, hydrolysis, benzylation, benzoylation and catalytic hydrogenation to give the key intermediate **6**. Subsequent reaction of **6** with various arylmethyl bromides (R^1-Br) or methyl iodide gave the target compounds **7a-g** [14,15]. To evaluate the influence on the glucose-decreasing activity of the configuration of the chiral carbon atom attached to the carboxy group, compounds **(+)-7a** and **(–)-7a** were resolved from **7a** *via* preparative chiral separation. Compounds **8a-h** were prepared from **3** *via* *N*-acylation with

aroyl chlorides, and aryl sulfonyl chlorides (R^2-Cl) or *N*-alkylation with arylmethyl bromides (R^2-Br) under strongly alkaline conditions (Scheme 2).

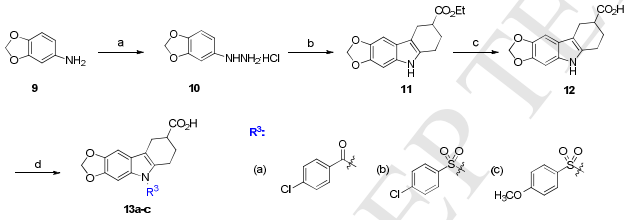


Scheme 1. (a) Ethyl 4-oxocyclohexanecarboxylate, H_3PO_4, EtOH, reflux, 6 h; (b) NaOH, THF/H_2O, reflux, 18 h; (c) BnBr, K_2CO_3, DMF, rt, 24 h; (d) 4-chlorobenzoyl chloride, NaH, DMF, -10 □, 30 min, then rt, 5 h; (e) Pd/C, H_2, EtOAc, rt, 4 h; (f) R^1-Br, NaH, DMF, -10 □, 10 min, then rt, 3-6 h (CH_3I for **7g**).



Scheme 2. (a) R^2-Cl or R^2-Br, NaH, DMF, -10 □, 10 min, then rt, 24 h.

Scheme 3 shows the detailed synthetic route for compounds **13a-c**. Starting from benzo[*d*][1,3]dioxol-5-amine (**9**), key intermediate **12** was synthesized *via* diazotization, a Fischer indole synthesis and hydrolysis, which was subjected to various aroyl chlorides or aryl sulfonyl chlorides (R^3-Cl) under alkaline conditions to afford the desired target compounds.



Scheme 3. (a) 37% HCl, 30% $NaNO_2$, $SnCl_2 \cdot 2H_2O$, H_2O, 0-5 □, 5 h; (b) ethyl 4-oxocyclohexanecarboxylate, H_3PO_4, EtOH, reflux, 6 h; (c) NaOH (s), THF/H_2O (v/v = 1:1), reflux, 7 h; (d) R^3-Cl, NaH, DMF, -10 □, 10 min, then rt, 5 h.

2.2 Biological evaluation

2.2.1 Glucose consumption assay

The glucose consumption activities of all the synthesized compounds were screened in human hepatoma cell lines (HepG2) at 10^{-5} M as previously reported [16]. As shown in Table 1 and Fig. 2a, when compared to the control group (DMSO), 10^{-3} M of metformin (Met) displayed an increase in glucose consumption in HepG2 cell lines by 63.7% ($P < 0.01$). 6-Benzyloxy-tetrahydrocarbazole-3-carboxyl acid (**3**) induced an increase in glucose consumption by 52.3%. Excitingly, a *p*-chloro-benzoyl substituent on the NH of the tetrahydrocarbazole scaffold can significantly improve the hypoglycemic activity (**7a**), with an increase of 82.5% in glucose consumption ($P < 0.01$). Various substituted benzyl groups on the C-6 position were taken into consideration and most of them showed a weaker glucose-decreasing activity than **7a**. Generally, substituents on the aromatic ring of the C-6 position exhibited negative effect on the glucose consuming activity (**7a** *versus* **7d**, **7e** and **7f**), and it may result from the steric hindrance, which blocked the π-sigma weak interaction of compound with AMPK (Fig. 7c and 7d). Considering the oxidation of the benzylic methylene group, a methyl substituted derivative **7b** was designed, which indicated a comparable effect on glucose consumption when compared with the positive control metformin. Both *N*-containing benzyl group derivatives **7c** and **7d** displayed moderate activity. A strong electron-withdrawing group attached to the benzene ring such as CN (**7f**), showed higher hypoglycemic activity than those with a halogen substituent (**7e**) (72.8% *versus* 68.8%). Next, various substituents on the NH were evaluated and it was found that compounds bearing an electron-withdrawing substituent showed higher activity in glucose consumption (**8a-c**) than those with electron-donating substituents (**8d-h**), with compound **8a** exhibiting the most potent efficiency (75.2%, $P < 0.01$). Besides, we also designed several C-6, C-7 cyclized derivatives **13a-c**. Disappointedly, none of them showed comparable activity with the positive control metformin. Comparing the glucose consumption data of **7a** with **8e**, **8a** with **8f**, **8b** with **8c**, **13b** with **13c**, we could conclude that compounds bearing an electron-withdrawing group on the N-9 position displayed higher activity than those with electron-donating substituents. To assess the effect of the chiral configuration, **(+)-7a** and **(–)-7a**, which were separated from **7a**, were evaluated, and the levo isomer **(–)-7a** showed an approximately 1.4-fold higher activity than the dextro isomer **(+)-7a**.

Table 1. Glucose consumption activity in the HepG2 cell lines over 24 h ($\overline{x} \pm s$, n = 4). [a]

Compound	Glucose consumption (mM)[b]	Glucose consumption increase (%)
3	2.63±0.61*	52.3
7a	**3.15±0.69****	**82.5**
7b	2.80±0.52**	62.1
7c	2.55±0.62*	47.5
7d	2.55±0.46*	47.5
7e	2.91±0.50**	68.8
7f	2.98±0.82*	72.8
7g	2.60±0.45*	50.5
8a	3.03±0.36**	75.2
8b	2.84±0.33**	64.5
8c	2.77±0.59	60.3
8d	2.26±0.73	30.4
8e	2.29±0.41	32.7
8f	2.32±0.48*	34.3
8g	2.46±0.52*	42.2
8h	2.21±0.46	27.5
13a	2.68±0.53	55.1
13b	2.55±0.41	47.5
13c	2.43±0.47*	40.3
(+)-7a	2.78±0.48**	60.6
(–)-7a	**3.21±0.53***	**85.5**
DMSO	1.73±0.25	-
Met[c]	2.83±0.32**	63.7

[a] All the compounds tested at a concentration of 10^{-5} M.
[b] Student's t test, * P <0.05, ** P <0.01 and *** P <0.001 versus the control sample.
[c] Met means metformin, test concentration: 10^{-3} M.

To evaluate the concentration-effect of our synthetic tetrahydrocarbazoles in HepG2 cell lines, compounds **3**, **7a**, **(+)-7a**, **(–)-7a**, **7b**, **7e** and **7f** were chosen and tested at three concentrations, 0.1 μM, 1 μM and 10 μM, respectively. As shown in Table 2 and Fig. 2b, all the tested compounds exhibited an obvious dose-dependent manner and the increase

in the glucose consumption induced by the test compounds was positively correlated to their concentration.

Table 2. Glucose consumption activity in the HepG2 cell lines over 24 h ($\bar{x} \pm s$, n = 3).

Compound	Concentration	Glucose consumption (mM)[a]	Glucose consumption increase (%)
DMSO	0.5% v/v	2.01±0.13	-
Met[b]	1 mM	3.22±0.35**	60.4
	0.1 μM	2.59±0.10**	29.0
3	1 μM	2.76±0.04***	37.2
	10 μM	2.98±0.16**	48.4
	0.1 μM	2.32±0.42	15.5
7a	1 μM	2.61±0.14**	30.2
	10 μM	3.47±0.17***	72.9
	0.1 μM	2.04±0.93	1.79
(+)-7a	1 μM	2.56±0.56	27.3
	10 μM	3.12±0.26**	55.3
	0.1 μM	2.27±0.87	12.9
(–)-7a	1 μM	2.72±0.94	35.6
	10 μM	3.54±0.73*	76.4
	0.1 μM	2.11±0.21	4.89
7b	1 μM	2.66±0.04**	32.2
	10 μM	3.39±0.32**	68.7
	0.1 μM	2.01±0.47	0.11
7e	1 μM	2.41±0.45	20.1
	10 μM	3.08±0.07***	53.4
	0.1 μM	2.20±0.63	9.65
7f	1 μM	2.55±0.15**	26.8
	10 μM	3.02±0.53*	50.2

[a] Student's t test, * P <0.05, ** P <0.01 and *** P <0.001 versus control.
[b] Met means metformin, test concentration: 10^{-3} M.

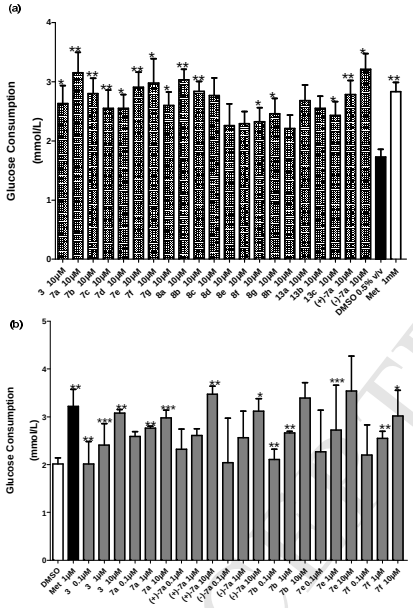


Fig. 2. (a) Glucose consumption activity in HepG2 cell lines; (b) Concentration-effect of the selected compounds in HepG2 cell lines.

2.2.2 Hypoglycemic and hypolipemic activities *in vivo*

To investigate the *in vivo* hypoglycemic activities of the novel tetrahydrocarbazoles derivatives, compounds **7a** and **8b**, which showed good effects on the glucose consumption in the HepG2 cell lines, were selected for further evaluation in db/db diabetic mice. As shown in Table 3 and Fig. 3, using a 0.5% CMC-Na treated group as the blank control and the insulin-sensitizer pioglitazone treated group as the positive control group, **7a** and **8b** were ingested *via* oral administration at a dose of 30 mg/kg. When compared with the pioglitazone-treated group, GY3 and **7a** showed more potent blood glucose decreasing ability, by 32.9% and 30.6%, respectively, but only GY3 showed a statistically significant difference (P <0.05). **7a** and **8b** reduced blood glucose by more than 25% after 1 week, which was more potent than GY3. After 3 weeks' continuous administration, **7a** exhibited a satisfactory anti hpyerglycemic activity with a blood glucose decreasing percentage of 28.0%, which was an approximately 2-fold higher activity than **8b** and GY3, but the result indicated no obvious difference versus the control group.

Table 3. The *in vivo* hyperglycemic activities of **7a** and **8b** in db/db mice ($\bar{x} \pm s$, n = 5).[a]

Group	Blood glucose decreasing percentage (%)[b]			
	Single dose	1 week	2 weeks	3 weeks
0.5% CMC-Na	0.0 ± 4.1	0.0 ± 8.6	0.0 ± 4.0	0.0 ± 6.8
Pioglitazone	21.9 ± 15.2	37.1 ± 8.1*	34.5 ± 16.0	37.4 ± 14.9
GY3	32.9 ±11.2*	25.7 ± 11.5	30.3 ± 8.5*	13.5 ± 9.0
7a	30.6 ± 13.9	29.3 ± 14.5	19.6 ± 12.9	28.0 ± 12.0
8b	15.5 ± 7.2	28.9 ± 17.0	16.3 ± 12.7	14.3 ± 12.1

[a] Oral dosage: 30 mg/kg; Student's t test, * P < 0.05 versus control.
[b] Values were determined 3 h after the final administration.

After 3 weeks' administration, the body-weights of the compounds-treated db/db mice were also measured (Table 4 and Fig. 4). Among which, pioglitazone significantly induced an increase in body-weight of 16.3% (P <0.001). **7a** showed an increasing percentage of 10.4% and **8b** exhibited the lowest body-weight increase effect of 3.7%, which indicated an obvious difference by statistical analysis versus the positive con trol pioglitazone (P <0.01). Notably, **7a** revealed an apparent time-dependence for the increase in body-weight.

Table 4. The effect of **7a** and **8b** on the body-weight of db/db mice ($\bar{x} \pm s$, n = 5).[a]

Group	Percentage body-weight increase (%)[b]			Compared with the solvent control (%)
	1 week	2 weeks	3 weeks	
0.5% CMC-Na	1.0 ± 4.4	-2.1 ± 2.9	-2.6 ± 2.6	-
Pioglitazone	6.7 ± 3.4	11.6 ± 0.9**	13.7 ± 1.5***	16.3
GY3	3.5 ± 3.3	6.5 ± 3.6	5.9 ± 4.7	8.5
7a	-1.6 ± 3.6	3.9 ± 3.4	7.8 ± 3.5*	10.4
8b	0.8 ± 3.1[#]	0.8 ± 3.1[##]	1.1 ± 3.3[##]	3.7

[a] Oral dosage: 30 mg/kg; Student's t test, * P <0.05, ** P <0.01 and *** P <0.001 versus the solvent control; [#] P < 0.05, [##] P < 0.01 versus the positive control.
[b] Values were determined 3 h after the final administration.

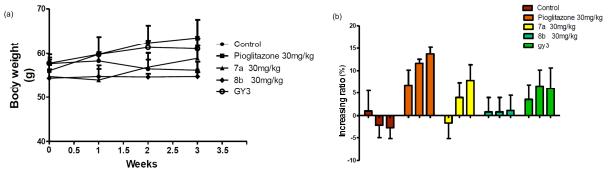


Fig. 4. (a) The effect of the tested compounds on the body-weight of db/db mice. (b) The increasing ratios of the tested compounds.

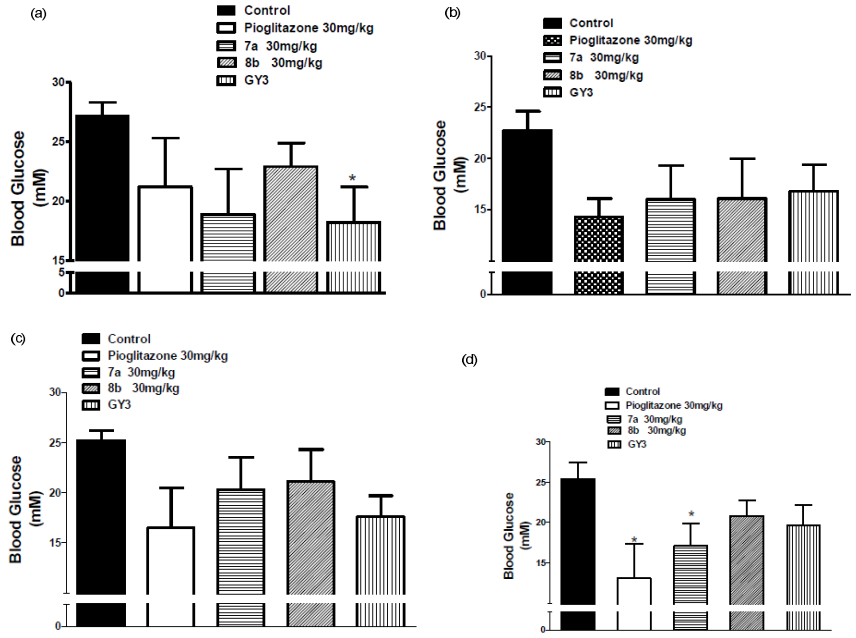


Fig. 3. (a) The hyperglycemic effects of the tested compounds after a single dose. (b) The hyperglycemic effects of the tested compounds after 1 week. (c) The hyperglycemic effects of the tested compounds after 2 weeks. (d) The hpyerglycemic effects of the tested compounds after 3 weeks.

Further, we have also evaluated the hypolipemic activities of **7a** and **8b** in db/db mice (Table 5 and Fig. 5a). After 3 weeks' continuous administration, pioglitazone could significantly reduce the level of fasting plasma triglycerides at a dose of 30 mg/kg when compared with the control group (P <0.05). Intriguingly, GY3 showed no effect on the plasma triglycerides, while compound **7a** revealed higher hypolipemic activity with a reducing plasma triglycerides percentage of 38.6% (P <0.05) than that of pioglitazone (32.9%). To our surprise, as a structural analogue of **7a**, **8b** showed no effect on triglycerides. Meanwhile, the effect of the tested compounds on the plasma total cholesterol in db/db mice was also investigated (Table 6 and Fig. 5b). When compared with the control, pioglitazole and GY3 showed no obvious activity on the total cholesterol, while **7a** and **8b** exhibited remarkable activity for improving the fasting plasma total cholesterol level after 3 weeks' continuous administration; however, the results indicated no statistical difference (P >0.05).

Table 5. The effect of the compounds on the plasma triglycerides in db/db mice ($\overline{X} \pm SE$, n = 5).[a]

Group	Plasma triglycerides (mM)	
	Pre-dose	3 weeks
Control	0.90 ± 0.08	0.74 ± 0.03
pioglitazone	0.76 ± 0.08	0.51 ± 0.05*
GY3	0.70 ± 0.07	0.79 ± 0.15
7a	0.88 ± 0.09	0.54 ± 0.05*
8b	0.97 ± 0.06	0.99 ± 0.14

[a] Oral dosage: 30 mg/kg, Student's t test, versus control and * P <0.05.

Table 6. The effect of the compounds on the plasma total cholesterol in db/db mice ($\overline{X} \pm SE$, n = 5).[a]

Group	Plasma total cholesterol (mM)	
	Pre-dose	3 weeks
Control	6.18 ± 0.37	7.35 ± 0.27
Pioglitazone	6.98 ± 0.24	7.89 ± 0.35
GY3	6.05 ± 0.24	7.78 ± 1.04
7a	6.47 ± 0.54	6.93 ± 0.48
8b	6.08 ± 0.45	6.91 ± 0.49

[a] Oral dosage: 30 mg/kg, Student's t test, versus control and * P <0.05.

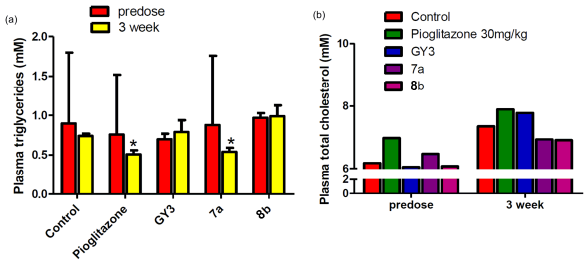


Fig. 5. (a) The effect of the tested compounds on the plasma triglycerides. (b) The effect of the tested compounds on the plasma total cholesterol.

2.2.3 Western blot assay

Western blot analysis was used to assess the potential mechanism of the tetrahydrocarbazoles on the hypoglycemic and hypolipemic activities. Our previous work has revealed that the lead compound GY3 was a potent AMPK activator [13]. Thus, in this study, the effects of the synthesized tetrahydrocarbazoles on the phosphorylation of AMPK and its substrate ACC were evaluated. As shown in Fig. 6, the tested compounds significantly stimulated the phosphorylation of p-AMPKα (Thr172) and p-ACC (Ser79) in HepG2 cell lines at 10 μM when compared with the solvent control. Therefore, bioactivities of this novel series of tetrahydrocarbazole derivatives may originate from the activation of the AMPK pathway.

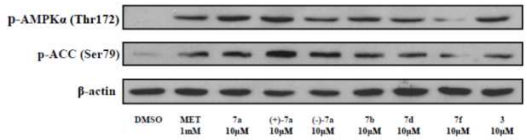


Fig. 6. The effect of the tested compounds on p-AMPK and p-ACC.

2.2.4 Molecular docking studies

To examine the possible binding modes of **7a** into AMPK, docking analysis was performed using Autodock and the protein crystal structure of AMPK α1β1γ1 (PDB code, 5KQ5) [2] was selected. As shown in Fig. 7, the lead compound GY3 forms a key hydrogen bond with the hinge of Arg10 from the α-subunit (Fig. 7a and 7b) and three hydrogen bonds occur between (*R*)-**7a** and AMPK (Fig. 7c). The carboxylic acid group forms two key hydrogen bonds with the hinges of Ala114 and Phe82 from the β-subunit, respectively [2] and the oxygen atom at the C-6 forms another hydrogen bond with the hinge of Asn111, which was also located in the β-subunit. Some non-hydrogen bonding interactions, such as π-anion and π-sigma, were observed in the 2D diagram (Fig. 7d). Surprisingly, (*S*)-**7a**, the other enantiomer of **7a**, showed no hydrogen bond interaction with the hinges of AMPK and only formed some non-hydrogen bonding interactions (Fig. 7e and 7f), which may explain the different hypoglycemic effects observed for the different configurational isomers (Table 1 and 2).

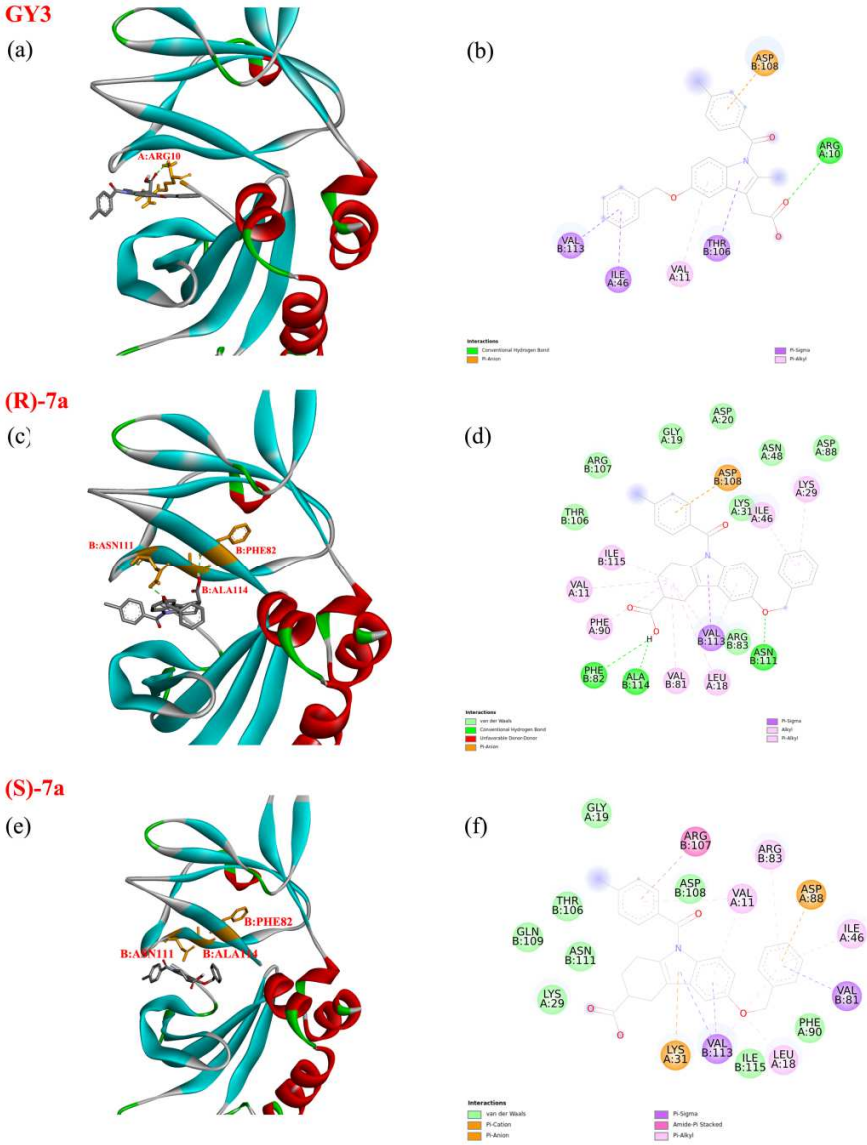


Fig. 7. The docking modes of GY3 and **7a** in the protein crystal structure of AMPK α1β1γ1 (PDB 5KQ5). (a) GY3 bound to AMPK. (b) 2D diagram of the interactions between GY3 and the binding site of AMPK. (c) (*R*)-**7a** bound to AMPK. (d) 2D diagram of the interactions between (*R*)-**7a** and the binding site of AMPK. (e) (*S*)-**7a** bound to AMPK. (f) 2D diagram of the interactions between (*S*)-**7a** and the binding site of AMPK.

2.3 Pharmacokinetic Assay

To further evaluate the *in vivo* pharmacokinetic profiles of the tetrahydrocarbazoles, **7a** was chosen and subjected to Sprague-Dawley (SD) rats *via* intravenous injection and intragastric administration at doses of 5 mg/kg and 20 mg/kg, respectively (Table 7, Fig. 8). As revealed in Table 7, **7a** showed a long half-life (6.46 h) and low oral bioavailability (20.2%). We concluded that the long half-time and low oral bioavailability may be caused by the high lipotropy and plasma protein binding (*C*logP: 5.75, PPB: 100%, ACD/Percepta 2015) and may provide new directions for our further structure optimization based on **7a**.

Table 7. The pharmacokinetic profiles of **7a** in SD rats ($\bar{x} \pm s$, n = 6).

Parameter	**iv** (5 mg/kg)	**ig** (20 mg/kg)
AUC_{0-t} (mg·h/L)	39.57±1.12	31.97±7.02
$AUC_{0-\infty}$ (mg·h/L)	70.99±7.13	48.28±13.48
Cl (L/h/kg)	0.07±0.01	0.45±0.13
Vd (L/kg)	2.70±0.13	9.98±2.84
C_{max} (mg/L)	-	2.32±0.17
t_{max} (h)	-	7.83±0.41
$t_{1/2}$ (h)	7.2±0.32	6.46±0.24
F (%)	-	20.2

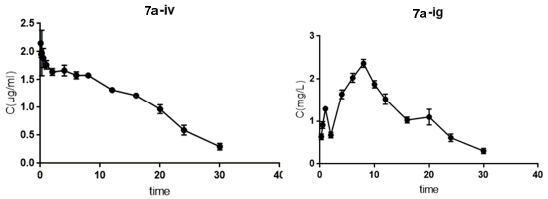


Fig. 8. The mean plasma concentration-time curve obtained for **7a** in SD rats (n = 6).

3. Conclusions

In this study, on a basis of our previous research results, we have designed and synthesized a series of tetrahydrocarbazole derivatives. These compounds were evaluated as potential AMPK activators. **7a** showed potent *in vitro* glucose consumption activity in the HepG2 cell lines, and good *in vivo* hypoglycemic and hypolipemic effects in db/db diabetic mice with a weaker body-weight increasing effect when compared with to tetrahydrocarbazoles exhibited their bioactivity *via* the AMPK pathway. Moreover, the pharmacokinetic profiles of **7a** were also evaluated. A further SAR study based on **7a** is presently being investigated.

4. Experimental Section

4.1 Chemistry

Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, Alfa Aesar, Fisher, Engergy Chemical, Aladin) and were used as such without further purification. The flash column chromatography was carried out over silica gel (200-300 mesh). ^{1}H and ^{13}C spectra were recorded on a INOVA 400 MHz spectrometer. Chemical shifts in ^{1}H NMR spectra were reported in parts per million (ppm) downfield from the internal standard Me_4Si (TMS). Chemical shifts in ^{13}C NMR spectra were reported relative to the central line of the chloroform signal (δ = 77.0 ppm). Low-resolution MS spectra were obtained on an Agilent LC-MS 6120 instrument with an ESI mass detector, and the data were obtained in the positive or negative ion mode. High resolution mass spectra were performed on Waters G2-XS QTof mass spectrometer. Melting points were determined on a Tektronix X-4 melting point apparatus. Analytical TLC was performed using EM separations percolated silica gel 0.2 mm layer UV 254 fluorescent sheets. Though the spectral data was not very normative and integral, most of the target compounds have been reported in our previous patent [14].

4.1.1 Preparation of 9-(4-Chlorobenzoyl)-6-hydroxy-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (6)

4.1.1.1 Ethyl 6-(benzyloxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (2)[17]

To a solution of (4-(benzyloxy)phenyl)hydrazine hydrochloride (**1**, 6.0 g, 0.02 mol) in absolute EtOH (150 mL) was added ethyl 4-oxocyclohexanecarboxylate (4.20 mL, 0.03 mol) and H_3PO_4 (3.00 mL, 0.05 mol). The reaction mixture was stirred at reflux for 6 h and then concentrated *in vacuo* to give a crude residue, which was redissolved in EtOAc (50 mL) and H_2O (50 mL), adjusted the pH to 8-9 with NaOH (aq. 2.0 M). The organic phase was collected and washed with H_2O (50 mL) and saturated NaCl (50 mL), dried over Na_2SO_4. The solution was concentrated *in vacuo* and the crude residue was recrystallized from absolute EtOH to give a light yellow solid **2** with a yield of 71.4%. Rf: 0.54 (PE/EA = 3:1); M.p. 118-120 ℃; ESI-MS (m/z): 350.2 $[M+H]^+$.

4.1.1.2 6-(Benzyloxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (3)[14]

2 (2.00 g, 0.06 mol) was dissolved THF/H_2O (20 mL, v/v, 1:1) and NaOH (4.50 g, 0.11 mol) was added. The resulting mixture was refluxed for 18 h. Removed the solvent and the residue was redissolved in H_2O (20 mL), adjusted the pH to 4-5 with HCl (aq. 1.0 M). The precipitation was collected and dried over vacuo to give **3** as a white solid with a yield of 94.5%. HPLC purity 98.60%; Rf: 0.42 (DCM/MeOH = 25:1); M.p. 215-217 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 10.51 (s, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 6.97 (d, *J* = 2.0 Hz, 1H), 6.70 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.06 (s, 2H), 2.86 (q, *J* = 9.8 Hz, 1H), 2.76-2.64 (m, 4H), 2.16 (d, *J* = 12.8 Hz, 1H), 1.83 (dd, *J* = 12.6, 8.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.6, 151.9, 138.0, 134.6, 131.1, 127.6, 127.5, 127.4, 111.2, 110.5, 106.7, 101.6, 69.8, 25.5, 23.7, 21.9; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{20}H_{18}NO_3]^-$: 320.1287; found: 320.1286.

4.1.1.3 Benzyl 6-(benzyloxy)-9-(4-chlorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (5)

To a solution of **3** (1.74 g, 5.42 mmol) in DMF (anhydrous, 10 mL) was added NaH (60%, 0.54 g, 13.55 mmol) slowly, and then BnBr (0.71 mL, 5.96 mmol) was introduced dropwise. The resulting mixture was stirred at ambient temperature for 24 h. Diluted with EtOAc (50 mL), the mixture was washed with H_2O (30 mL×3) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated in vacuo and crude product **4** was used for the next step directly without further purification. Rf: 0.35 (PE/EA = 5:1); M.p. 98-100 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.40-7.27 (m, 8H), 7.13 (d, *J* = 8.7 Hz, 1H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.20-5.11 (m, 2H), 5.06 (s, 2H), 2.96-2.82 (m, 2H), 2.76 (dd, *J* = 13.1, 8.3 Hz,

3H), 2.19 (d, *J* = 12.1 Hz, 1H), 1.95-1.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.7, 152.0, 138.0, 136.4, 134.5, 128.5, 128.0, 127.6, 127.3, 111.2, 110.5, 106.4, 101.2, 69.8, 65.5, 25.5, 23.6, 21.8; HRMS (ESI): m/z $[M+H]^+$ calcd for $[C_{27}H_{26}NO_3]^+$: 412.1904; found: 412.1904.

To a solution of crude product **4** in DMF (anhydrous, 10 mL) was added 60% NaH (0.54 g, 13.55 mmol) at -10 ℃. The mixture was stirred at ambient temperature for 30 min and then 4-chlorobenzoyl chloride (2.50 mL, 20.00 mmol) was introduced. Maintained the reaction temperature for 5 h. Diluted with EtOAc (50 mL), the mixture was washed with H_2O (30 mL×3) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated in vacuo and purified by column chromatography (PE/EtOAc = 4/1) to afford **5** as light brown oil with yield of 25.7%. Rf: 0.73 (PE/EA = 4:1); M.p. 88-91 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66-7.52 (m, 5H), 7.47-7.28 (m, 9H), 7.13 (dd, *J* = 9.1, 5.7 Hz, 2H), 6.82 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.13 (dd, *J* = 13.5, 7.3 Hz, 4H), 2.93 (d, *J* = 12.8 Hz, 2H), 2.83-2.80 (m, 1H), 2.75-2.63 (m, 2H),2.11-2.04 (m, 1H), 1.76 (dt, *J* = 14.7, 7.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 167.3, 154.8, 137.3, 137.1, 136.3, 135.4, 134.3, 130.8, 128.7, 128.4, 127.8, 127.7, 116.0, 115.3, 112.3, 102.5, 69.6, 65.7, 38.1, 25.6, 24.2, 23.2; HRMS (ESI): m/z $[M+H]^+$ calcd for $[C_{34}H_{29}ClNO_4]^+$: 550.1777; found: 550.1777.

4.1.1.4 9-(4-Chlorobenzoyl)-6-hydroxy-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (6)

A mixture of **5** (0.50 g, 1.09 mmol) and 10% Pd/C (0.10 g) in EtOAc (5.0 mL) was stirred under H_2 atmosphere at rt for 4 h. Then the catalyst was filtered off, the solvent was removed to give a crude residue, which was recrystallized from EtOAc/CH_2Cl_2 (v/v : 1/10) to afford title compound **6** as white solid with yield of 69.7%. Rf: 0.48 (DCM/MeOH = 20:1); M.p. 213-215 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 9.24 (s, 1H), 7.62 (q, *J* = 8.6 Hz, 4H), 7.05 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 2.1 Hz, 1H), 6.58 (dd, *J* = 8.9, 2.2 Hz, 1H), 2.86-2.58 (m, 3H), 2.49 (m, 2H), 2.04 (d, *J* = 11.4 Hz, 1H), 1.67 (d, *J* = 21.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.0, 167.2, 153.6, 136.9, 135.1, 134.6, 130.7, 129.7, 128.9, 116.1, 115.3, 112.2, 103.0, 59.8, 25.7, 24.5, 23.2; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{20}H_{15}ClNO_4]^-$: 368.0690; found: 368.0696.

4.1.2 General procedure for the preparation of compounds 7a-g

To a solution of **6** (1.00 mmol) in DMF (anhydrous, 5.0 mL) was added 60% NaH (3.0 mmol) slowly at -10 ℃ under N_2 atmosphere, and the resulting mixture was stirred at this temperature for 10 min. Then various benzyl bromide R^1-Br (1.00 mmol) was introduced dropwise. After the addition, the mixture was stirred at rt for 3.0-6.0 h (indicated by TLC). Quenched with NH_4Cl (aq.), the mixture was diluted with EtOAc (30 mL) and then adjusted the pH to 4-5 with HCl (1.0 M). The organic phase was washed with H_2O (30 mL×3) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated in vacuo and recrystallized from EtOAc to give desired compounds **7a-g**.

4.1.2.1 6-(Benzyloxy)-9-(4-chlorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7a)

White solid; Yield 41.7%; HPLC purity 99.82%; Rf: 0.32 (DCM/MeOH = 35:1); M.p. 180-182 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.5 Hz, 2H), 7.50-7.42 (m, 4H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.32 (dd, *J* = 8.5, 5.8 Hz, 1H), 6.99 (d, *J* = 9.2 Hz, 2H), 6.77 (dd, *J* = 8.9, 2.6 Hz, 1H), 5.09 (s, 2H), 3.01 (dd, *J* = 19.8, 9.2 Hz, 1H), 2.95-2.85 (m, 2H), 2.80 (d, *J* = 5.3 Hz, 2H), 2.32-2.24 (m, 1H), 1.94 (dq, *J* = 17.0, 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 181.0, 167.8, 155.3, 138.8, 137.1, 135.9, 134.0, 131.2, 130.8, 130.5, 129.1, 128.6, 128.0, 127.5, 116.3, 115.5, 112.5, 102.4, 70.5, 38.9, 25.8, 24.6, 23.5; HRMS (ESI): m/z $[M-H]^-$ calcd. for $[C_{27}H_{21}ClNO_4]^-$: 458.1169, found: 458.1165;

4.1.2.2 9-(4-Chlorobenzoyl)-6-(1-phenylethoxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7b)[14]

White solid; Yield 56.5%; HPLC purity 99.10%; Rf: 0.40 (DCM/MeOH = 35:1); M.p. 192-195 ℃; ^{1}H NMR (400 MHz, CD_3OD) *δ* 7.61-7.52 (m, 4H), 7.39 (d, *J* = 6.0 Hz, 2H), 7.31 (t, *J* = 6.0 Hz, 2H), 7.22 (d, *J* = 6.0 Hz, 1H), 7.02-6.99 (m, 1H), 6.86-6.85 (m, 1H), 6.69 (d, *J* = 7.2 Hz, 1H), 5.42-5.39 (m, 1H), 2.77-2.65 (m, 5H), 2.17-2.11 (m, 1H), 1.84-1.78 (m, 1H), 1.59 (d, *J* = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) *δ* 168.6, 155.1, 144.5, 138.6, 135.5, 131.8, 130.0, 129.9, 129.5, 129.5, 128.4, 126.9, 117.3, 100.0, 89.6, 76.5, 26.8, 25.5, 24.9, 24.7; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{28}H_{23}ClNO_4]^-$: 472.1314; found: 472.1315.

4.1.2.3 9-(4-Chlorobenzoyl)-6-(pyridin-2-ylmethoxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7c)[14]

White solid; Yield 66.9%; HPLC purity 96.50%; Rf: 0.31 (DCM/MeOH = 35:1); M.p. 200-203 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.63 (d, *J* = 4.8 Hz, 1H), 7.78 (t, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.48-7.38 (m, 5H), 7.29 (t, *J* = 6.0 Hz, 1H), 7.03 (d, *J* = 1.6 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 5.32 (s, 2H), 3.04 (dd, *J* = 4.4, 15.6 Hz, 1H), 2.92-2.77 (m, 2H), 2.62-2.49 (m, 2H), 2.23 (d, *J* = 10.4 Hz, 1H), 1.85 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.0, 167.3, 156.9, 154.6, 149.1, 137.1, 169.9, 135.6, 134.3, 130.8, 130.7, 128.9, 122.9, 121.6, 116.3, 115.3, 112.3, 102.5, 70.6, 38.2, 25.7, 24.4, 23.3; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{26}H_{20}ClN_2O_4]^-$: 459.1112; found: 459.1104.

4.1.2.4 9-(4-Chlorobenzoyl)-6-((3,5,6-trimethylpyrazin-2-yl)methoxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7d)[14]

White solid; Yield 43.4%; HPLC purity 97.10%; Rf: 0.60 (DCM/MeOH = 35:1); M.p. 93-95 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.48-7.46 (m, 3H), 6.82 (d, *J* = 2.8 Hz, 2H), 6.65 (dd, *J* = 2.4, 8.8 Hz, 2H), 5.04 (s, 2H), 2.91-2.87 (m, 2H), 2.82 (d, *J* = 10.0 Hz, 1H), 2.77-2.72 (m, 2H), 2.45 (s, 2H), 2.14 (d, *J* = 10.0 Hz, 2H), 1.82 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 175.1, 165.9, 152.5, 136.4, 135.1, 131.7, 131.4, 131.3, 130.8, 130.2, 128.9, 127.3, 117.6, 116.2, 112.5, 103.5, 60.8, 39.0, 31.9, 29.7, 29.4, 26.2, 23.7, 14.2; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{28}H_{25}ClN_3O_4]^-$: 502.1539; found: 502.1538.

4.1.2.5 9-(4-Chlorobenzoyl)-6-((4-fluorobenzyl)oxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7e)

White solid; Yield 65.0%; HPLC purity 98.25%; Rf: 0.33 (DCM/MeOH = 35:1); M.p. 187-190 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (q, *J* = 8.7 Hz, 4H), 7.51 (dd, *J* = 8.5, 5.7 Hz, 2H), 7.23-7.19 (m, 2H), 7.14-7.10 (m, 2H), 6.81 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.11 (s, 2H), 2.90 (m, 1H), 2.72 (d,

J = 9.9 Hz, 2H), 2.51 (dd, *J* = 6.8, 5.1 Hz, 2H), 2.18-1.95 (m, 1H), 1.73 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.9, 167.4, 162.8, 160.8, 150.0, 137.2, 135.9, 134.0, 133.1, 129.7, 128.9, 117.9, 116.2, 115.9, 115.4, 115.2, 103.0, 69.6, 38.1, 25.6, 24.5, 23.4; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{27}H_{20}ClFNO_4]^-$: 476.1063; found: 476.1064.

4.1.2.6
9-(4-Chlorobenzoyl)-6-((4-cyanobenzyl)oxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7f)[14]

White solid; Yield 14.0%; HPLC purity 99.25%; Rf: 0.22 (DCM/MeOH = 35:1); M.p. 199-203 ℃; ^{1}H NMR (400 MHz, CD_3OD) δ 7.69-7.64 (m, 2H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.49-7.29 (m, 5H), 6.95-6.88 (m, 1H), 6.80 (d, *J* = 9.2 Hz, 1H), 5.15 (s, 2H), 2.97-2.79 (m, 3H), 2.64-2.51 (m, 2H), 2.19 (d, *J* = 13.2 Hz, 1H), 1.87 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 155.6, 152.2, 144.3, 143.5, 136.5, 134.4, 132.1, 132.0, 131.9, 130.8, 129.9, 128.9, 127.8, 127.7, 127.7, 127.6, 127.5, 118.3, 112.4, 111.1, 110.8, 106.9, 102.3, 101.9, 101.2, 100.0, 68.9, 40.3, 25.9, 23.3, 21.9, 13.1; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{28}H_{20}ClN_2O_4]^-$: 483.1112; found: 483.1125.

4.1.2.7
9-(4-Chlorobenzoyl)-6-methoxy-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (7g)[15]

White solid; Yield 28.9%; HPLC purity 98.90%; Rf: 0.23 (DCM/MeOH = 35:1); M.p. 190-193 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.39 (m, 5H), 6.88 (d, *J* = 2.4 Hz, 1H), 6.76-6.73 (m, 1H), 3.84 (s, 3H), 3.02-2.86 (m, 3H), 2.65-2.55 (m, 2H), 2.36-2.21 (m, 1H), 1.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 165.8, 156.6, 136.4, 135.0, 131.8, 131.4, 131.0, 130.7, 130.2, 128.9, 127.4, 117.4, 116.0, 112.1, 101.2, 55.7, 38.5, 29.7, 25.9, 23.6; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{21}H_{17}NO_4Cl]^-$: 382.0846; found: 382.0846.

4.1.3 General procedure for the preparation of compounds 8a-h

To a solution of **3** (1.00 mmol) in DMF (anhydrous, 5.0 mL) was added 60% NaH (2.45 mmol) slowly at -10 ℃ under N_2 atmosphere, stirred at this temperature for 10 min. Then various acyl chloride, sulphonyl chloride or benzyl bromide (R^2-Cl or R^2-Br) (1.20 mmol) was introduced dropwise. After the addition, the mixture was stirred at rt for 24 h (indicated by TLC). The mixture was quenched with NH_4Cl (aq.), diluted with EtOAc (30 mL) and then adjusted the pH to 4-5 with HCl (1 M). The organic phase was washed with H_2O (30 mL×3) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated *in vacuo* and purified by column chromatography (PE/EtOAc = 4/1 to 2/1) to give corresponding title compounds **8a-h**.

4.1.3.1
6-(Benzyloxy)-9-(4-fluorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8a)[14]

White solid; Yield 29.4%; HPLC purity 98.36%; Rf: 0.32 (DCM/MeOH = 35:1); M.p. 199-201 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.65 (m, 2H), 7.50-7.28 (m, 5H), 7.21-7.12 (m, 2H), 7.04-6.90 (m, 2H), 6.84-6.72 (m, 1H), 5.11 (s, 2H), 3.00 (t, *J* = 8.8 Hz, 1H), 2.93-2.81 (m, 4H), 2.36-2.22 (m, 1H), 2.05-1.95 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.7, 167.8, 164.2, 155.2, 137.0, 135.9, 132.0, 131.9, 131.7, 131.3, 130.4, 128.6, 127.9, 127.5, 116.0, 115.9, 115.4, 112.42, 102.3, 70.5, 38.9, 29.7, 24.5, 23.5; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{27}H_{22}FNO_4]^-$: 442.1455; found: 442.1456.

4.1.3.2
6-(Benzyloxy)-9-((4-chlorophenyl)sulfonyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8b)[14]

White solid; Yield 74.0%; HPLC purity 98.80%; Rf: 0.36 (DCM/MeOH = 35:1); Mp: 215-218℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 7.1 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.36-7.30 (m, 1H), 7.11 (d, *J* = 2.4 Hz, 1H), 6.97 (dt, *J* = 8.6, 4.3 Hz, 1H), 5.12 (d, *J* = 11.9 Hz, 2H), 3.11 (d, *J* = 17.9 Hz, 1H), 3.03-2.91 (m, 1H), 2.83 (dd, *J* = 15.3, 4.2 Hz, 1H), 2.78-2.61 (m, 2H), 2.27-2.16 (m, 1H), 1.93-1.81 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.1, 164.8, 148.6, 146.4, 145.4, 144.5, 140.2, 139.3, 137.7, 137.4, 137.1, 137.0, 127.5, 124.1, 122.7, 112.1, 78.9, 47.2, 34.7, 32.7, 32.6; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{26}H_{21}ClNO_5S]^-$: 4994.0827; found: 490.0828.

4.1.3.3
6-(Benzyloxy)-9-((4-methoxyphenyl)sulfonyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8c)[14]

White solid; Yield 67.3%; HPLC purity 97.32%; Rf: 0.64 (DCM/MeOH = 35:1); M.p. 203-206 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 9.2 Hz, 1H), 7.67 (dd, *J* = 2.0, 6.8 Hz, 2H), 7.46-7.43 (m, 2H), 7.41-7.37 (m, 2H), 7.34-7.32 (m, 1H), 6.96 (dd, *J* = 2.8, 8.8 Hz, 1H), 6.89 (d, *J* = 2.8 Hz, 1H), 6.86-6.83 (m, 2H), 5.30 (s, 2H), 3.78 (s, 3H), 3.30-3.24 (m, 1H), 2.99-2.78 (m, 4H), 2.35-2.31 (m, 1H), 2.05-1.95 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.9, 163.5, 155.7, 140.0, 135.1, 131.1, 130.7, 130.4, 128.6, 128.5, 128.0, 127.5, 116.8, 115.4, 114.3, 113.3, 102.2, 70.5, 55.6, 38.7, 25.6, 23.8, 23.5; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{27}H_{24}NO_6S]$-: 490.1324; found: 490.1326.

4.1.3.4
9-Benzyl-6-(benzyloxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8d)[14]

White solid; Yield 45.5%; HPLC purity 96.60%; Rf: 0.29 (DCM/MeOH = 35:1); M.p. 189-190 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.4 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.32-7.19 (m, 4H), 7.10-7.07 (m, 2H), 6.96 (d, *J* = 7.1 Hz, 2H), 6.85 (dd, *J* = 8.8, 2.2 Hz, 1H), 5.25-5.14 (m, 2H), 5.10 (s, 2H), 3.11 (dd, *J* = 15.2, 5.0 Hz, 1H), 3.01-2.92 (m, 1H), 2.90-2.74 (m, 2H), 2.67 (d, *J* = 8.3 Hz, 1H), 2.33 (d, *J* = 13.2 Hz, 1H), 2.09-1.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 153.1, 138.0, 137.7, 135.2, 132.2, 128.7, 128.5, 127.7, 127.5, 127.2, 127.2, 126.0, 111.5, 109.8, 107.6, 101.7, 70.9, 46.4, 39.9, 25.4, 23.9, 21.3; HRMS (ESI): m/z [M-H]$^-$ calcd for $[C_{27}H_{24}NO_3]^-$: 432.0309; found: 410.1756.

4.1.3.5
6-(Benzyloxy)-9-(4-chlorobenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8e)[14]

White solid; Yield 45.5%; HPLC purity 98.50%; Rf: 0.26 (DCM/MeOH = 35:1); M.p. 178-180 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.2 Hz, 2H), 7.38 (dd, *J* = 12.8, 3.6 Hz, 2H), 7.35-7.28 (m, 1H), 7.25-7.19 (m, 2H), 7.06 (t, *J* = 5.6 Hz, 2H), 6.91-6.83 (m, 3H), 5.17 (d, *J* = 3.6 Hz, 2H), 5.10 (s, 2H), 3.10 (dd, *J* = 12.0, 5.6 Hz, 1H), 2.96 (dd, *J* = 15.2, 9.6 Hz, 1H), 2.90-2.82 (m, 1H), 2.76 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.70-2.59 (m, 1H), 2.37-2.29 (m, 1H), 2.10-1.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 153.2, 137.6, 136.4, 135.0, 133.0, 132.0, 128.9, 128.5, 127.7, 127.5,

127.4, 127.3, 111.7, 109.7, 107.9, 101.8, 70.8, 45.8, 39.8, 25.3, 23.8, 21.2; HRMS (ESI): m/z [M-H]$^-$ calcd for [$C_{27}H_{23}NO_3Cl$]$^-$: 444.1366; found: 444.1367.

4.1.3.6
6-(Benzyloxy)-9-(4-fluorobenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8f)[14]

White solid; Yield 45.5%; HPLC purity 98.45%; Rf: 0.41 (DCM/MeOH = 35:1); M.p. 189-190 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.2 Hz, 2H), 7.38 (t, J = 6.0 Hz, 2H), 7.31 (t, J = 5.6 Hz, 1H), 7.08-7.07 (m, 2H), 6.96-6.91 (m, 4H), 6.86 (dd, J = 7.2 Hz, 1.6 Hz, 1H), 5.20-5.16 (m, 2H), 5.11 (s, 2H), 3.10 (dd, J = 8.0 Hz, 4.0 Hz, 1H), 2.99-2.94 (m, 1H), 2.88-2.86 (m, 1H), 2.80-2.76 (m, 1H), 2.69-2.63 (m, 1H), 2.35-2.32 (m, 1H), 2.08-2.01 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 163.0, 161.0, 153.2, 137.7, 135.0, 133.7, 132.1, 128.5, 127.7, 127.7, 127.6, 127.6, 127.3, 115.7, 115.5, 111.7, 109.7, 107.8, 101.8, 70.9, 45.8, 39.8, 25.4, 23.8, 21.2; HRMS (ESI): m/z [M-H]$^-$ calcd for [$C_{27}H_{23}NO_3F$]$^-$: 428.1662; found: 428.1662.

4.1.3.7
6-(Benzyloxy)-9-(4-methylbenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8g)[14]

White solid; Yield 45.5%; HPLC purity 98.32%; Rf: 0.17 (DCM/MeOH = 35:1); M.p. 171-172 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.2 Hz, 2H), 7.38 (dd, J = 12.8, 7.6 Hz, 2H), 7.34-7.28 (m, 1H), 7.13-7.04 (m, 4H), 6.89-6.83 (m, 3H), 5.18 (d, J = 6.4 Hz, 2H), 5.10 (s, 2H), 3.11 (dd, J = 15.2, 4.8 Hz, 1H), 3.00-2.91 (m, 1H), 2.89-2.76 (m, 2H), 2.72-2.61 (m, 1H), 2.37-2.28 (m, 4H), 2.09-1.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 153.1, 137.7, 136.9, 135.2, 134.9, 132.2, 129.4, 128.5, 127.7, 127.5, 127.2, 126.0, 111.5, 109.8, 107.4, 101.6, 70.9, 46.2, 39.9, 25.4, 23.9, 21.3, 21.0; HRMS (ESI): m/z [M-H]$^-$ calcd for [$C_{28}H_{26}NO_3$]$^-$: 424.1913; found: 424.1913.

4.1.3.8
6-(Benzyloxy)-9-((2'-carboxy-[1,1'-biphenyl]-4-yl)methyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (8h)[14]

Light yellow oil; Yield 29.6%; HPLC purity 95.56%; Rf: 0.35 (DCM/MeOH = 35:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 5.6 Hz, 1H), 7.56-7.53 (m, 1H), 7.48 (d, J = 6.0 Hz, 2H), 7.43-7.24 (m, 6H), 7.20 (d, J = 6.4 Hz, 2H), 7.07 (d, J = 2.0 Hz, 1H), 6.98 (d, J = 6.4 Hz, 2H), 6.93 (dd, J = 2.0 Hz, 7.2 Hz, 1H), 5.31 (d, J = 13.2 Hz, 1H), 5.18 (d, J = 13.2 Hz, 1H), 5.11 (s, 2H), 3.06 (dd, J = 12.0, 4.0 Hz, 1H), 2.93-2.89 (m, 1H), 2.80-2.78 (m, 1H), 2.65 (d, J = 8.4 Hz, 1H), 2.48-2.42 (m, 1H), 2.28-2.25 (m, 1H), 1.97-1.88 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4, 173.5, 153.1, 143.3, 140.3, 137.7, 137.1, 135.5, 132.6, 132.2, 130.7, 130.7, 129.3, 128.8, 128.5, 127.7, 127.6, 127.3, 127.0, 126.1, 111.6, 109.4, 107.9, 101.7, 70.9, 46.4, 39.9, 29.7, 25.4, 23.9, 21.6; HRMS (ESI): m/z [M-H]$^-$ calcd for [$C_{34}H_{28}NO_5$]$^-$: 530.1967; found: 530.1968.

4.1.4 Preparation of key intermediate 6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (12)

4.1.4.1 Benzo[d][1,3]dioxol-5-ylhydrazine hydrochloride (10) [18]

A mixture of benzo[*d*][1,3]dioxol-5-amine (**9**) (2.74 g, 0.02 mmol), HCl (conc. 8.90 mL) in H_2O (30 mL) was stirred at ambient temperature for 1.0 h. Then cooled to 0-5 ℃ and 30% $NaNO_2$ (aq.) was added dropwise. After the addition, the reaction mixture was stirred below 10 ℃ for 4 h. Another pre-prepared solution of $SnCl_2 \cdot 2H_2O$ in HCl (conc., 13.0 mL) was injected slowly at 0 ℃, followed by a stirring at ambient temperature for 24 h. Filtered and the filter mass was collected, washed with cool water, EtOH and ethyl ether in turn, and then dried *in vacuo* to afford **10** as a yellow solid in 70.0% yield. Rf: 0.57 (DCM/MeOH = 30:1); M.p. 159-162 ℃; ESI-MS (m/z): 153.0 [M+H]$^+$.

4.1.4.2
6,7,8,9-Tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (12)

To a solution of **10** (1.52 g, 0.01 mmol) in absolute EtOH (60 mL) was added ethyl 4-oxocyclohexanecarboxylate (2.00 mL, 0.03 mol) and H_3PO_4 (1.60 mL, 0.03 mol). The reaction mixture was stirred at reflux for 6 h and then concentrated *in vacuo* to give a crude residue, which was redissolved in EtOAc (40 mL) and H_2O (30 mL), adjusted the pH to 8-9 with NaOH (aq. 2 M). The organic phase was collected and washed with H_2O (30 mL) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated *in vacuo* and the crude residue was recrystallized from absolute EtOH to give **11** (2.59 g, light yellow solid) with a yield of 90.4%. Rf: 0.57 (DCM/MeOH = 30:1); M.p. 151-153 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.64 (s, 1H), 6.85 (s, 1H), 6.85 (s, 1H), 6.76 (s, 1H), 6.76 (s, 1H), 5.90 (s, 2H), 5.90 (s, 2H), 4.25-4.14 (m, 2H), 4.23-4.16 (m, 2H), 3.00-2.92 (m, 1H), 3.04-2.51 (m, 6H), 2.86-2.73 (m, 4H), 2.28 (dd, J = 9.8, 3.7 Hz, 1H), 2.28 (dd, J = 9.8, 3.7 Hz, 1H), 2.02-1.93 (m, 1H), 2.06-1.89 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H), 1.30 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 144.0, 142.3, 130.4, 121.2, 108.7, 100.4, 96.9, 91.9, 60.5, 40.3, 25.7, 23.9, 22.4, 14.2, 0.99; HRMS (ESI): m/z [M+H]$^+$ calcd for [$C_{16}H_{18}NO_4$]$^+$: 288.1236; found: 288.1238.

11 (0.86 g, 3.0 mmol) was dissolved THF/H_2O (20 mL, v/v, 1:1) and NaOH (0.24 g, 6.0 mmol) was added. The resulting mixture was refluxed for 7.0 h. Removed the solvent and the residue was redissolved in H_2O (15 mL), adjusted the pH to 4-5 with HCl (aq. 1.0 M). The precipitation was collected and dried over *vacuo* to give **12** (0.56 g, white solid) with a yield of 71.1%. Rf: 0.55 (DCM/MeOH = 30:1); M.p. 208-210 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 10.48 (s, 2H), 6.83 (s, 1H), 6.78 (s, 1H), 5.87 (d, J = 0.5 Hz, 2H), 2.82 (q, J = 9.8 Hz, 1H), 2.72-2.61 (m, 4H), 2.13 (d, J = 13.3 Hz, 1H), 1.85-1.73 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.0, 143.4, 141.7, 132.6, 130.8, 121.2, 107.5, 100.2, 96.9, 92.4, 26.0, 24.1, 22.3; HRMS (ESI): m/z [M-H]$^-$ calcd for [$C_{14}H_{12}NO_4$]$^-$: 258.0766; found: 258.0771.

4.1.5 General procedure for the preparation of compounds 13a-c

To a solution of **12** (2.00 mmol) in DMF (anhydrous, 10 mL) was added 60% NaH (5.00 mmol) slowly at -10 ℃ under N_2 atmosphere, stirred at this temperature for 10 min. Then various acyl chloride or benzyl bromide (R^3-Cl) (2.40 mmol) was introduced dropwise. After the addition, the mixture was stirred at rt for 5 h (indicated by TLC). The mixture was quenched with NH_4Cl (aq.), diluted with EtOAc (30 mL) and then adjusted the pH to 4-5 with HCl (1.0 M). The organic phase was washed with H_2O (30 mL×3) and saturated NaCl (30 mL), dried over Na_2SO_4. The solution was concentrated *in vacuo* and purified by column chromatography (PE/EtOAc = 4/1 to 2/1) to give corresponding title compounds **13a-c**.

4.1.5.1
5-(Chlorobenzoyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (13a)[14]

White solid; Yield 0.69 g; HPLC purity 98.66%; Rf: 0.35 (DCM/MeOH = 35:1); M.p. 269-271 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 3.6 Hz, 2H), 5.95 (d, J = 1.6 Hz, 2H), 2.98-2.83 (m, 3H), 2.62 (d, J = 5.6 Hz, 2H), 2.25-2.21 (m, 1H), 1.94-1.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 168.8, 145.9, 145.0, 139.3, 134.3, 129.5, 124.3, 117.5, 101.5, 97.6, 97.3, 39.4, 26.5, 25.3, 24.1, 20.2; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{21}H_{15}NO_5Cl]^-$: 396.0639; found: 396.0635.

4.1.5.2
5-((4-Chlorophenyl)sulfonyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (13b)[14]

White solid; Yield 36.5%; HPLC purity 96.20%; Rf: 0.46 (DCM/MeOH = 35:1); M.p. 219-220 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 7.83-7.78 (m, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.48 (s, 1H), 6.98 (s, 1H), 6.02 (dd, J = 3.0, 0.8 Hz, 2H), 3.05 (d, J = 16.8 Hz, 1H), 2.91 (d, J = 8.7 Hz, 1H), 2.80-2.52 (m, 3H), 2.15 (d, J = 13.6 Hz, 1H), 1.87-1.72 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.6, 147.6, 146.9, 141.3, 138.2, 134.6, 132.2, 130.7, 129.1, 120.0, 102.7, 98.5, 97.5, 40.4, 40.0, 27.0, 25.0, 24.7; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{20}H_{15}NO_6SCl]^-$: 432.0309; found: 432.0310.

4.1.5.3
5-((4-Methoxyphenyl)sulfonyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (13c)

White solid; Yield 31.2%; HPLC purity 98.52%; Rf: 0.36 (DCM/MeOH = 35:1); M.p. 255-257 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.37 (s, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.52 (s, 1H), 7.05 (d, J = 8.8 Hz, 2H), 6.97 (s, 1H), 6.03 (d, J = 4.0 Hz, 2H), 3.78 (s, 3H), 3.09 (d, J = 18.2 Hz, 1H), 2.97-2.87 (m, 1H), 2.76 (d, J = 15.3 Hz, 1H), 2.61 (dd, J = 32.9, 17.7 Hz, 2H), 2.17 (d, J = 12.6 Hz, 1H), 1.82 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.4, 163.9, 145.9, 145.1, 133.5, 130.2, 129.5, 129.1, 124.3, 117.9, 115.4, 101.7, 98.3, 96.1, 56.2, 38.5, 25.9, 23.9, 23.8; HRMS (ESI): m/z $[M-H]^-$ calcd for $[C_{21}H_{18}NO_7S]^-$: 428.0804; found: 428.0804.

4.1.5.4
(+)-6-(Benzyloxy)-9-(4-chlorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid [(+)7a]

The title compound was obtained *via* chiral preparative column (CHIRALPAK IE, 25 cm × 0.46 cm, 5 μm). Flow rate: 1 mL/min; Mobile phase: THF/DCM/EtOAc/DEA=10/85/5/0.1; HPLC purity 99.32%; Retention time 11.93 min; HRMS (ESI): m/z $[M-H]^-$ calcd. for $[C_{27}H_{21}ClNO_4]^-$: 458.1169, found: 458.1170; $[\alpha]_D^{20}$ = 39.7 (c = 0.10, $CHCl_3$).

4.1.5.5
(-)-6-(Benzyloxy)-9-(4-chlorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid [(-)7a]

The title compound was obtained *via* chiral preparative column (CHIRALPAK IE, 25 cm × 0.46 cm, 5 μm). Flow rate: 1 mL/min; Mobile phase: THF/DCM/EtOAc/DEA=10/85/5/0.1; HPLC purity 98.54%; Retention time 13.06 min; HRMS (ESI): m/z $[M-H]^-$ calcd. for $[C_{27}H_{21}ClNO_4]^-$: 458.1169, found: 458.1167; $[\alpha]_D^{20}$ = -38.7 (c = 0.21, $CHCl_3$).

4.2 Biological evaluation

Metformin was supplied by Zhejiang Octopus Pharmaceutical Co., Ltd (Zhejiang, China), pioglitazone was obtained from Jiangsu Deyuan Pharmaceutical Co., Ltd (Jiangsu, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and BSA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucose reagent kits, triglyceride reagent kit and total cholesterol assay Kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). In the *in vitro* studies, Metformin, GY3 and test compounds were dissolved in DMSO and formulated into 0.2 mM, 2 mM and 10 mM stock solution, respectively. In the *in vivo* studies, pioglitazone and test compounds were prepared with 0.5% sodium carboxymethyl cellulose for animal experiments.

4.2.1 Cell culture and differentiation

HepG2 cells was obtained from the Cell Bank of the Chinese Science Academy (Shanghai, China). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (25 mM D-glucose) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY) or newborn bovine serum (Gibco BRL, Grand Island, NY) at 37 ℃ in a humidified atmosphere with 5% CO_2. Penicillin (100 units/mL) and streptomycin (100 mg/mL) were added to all media

4.2.2 Glucose consumption assay

HepG2 cells were grown on 96-well plates with 6 wells left without cells as blank wells. After the cells reached 80-90% confluence, the medium was replaced by RPMI-1640 containing 11.1 mM glucose and 0.2% BSA instead of serum. The assay was divided into three groups: DMSO black wells group, metformin treated groups and tetrahydrocarbazoles treated group. HepG2 cells were then challenged with the tested compounds with a treat of 24 h, the glucose content in the medium was determined by the glucose oxidase method according to the protocol provided by the manufacturer (glucose consumption = glucose concentration of blank wells - glucose concentration of wells plated with cells).

4.2.3 Hypoglycemic and hypolipemic activities *in vivo*

Ethics approval for animal experimentation was obtained from the Animal Ethical Committee of Zhejiang University, Zhejiang, China (SYXK/Zhe/2012-0178). The animals were treated in accordance with internationally accepted protocols. Female db/db mice (SPF) were obtained from the Shanghai SLAC laboratory Animal Co., Ltd (Shanghai, China) (Approval number SCXK/Hu/2012-0002) were housed at 22 ± 2 °C and 55% ± 5% relative humidity, with a dark cycle of 12 h (19:00 to 7:00) and a light cycle of 12 h (07:00 to 19:00). Eight-week-old db/db mice were used in the experiment. The db/db mice were randomly divided into five groups, namely, model control group, pioglitazone-treated group, GY3 group, compounds **7a**, **8b** groups. Each group comprised five mice. pioglitazone-treated group (30 mg/kg), GY3 group (30 mg/kg), **7a** group (30 mg/kg), **8b** group (30 mg/kg) or water was intragastrically administered to the mice for 3 weeks, the model control (Model) groups were treated with water. Body weight, randomly blood sugar, fasting plasma triglyceride and fasting plasma total cholesterol were recoded weekly to monitor any changes.

4.2.4 Western blot assay

The cells challenged with corresponding agents were collected, lysed in a lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 25 mM NaF, 25 mM β-glycerol phosphate, pH 7.5, 0.2% Triton X-100, 1 mM PMSF, 10 mg ml^{-1} leupeptin and 10 mg ml^{-1} aprotinin) and centrifuged at 10,000 × g for 30 min at 4 ℃, as previously described. Equal volumes of cell lysates were resolved on 8% SDS-PAGE gels, and then proteins were transferred to 0.45 mm pore PVDF membranes (Pierce Chemical) and blocked for 1 h. The PVDF membranes were exposed to the corresponding primary antibodies followed by the appropriate secondary anti-bodies conjugated to horseradish peroxidase. The proteins were visualized autoradiographically using the ECL Plus western blotting detection system (Pierce Chemical).

4.2.5 Molecular modeling

The AMPK-PF06409577 protein-ligand complex crystal structure (PDB ID: 5KQ5; 3.41 Å) was chosen as the template to compare the docking mode among compounds GY3, (*R*)-**7a** and (*S*)-**7a** bound to AMPK. For ligand preparation, the 3D structures of compounds GY3, (*R*)-**7a** and (*S*)-**7a** were generated and minimized using sybyl X2.0 (Gaussian 09W). The molecular dockings were performed by Autodock 4.26 and Autodocktools (ADT) programs. The polar hydrogen atoms were added to AMPK and Kollman united atom charges were assigned before docking. During the docking process, AMPK was taken as rigid while positions and torsional bonds of each ligand were kept free for flexible docking. Before grid maps were calculated by AutoGrid, the three dimensions of the grid were set to 50 Å×50 Å×50 Å with 0.375 Å spacing value and the center of grid box was set to 46.77×-81.64×-39.08. During the docking process of ligands into AMPK, the empirical free energy function and the Lamarckian genetic algorithm were employed. 100 independent runs were carried out with maximum of energy evaluations to 25,000,000 performed for each ligand and population size to 300. After clustering analysis, the structures with lower mean binding energy and the larger number of conformations were chosen as the preferred docking conformations.

4.3 Pharmacokinetic Assay

Pharmacokinetic studies were performed in Sprague-Dawley (SD) rats (three male and three female) following Institutional Animal Care and Use Committee guidelines. Oral dosing was administered by gavage in a vehicle containing 20% 1,2-propanediol and 80% water. Blood samples were collected over a 32 h period post dose into vacutainer tubes containing sodium heparin. Plasma was isolated, and the concentration of compound **7a** in plasma was determined by LC/MS/MS after protein extraction with EtOAc. Non-compartmental PK analysis was performed on plasma concentration data to calculate PK parameters using DAS 2.0. The concentration time curve of **7a** were plotted using Prism 7.0 Dose-response curves (GraphPad Software Inc., USA).

4.4 Statistical Analysis

Potential percentages of glucose consumption and triglyceride content were obtained by normalizing the values obtained with that of the control; the values obtained for the control were considered to be 100%.

Data are shown as the mean ± standard deviation (S.D.). Statistical analysis was performed using one-way ANOVA or Student's *t*-test. *P*-value of <0.05 was considered to be statistically significant.

Supporting Information

^{1}H NMR and ^{13}C NMR spectra for the target compounds.

Acknowledgements

This work was financially supported by the National Science Fundation of China (No. 81660573, 81703356, 81360470), the Engineering Research Center of Chemical Synthetic Drugs of Guizhou Province (No. [2016]5402), the Medicinal Chemistry Engineering Research Center of High Education of Guizhou Province (No. KY[2014]219), the Social Development of Technical Supporting Foundation of Guizhou Province (No. [2016]2819), the Joint Foundation of the Science and Technology Department of Guizhou Province & Guizhou Medical University (No. LH[2016]7349, LH[2016]7379) and the Undergraduate Training Program for Innovation & Entrepreneurship of Guizhou Province (No. 201610660037).

References

[1] Oh, S.; Kim, S. J.; Hwang, J. H.; Lee, H. Y.; Ryu, M. J.; Park, J.; Kim, S. J.; Jo, Y. S.; Kim, Y. K.; Lee, C.-H.; Kweon, K. R.; Shong, M.; Park, S. B. Antidiabetic and antiobesity effects of Ampkinone (**6f**), a novel small molecule activator of AMP-activated protein kinase. *J. Med. Chem.* **2010**, 53, 7405-7413.

[2] Cameron, K. O.; Kung, D. W.; Kalgutkar, A. S.; Kurumbail, R. G.; Miller, R.; Salatto, C. T.; Ward, J.; Withka, J. M.; Bhattacharya, S. K.; Boehm, M.; Borzilleri, K. A.; Brown, J. A.; Calabrese, M.; Caspers, N. L.; Cokorinos, E.; Conn, E. L.; Dowling, M. S.; Edmonds, D. J.; Eng, H.; Fernando, D. P.; Frisbie, R.; Hepworth, D.; Landro, J.; Mao, Y.; Rajamohan, F.; Reyes, A. R.; Rose, C. R.; Ryder, T.; Shavnya, A.; Smith, A. C.; Tu, M.; Wolford, A. C.; Xiao, J. Discovery and preclinical characterization of 6-chloro-5-[4-(1-hydroxycyclobutyl)phenyl]-1*H*-indole-3-carboxylic acid (PF-06409577), a direct activator of adenosine monophosphate-activated protein kinase (AMPK), for the potential treatment of diabetic nephropathy. *J. Med. Chem.* **2016**, 59, 8068-8081.

[3] Diabetes, 7th ed.; International Diabetes Federation, Brussels, Belgium, 2015; http://www.diabetesatlas.org (accessed March 29, 2016).

[4] Wing, R. R.; Goldstein, M. G.; Acton, K. J.; Birch, L. L.; Jakicic, J. M.; Sallis, J. F., Jr.; Smith-West, D.; Jeffery, R. W.; Surwit, R. S. Behavioral science research in diabetes: lifestyle changes related to obesity, eating behavior, and physical activity. *Diabetes Care* **2001**, 24, 117-23.

[5] Hwang, J. H.; Kim, D. W.; Jo, E. J.; Kim, Y. K.; Jo, Y. S.; Park, J. H.; Yoo, S. K.; Park, M. K.; Kwak, T. H.; Kho, Y. L.; Han, J.; Choi, H.-S.; Lee, S.-H.; Kim, J. M.; Le, I.; Kyung, T.; Jang, C.; Chung, J.; Kweon, G. R.; Shong, M. Pharmacological stimulation of NADH oxidation ameliorates obesity and related phenotypes in mice. *Diabetes* **2009**, 58, 965-974.

[6] Fujii, N.; Jessen, N.; Goodyear, L. J. AMP-activated protein kinase and the regulation of glucose transport. *Am. J. Physiol. Endocrinol. Metab.* **2006**, 291, E867-E877.

[7] Ross, F. A.; MacKintosh, C.; Hardie, D. G. AMP-activated protein kinase: a cellular energy sensor that comes in 12 flavours. *FEBS J.* **2016**, 283, 2987-3001.

[8] Steinberg, G. R.; Kemp, B. E. AMPK in health and disease. *Physiol. Rev.* **2009**, 89, 1025-1078.

[9] Rana, S.; Blowers, E. C.; Natarajan, A. Small molecule adenosine 5'-monophosphate activated protein kinase (AMPK) modulators and human diseases. *J. Med. Chem.* **2015**, 58, 2-29.
[10] Suter, M.; Riek, U.; Tuerk, R.; Schlattner, U.; Wallimann, T.; Neumann, D. Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J. Biol. Chem.* **2006**, 281, 32207-32216.
[11] Carling, D. AMP-activated protein kinase: balancing the scales. *Biochimie* **2005**, 87, 87-91.
[12] Zhang, J.; Wang, J.; Wu, H.; He, Y.; Zhu, G.; Cui, X.; Tang, L. Design, synthesis and insulin-sensitizing activity of indomethacin and diclofenac derivatives. *Bioorg. Med. Chem. Lett.* **2009**, 19, 3324-3327.
[13] Si, M.; Yan, Y.; Tang, L.; Wu, H.; Yang, B.; He, Q.; Wu, H. A novel indole derivative compound GY3 improves glucose and lipid metabolism via activation of AMP-activated protein kinase pathway. *Eur. J. Pharmacol.* **2013**, 698, 480-488.
[14] Tang, L.; Zhang, J.; Zhong, G.; Ma, X.; Wang, J.; Chen, R. Preparation of aromatic heterocyclic derivatives as antidiabetic agents. *CN105646330A*, **2016**.
[15] Liedtke, A. J.; Adeniji, A. O.; Chen, M.; Byrns, M. C.; Jin, Y.; Christianson, D. W.; Marnett, L. J.; Penning, T. M. Development of Potent and Selective Indomethacin Analogues for the Inhibition of AKR1C3 (Type 5 17β-Hydroxysteroid Dehydrogenase/Prostaglandin F Synthase) in Castrate-Resistant Prostate Cancer. *J. Med. Chem.* **2013,** 56, 2429-2446.
[16] Rosa Lovati, M.; Manzoni, C.; Castiglioni, S.; Parolari, A.; Magni, C.; Duranti, M. Lupin seed γ-conglutin lowers blood glucose in hyperglycaemic rats and increases glucose consumption of HepG2 cells. *Br. J. Nutr.* **2012**, 107, 67-73.
[17] Johnson, K. W.; Phebus, L. A. Preparation of indole and carbazole derivatives as serotonin agonists. *WO9806402A1*, **1998**.
[18] Shirai, F.; Azami, H.; Kayakiri, N.; Okumura, K.; Nakamura, K. Preparation of pyrazole derivatives useful as COX-1 inhibitors. *WO2004050632A1*, **2004**.

Highlights in this manuscript

1) a series of novel tetrahydrocarbazoles were designed and synthesized. The target compounds were screened for glucose consumption activities in HepG2 cell lines, and most of them showed potent glucose decreasing activities.

2) Compounds **7a** and **8b** were selected for further *in vivo* evaluation of hypoglycemic and hypolipemic effects, and **7a** exhibited comparable activities with positive control pioglitazone, but with a weaker body-weight increasing effect.

3) The western blot assay indicated these tetrahydrocarbazoles exhibited the bioactivities *via* the AMPK pathway. The pharmacokinetic profiles of **7a** were also evaluated.

Discovery of Tetrahydrocarbazoles with Potent Hypoglycemic and Hypolipemic Activities

Ji-Quan Zhang,[a,b,c] Shu-Min Li,[a] Xiao Ma,[a] Gang Zhong,[a] Rui Chen,[a] Xiao-Shuang Li,[a] Gao-Feng Zhu,[a] Bo Zhou,[c] Bing Guo,[b] Hao-Shu Wu,[d] and Lei Tang*[,a,b,c]

[a] College of Pharmacy, Guizhou Medical University, Guiyang, 550025, PR China
[b] State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, PR China
[c] Guizhou Provincial Engineering Technology Research Center for Chemical Drug R&D, Guizhou Medical University, Guiyang, 550004, PR China
[d] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, PR China

Fax: (+86)-851-86908318

E-mail: tlei1974@hotmail.com

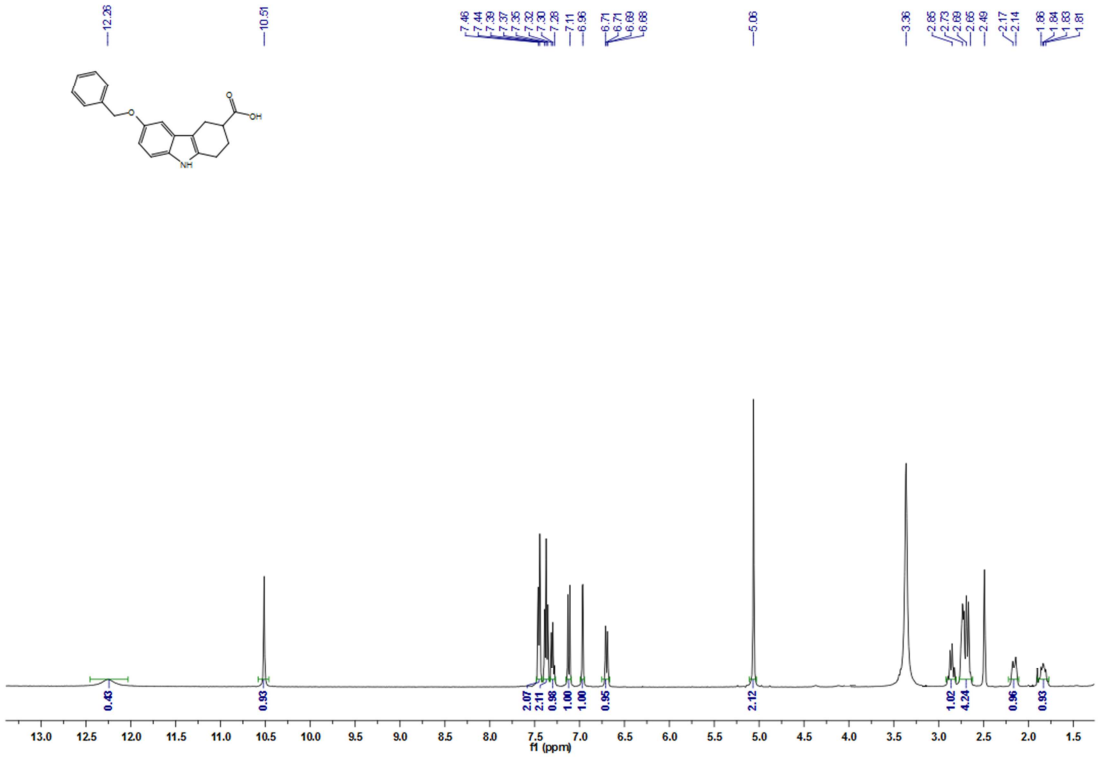
Supporting information

1. ^{1}H NMR and ^{13}C NMR spectra for the target compounds

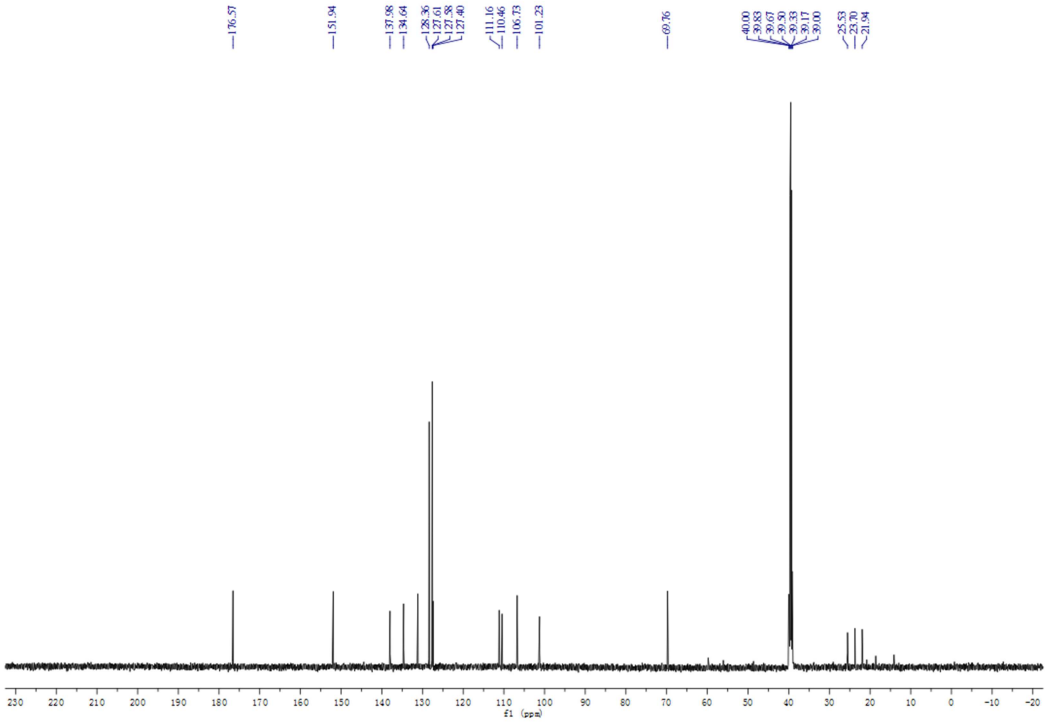
1. ^{1}H NMR and ^{13}C NMR spectra for selected compounds

*6-(Benzyloxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**3**)*

^{1}H NMR

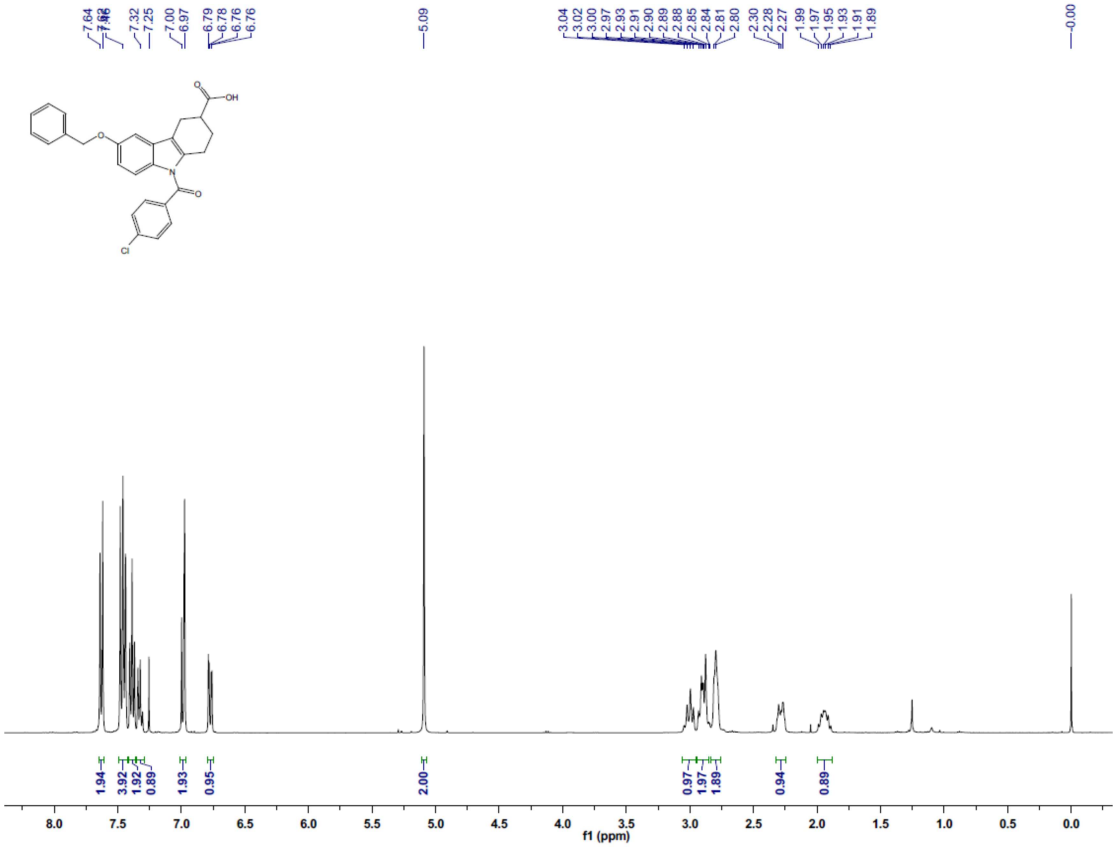


^{13}C NMR

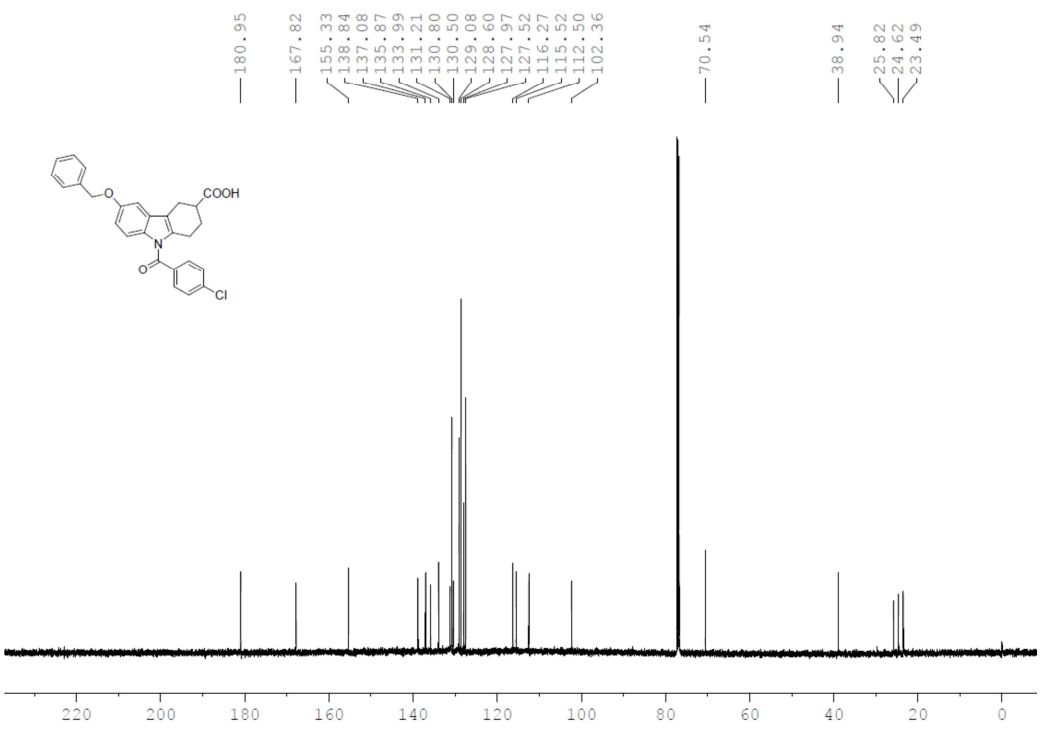


*6-(Benzyloxy)-9-(4-chlorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**7a**)*

^{1}H NMR

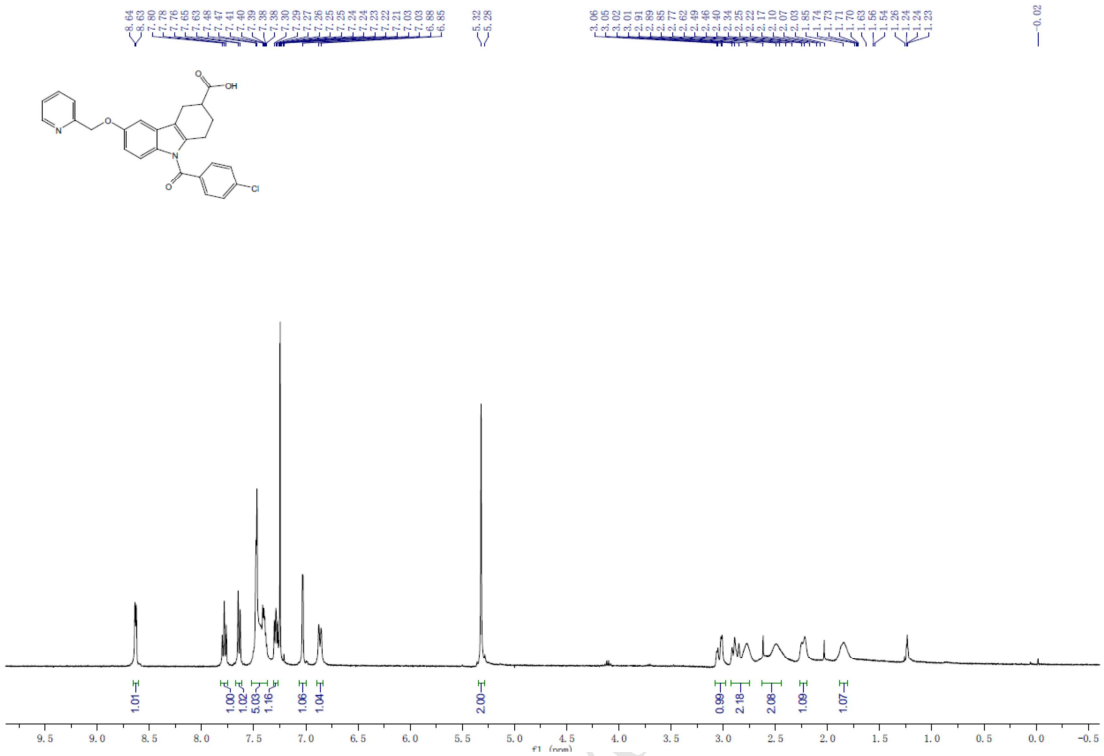


^{13}C NMR

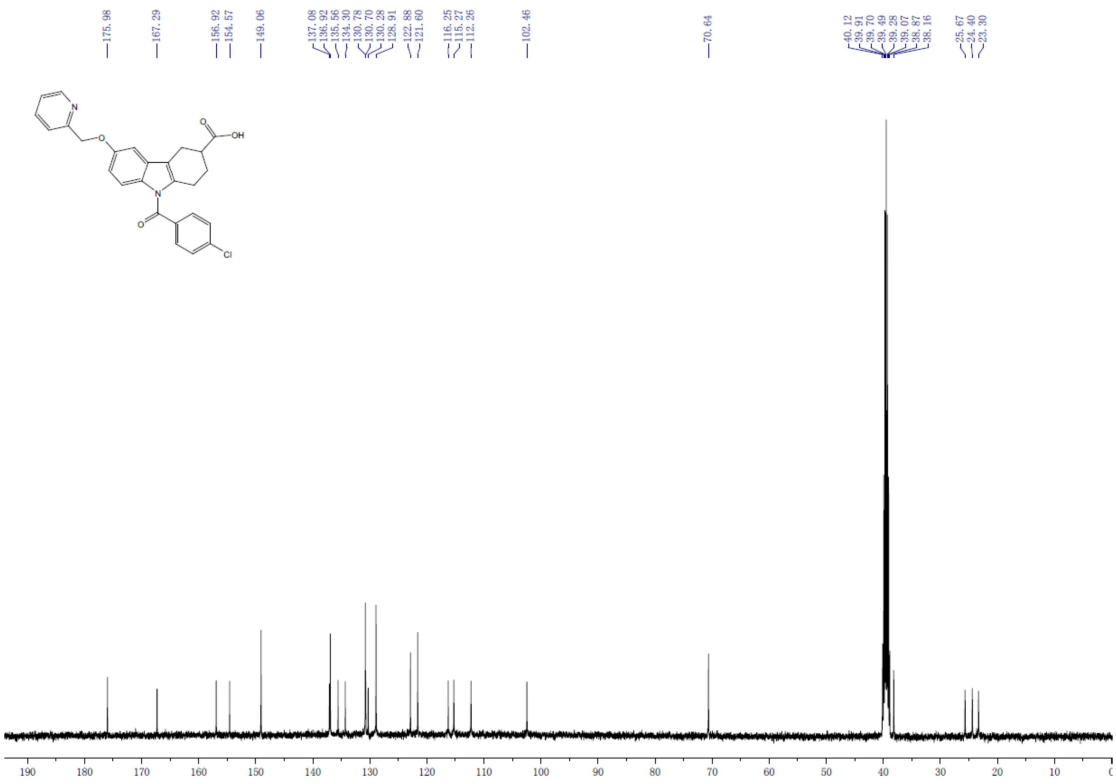


*9-(4-Chlorobenzoyl)-6-(pyridin-2-ylmethoxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**7c**)*

^{1}H NMR

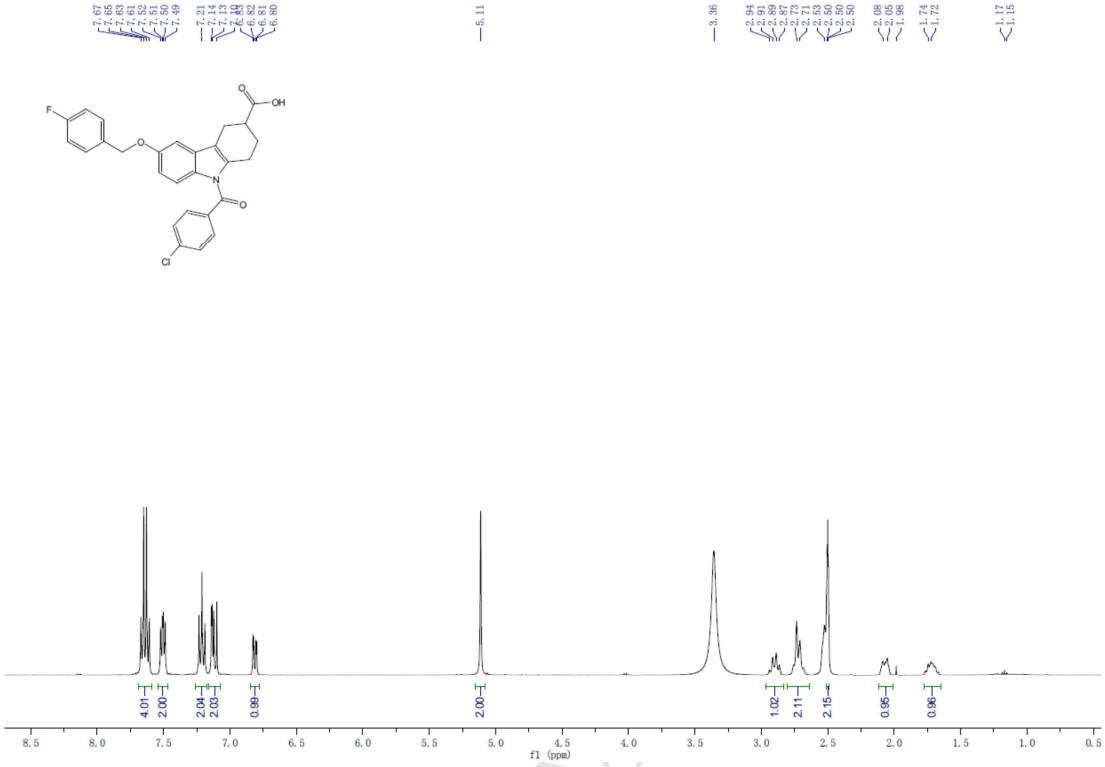


^{13}C NMR

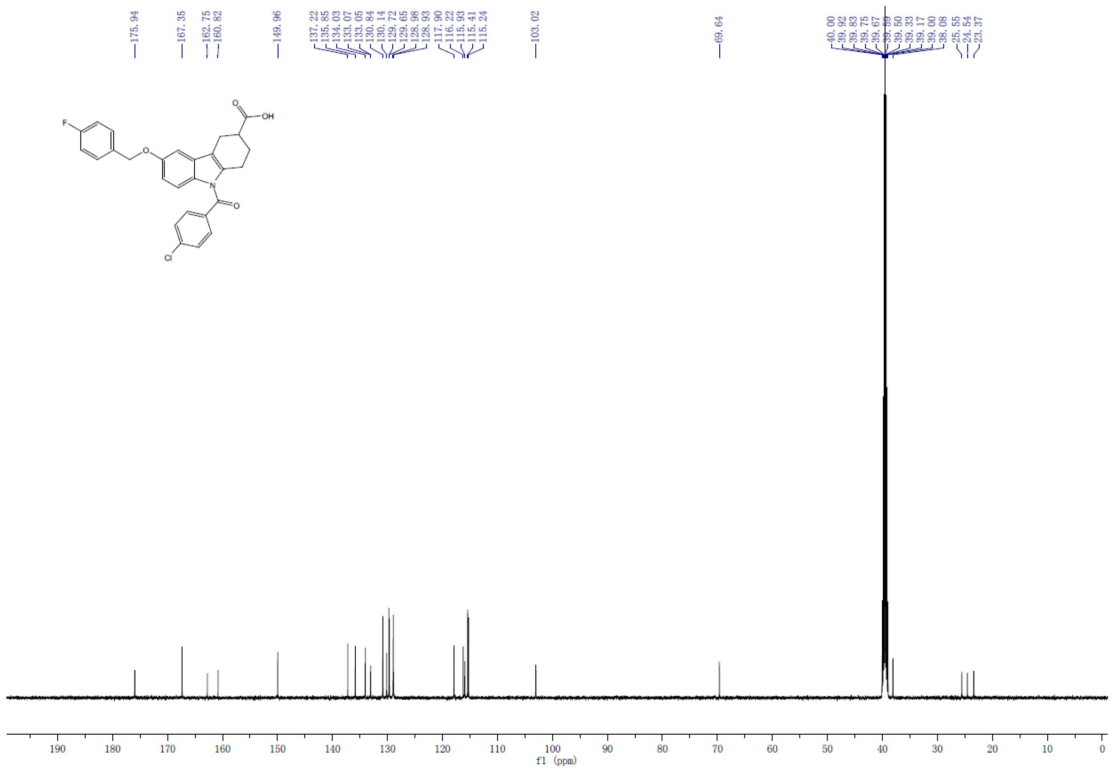


*9-(4-Chlorobenzoyl)-6-((4-fluorobenzyl)oxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**7e**)*

^{1}H NMR

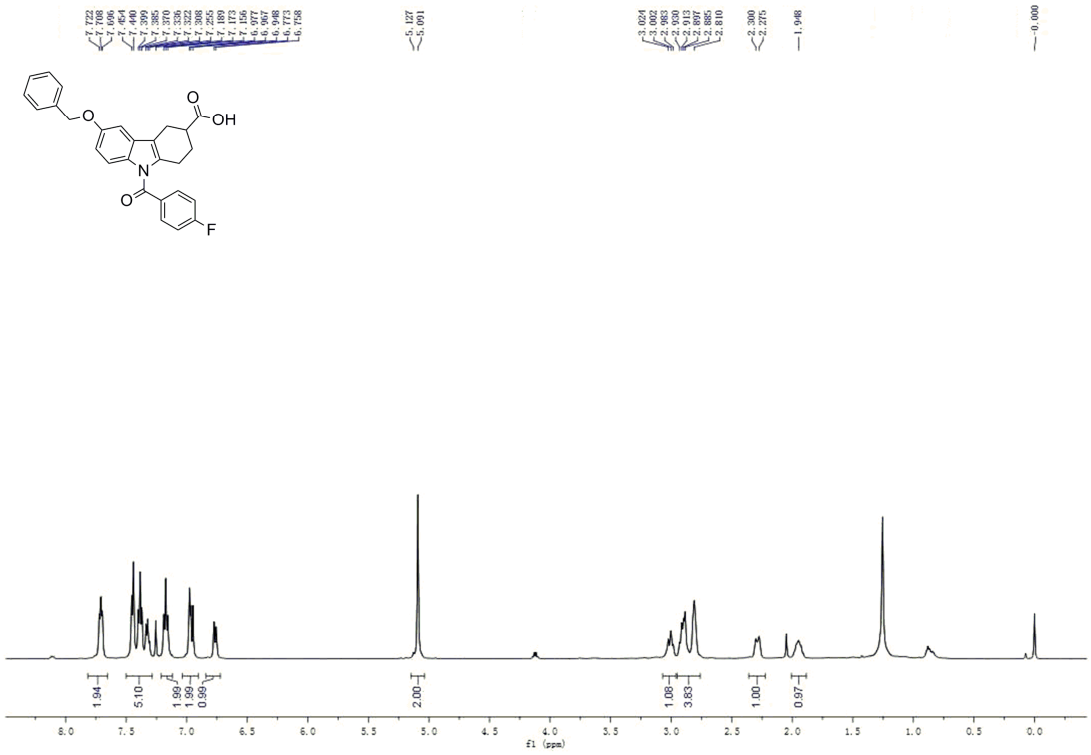


^{13}C NMR

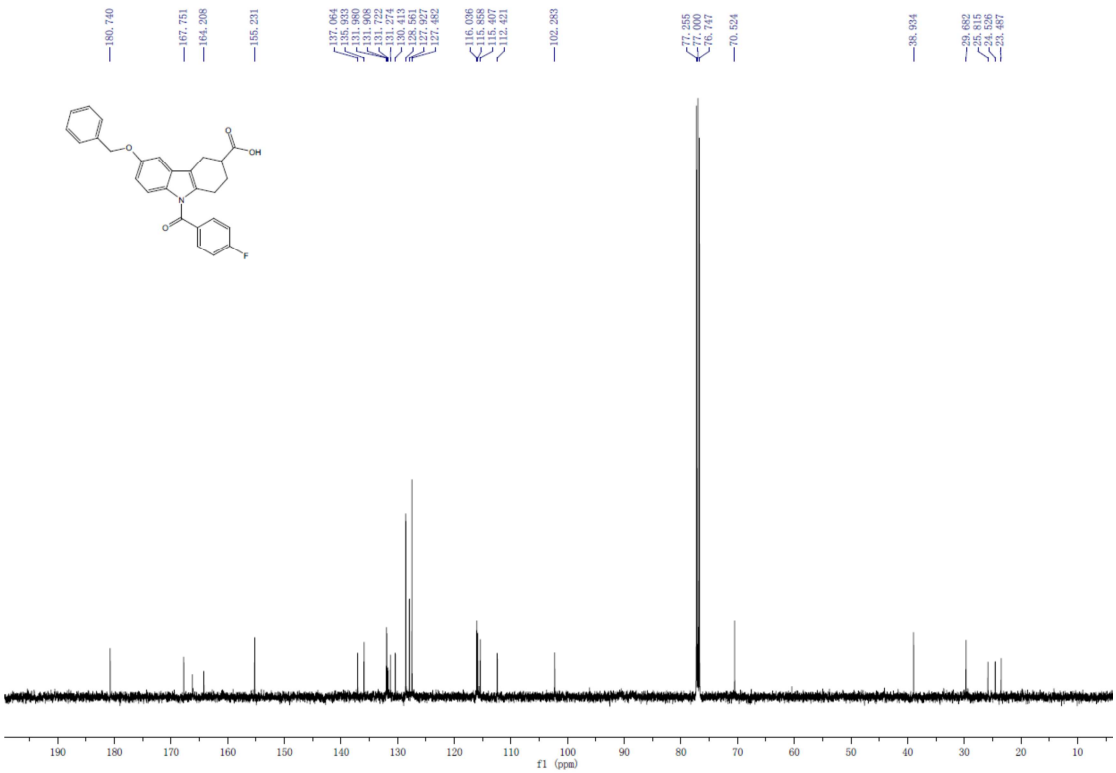


*6-(Benzyloxy)-9-(4-fluorobenzoyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid **(8a)***

^{1}H NMR

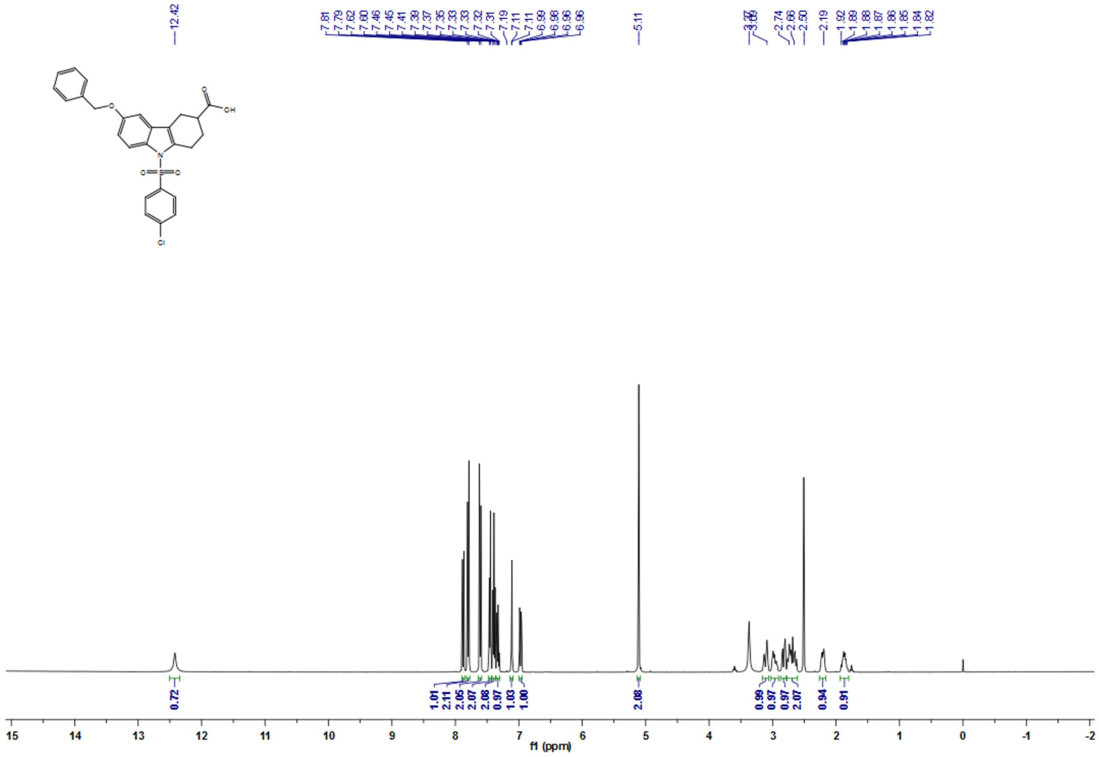


^{13}C NMR

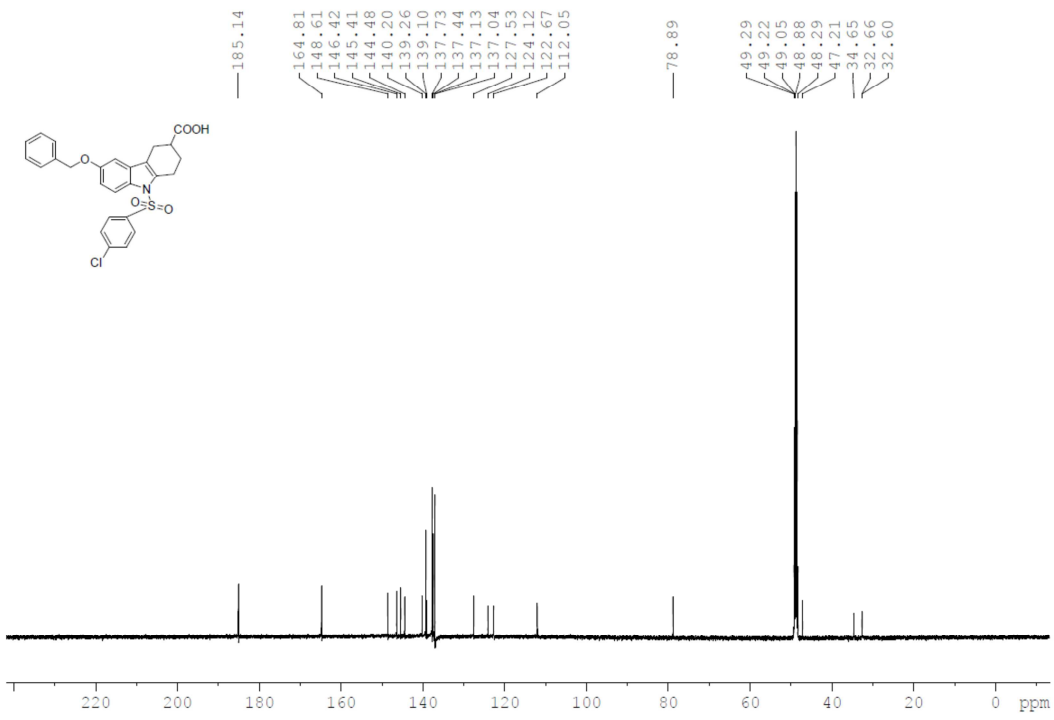


*6-(Benzyloxy)-9-((4-chlorophenyl)sulfonyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**8b**)*

^{1}H NMR

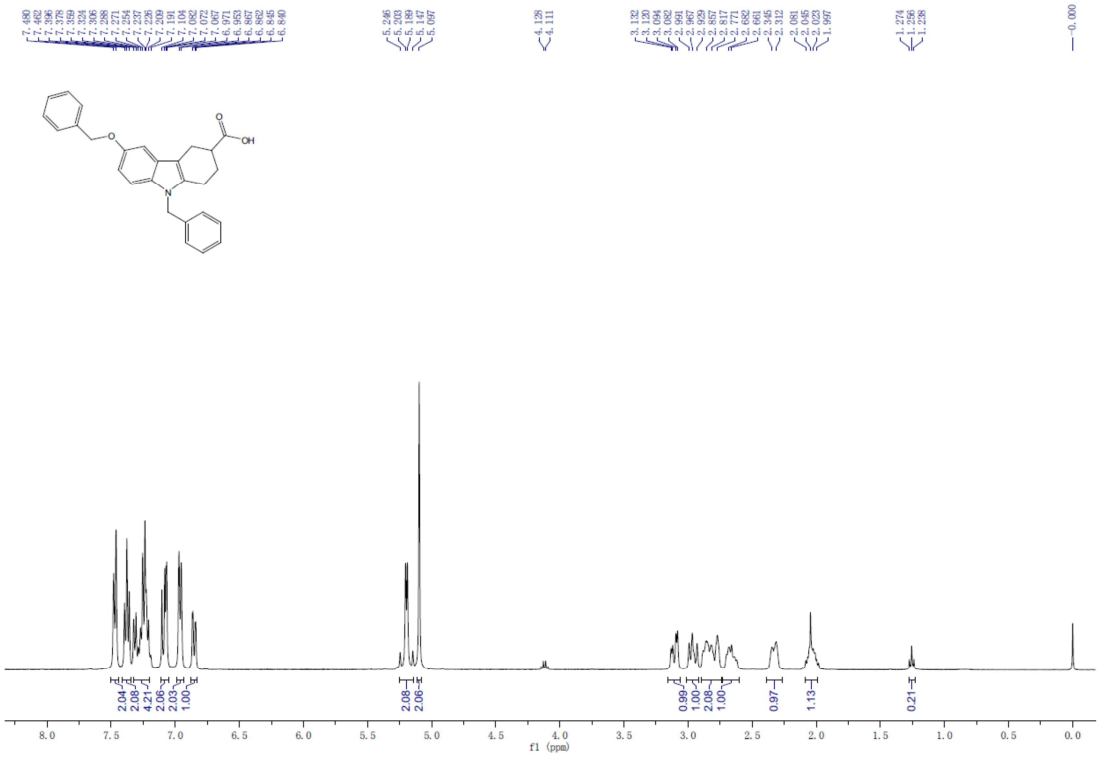


^{13}C NMR

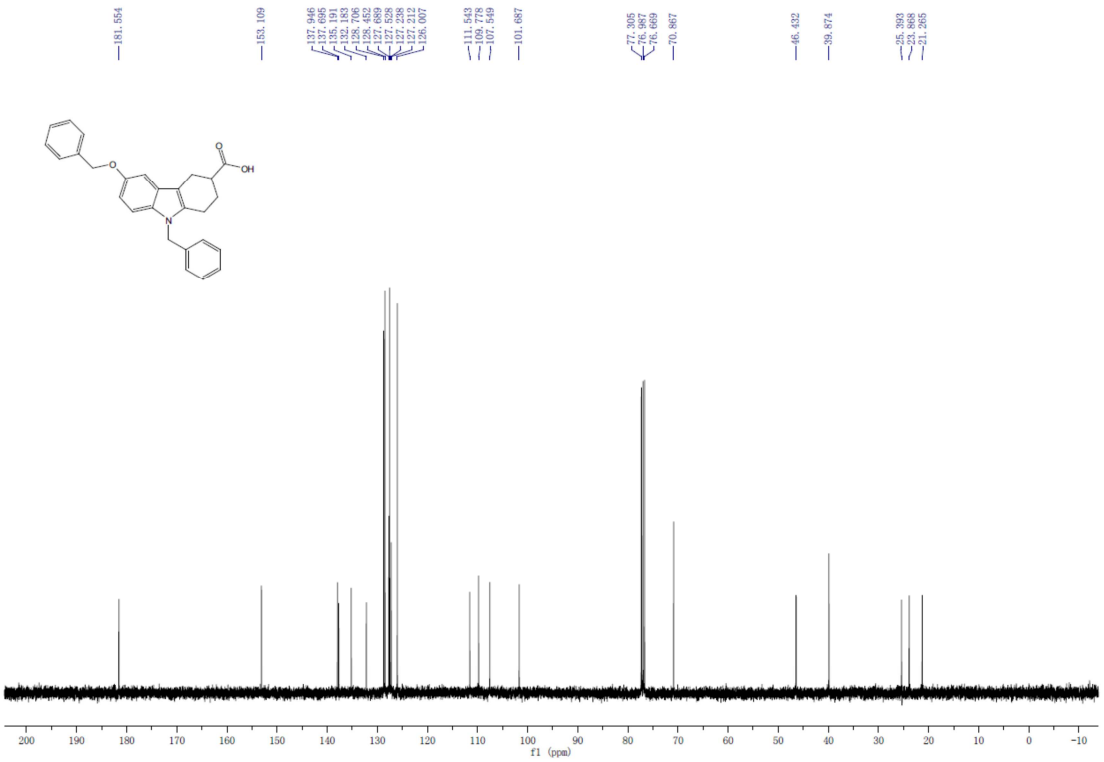


*9-Benzyl-6-(benzyloxy)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**8d**)*

^{1}H NMR

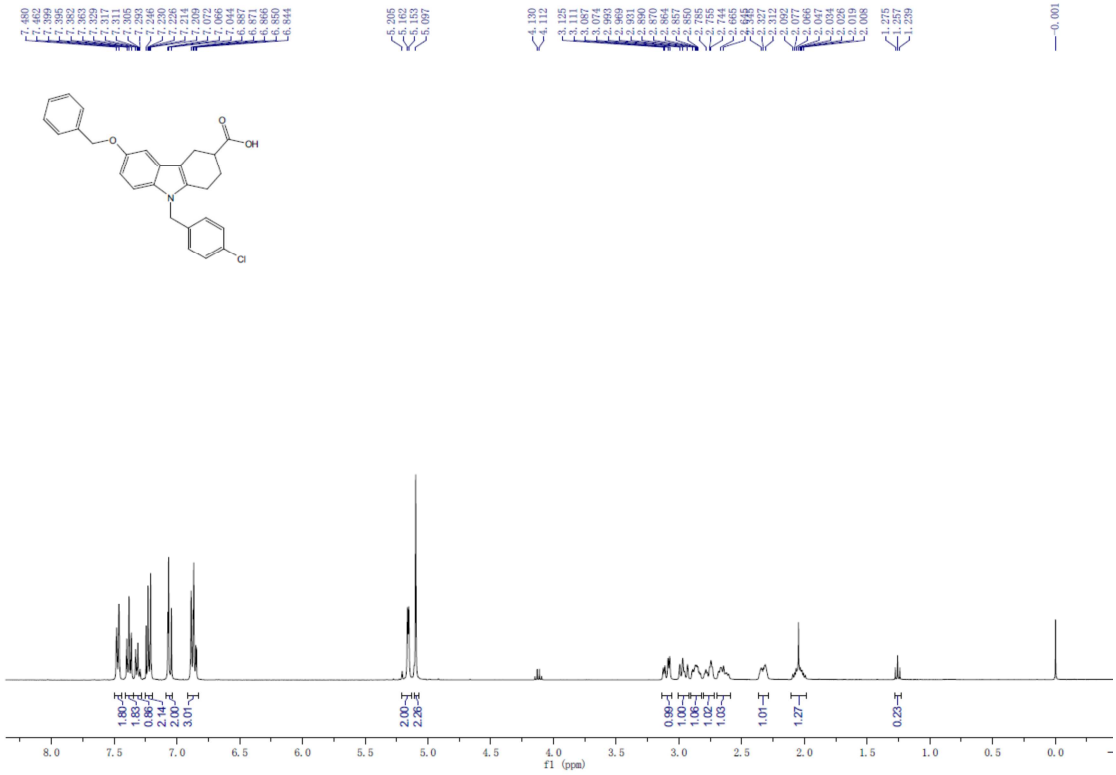


^{13}C NMR

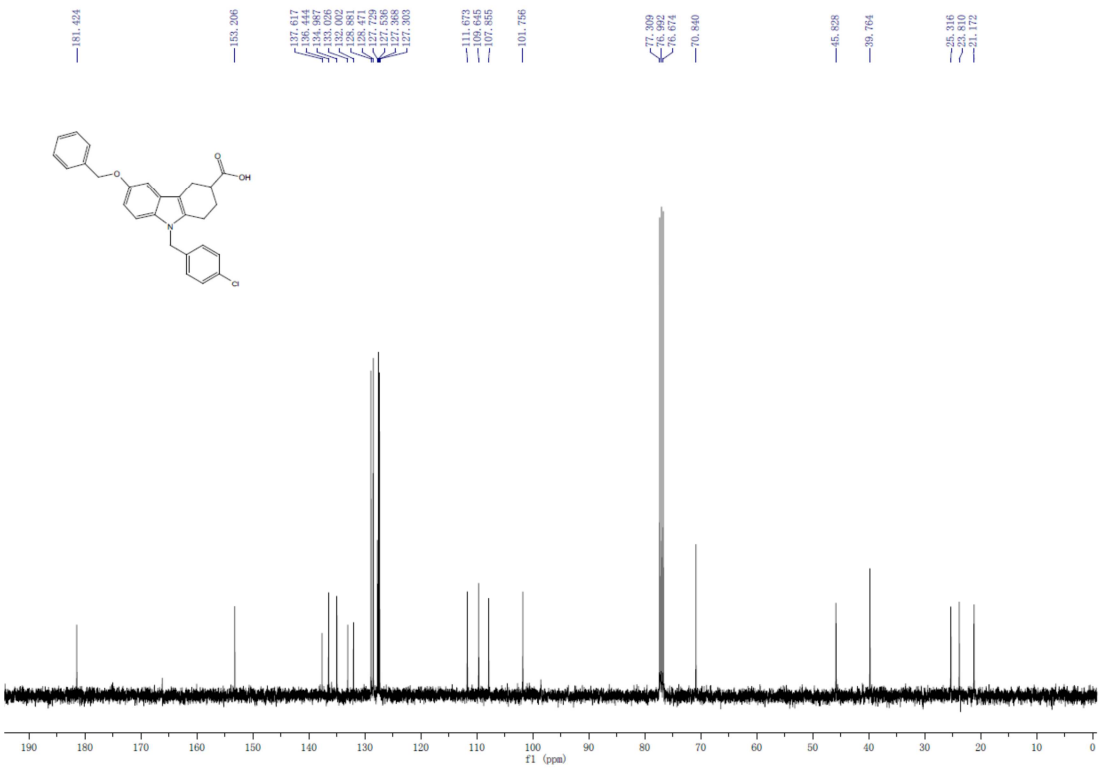


6-(Benzyloxy)-9-(4-chlorobenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid ***(8e)***

^{1}H NMR

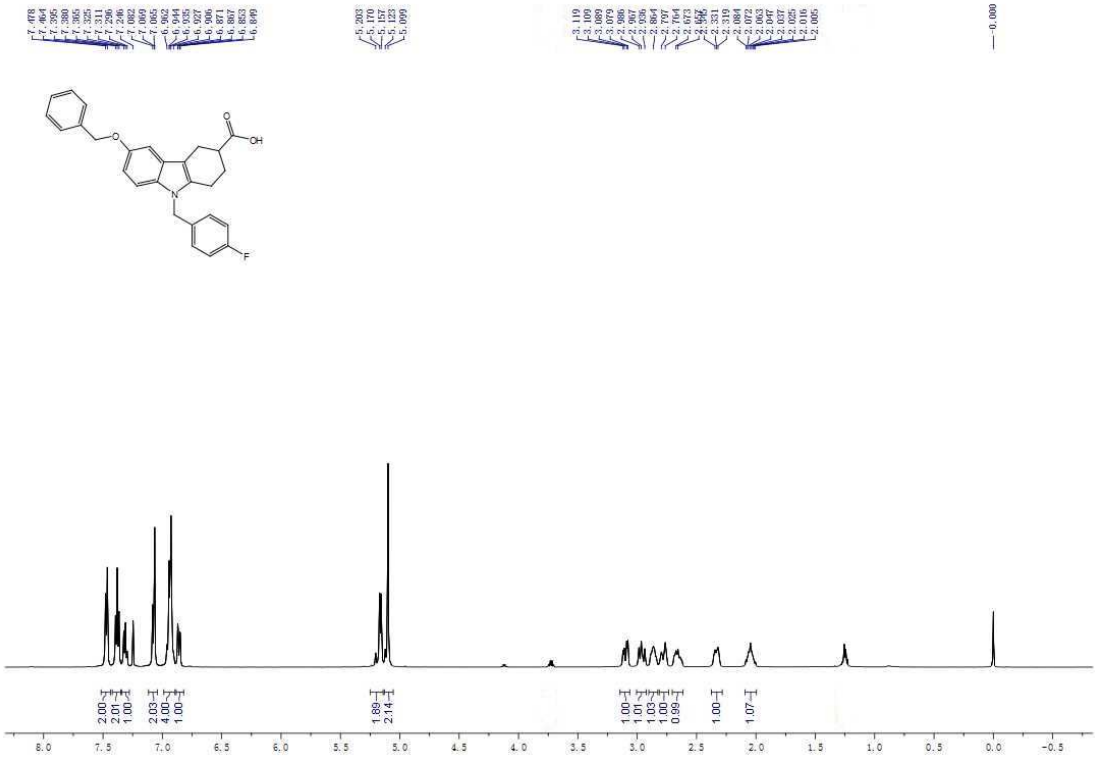


^{13}C NMR

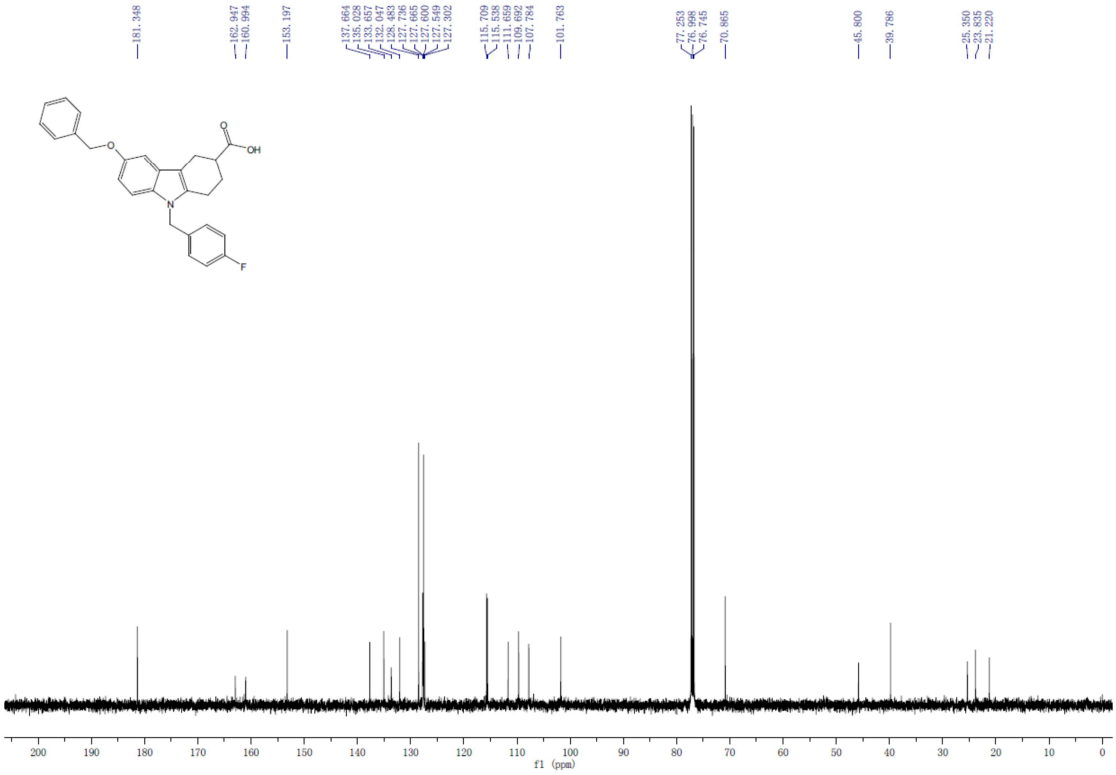


6-(Benzyloxy)-9-(4-fluorobenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid ***(8f)***

^{1}H NMR

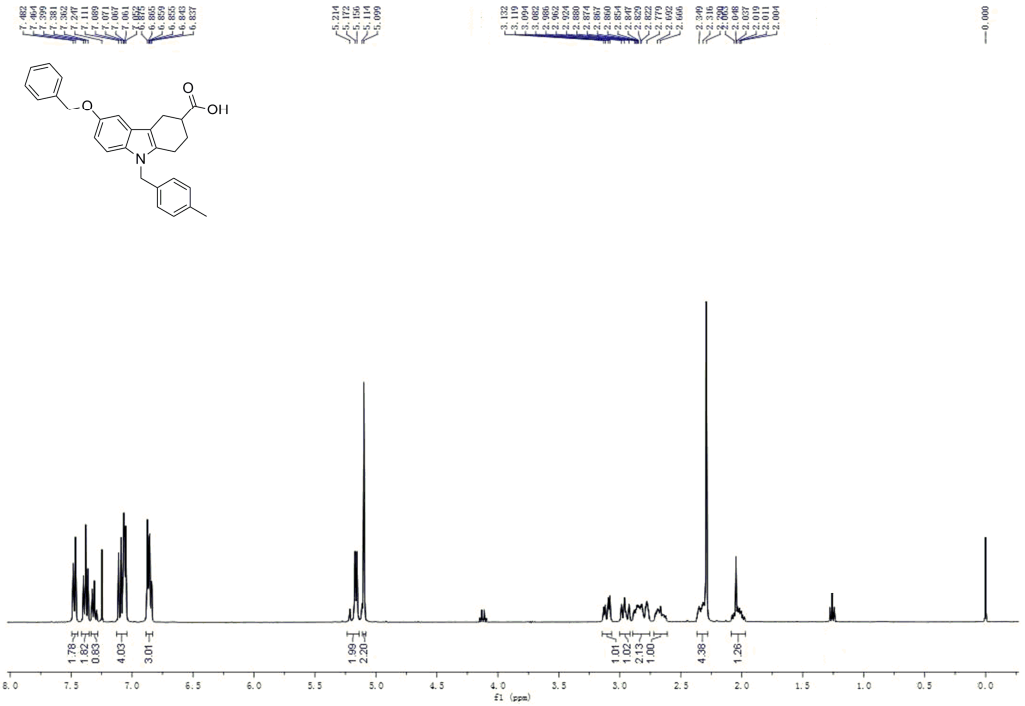


^{13}C NMR

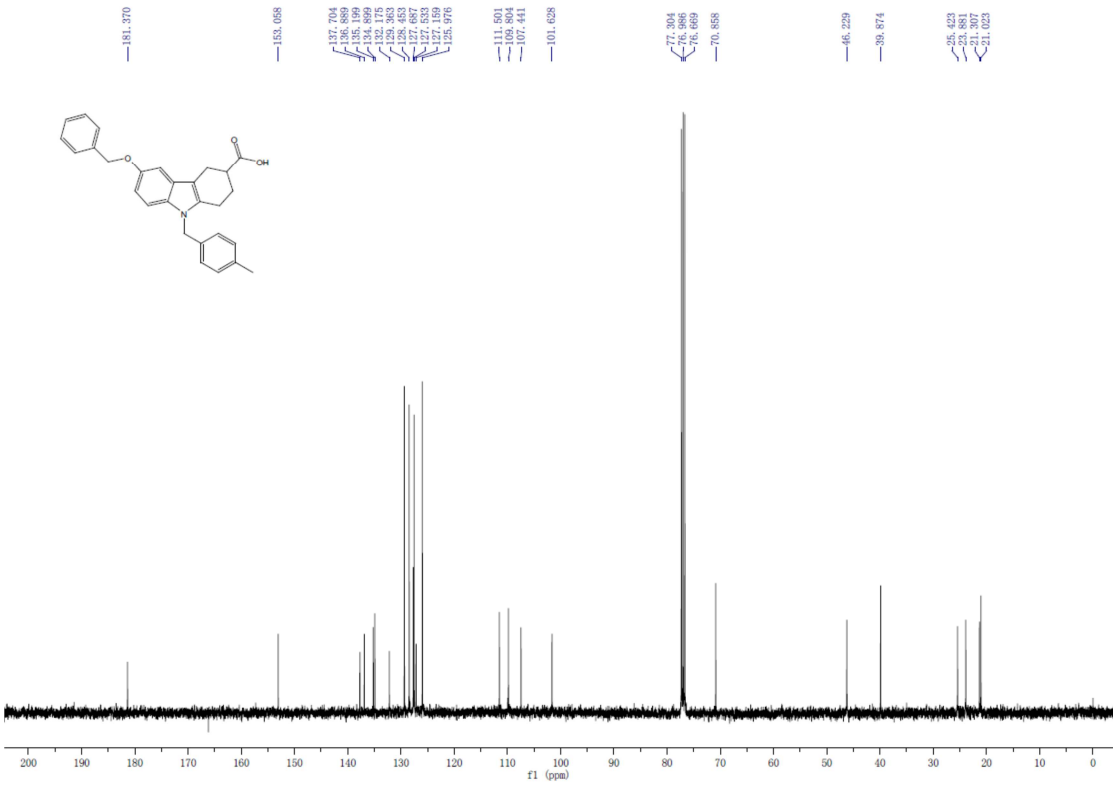


6-(Benzyloxy)-9-(4-methylbenzyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid ***(8g)***

^{1}H NMR

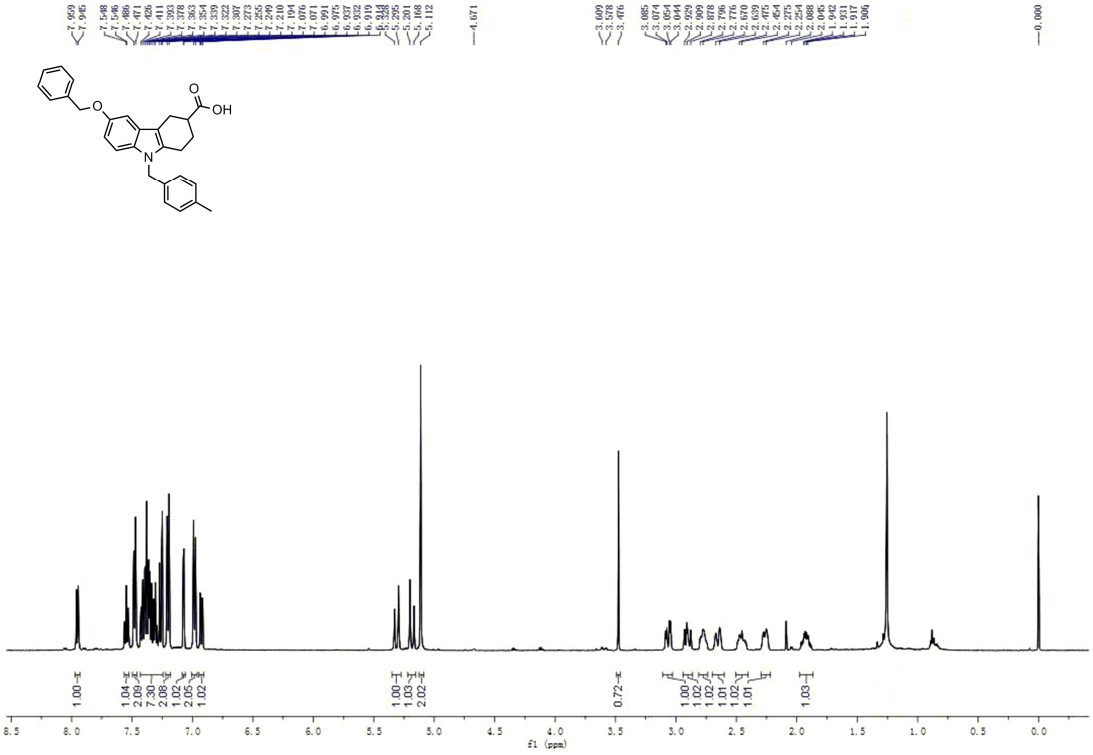


^{13}C NMR

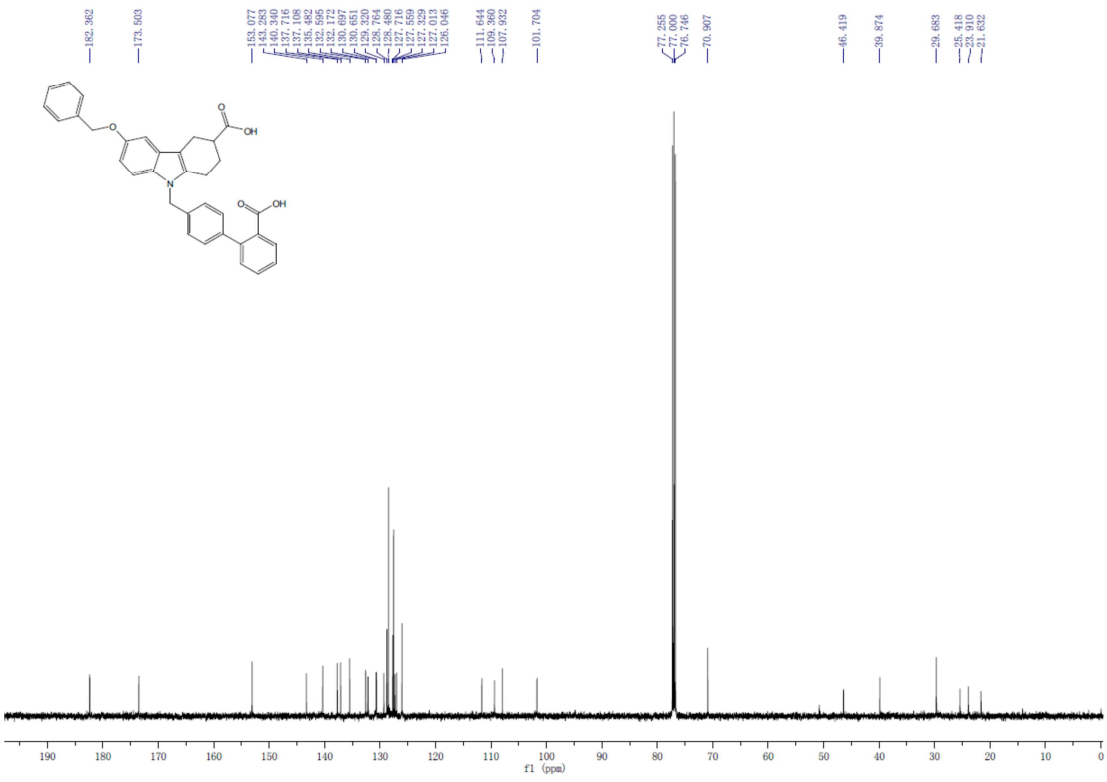


*6-(Benzyloxy)-9-((2'-carboxy-[1,1'-biphenyl]-4-yl)methyl)-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (**8h**)*

^{1}H NMR

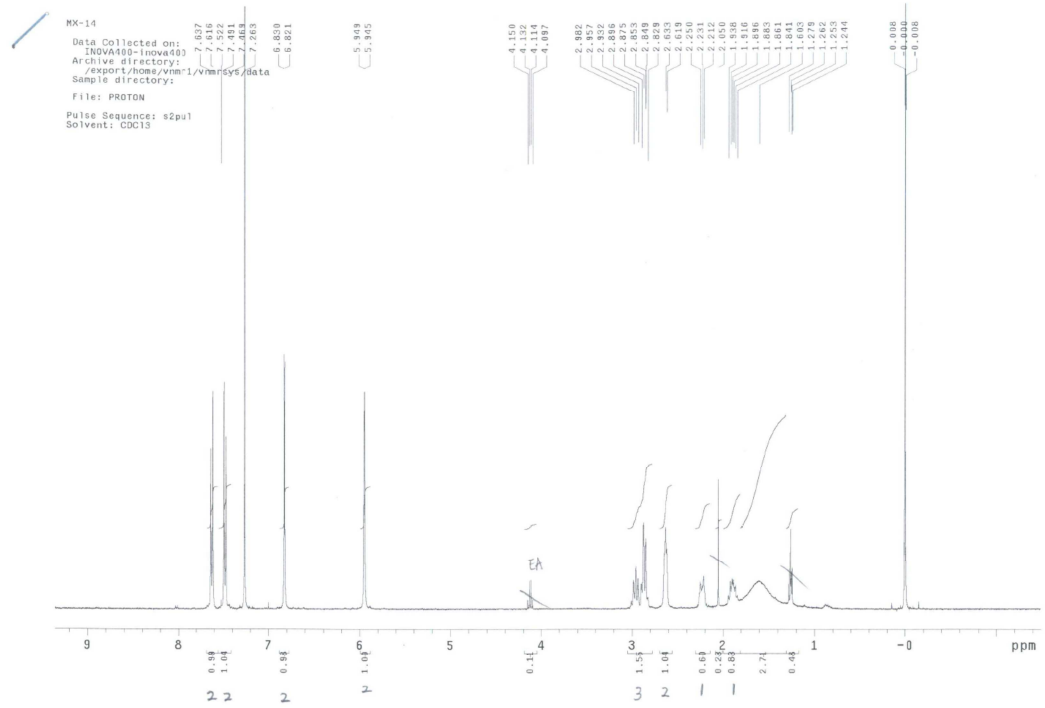


^{13}C NMR

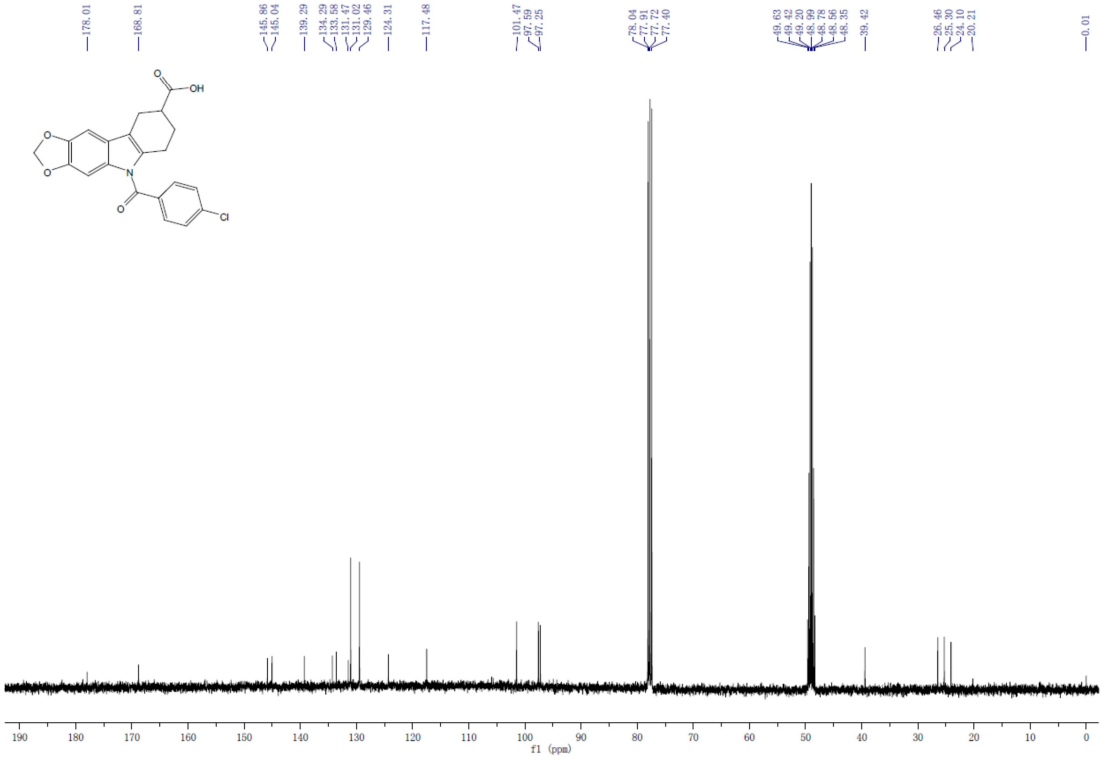


*5-(Chlorobenzoyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (**13a**)*

^{1}H NMR

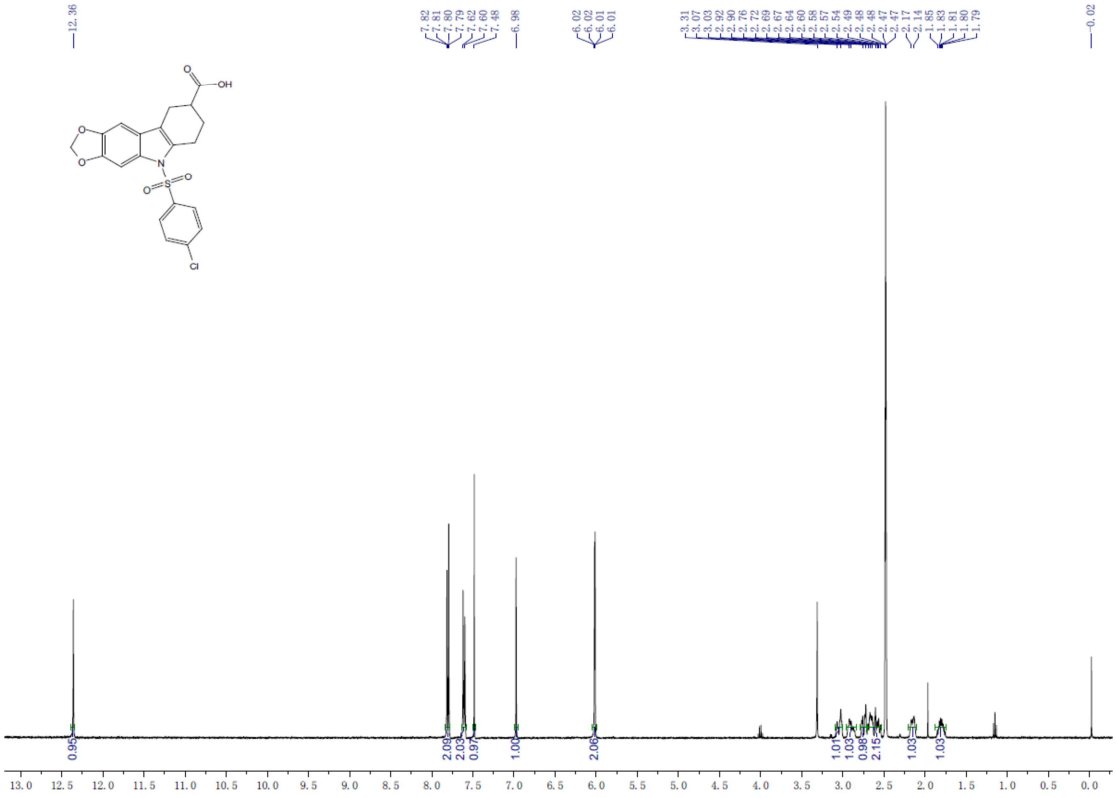


^{13}C NMR

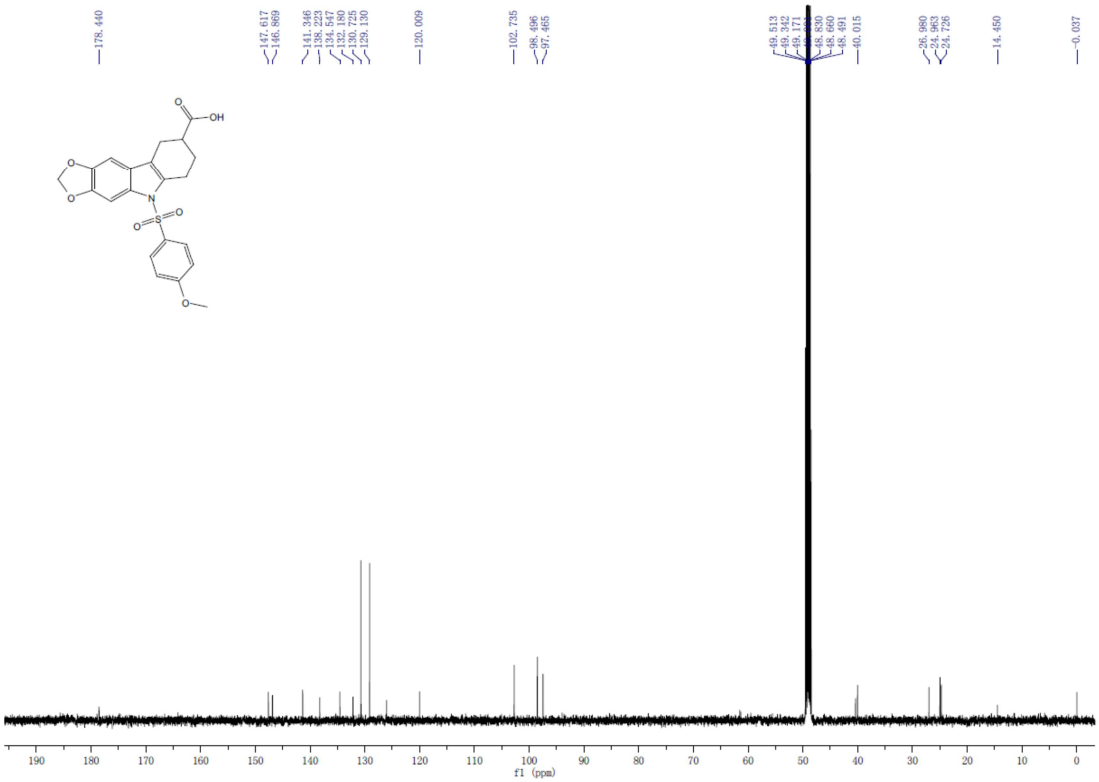


*5-((4-Chlorophenyl)sulfonyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (**13b**)*

^{1}H NMR

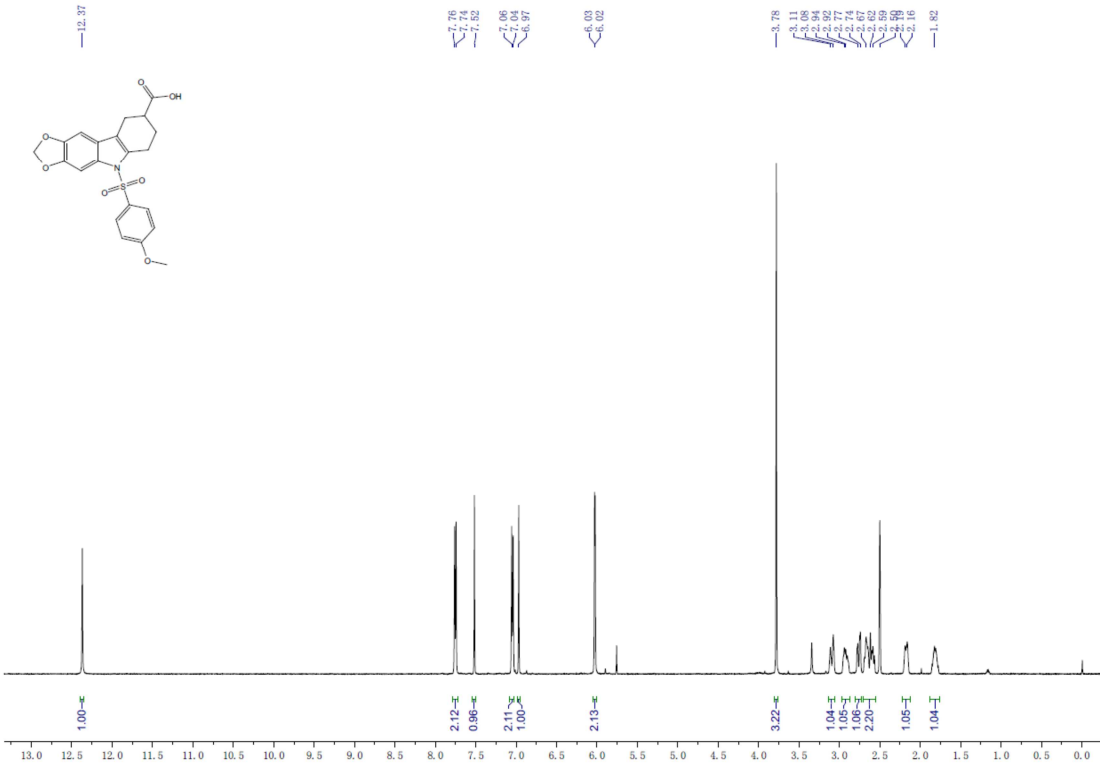


^{13}C NMR



*5-((4-Methoxyphenyl)sulfonyl)-6,7,8,9-tetrahydro-5H-[1,3]dioxolo[4,5-b]carbazole-8-carboxylic acid (**13c**)*

^{1}H NMR



^{13}C NMR