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Synthesis of 3,8,9-trisubstituted-1,7,9-triaza-fluorene-6-carboxylic acid derivatives as a new class of insulin secretagogues☆

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Abstract—β-Carbolines stimulate insulin secretion in a glucose-dependent manner, probably by acting on I_3-binding site. Knowing the in vitro glucose-dependent insulinotropic potential of β-carbolines, in this project, three series of substituted-triaza-fluorene-6-carboxylic acids (**5a**–**v**, **6a**–**t**, and **7a**–**t**) were designed (analogs of β-carboline) as a new class of insulinotropic agents. The in vitro glucose-dependent insulinotropic activities of test compounds were evaluated using RIN5F assay. Interestingly, with respect to the control, test compounds showed concentration-dependent insulin release, only in presence of glucose load (16.7 mmol). Some of the test compounds from each series were found to be equipotent to standard compound (Harmane), indicating that the pyridine ring systems of substituted-triaza-fluorenes act as bioisosteres of benzene ring in β-carbolines.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common, chronic, and life threatening diseases. Every year, the prevalence of T2DM is increasing worldwide and recently, World Health Organization (WHO) predicted that by 2030, the number of patients diagnosed with T2DM will be more than 366 millions.[1,2] Clinically T2DM is characterized by increased blood glucose levels, either because of defect in insulin secretion, insulin resistance or both.[3] Despite large efforts to discover new antidiabetic drugs, only three classes of oral hypoglycemic agents (sulfonylureas, biguanides, and insulin sensitizers) are available for the treatment of T2DM.[4] Sulfonylureas exhibit antidiabetic activity by blocking the ATP sensitive K^+ channel (K_{ATP} channel) in the pancreatic β-cells and stimulate insulin secretion both at low and high glucose concentration.[5,6] This glucose-independent insulinotropic effect of sulfonylureas often led to hypoglycemia.[7,8] Thus, there is an urgent need to develop some novel therapeutic approaches for glycemic control that can complement with the existing therapies and possibly attempt to preserve normal physiological response to meal intake. One such approach is based on the development of insulin secretagogues, which do not cause glucose secretion under basal blood glucose levels but show only glucose-dependent insulin release.

The pancreatic β-cells express an imidazoline-binding site (IBS) that is involved in the regulation of insulin secretion.[9–11] This site is pharmacologically different in composition with the I_1 and I_2 imidazoline sites described in other tissues and it has been classified as third imidazoline-binding site or I_3 receptor.[12] It is well established that a variety of imidazoline derivatives, such as a BL 11282 (**1**), stimulate glucose-dependent insulin secretion, by binding to I_3 site.[13–15] Recently, it has been proposed that β-carboline may be endogenous ligand for I_3 receptor.[16] The β-carbolines and its structural analogs (Fig. 1) such as Harmane (**2a**), Norharmane (**2b**) and Harmine (**2c**) are reported in the literature to cause a concentration- and glucose-dependent insulin secretion in isolated human islet.[17] Thus, β-carbolines represent a new class of insulin secretagogues, which mediate its action by I_3-binding sites in the β-cells. However, β-carbolines exhibit high binding affinity for several other receptors such as I_1 (hypotension) and I_2 (CNS effect) imidazoline-binding sites (IBS) and α_2-adrenoreceptors (α_2-ARs, hypotension), which often lead to CNS and CVS related side-effects.[18–20]

Keywords: Insulin secretion; β-Carboline; Harmane; Triazafluorene; I_3 receptor; Type 2 diabetes.
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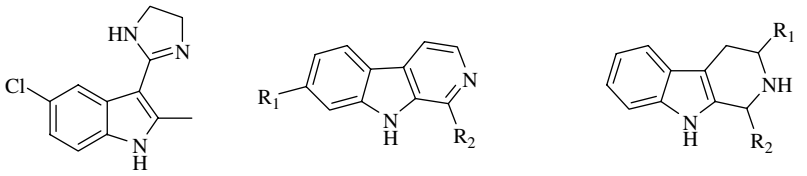


BL11282 (**1**)

β–Carbolines
2a: Harmane (R_1= H; R_2 = CH_3)
2b: Norharmane (R_1 & R_2 = H)
2c: Harmine (R_1 = OCH_3; R_2 = CH_3)

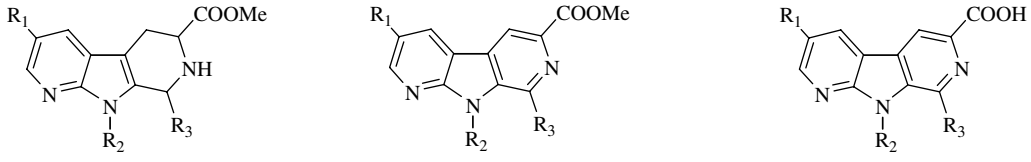
Tetrahydro-β-carboline (**3**)
R_1 = COOH / H; R_2 = alkyl / aryl

Figure 1. Structures of compounds having binding affinity for imidazoline receptors.

In order to develop safe and effective β-carboline based new classes of secretagogues, it is essential to abolish α_2, I_1, and I_2 binding affinities of β-carbolines and retain selective I_3-receptor-binding affinity, which might be possible by carrying out suitable structural modifications. The I_1, I_2, and α_2 receptor site binding affinities for a tetrahydro-β-carboline (**3**; Fig. 1) have been reported in the literature and it was found that compound **3** exhibited no binding at I_1, I_2 (R_1 = COOH) and α_2 receptor (R_1 = H) sites (K_i/IC_{50} > 10 μM).[18] The structure–activity relationship (SAR) study of tetrahydro-β-carbolines reveals that introduction of a carboxylate group (–COOH) at R_1 position abolishes I_1- and I_2-binding affinity, and the I_2 affinity was dramatically lowered if R_2 is out of the plane of the ring.[19] Furthermore, substitution of R_2 with α/β-methyl group (α-below the plane of ring and β group above the plane) diminishes I_1 and α_2 affinities.[18] Based upon the SAR study of tetrahydro-β-carbolines and knowing the I_3 receptor mediated insulin secretagogue properties of β-carbolines, in this project, series of substituted-triaza-fluorene-6-carboxylic acids were designed as analogs of β-carboline (Fig. 2). While designing the new series of substituted-triaza-fluorene-6-carboxylic acids, favorable attributes of tetrahydro-β-carboline derivatives were taken into consideration so as to abolish I_1-, I_2-, and α_2-binding affinity of triaza-fluorenes and retained the I_3 receptor mediated insulin secretagogue properties of β-carbolines. The in vitro glucose-dependent insulinotropic activity of all the test compounds was evaluated using RIN5F (Rat Insulinoma) cell assay. Furthermore, most potent compounds from each series were tested for in vitro binding affinities at I_1, I_2, and α_2 sites.

2. Chemistry

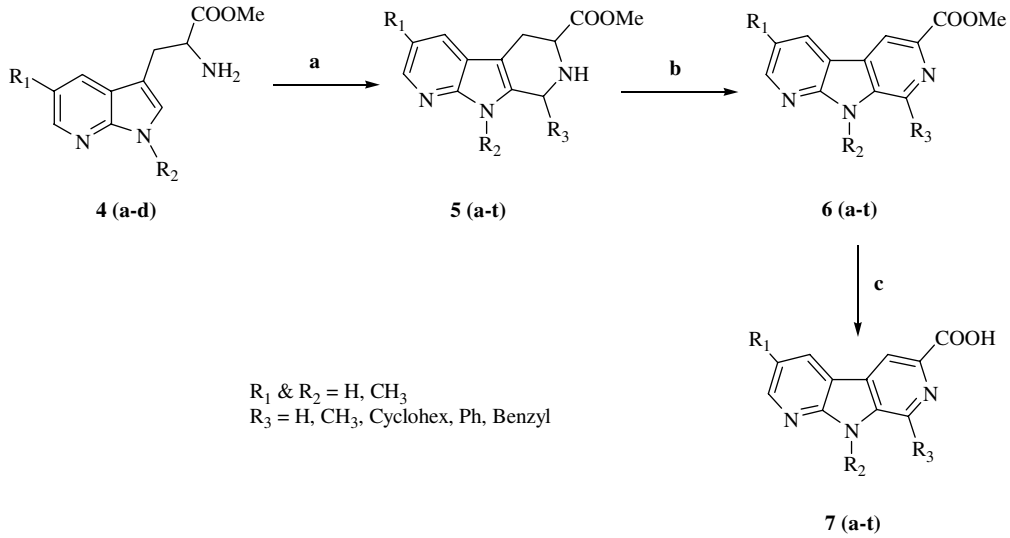
As shown in Scheme 1, synthesis of titled compounds was carried out by modified methods, which are available for the synthesis of β-carbolines. Starting material 1,5-disubstituted-7-azatryptophan (**4a**–**d**) was prepared from substituted-7-azaindoles, using literature method.[21] Synthesis of *cis* and (±) *trans* isomers of 3,8,9-trisubstituted-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-6-carboxylic acid methyl ester (**5a**–**t**) was carried out by cyclization of 1,5-disubstituted-7-azatryptophan (**4a**–**d**) by Pictet–Spingler cyclization, in the presence of appropriate aldehydes.[22] The reaction was performed in non-acidic/non-basic aprotic media (refluxing in benzene) or acidic/basic protic media (aqueous HCl or NaOH reflux) and the best results were obtained in non-acidic/non-basic aprotic media.[23,24] When the cyclization of compounds **4a**–**d** was carried out in the presence of *p*-toluenesulfonic acid and toluene (48 h reflux), it resulted in the formation of fully aromatized compounds 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid



5 (a-t) **6 (a-t)** **7 (a-t)**

5, 6 & 7a: R_1, R_2, R_3= H;
5, 6 & 7b: R_1, R_2= H & R_3= CH_3;
5, 6 & 7c: R_1, R_2= H & R_3= Cyclohex;
5, 6 & 7d: R_1, R_2= H & R_3= Ph;
5, 6 & 7e: R_1, R_2 = H & R_3= Benzyl ;
5, 6 & 7f: R_1= H, R_2= CH_3 & R_3= H;
5, 6 & 7g: R_1= H, R_2= CH_3 & R_3= CH_3;
5, 6 & 7h: R_1= H, R_2= CH_3 & R_3= Cyclohex;
5, 6 & 7i: R_1= H, R_2= CH_3 & R_3= Ph;
5, 6 & 7j: R_1= H, R_2= CH_3 & R_3= Benzyl;
5, 6 & 7k: R_1= CH_3, R_2= H & R_3= H;
5, 6 & 7l: R_1= CH_3, R_2= H & R_3= CH_3;
5, 6 & 7m: R_1= CH_3, R_2= H & R_3= Cyclohex;
5, 6 & 7n: R_1= CH_3, R_2= H & R_3= Ph;
5, 6 & 7o: R_1= CH_3, R_2= H & R_3= Benzyl;
5, 6 & 7p: R_1, R_2= CH_3 & R_3= H;
5, 6 & 7q: R_1, R_2 & R_3= CH_3;
5, 6 & 7r: R_1, R_2= CH_3 & R_3= Cyclohex;
5, 6 & 7s: R_1, R_2= CH_3 & R_3= Ph;
5, 6 & 7t: R_1, R_2= CH_3 & R_3= Benzyl;

Figure 2. Structures of 3,8,9-trisubstituted-1,7,9-triaza-fluorene-6-carboxylic acid derivatives.



Scheme 1. Reagents and conditions: (a) aldehydes, benzene, reflux; (b) sulfur powder, xylene, reflux; (c) $NaOH_{(aq)}$, ethanol, reflux.

methyl esters (**6a**–**t**) and the overall yield via one-pot aromartization was found to be less (~10%). The Pictet–Spingler condensation of compounds **4a**–**d** with the appropriate aldehyde in refluxing benzene gave 60–90% yield of compounds **5a**–**t**, as a mixture of diastereoisomers. Cyclization in acidic or basic solution gave significantly lower yields (~20%). Further compounds **5a**–**t** as a mixture of diastereomers were subjected to aromatization by refluxing with sulfur powder in dry xylene and compounds **6a**–**t** were obtained.[25] Attempts were also made to carry out dehydrogenation of compounds **5a**–**t** with 5% Pd/C, in refluxing cumene (with lead tetraacetate) or in glacial acetic acid, but it mainly gave poor yield (~20%).[26] Finally compounds 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acids (**7a**–**t**) were obtained by the hydrolysis of compounds **6a**–**t** with sodium hydroxide.[26]

Total 60 compounds (**5a**–**t**, **6a**–**t**, and **7a**–**t**) were prepared in good yield, under the mild reaction condition and the overall percentage yield was found to be in the range of 60–75%. The Infrared (IR) spectra of compounds **5a**–**t** showed peaks in the region of 3350–3250 due to N–H stretching of triaza-fluorenes and 1740–1730 due to C=O stretching of –COOMe group. Compounds **6a**–**t** showed absorption peaks in the region of 1725–1705 (C=O stretching) and compounds **7a**–**t** showed peaks at 1640–1625 due to C=O stretching of COOH groups. The ^{1}H NMR spectra of compounds **5** and **6** showed chemical shift (δ ppm) in the range of ~3.7–4.0 (s, 3H) due to –COOMe group, while compounds **7a**–**t** showed disappearance of signals due to –COOMe group and appearance of additional signals in the range of ~11.9–12.2 (bs, 1H) due to COOH group. The elemental analyses of all the compounds were found within the limit of ±0.4% of theoretical values. The corresponding spectral data are presented in experimental Section 5.2 and were found to be in confirmatory with the structure assigned. Synthesis of Harmane was carried out by literature procedure.[27,28]

3. Results and discussion

3.1. In vitro insulin secretion assay results

In vitro glucose-dependent or -independent insulin secretion properties of test compounds, 3,8,9-trisubstituted-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (**5a**–**t**), 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (**6a**–**t**), and 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (**7a**–**t**), were determined using a RIN5F cell assay.[29,30] Fold insulin release, both in the presence and absence of 16.7 mM glucose, was measured for Harmane and test compounds (0.1, 1, and 10 μM concentrations). At 0 μM glucose load, incubation of test and standard compounds with RIN5F cells showed basal insulin secretion. However in presence of 16.7 mM glucose load, significant concentration-dependent insulin secretion was observed, both for standard and test compounds. Further, to validate the in vitro assay, RIN5F cells were incubated with Tolbutamide (sulfonylurea), both in presence or absence of glucose load (0 and 16.7 mM), at 0.1 and 1 μM concentration. Tolbutamide showed significant insulin secretion both in presence or absence of glucose load (Tables 1–3).

In 3,8,9-trisubstituted-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (**5a**–**t**) series, preliminary glucose-dependent insulinotropic effect of compounds was assessed as racemic mixture (Table 1). The compounds **5p**–**t** showed highest insulin secretion among all the compounds tested in this series. With respect to Harmane compounds **5a**–**t** showed mild glucose-dependent insulinotropic effect. Among compounds **5p**–**t**, compound **5t** (racemate) showed highest activity in this series. In 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (**6a**–**t**) series, compounds **6p**–**t** showed highest insulin secretion. Among compounds **6p**–**t**, compound **6t** showed highest glucose-dependent insulin secretion

Table 1. In vitro glucose-dependent insulin secretion activity of compounds **5a–t**

Compound	R_1	R_2	R_3	Conc. (μM)	Insulin secretion[a] (pg/μg/h)
Control 1 (0 mM glucose)					6 ± 0.66
Control 2 (16.7 mM glucose)					10 ± 0.62
Tolbutamide (0 mM glucose)				0.1/1	19.6 ± 0.61/26.0 ± 0.52
Tolbutamide (16.7 mM glucose)				0.1/1	19.9 ± 0.56/27.1 ± 0.59
Harmane				0.1/1/10	20.0 ± 0.61/27.0 ± 0.52/37.0 ± 0.36
5a	H	H	H	0.1/1/10	10.1 ± 0.22/10.6 ± 0.11/10.9 ± 0.16
5b	H	H	CH_3	0.1/1/10	10.1 ± 0.06/10.6 ± 0.16/10.9 ± 0.12
5c	H	H	Cyclohex	0.1/1/10	10.2 ± 0.22/10.7 ± 0.24/11.0 ± 0.29
5d	H	H	Ph	0.1/1/10	10.8 ± 0.11/11.2 ± 0.13/11.5 ± 0.42
5e	H	H	Benzyl	0.1/1/10	10.9 ± 0.23/11.5 ± 0.36/11.9 ± 0.46
5f	H	CH_3	H	0.1/1/10	10.8 ± 0.44/11.2 ± 0.34/11.5 ± 0.31
5g	H	CH_3	CH_3	0.1/1/10	10.9 ± 0.41/ 11.5 ± 0.45/11.9 ± 0.43
5h	H	CH_3	Cyclohex	0.1/1/10	10.9 ± 0.33/11.5 ± 0.36/11.9 ± 0.31
5i	H	CH_3	Ph	0.1/1/10	11.0 ± 0.51/11.9 ± 0.56/12.2 ± 0.50
5j	H	CH_3	Benzyl	0.1/1/10	11.5 ± 0.10/12.2 ± 0.12/12.9 ± 0.16
5k	CH_3	H	H	0.1/1/10	10.8 ± 0.06/11.2 ± 0.16/11.5 ± 0.08
5l	CH_3	H	CH_3	0.1/1/10	10.9 ± 0.09/11.5 ± 0.06/11.9 ± 0.12
5m	CH_3	H	Cyclohex	0.1/1/10	10.9 ± 0.13/11.5 ± 0.22/11.9 ± 0.11
5n	CH_3	H	Ph	0.1/1/10	11.0 ± 0.22/11.9 ± 0.15/12.2 ± 0.14
5o	CH_3	H	Benzyl	0.1/1/10	11.5 ± 0.33/12.2 ± 0.43/12.9 ± 0.42
5p	CH_3	CH_3	H	0.1/1/10	12.2 ± 0.26/13.1 ± 0.46/14.1 ± 0.18
5q	CH_3	CH_3	CH_3	0.1/1/10	12.3 ± 0.25/13.2 ± 0.22/14.0 ± 0.18
5r	CH_3	CH_3	Cyclohex	0.1/1/10	12.4 ± 0.16/13.4 ± 0.26/14.2 ± 0.22
5s	CH_3	CH_3	Ph	0.1/1/10	13.5 ± 0.23/14.9 ± 0.34/15.2 ± 0.27
5t	CH_3	CH_3	Benzyl	0.1/1/10	15.1 ± 0.52/15.9 ± 0.42/16.8 ± 0.59

[a] In vitro glucose-dependent (16.7 mM glucose load) insulin secretion with various concentrations of test and standard compounds was measured using Rat Insulinoma (RIN5F) cells. The total insulin content obtained in pg was divided by total protein (μg) to normalize difference in cell density between wells. $n = 3$, values represent means ± SD.

Table 2. In vitro glucose-dependent insulin secretion activity of compounds **6a–t**

Compound	R_1	R_2	R_3	Conc. (μM)	Insulin secretion[a] (pg/μg/h)
Control 1 (0 mM glucose)					6 ± 0.66
Control 2 (16.7 mM glucose)					10 ± 0.62
Tolbutamide (0 mM glucose)				0.1/1	19.6 ± 0.61/26.0 ± 0.52
Tolbutamide (16.7 mM glucose)				0.1/1	19.9 ± 0.56/27.1 ± 0.59
Harmane				0.1/1/10	20.0 ± 0.61/27.0 ± 0.52/37.0 ± 0.36
6a	H	H	H	0.1/1/10	11.0 ± 0.52/11.9 ± 0.42/12.0 ± 0.59
6b	H	H	CH_3	0.1/1/10	11.0 ± 0.12/12.1 ± 0.18/12.5 ± 0.10
6c	H	H	Cyclohex	0.1/1/10	11.9 ± 0.11/12.1 ± 0.17/12.5 ± 0.23
6d	H	H	Ph	0.1/1/10	12.0 ± 0.22/12.6 ± 0.12/12.9 ± 0.06
6e	H	H	Benzyl	0.1/1/10	12.9 ± 0.52/13.0 ± 0.42/13.5 ± 0.59
6f	H	CH_3	H	0.1/1/10	13.0 ± 0.23/13.1 ± 0.34/13.9 ± 0.27
6g	H	CH_3	CH_3	0.1/1/10	13.4 ± 0.16/13.9 ± 0.26/14.1 ± 0.22
6h	H	CH_3	Cyclohex	0.1/1/10	13.4 ± 0.09/13.9 ± 0.06/14.2 ± 0.12
6i	H	CH_3	Ph	0.1/1/10	13.9 ± 0.22/14.1 ± 0.15/14.8 ± 0.14
6j	H	CH_3	Benzyl	0.1/1/10	14.0 ± 0.13/14.6 ± 0.22/15.0 ± 0.11
6k	CH_3	H	H	0.1/1/10	13.1 ± 0.44/13.2 ± 0.34/13.9 ± 0.31
6l	CH_3	H	CH_3	0.1/1/10	13.5 ± 0.23/13.9 ± 0.36/14.2 ± 0.43
6m	CH_3	H	Cyclohex	0.1/1/10	13.5 ± 0.11/14.0 ± 0.13/14.3 ± 0.42
6n	CH_3	H	Ph	0.1/1/10	13.9 ± 0.51/14.2 ± 0.56/14.9 ± 0.50
6o	CH_3	H	Benzyl	0.1/1/10	14.1 ± 0.61/14.8 ± 0.52/15.1 ± 0.36
6p	CH_3	CH_3	H	0.1/1/10	14.3 ± 0.11/14.9 ± 0.14/15.6 ± 0.51
6q	CH_3	CH_3	CH_3	0.1/1/10	14.8 ± 0.26/15.2 ± 0.41/15.9 ± 0.17
6r	CH_3	CH_3	Cyclohex	0.1/1/10	14.8 ± 0.10/15.2 ± 0.12/15.9 ± 0.16
6s	CH_3	CH_3	Ph	0.1/1/10	15.1 ± 0.22/15.6 ± 0.32/16.2 ± 0.29
6t	CH_3	CH_3	Benzyl	0.1/1/10	15.9 ± 0.18/16.1 ± 0.22/16.9 ± 0.27

[a] In vitro glucose-dependent (16.7 mM glucose load) insulin secretion with various concentrations of test and standard compounds was measured using Rat Insulinoma (RIN5F) cells. The total insulin content obtained in pg was divided by total protein (μg) to normalize difference in cell density between wells. $n = 3$, values represent means ± SD.

(Table 2). With respect to the standard compounds, compound **5t** showed moderate insulin secretion. In 3,8,9-trisubstituted-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (**7a–t**) series, compounds **7p–t** showed highest insulin secretion (Table 3). Among compounds **7p–t**, compound **7t** showed highest glucose-dependent

Table 3. In vitro glucose-dependent insulin secretion activity of compounds **7a–t**

Compound	R_1	R_2	R_3	Conc. (μM)	Insulin secretion (pg/mg/h)[a]
Control 1 (0 mM glucose)					6 ± 0.66
Control 2 (16.7 mM glucose)					10 ± 0.62
Tolbutamide (0 mM glucose)				0.1/1	19.6 ± 0.61/26.0 ± 0.52
Tolbutamide (16.7 mM glucose)				0.1/1	19.9 ± 0.56/27.1 ± 0.59
Harmane				0.1/1/10	20.0 ± 0.61/27.0 ± 0.52/37.0 ± 0.36
7a	H	H	H	0.1/1/10	14.1 ± 0.52/16.2 ± 0.42/18.0 ± 0.59
7b	H	H	CH_3	0.1/1/10	15.4 ± 0.23/17.4 ± 0.34/20.1 ± 0.27
7c	H	H	Cyclohex	0.1/1/10	15.5 ± 0.16/17.1 ± 0.22/20.4 ± 0.26
7d	H	H	Ph	0.1/1/10	16.2 ± 0.25/18.1 ± 0.22/20.6 ± 0.18
7e	H	H	Benzyl	0.1/1/10	17.1 ± 0.26/18.3 ± 0.46/21.5 ± 0.18
7f	H	CH_3	H	0.1/1/10	15.2 ± 0.33/17.4 ± 0.43/19.1 ± 0.42
7g	H	CH_3	CH_3	0.1/1/10	16.2 ± 0.22/18.1 ± 0.14/21.5 ± 0.15
7h	H	CH_3	Cyclohex	0.1/1/10	16.1 ± 0.14/18.4 ± 0.22/21.4 ± 0.11
7i	H	CH_3	Ph	0.1/1/10	17.2 ± 0.22/19.3 ± 0.11/21.1 ± 0.16
7j	H	CH_3	Benzyl	0.1/1/10	18.5 ± 0.16/19.4 ± 0.12/22.2 ± 0.14
7k	CH_3	H	H	0.1/1/10	15.1 ± 0.13/17.3 ± 0.22/19.2 ± 0.11
7l	CH_3	H	CH_3	0.1/1/10	16.2 ± 0.44/18.5 ± 0.34/21.1 ± 0.11
7m	CH_3	H	Cyclohex	0.1/1/10	16.3 ± 0.23/18.4 ± 0.35/21.2 ± 0.45
7n	CH_3	H	Ph	0.1/1/10	17.1 ± 0.10/19.3 ± 0.12/21.4 ± 0.16
7o	CH_3	H	Benzyl	0.1/1/10	18.0 ± 0.51/19.2 ± 0.43/22.1 ± 0.24
7p	CH_3	CH_3	H	0.1/1/10	17.3 ± 0.41/20.0 ± 0.44/24.2 ± 0.32
7q	CH_3	CH_3	CH_3	0.1/1/10	18.1 ± 0.22/22.0 ± 0.14/28.3 ± 0.19
7r	CH_3	CH_3	Cyclohex	0.1/1/10	18.2 ± 0.42/23.1 ± 0.18/28.2 ± 0.14
7s	CH_3	CH_3	Ph	0.1/1/10	19.1 ± 0.16/26.0 ± 0.12/32.2 ± 0.31
7t	CH_3	CH_3	Benzyl	0.1/1/10	20.0 ± 0.22/28.1 ± 0.15/38.3 ± 0.41

[a] In vitro glucose-dependent (16.7 mM glucose load) insulin secretion with various concentrations of test and standard compounds was measured using Rat Insulinoma (RIN5F) cells. The total insulin content obtained in pg was divided by total protein (μg) to normalize difference in cell density between wells. $n = 3$, values represent means ± SD.

insulin secretion and was found to be equipotent to Harmane.

In general, compared to tetrahydro-triaza-fluorene ring system (compounds **5a–t**), aromatized ring systems (triaza-fluorene-6-carboxylic acid methyl esters **6a–t**, and triaza-fluorene-6-carboxylic acids **7a–t**) showed good insulinotropic activities. Within aromatized ring systems (compounds **6a–t** and **7a–t**), compounds (**7a–t**) with free carboxylate (–COOH) group were found to be more potent than methyl esters of triaza-fluorene-6-carboxylic acid, indicating that free acidic group at sixth position favors insulinotropic effect. Various substituents at R_1, R_2, and R_3 position showed critical role, in terms of their structure–activity relationship (Tables 1–3). Substitution of either of R_1 and R_2 with hydrogen atom drastically reduced potency of test compounds in all the three series. Substitution of both the R_1 and R_2 with methyl group was found to be most active in all the three series. Substitution of R_3 with hydrogen atom showed least activity, while compounds in which R_3 is substituted with methyl or cyclohexyl group showed moderate activity. Substitution of R_3 with aromatic or alkyl–aryl groups showed highest activity indicating that hydrophobic bulky substitutions at R_3 position are favorable for glucose-dependent insulinotropic activity.

3.2. In vitro assay results of radioligand binding affinities at I_1-IBS, I_2-IBS, and α_2-ARs sites

Along with the binding at I_3 sites, β-carboline is known to exhibit high binding affinity for I_1-IBS, I_2-IBS, and α_2-ARs, which often lead to CNS and CVS related side-effects.[18–20] Thus, binding affinities (K_i) of most potent compounds from each series (**5t**, **6t**, and **7t**) for I_1-IBS, I_2-IBS, and α_2-ARs were assessed by measuring the ability of the test compounds to displace [^{3}H]clonidine (from adrenal medulla plasma membrane), [^{3}H]idazoxan (from rabbit kidney membrane), and [^{3}H]clonidine (from cerebral cortex of rat brain), respectively. In order to validate the assay system, along with the three test compounds, Harmane was taken as reference standard compound. As shown in Table 4, Harmane showed significant binding affinity at I_1 and I_2 sites in the nano-molar range, while at α_2 site, binding affinity was found to be in μM range. With respect to Harmane, none of the test compounds assayed displayed significant binding affinity at I_1, I_2, and α_2, in the μM range or higher (>100 μM). Combined evaluation of in vitro results indicated that the new class of substituted triazafluorenes, which were designed as analogs of β-carboline, exhibit selective glucose-dependent

Table 4. In vitro binding affinities of triazafluorenes for I_1, I_2 and α_2 sites

Compound	K_i (nM) I_1	K_i (nM) I_2	K_i (nM) α_2
Harmane	36 ± 3.2	54 ± 4.6	>12 000
5t	NB	NB	NB
6t	NB	NB	NB
7t	NB	NB	NB

K_i affinity for I_1-IBS, I_2-IBS and α_2-ARs were assessed by measuring the ability of the test compounds to displace [^{3}H]clonidine (from adrenal medulla plasma membrane), [^{3}H]idazoxan (from rabbit kidney membrane) and [^{3}H]clonidine from cerebral cortex of rat brain respectively. Experiments performed in triplicate with $n = 3$, values represent mean ± SEM, NB represents no binding >100 mM concentration of test compounds.

insulin secretion and will be expected to be devoid of CNS and CVS related side-effects due to non-specificity toward I_1, I_2, and α_2 sites.

4. Conclusions

The glucose-independent insulinotropic activity of sulfonylurea often led to hypoglycemic effect, thus it is essential to develop novel insulin secretagogues, which show only glucose-dependent insulin secretion. In this regard, β-carboline derivatives were found to be promising compounds. All the three series of triaza-fluorenes (**5a**–**t**, **6a**–**t**, and **7a**–**t**), which were designed as analogs of β-carbolines showed concentration- and glucose-dependent insulin secretion, indicating that the pyridine ring systems of triaza-fluorenes act as bioisosteres of benzene ring in β-carbolines. In general, series 1 (compounds **5a**–**t**) showed mild insulinotropic activity, series 2 (compounds **6a**–**t**) showed moderate insulinotropic activity, and series 3 (compounds **7a**–**t**) showed highest insulinotropic activity. Among all the three series tested in vitro, 8-benzyl-3,9-dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (**7t**) showed highest activity and the glucose-dependent insulin secretion profile of compound **7t** was found to be identical to that of standard compound (Harmane). Further mechanistic studies to investigate the mode of action and in vivo pharmacological studies are in progress and it will be published elsewhere.

5. Experimental

5.1. Chemistry

Melting points were recorded on open glass capillaries, using a scientific melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (V_{max} in cm^{-1}, using KBr pellets). The ^1H NMR spectra were recorded on a Brucker Avanc-300 spectrometer (300 MHz). The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer. Elemental analyses were carried out, using a Perkin-Elmer 2400 CHN analyzer, and values within limit of ±0.4 % of the theoretical values were taken into consideration. Purity of synthesized compounds was checked by precoated TLC plates (E. Merck Kieselgel 60 F_{254}) and the spots were visualized by iodine vapors. The chromatographic purification was performed on silica gel (100–200 or 200–400 mesh). All the chemicals used for the synthesis were purchased from Aldrich Company Limited, Dorset (UK).

5.2. Experimental details

5.2.1. General method for the synthesis of substituted-triaza-fluorene-6-carboxylic acids (Scheme 1).

5.2.1.1. Synthesis of 6,7,8,9-Tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5a). A mixture of **4a** (0.01 mol), paraformaldehyde (0.01 mol), and benzene (50 mL) was refluxed for 48 h. The reaction mixture was distilled under reduced pressure to obtain the yellow solid. The crude product obtained was purified by column chromatography, using a mixture of dichloromethane and ethyl acetate (8:2) as an eluent system, fractions were evaporated, and white solid compound was isolated as a mixture of *cis* and *trans* isomers of compound **5a**. Yield: 65%; white solid; mp 165–166 °C; IR (KBr): 3323, 3105, 2837, 1739, 1438, 742 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.71–10.68 (bs, 1H, pyrrole ring –*NH*), 8.59 (d, 1H, J = 7.05 Hz, pyridine ring), 7.75 (d, 1H, J = 7.65 Hz, pyridine ring), 7.38 (t, 1H, J = 7.11 Hz, pyridine ring), 4.12–3.93 (m, 2H, piperidine ring –*CH*$_2$–NH), 3.74–3.70 (m, 1H, piperidine ring –*CH*–$COOCH_3$), 3.67 (s, 3H, –COO*CH*$_3$), 2.96–2.72 (m, 3H, piperidine ring –*CH*$_2$ and –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.9, 41.3, 51.5, 55.1, 105.4, 117.1, 120.4, 126.9, 135.7, 142.1, 147.3, 173.6. MS (ESI) m/z 232 $[M+1]^+$; Anal. Calcd for $C_{12}H_{13}N_3O_2$: C 62.33, H 5.67, N 18.17. Found: C 62.29, H 5.64, N 18.15.

5.2.1.2. 8-Methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5b). Yield: 69%; white solid; mp 85–87 °C; IR (KBr): 3296, 2950, 2846, 1738, 1624, 1382, 1315, 744 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.79–7.68 (bs, 1H, pyrrole ring –*NH*), 7.46 (d, 1H, J = 7.05 Hz, pyridine ring), 7.31 (d, 1H, J = 7.65 Hz, pyridine ring), 7.08 (t, 1H, J = 7.11 Hz, pyridine ring), 4.29–4.24 (m, 1H, piperidine ring –*CH*–NH), 3.85–3.84 (m, 1H, piperidine ring, –*CH*–$COOCH_3$), 3.67 (s, 3H, –COO*CH*$_3$), 2.96–2.72 (m, 3H, piperidine ring –*CH*$_2$ and –*NH*), 1.47 (d, 3H, J = 6.61 Hz, piperidine ring –CH(*CH*$_3$)–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.9, 23.2, 48.9, 51.5, 57.6, 111.2, 115.6, 124.6, 129.0, 136.7, 142.1, 149.6, 173.0. MS (ESI) m/z 246 $[M+1]^+$ Anal. Calcd for $C_{13}H_{15}N_3O_2$: C 63.66, H 6.16, N 17.13. Found: C 63.62, H 6.20, N 17.12.

5.2.1.3. 8-Cyclohexyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5c). Yield: 66%; white solid; mp 182–184 °C; IR (KBr): 3293, 2953, 2806, 1732, 1598, 1382, 1353, 1332, 1315, 744 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.75–7.72 (bs, 1H, pyrrole ring –*NH*), 7.44 (d, 1H, J = 7.15 Hz, pyridine ring), 7.32 (d, 1H, J = 7.61 Hz, pyridine ring), 7.06 (t, 1H, J = 7.08 Hz, pyridine ring), 4.28–4.24 (m, 1H, piperidine ring –*CH*–NH), 3.68 (s, 3H, –COO*CH*$_3$), 3.78 (m, 1H, piperidine ring –*CH*–$COOCH_3$), 2.96–2.72 (m, 3H, piperidine ring –*CH*$_2$ and –*NH*), 2.16–2.15 (m, 1H, cyclohexyl ring), 1.28–1.24 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.9, 24.9, 26.3, 27.7, 38.9, 50.4, 53.7, 64.2, 112.2, 115.6, 125.6, 129.0, 136.7, 142.4, 148.6, 172.3. MS (ESI) m/z 314 $[M+1]^+$; Anal. Calcd for $C_{18}H_{23}N_3O_2$: C 68.98, H 7.40, N 13.41. Found: C 68.95, H 7.36, N 13.38.

5.2.1.4. 8-Phenyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5d). Yield: 68%; white solid; mp 215–217 °C; IR (KBr): 3058, 2953, 1735, 1685, 1413, 1340, 704 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.26–11.02 (bs, 1H, pyridine ring –*NH*),

8.11 (t, 1H, J = 7.57 Hz, pyridine ring), 7.85 (d, 1H, J = 7.53 Hz, pyridine ring), 7.33–7.21 (m, 5H, benzene ring), 7.04–6.98 (m, 1H, pyridine ring), 5.29–5.22 (m, 1H, piperidine ring –*CH*–NH), 3.86–3.85 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –$COOCH_3$), 3.20–2.88 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 49.7, 54.8, 56.1, 64.3, 68.1, 112.1, 115.3, 124.6, 126.8, 128.3, 128.9, 129.0, 136.7, 137.2, 142.2, 149.6, 148.9, 172.9. MS (ESI) *m*/*z* 308 $[M+1]^+$; Anal. Calcd for $C_{18}H_{17}N_3O_2$: C 70.34, H 5.58, N 13.67. Found: C 70.30, H 5.59, N 13.64.

5.2.1.5. 8-Benzyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5e). Yield: 69%; white solid; mp 232–234 °C; IR (KBr): 3058, 2933, 1732, 1660, 1560, 1442, 1340, 738 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41–10.35 (bs, 1H, pyrrole ring –*NH*), 8.29 (d, 1H, J = 7.79 Hz, pyridine ring), 8.06 (d, 1H, J = 7.47 Hz, pyridine ring), 7.30 (t, 1H, J = 7.38 Hz, pyridine ring), 7.26–7.18 (m, 5H, benzene ring), 4.65–4.62 (m, 1H, –*CH*–NH), 3.89–3.86 (m, 1H, –*CH*–$COOCH_3$), 3.70 (s, 3H, –$COOCH_3$), 2.98 (d, 2H, J = 6.8 Hz, benzyl –CH_2), 2.88–2.80 (m, 2H, piperidine ring –CH_2), 2.71–2.69 (bs, 1H, piperidine ring –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 45.2, 49.6, 54.7, 54.9, 64.9, 64.6, 112.1, 115.3, 124.6, 126.8, 128.3, 128.9, 129.0, 136.7, 137.2, 142.2, 149.6, 148.9, 172.9. MS (ESI) *m*/*z* 322 $[M+1]^+$; Anal. Calcd for $C_{19}H_{19}N_3O_2$: C 71.01, H 5.96, N 13.08. Found: C 69.98, H 5.93, N 13.03.

5.2.1.6. 9-Methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5f). Yield: 68%; white solid; mp 95–96 °C; IR (KBr): 3105, 2837, 1739, 1473, 1438, 788 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.59 (d, 1H, J = 6.98 Hz, pyridine ring), 7.72 (d, 1H, J = 7.09 Hz, pyridine ring), 7.32 (t, 1H, J = 6.99 Hz, pyridine ring), 3.93–3.87 (m, 2H, –CH_2–NH), 3.74–3.71 (m, 1H, piperidine ring –*CH*–$COOCH_3$), 3.68 (s, 3H, –$COOCH_3$), 3.48 (s, 3H, pyrrole ring N–CH_3), 2.96–2.92 (m, 2H, piperidine ring–CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.9, 34.9, 41.3, 51.5, 55.1, 105.4, 117.1, 120.4, 126.9, 135.7, 142.1, 147.3, 173.6. MS (ESI) *m*/*z* 246 $[M+1]^+$; Anal. Calcd for $C_{13}H_{15}N_3O_2$: C 63.66, H 6.16, N 17.13. Found: C 63.64, H 6.15, N 17.09.

5.2.1.7. 8,9-Dimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5g). Yield: 69%; white solid; mp 68–71 °C; IR (KBr): 3284, 2906, 2833, 1738, 1624, 1452, 1382, 1315, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.34 (d, 1H, J = 7.52 Hz, pyridine ring), 7.27 (d, 1 H, J = 7.86 Hz, pyridine ring), 6.99 (t, 1H, J = 7.12 Hz, pyridine ring), 4.01 (q, 1H, J = 6.51 Hz, piperidine ring –*CH*(CH_3)–NH), 3.85–3.84 (m, 1H, –*CH*–$COOCH_3$), 3.62 (s, 3H, –$COOCH_3$), 3.48 (s, 3H, pyrrole ring N–CH_3), 2.96–2.94 (m, 2H, piperidine ring–CH_2), 2.70–2.69 (bs, 1H, piperidine ring –*NH*), 1.47 (d, 3H, J = 6.6 Hz, piperidine ring –CH(CH_3)–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.9, 23.2, 35.8, 48.9, 51.5, 57.6, 111.2, 115.6, 124.6, 129.0, 136.7, 142.1, 149.6, 173.0. MS (ESI) *m*/*z* 260 $[M+1]^+$; Anal. Calcd for $C_{14}H_{17}N_3O_2$: C 64.85, H 6.16, N 16.21. Found: C 64.83, H 6.12, N 16.18.

5.2.1.8. 8-Cyclohexyl-9-methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5h). Yield: 68%; white solid; mp 152–153 °C; IR (KBr): 3293, 2953, 2806, 1738, 1598, 1382, 1353, 1332, 1315, 744 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, 1H, J = 7.13 Hz, pyridine ring), 7.28 (d, 1H, J = 7.49 Hz, pyridine ring), 7.06 (t, 1H, J = 7.11 Hz, pyridine ring), 4.07–3.98 (m, 1H, piperidine ring –*CH*–NH), 3.78 (m, 1H, –*CH*–$COOCH_3$), 3.68 (s, 3H, –$COOCH_3$), 3.46 (s, 3H, pyrrole ring N–CH_3), 2.96–2.72 (m, 3H, piperidine ring –CH_2 and –*NH*), 2.12–2.11 (m, 1H, cyclohexyl ring), 1.27–1.24 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.9, 24.9, 26.3, 27.7, 38.9, 50.4, 53.7, 64.2, 112.2, 115.6, 125.6, 129, 136.7, 142.4, 148.6, 172.3. MS (ESI) *m*/*z* 328 $[M+1]^+$; Anal. Calcd for $C_{19}H_{25}N_3O_2$: C 69.70, H 7.70, N 12.83. Found; C 69.68, H 7.69, N 12.78.

5.2.1.9. 9-Methyl-8-phenyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5i). Yield: 68%; white solid; mp 147–149 °C; IR (KBr): 3058, 2953, 1735, 1685, 1452, 1413, 1342, 724 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.09 (t, 1H, J = 5.67 Hz, pyridine ring), 7.85 (d, 1H, J = 7.51 Hz, pyridine ring), 7.33–7.21 (m, 5H, benzene ring), 7.04–6.98 (m, 1H, pyridine ring), 5.29–5.22 (m, 1H, piperidine ring –*CH*–NH), 3.86–3.85 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –$COOCH_3$), 3.49 (s, 3H, pyrrole ring N–CH_3), 3.20–2.83 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 35.8, 49.7, 54.8, 56.1, 64.3, 68.1, 112.1, 115.3, 124.6, 126.8, 128.3, 128.9, 129.8, 136.2, 137.2, 142.2, 149.6, 148.9, 176.0. MS (ESI) *m*/*z* 322 $[M+1]^+$; Anal. Calcd for $C_{19}H_{19}N_3O_2$: C 71.01, H 5.96, N 13.08. Found: C 70.98, H 6.01, N 13.06.

5.2.1.10. 8-Benzyl-9-methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5j). Yield: 66%; white solid; mp 232–234 °C; IR (KBr): 3048, 2931, 1732, 1660, 1560, 1453, 1432, 1340, 738 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.29 (d, 1H, J = 7.67 Hz, pyridine ring), 8.05 (d, 1H, J = 7.52 Hz, pyridine ring), 7.29 (t, 1H, J = 7.41 Hz, pyridine ring), 7.24–7.18 (m, 5H, benzene ring), 4.58–4.57 (m, 1H, piperidine ring –*CH*–NH), 3.58 (s, 3H, pyrrole ring N–CH_3), 3.79–3.76 (m, 1H, –*CH*–$COOCH_3$), 3.70 (s, 3H, –$COOCH_3$), 3.07–2.98 (m, 2H, piperidine ring –CH_2), 2.88 (d, 2H, J = 6.8 Hz, benzyl –CH_2), 2.71–2.69 (bs, 1H, piperidine ring –*NH*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 45.2, 49.6, 54.7, 54.9, 64.9, 64.6, 112.1, 115.3, 124.6, 126.8, 128.3, 128.9, 129.0, 136.7, 137.2, 142.2, 149.6, 148.9, 172.9. MS (ESI) *m*/*z* 336 $[M+1]^+$; Anal. Calcd for $C_{20}H_{21}N_3O_2$: C 71.62, H 6.31, N 12.53. Found: C 71.58, H 6.29, N 12.48.

5.2.1.11. 3-Methyl-6,7,8,9-Tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5k). Yield: 70%; white solid; mp 187–189 °C; IR (KBr): 3321, 3105, 2837, 1739, 1438, 1334, 742 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.67–10.65 (bs, 1H, pyrrole ring –*NH*), 8.43 (s, 1H, pyridine ring), 7.73 (s, 1H, pyridine ring), 3.92–3.86 (m, 2H, piperidine ring –CH_2–NH), 3.74–3.70 (m, 1H, –*CH*–$COOCH_3$), 3.70 (s, 3H, –$COOCH_3$),

2.96–2.72 (m, 3H, piperidine ring –CH_2 and –*NH*), 2.31 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2, 24.9, 41.3, 51.5, 55.1, 117.1, 123.2, 124.4, 129.9, 135.7, 142.1, 147.3, 173.6. MS (ESI) *m*/*z* 246 $[M+1]^+$; Anal. Calcd for $C_{13}H_{15}N_3O_2$: C 63.66, H 6.16, N 17.13. Found: C 63.67, H 6.12, N 17.10.

5.2.1.12. 3,8-Dimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5l). Yield: 69%; white solid; mp 112–113 °C; IR (KBr): 3296, 2950, 2846, 1738, 1624, 1436, 1382, 1354, 1315, 744 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76–7.74 (bs, 1H, pyrrole ring –*NH*), 7.39 (s, 1H, pyridine ring), 7.31 (s, 1H, pyridine ring), 4.26–4.24 (m, 1H, piperidine ring –*CH*(CH_3)–NH), 3.83–3.82 (m, 1H, –*CH*–$COOCH_3$), 3.67 (s, 3H, –COO*CH*$_3$), 2.96–2.72 (m, 3H, piperidine ring –CH_2 and –*NH*), 2.32 (s, 3H, –CH_3), 1.47 (d, 3H, J = 6.6 Hz, piperidine ring –CH(*CH*$_3$)–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 24.8, 26.7, 48.1, 51.3, 64.3, 111.2, 123.8, 124.6, 129.2, 136.7, 142.1, 147.3, 171.8. MS (ESI) *m*/*z* 260 $[M+1]^+$; Anal. Calcd for $C_{14}H_{17}N_3O_2$: C 64.85, H 6.61, N 16.21. Found: C 64.81, H 6.59, N 16.18.

5.2.1.13. 8-Cyclohexyl-3-methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5m). Yield: 66%; white solid; mp 168–171 °C (dec.); IR (KBr): 3293, 2953, 2806, 1732, 1598, 1436, 1382, 1349, 1334, 1316, 778 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72–7.70 (bs, 1H, pyrrole ring –*NH*), 7.39 (s, 1H, pyridine ring), 7.32 (s, 1H, pyridine ring), 4.21–3.98 (m, 1H, piperidine ring –*CH*–NH), 3.78 (m, 1H, –*CH*–$COOCH_3$), 3.67 (s, 3H, –COO*CH*$_3$), 2.96–2.72 (m, 3H, piperidine ring –CH_2 and –*NH*), 2.38 (s, 3H, –CH_3), 2.16–2.15 (m, 1H, cyclohexyl ring), 1.28–1.24 (m, 10H,, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.8, 23.9, 24.9, 26.3, 27.7, 38.9, 50.4, 53.7, 64.2, 112.2, 123.4, 125.6, 129, 136.7, 142.4, 148.6, 172.3. MS (ESI) *m*/*z* 328 $[M+1]^+$; Anal. Calcd for $C_{19}H_{25}N_3O_2$: C 69.70, H 7.70, N 12.83. Found: C 69.69, H 7.69, N 12.81.

5.2.1.14. 3-Methyl-8-phenyl-6,7,8,9-tetrahydro-5 H-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5n). Yield: 69%; white solid; mp 230–232 °C; IR (KBr): 3045, 2937, 1733, 1685, 1413, 1340, 1336, 767 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.18–11.09 (bs, 1H, pyrrole ring –*NH*), 8.12 (s, 1H, pyridine ring), 7.83 (s, 1H, pyridine ring), 7.33–7.21 (m, 5H, benzene ring), 5.18–5.12 (m, 1H, piperidine ring –*CH*–NH), 3.86 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –COO*CH*$_3$), 3.20–2.83 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*), 2.32 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.8, 26.3, 49.7, 54.8, 56.1, 64.3, 68.1, 112.1, 123.8, 124.6, 126.8, 128.3, 128.9, 129, 136.7, 137.2, 142.2, 149.6, 147.2, 173.3. MS (ESI) *m*/*z* 322 $[M+1]^+$; Anal. Calcd for $C_{19}H_{19}N_3O_2$: C 71.01, H 5.96, N 13.08. Found: C 70.97, H 5.98, N 13.05.

5.2.1.15. 8-Benzyl-3-methyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5o). Yield: 67%; white solid; mp 243–244 °C; ESI-MS: 336 $(M+H)^+$; IR (KBr): 3057, 2933, 1732, 1660, 1560, 1442, 1340, 1335, 744 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.32–10.28 (bs, 1H,, pyrrole ring –*NH*), 8.27 (s, 1H, pyridine ring), 8.1 (s, 1H, pyridine ring), 7.25–7.18 (m, 5H, benzene ring), 4.39–4.37 (m, 1H, piperidine ring –*CH*–NH), 3.89–3.86 (m, 1H, –*CH*–$COOCH_3$), 3.70 (s, 3H, –COO*CH*$_3$), 3.07–2.98 (m, 2H, piperidine ring –CH_2), 2.92 (d, 2H, J = 6.8 Hz, benzyl –CH_2), 2.71–2.69 (bs, 1H, piperidine ring –*NH*), 2.32 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.2, 26.3, 45.2, 49.6, 54.7, 54.9, 64.9, 64.6, 112.1, 123.4, 124.6, 126.8, 128.3, 128.9, 129.7, 136.7, 137.2, 142.2, 149.6, 148.9, 172.9. MS (ESI) *m*/*z* 336 $[M+1]^+$; Anal. Calcd for $C_{20}H_{21}N_3O_2$: C 71.62, H 6.31, N 12.53. Found: C 71.60, H 6.27, N 12.51.

5.2.1.16. 3,9-Dimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5p). Yield: 69%; white solid; mp 134–136 °C; IR (KBr): 2959, 2837, 1739, 1472, 1438, 1336, 767 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49 (s, 1H, pyridine ring), 7.69 (s, 1H, pyridine ring), 3.89–3.87 (m, 2H, piperidine ring –CH_2–NH), 3.74–3.71 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –COO*CH*$_3$), 3.48 (s, 3H, pyrrole ring N–CH_3), 2.96–2.92 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*), 2.31 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.6, 24.8, 34.9, 41.4, 51.6, 55.1, 105.4, 123.4, 124.6, 126.9, 135.7, 142.1, 147.3, 172.4. MS (ESI) *m*/*z* 260 $[M+1]^+$; Anal. Calcd for $C_{14}H_{17}N_3O_2$: C 64.85, H 6.61, N 16.21. Found: C 64.82, H 6.57, N 16.19.

5.2.1.17. 3,8,9-Trimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5q). Yield: 65%; thick oil; IR (Nujol): 3284, 2906, 2833, 1738, 1624, 1452, 1382, 1357, 1315, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.42 (s, 1H, pyridine ring), 7.26 (s, 1H, pyridine ring), 4.01–4.00 (m, 1H, piperidine ring –*CH*(CH_3)–NH), 3.85–3.84 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –COO*CH*$_3$), 3.49 (s, 3H, pyrrole ring N–CH_3), 2.95–2.92 (m, 2H, piperidine ring –CH_2), 2.70–2.68 (bs, 1H, piperidine ring –*NH*), 2.29 (s, 3H, –CH_3), 1.47 (d, 3H, J = 6.75 Hz, piperidine ring –CH(*CH*$_3$)–NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2, 23.2, 25.2, 35.8, 48.9, 51.5, 57.6, 112.2, 124.6, 124.1, 129.2, 140.2, 143.1, 149.6, 172.9. MS (ESI) *m*/*z* 274 $[M+1]^+$; Anal. Calcd for $C_{15}H_{19}N_3O_2$: C 65.91, H 7.01, N 15.73. Found: C 65.87, H 6.98, N 15.71.

5.2.1.18. 8-Cyclohexyl-3,9-dimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5r). Yield: 68%; white solid; mp 215–217 °C (dec.); IR (KBr): 3291, 2953, 2817, 1741, 1598, 1382, 1353, 1332, 1315, 744 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (s, 1H, pyridine ring), 7.29 (s, 1H, pyridine ring), 3.89–3.85 (m, 1H, piperidine ring –*CH*–NH), 3.72–3.71 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –COO*CH*$_3$), 3.48 (s, 3H, pyrrole ring N–CH_3), 2.95–2.92 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*), 2.29 (s, 3H, –CH_3), 2.12–2.11 (m, 1H, cyclohexyl ring), 1.27–1.24 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.9, 23.9, 24.9, 26.3, 27.7, 38.9, 50.4, 53.7, 64.2, 112.2, 124.1, 125.6, 128.9, 136.7, 142.4, 148.6, 173.2. MS (ESI) *m*/*z* 342 $[M+1]^+$; Anal. Calcd for $C_{20}H_{27}N_3O_2$: C 70.35, H 7.97, N 12.31. Found: C 70.31, H 7.94, N 12.28.

5.2.1.19. 3,9-Dimethyl-8-phenyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5s). Yield: 66%; white solid; mp 168–169 °C; IR (KBr): 3058, 2953, 1735, 1685, 1452, 1413, 1342, 1337, 724 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.09 (s, 1H, pyridine ring), 7.85 (s, 1H, pyridine ring), 7.31–7.21 (m, 5H, benzene ring), 5.29–5.22 (m, 1H, piperidine ring –*CH*–NH), 3.86–3.85 (m, 1H, –*CH*–$COOCH_3$), 3.69 (s, 3H, –$COOCH_3$), 3.49 (s, 3H, pyrrole ring N–CH_3), 3.20–2.83 (m, 2H, piperidine ring –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*), 2.29 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.3, 26.3, 35.8, 49.7, 54.8, 56.1, 64.3, 68.1, 112.1, 124.6, 124.1, 126.8, 128.3, 128.9, 129.8, 136.2, 137.2, 142.2, 149.6, 148.9, 172.8. MS (ESI) *m*/*z* 336 $[M+1]^+$; Anal. Calcd for $C_{20}H_{21}N_3O_2$: C 71.62, H 6.31, N 12.53. Found: C 71.58, H 6.29, N 12.49.

5.2.1.20. 8-Benzyl-3,9-dimethyl-6,7,8,9-tetrahydro-5*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (5t). Yield: 68%; white solid; mp 182–183 °C; IR (KBr): 3047, 2939, 1734, 1659, 1560, 1453, 1431, 1340, 1336, 742 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.27 (s, 1H, pyridine ring), 8.05 (s, 1H, pyridine ring), 7.24–7.18 (m, 5H, benzene ring), 4.58–4.56 (m, 1H, , piperidine ring –*CH*–NH), 3.76–3.86 (m, 1H, –*CH*–$COOCH_3$), 3.70 (s, 3H, –$COOCH_3$), 3.58 (s, 3H, pyrrole ring N–CH_3), 3.07–2.95 (m, 2H, piperidine ring –CH_2), 2.89 (d, 2H, J = 6.84 Hz, benzyl –CH_2), 2.72–2.70 (bs, 1H, piperidine ring –*NH*), 2.31 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 25.5, 35.2, 45.5, 51.7, 56.2, 57.8, 112.1, 124.2, 124.6, 126.6, 128.3, 128.9, 129.2, 136.7, 137.2, 142.2, 149.6, 148.9, 173.2. MS (ESI) *m*/*z* 350 $[M+1]^+$; Anal. Calcd for $C_{21}H_{23}N_3O_2$: C 72.18, H 6.63, N 12.03. Found: C 72.17, H 6.61, N 11.98.

5.2.1.21. 9*H*-1,7,9-Triaza-fluorene-6-carboxylic acid methyl ester (6a). A mixture of compound **5a** (50 mmol) and sulfur powder (105 mmol) in dry xylene (100 mL) was heated under reflux for 4 h. The reaction mixture was cooled (5 °C), the pink solid obtained was filtered and washed with excess of xylene (ice cool) and petroleum ether. The solid obtained was recrystallized from methanol to get the pure compound **6a**. Yield: 69%; white solid; mp 246–247 °C; IR (KBr): 3246, 2945, 1710, 1626, 1342, 731 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.06–12.04 (bs, 1H, pyrrole ring –*NH*), 8.96 (s, 1H, pyridine ring), 8.91 (s, 1H, pyridine ring), 8.40 (d, 1H, J = 7.83 Hz, pyridine ring), 7.67 (d, 1H, J = 8.1 Hz, pyridine ring), 7.32 (t, 1H, J = 7.30 Hz, pyridine ring), 3.89 (s, 3H, –$COOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.9, 105.3, 115.6, 121.2, 124.8, 125.4, 129.0, 142.1, 148.2, 148.9, 149.3, 166.0. MS (ESI) *m*/*z* 228 $[M+1]^+$; Anal. Calcd for $C_{12}H_9N_3O_2$: C 63.43, H 3.99, N 18.49. Found: C 63.39, H 3.96, N 18.46.

5.2.1.22. 8-Methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6b). Yield: 67%; white solid; mp 247–249 °C; IR (KBr): 3328, 2949, 1716, 1625, 1352, 740 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.01–11.98 (bs, 1H, pyrrole ring –*NH*), 8.76 (s, 1H, pyridine ring), 8.36 (d, 1H, J = 7.83 Hz, pyridine ring), 7.65 (d, 1H, J = 8.13 Hz, pyridine ring), 7.28 (t, 1H, J = 7.67 Hz, pyridine ring), 3.81 (s, 3H, –$COOCH_3$), 2.81 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.3, 51.9, 105.3, 115.6, 121.2, 124.8, 125.4, 129.0, 142.1, 148.2, 148.9, 157.8, 166.0. MS (ESI) *m*/*z* 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.69, H 4.61, N 17.44.

5.2.1.23. 8-Cyclohexyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6c). Yield: 66%; white solid; mp 156–157 °C; IR (KBr): 3337, 2938, 1725, 1625, 1452, 1352, 1332, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.06–12.01 (bs, 1H, pyrrole ring –*NH*), 8.72 (s, 1H, pyridine ring), 8.32 (d, 1H, J = 7.81 Hz, pyridine ring), 7.63 (d, 1H, J = 8.1 Hz, pyridine ring), 7.31 (t, 1H, J = 7.49 Hz, pyridine ring), 3.83 (s, 3H, –$COOCH_3$), 2.76–2.75 (m, 1H, cyclohexyl ring), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 27.4, 29.1, 34.2, 51.9, 102.3, 114.8, 120.7, 123.6, 125.4, 129.3, 142.1, 147.6, 148.9, 161.8, 168.0. MS (ESI) *m*/*z* 242 $[M+1]^+$; Anal. Calcd for $C_{18}H_{19}N_3O_2$: C 69.88, H 6.19, N 13.58. Found: C 69.83, H 6.21, N 13.53.

5.2.1.24. 8-Phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6d). Yield: 68%; white solid; mp 254–256 °C; IR (KBr): 3315, 2949, 1722, 1625, 1458, 1352, 738 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.95–11.92 (bs, 1H, pyrrole ring –*NH*), 8.94 (s, 1H, pyridine ring), 8.43 (d, 1H, J = 7.86 Hz, pyridine ring), 8.01 (d, 1H, J = 7.38 Hz, pyridine ring), 7.71–7.57 (m, 5H, benzene ring), 7.34 (t, 1H, J = 7.48 Hz, pyridine ring), 3.94 (s, 3H, –$COOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.9, 102.3, 114.8, 120.7, 123.6, 125.4, 127.3, 127.8, 128.4, 129.3, 139.6, 142.1, 147.6, 148.9, 156.8, 168.0. MS (ESI) *m*/*z* 304 $[M+1]^+$; Anal. Calcd for $C_{18}H_{13}N_3O_2$: C 71.28, H 4.32, N 13.85. Found: C 71.22, H 4.29, N 13.82.

5.2.1.25. 8-Benzyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6e). Yield: 67%; white solid; mp 205–206 °C; IR (KBr): 3328, 2937, 1731, 1624, 1458, 1437, 1352, 744 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.03–12.0 (bs, 1H, pyrrole ring –*NH*), 8.91 (s, 1H, pyridine ring), 8.39 (d, 1H, J = 7.82 Hz, pyridine ring), 8.02 (d, 1H, J = 7.37 Hz, pyridine ring), 7.34 (t, 1H, J = 7.45 Hz, pyridine ring), 7.26–7.17 (m, 5H, benzene ring), 4.29 (s, 2H, benzyl –CH_2), 3.92 (s, 3H, –$COOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.4, 51.9, 102.3, 114.8, 120.7, 123.6, 125.4, 127.3, 127.8, 129.1, 129.8, 137.6, 142.1, 147.6, 148.9, 157.4, 166.0. MS (ESI) *m*/*z* 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.87, H 4.73, N 13.21.

5.2.1.26. 9-Methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6f). Yield: 66%; white solid; mp 198–199 °C; IR (KBr): 2949, 1707, 1626, 1438, 1363, 1342, 754 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.94 (s, 1H, pyridine ring), 8.90 (s, 1H, pyridine ring), 8.23 (d, 1H, J = 7.87 Hz, pyridine ring), 7.67 (t, 1H, J = 7.71 Hz, pyridine ring), 7.35 (d, 1H, J = 7.49 Hz, pyridine ring), 4.07 (s, 3H, –$COOCH_3$), 4.01 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.2, 51.9, 100.3, 113.6, 120.9, 124.8, 125.4, 129.0,

139.8, 148.2, 149.2, 152.5, 168.0. MS (ESI) *m/z* 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.73, H 4.59, N 17.40.

5.2.1.27. 8,9-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6g). Yield: 68%; white solid; mp 221–223 °C; IR (KBr): 2949, 1726, 1626, 1352, 1338, 740 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.72 (s, 1H, pyridine ring), 8.35 (d, 1H, J = 7.81 Hz, pyridine ring), 7.72 (d, 1H, J = 8.1 Hz, pyridine ring), 7.29 (t, 1H, J = 7.08 Hz, pyridine ring), 3.83 (s, 3H, –COOCH_3), 3.78 (s, 3H, pyrrole ring N–CH_3), 2.81 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.3, 43.8, 51.9, 105.3, 115.6, 121.2, 124.8, 125.4, 129.0, 142.1, 147.8, 148.9, 157.8, 166.0. MS (ESI) *m/z* 256 $[M+1]^+$; Anal. Calcd for $C_{14}H_{13}N_3O_2$: C 65.87, H 5.13, N 16.46. Found: C 65.84, H 5.14, N 16.39.

5.2.1.28. 8-Cyclohexyl-9-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6h). Yield: 66%; white solid; mp 148–149 °C; IR (KBr): 2938, 1718, 1629, 1451, 1352, 1332, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.69 (s, 1H, pyridine ring), 8.26 (d, 1H, J = 7.79 Hz, pyridine ring), 7.61 (d, 1H, J = 7.86 Hz, pyridine ring), 7.36 (t, 1H, J = 7.09 Hz, pyridine ring), 3.91 (s, 3H, –COOCH_3), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.74–2.73 (m, 1H, cyclohexyl ring), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 27.4, 29.1, 34.2, 41.8, 51.9, 101.3, 114.8, 121.7, 123.6, 125.4, 129.3, 142.1, 147.6, 148.9, 161.8, 166.0. MS (ESI) *m/z* 324 $[M+1]^+$; Anal. Calcd for $C_{19}H_{21}N_3O_2$: C 70.57, H 6.55, N 12.99. Found: C 70.51, H 6.50, N 12.97.

5.2.1.29. 9-Methyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6i). Yield: 68%; white solid; mp 217–218 °C; IR (KBr): 2949, 1723, 1625, 1456, 1352, 1336, 746 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.67 (s, 1H, pyridine ring), 8.39 (d, 1H, J = 7.82 Hz, pyridine ring), 8.09 (d, 1H, J = 7.41 Hz, pyridine ring), 7.71–7.57 (m, 5H, benzene ring), 7.34 (t, 1H, J = 7.43 Hz, pyridine ring), 3.94 (s, 3H, –COOCH_3), 3.68 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.9, 51.7, 102.3, 114.8, 120.7, 123.6, 125.4, 126.9, 127.8, 128.4, 129.3, 139.6, 142.1, 147.6, 148.9, 156.8, 168.0. MS (ESI) *m/z* 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.87, H 4.72, N 13.20.

5.2.1.30. 8-Benzyl-9-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6j). Yield: 69%; white solid; mp 186–187 °C; IR (KBr): 2938, 1719, 1617, 1458, 1437, 1348, 740 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.93 (s, 1H, pyridine ring), 8.37 (d, 1H, J = 7.78 Hz, pyridine ring), 8.04 (d, 1H, J = 7.42 Hz, pyridine ring), 7.34 (t, 1H, J = 7.39 Hz, pyridine ring), 7.26–7.17 (m, 5H, benzene ring), 4.29 (s, 2H, benzyl –CH_2), 3.87 (s, 3H, –COOCH_3), 3.72 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.4, 51.9, 102.3, 114.8, 120.7, 123.6, 125.4, 127.3, 127.8, 129.8, 129.1, 137.6, 142.1, 147.6, 148.9, 157.4, 166.0. MS (ESI) *m/z* 332 $[M+1]^+$; Anal. Calcd for $C_{20}H_{17}N_3O_2$: C 72.49, H 5.17, N 12.68. Found: C 72.48, H 5.15, N 12.65.

5.2.1.31. 3-Methyl-9*H*-1,7,9-Triaza-fluorene-6-carboxylic acid methyl ester (6k). Yield: 65%; white solid; mp 169–171 °C; IR (KBr): 3241, 2945, 1730, 1626, 1342, 1321, 746 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.02–11.98 (bs, 1H, pyrrole ring –NH), 8.95 (s, 1H, pyridine ring), 8.89 (s, 1H, pyridine ring), 8.39 (s, 1H, pyridine ring), 7.62 (s, 1H, pyridine ring), 3.89 (s, 3H, –COOCH_3), 2.36 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 51.9, 105.3, 119.9, 124.4, 124.8, 125.4, 129.3, 143.1, 148.2, 148.9, 149.3, 167.0. MS (ESI) *m/z* 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.68, H 4.57, N 17.38.

5.2.1.32. 3,8-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6l). Yield: 69%; white solid; mp 209–211 °C; IR (KBr): 3336, 2943, 1716, 1625, 1348, 1334, 740 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.98–11.95 (bs, 1H, pyrrole ring –NH), 8.75 (s, 1H, pyridine ring), 8.29 (s, 1H, pyridine ring), 7.64 (s, 1H, pyridine ring), 3.91 (s, 3H, –COOCH_3), 2.81 (s, 3H, –C(CH_3) = N), 2.29 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.3, 24.7, 52.0, 105.3, 120.4, 121.2, 124.8, 125.4, 129.0, 143.6, 148.2, 148.9, 158.1, 167.1. MS (ESI) *m/z* 256 $[M+1]^+$; Anal. Calcd for $C_{14}H_{13}N_3O_2$: C 65.87, H 5.13, N 16.46. Found: C 65.58, H 5.12, N 16.42.

5.2.1.33. 8-Cyclohexyl-3-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6m). Yield: 65%; white solid; mp 148–150 °C; IR (KBr): 3346, 2947, 1721, 1625, 1452, 1352, 1338, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.01–11.99 (bs, 1H, pyrrole ring –NH), 8.68 (s, 1H, pyridine ring), 8.31 (s, 1H, pyridine ring), 7.61 (s, 1H, pyridine ring), 3.83 (s, 3H, –COOCH_3), 2.76–2.75 (m, 1H, cyclohexyl ring), 2.31 (s, 3H, –CH_3), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 23.8, 27.4, 29.1, 34.2, 51.9, 102.3, 123.6, 124.8, 125.4, 129.3, 142.1, 147.6, 148.9, 161.8, 168.0. MS (ESI) *m/z* 324 $[M+1]^+$; Anal. Calcd for $C_{19}H_{21}N_3O_2$: C 70.57, H 6.55, N 12.99. Found: C 70.53, H 6.56, N 13.01.

5.2.1.34. 3-Methyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6n). Yield: 69%; white solid; mp 269–270 °C (dec.); IR (KBr): 3328, 2949, 1722, 1625, 1458, 1352, 1337, 747 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.03–12.00 (bs, 1H, pyrrole ring –NH), 8.94 (s, 1H, pyridine ring), 8.41 (s, 1H, pyridine ring), 8.03 (s, 1H, pyridine ring), 7.72–7.56 (m, 5H, benzene ring), 3.94 (s, 3H, –COOCH_3), 2.32 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 51.9, 102.3, 120.7, 121.7, 123.6, 124.8, 125.4, 127.8, 128.4, 129.3, 139.6, 142.1, 147.6, 148.9, 156.8, 168.0. MS (ESI) *m/z* 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.92, H 4.79, N 13.19.

5.2.1.35. 8-Benzyl-3-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6o). Yield: 67%; white solid; mp 258–260 °C; IR (KBr): 3329, 2948, 1721, 1624, 1438, 1352, 1336, 744 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.06–12.02 (bs, 1H, pyrrole ring –NH), 8.91 (s, 1H, pyridine ring), 8.39 (s, 1H, pyridine ring), 7.99 (s, 1H, pyridine ring), 7.26–7.17 (m, 5H, benzene

ring), 4.29 (s, 2H, benzyl –CH_2), 3.92 (s, 3H, –COOCH_3), 2.31 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.9, 38.4, 51.9, 102.3, 120.7, 123.6, 124.8, 125.4, 127.3, 127.8, 128.6, 129.8, 137.6, 142.1, 147.6, 148.9, 157.4, 166.0. MS (ESI) *m/z* 332 $[M+1]^+$; Anal. Calcd for $C_{20}H_{17}N_3O_2$: C 72.49, H 5.17, N 12.68. Found: C 72.44, H 5.15, N 12.64.

5.2.1.36. 3,9-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6p). Yield: 68%; white solid; mp 162–163 °C; IR (KBr): 2948, 1723, 1626, 1438, 1363, 1342, 754 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.96 (s, 1H, pyridine ring), 8.89(s, 1H, pyridine ring), 8.23 (s, 1H, pyridine ring), 7.67 (s, 1H, pyridine ring), 4.07 (s, 3H, –COOCH_3), 4.01 (s, 3H, pyrrole ring N–CH_3), 2.32 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.4, 45.2, 51.9, 100.3, 120.9, 124.6, 124.8, 129.8, 129.0, 139.8, 147.2, 149.2, 150.1, 166.8. MS (ESI) *m/z* 256 $[M+1]^+$; Anal. Calcd for $C_{14}H_{13}N_3O_2$: C 65.87, H 5.13, N 16.46. Found: C 65.85, H 5.12, N 16.41.

5.2.1.37. 3,8,9-Trimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6q). Yield: 66%; white solid; mp 234–235 °C; IR (KBr): 2949, 1724, 1629, 1352, 1348, 1336, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.75 (s, 1H, pyridine ring), 8.37 (s, 1H, pyridine ring), 7.72 (s, 1H, pyridine ring), 3.81 (s, 3H, –COOCH_3), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.78 (s, 3H, –C(CH_3)=N), 2.28 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.4, 21.3, 43.8, 51.9, 103.3, 120.2, 121.7, 124.8, 129.4, 129.0, 142.1, 148.8, 149.7, 158.1, 166.5. MS (ESI) *m/z* 270 $[M+1]^+$; Anal. Calcd for $C_{15}H_{15}N_3O_2$: C 66.90, H 5.61, N 15.60. Found: C 66.87, H 5.58, N 15.56.

5.2.1.38. 8-Cyclohexyl-3,9-dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6r). Yield: 68%; white solid; mp 210–212 °C; IR (KBr): 2936, 1716, 1628, 1451, 1352, 1343, 1334, 747 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (s, 1H, pyridine ring), 8.32 (s, 1H, pyridine ring), 7.63 (s, 1H, pyridine ring), 3.87 (s, 3H, –COOCH_3), 3.67 (s, 3H, pyrrole ring N–CH_3), 2.78 (m, 1H, cyclohexyl ring), 2.32 (s, 3H, –CH_3), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2, 23.6, 27.2, 28.9, 34.2, 41.7, 51.9, 100.9, 120.7, 123.7, 125.1, 128.9, 129.3, 143.1, 147.6, 147.9, 159.8, 166.7. MS (ESI) *m/z* 338 $[M+1]^+$; Anal. Calcd for $C_{20}H_{23}N_3O_2$: C 71.19, H 6.87, N 12.45. Found: C 71.14, H 6.89, N 12.39.

5.2.1.39. 3,9-Dimethyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester(6s). Yield: 70%; white solid; mp 286–288 °C; IR (KBr): 2952, 1724, 1625, 1567, 1456, 1351, 1343, 1332, 738 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.62 (s, 1H, pyridine ring), 8.31 (s, 1H, pyridine ring), 7.89 (s, 1H, pyridine ring), 7.71–7.57 (m, 5H, benzene ring), 3.89 (s, 3H, –COOCH_3), 3.62 (s, 3H, pyrrole ring N–CH_3), 2.34 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 43.2, 51.4, 101.8, 120.3, 122.1, 124.4, 126.9, 127.6, 128.4, 129.1, 129.3, 139.6, 142.1, 147.6, 148.9, 156.8, 168.0. MS (ESI) *m/z* 332 $[M+1]^+$; Anal. Calcd for $C_{20}H_{17}N_3O_2$: C 72.49, H 5.17, N 12.68. Found: C 72.47, H 5.15, N 12.63.

5.2.1.40. 8-Benzyl-3,9-dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid methyl ester (6t). Yield: 69%; white solid; mp 231–232 °C; IR (KBr): 2939, 1719, 1614, 1452, 1346, 1338, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1 H, pyridine ring), 8.35 (s, 1H, pyridine ring), 7.79 (s, 1H, pyridine ring), 7.25–7.18 (m, 5H, benzene ring), 4.24 (s, 2H, benzyl –CH_2), 3.84 (s, 3H, –COOCH_3), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.31 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.4, 38.3, 51.8, 102.3, 120.7, 121.7, 124.3, 125.4, 127.3, 127.8, 129.8, 129.6, 129.1, 137.2, 143.1, 147.6, 149.6, 158.1, 167.2. MS (ESI) *m/z* 346 $[M+1]^+$; Anal. Calcd for $C_{21}H_{19}N_3O_2$: C 73.03, H 5.54, N 12.17. Found: C 72.99, H 5.59, N 12.14.

5.2.1.41. Synthesis of 9*H*-1,7,9-Triaza-fluorene-6-carboxylic acid (7a). A solution of compound **6a** (50 mmol) and NaOH (200 mmol), dissolved in a mixture of ethanol (120 mL) and water (240 mL), was refluxed for 30 min. Excess of ethanol was removed on the rotary evaporator. The reaction mixture was neutralized (pH 5) with HCl (6 N, 33 mL) and cooled. The solid product obtained was filtered, washed with water (500 mL), and dried to get the pure compound **7a**.Yield: 70%; white solid; mp >290 °C; IR (KBr): 3396, 2949, 1654, 1616.2, 731 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.06–12.02 (bs, 1H, pyrrole ring –*NH*), 9.03 (s, 1H, pyridine ring), 8.96 (s, 1H, pyridine ring), 8.41 (d, 1H, J = 7.81 Hz, pyridine ring), 7.65 (d, 1H, J = 7.98 Hz, pyridine ring), 7.32 (t, 1H, J = 7.30 Hz, pyridine ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 102.4, 115.2, 120.7, 124.6, 125.4, 129.0, 142.1, 148.2, 148.9, 149.3, 172.0. MS (ESI) *m/z* 214 $[M+1]^+$; Anal. Calcd for $C_{11}H_7N_3O_2$: C 61.97, H 3.31, N 19.71. Found: C 61.95, H 3.28, N 19.68.

5.2.1.42. 8-Methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7b). Yield: 72%; white solid; mp >290 °C; IR (KBr): 3389, 2947, 1648, 1589, 1352, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.07–12.04 (bs, 1H, pyrrole ring –*NH*), 9.01 (s, 1H, pyridine ring), 8.32 (d, 1H, J = 7.74 Hz, pyridine ring), 7.64 (d, 1H, J = 7.92 Hz, pyridine ring), 7.32 (t, 1H, J = 7.25 Hz, pyridine ring), 2.82 (s, 3H, –C(CH_3)=N). ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.2, 103.1, 115.6, 121.2, 124.7, 125.4, 129.2, 143.0, 148.2, 148.9, 157.2, 173.5. MS (ESI) *m/z* 228 $[M+1]^+$; Anal. Calcd for $C_{12}H_9N_3O_2$: C 63.43, H 3.99, N 18.49. Found: C 63.39, H 3.97, N 18.46.

5.2.1.43. 8-Cyclohexyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7c). Yield: 75%; white solid; mp 280–282 °C(dec.); IR (KBr): 3387, 2938, 1648, 1575, 1452, 1348, 1332, 743 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.97–11.94 (bs, 1H, pyrrole ring –*NH*), 9.00 (s, 1H, pyridine ring), 8.31 (d, 1H, J = 7.80 Hz, pyridine ring), 7.76 (d, 1H, J = 7.78 Hz, pyridine ring), 7.32 (t, 1H, J = 7.30 Hz, pyridine ring), 2.76–2.75 (m, 1H, cyclohexyl ring), 1.27–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 27.4, 29.1, 34.2, 101.9, 114.7, 121.0, 123.5, 125.7, 129.3, 142.1, 147.6, 148.9, 160.7, 173.1. MS (ESI) *m/z* 296 $[M+1]^+$; Anal. Calcd for $C_{17}H_{17}N_3O_2$: C 69.14, H 5.08, N 14.23. Found: C 69.10, H 5.05, N 14.19.

5.2.1.44. 8-Phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7d). Yield: 74%; white solid; mp >290 °C; IR

(KBr): 3389, 2943, 1664, 1625, 1458, 738 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.01–11.98 (bs, 1H, pyrrole ring –*NH*), 8.93 (s, 1H, pyridine ring), 8.36 (d, 1H, J = 7.82 Hz, pyridine ring), 8.02 (d, 1H, J = 7.34 Hz, pyridine ring), 7.72–7.56 (m, 5H, benzene ring), 7.34 (t, 1H, J = 7.42 Hz, pyridine ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 102.3, 115.2, 120.5, 123.5, 125.2, 127.3, 127.8, 128.4, 129.3, 139.5, 142.1, 147.6, 148.9, 156.8, 173.4. MS (ESI) m/z 290 $[M+1]^+$; Anal. Calcd for $C_{17}H_{11}N_3O_2$: C 70.58, H 3.83, N 14.53. Found: C 70.53, H 3.79, N 14.49.

5.2.1.45. 8-Benzyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7e). Yield: 75%; white solid; mp >290 °C; IR (KBr): 3382, 2947, 1647, 1589, 1458, 1437, 739 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.01–11.97 (bs, 1H, pyrrole ring –*NH*), 9.01 (s, 1H, pyridine ring), 8.36 (d, 1H, J = 7.78 Hz, pyridine ring), 8.06 (d, 1H, J = 7.38 Hz, pyridine ring), 7.38 (t, 1H, J = 7.42 Hz, pyridine ring), 7.26–7.17 (m, 5H, benzene ring), 4.29 (s, 2H, benzyl –CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 39.0, 102.3, 114.8, 120.7, 123.7, 125.5, 127.7, 127.3, 129.1, 129.9, 137.6, 142.1, 147.6, 149.2, 158.1, 172.7. MS (ESI) m/z 304 $[M+1]^+$; Anal. Calcd for $C_{18}H_{13}N_3O_2$: C 71.28, H 4.32, N 13.85. Found: C 71.25, H 4.33, N 13.86.

5.2.1.46. 9-Methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7f). Yield: 73%; white solid; mp 248–249 °C; IR (KBr): 3341, 2949, 1648, 1626, 1438, 1338, 754 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.96 (s, 1H, pyridine ring), 8.94 (s, 1H, pyridine ring), 8.28 (d, 1H, J = 7.83 Hz, pyridine ring), 7.67 (t, 1H, J = 7.67 Hz, pyridine ring), 7.36 (d, 1H, J = 7.43 Hz, pyridine ring), 4.01 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.3, 100.3, 113.7, 120.9, 124.8, 125.4, 129.2, 139.8, 148.2, 149.2, 152.5, 173.6. MS (ESI) m/z 228 $[M+1]^+$; Anal. Calcd for $C_{12}H_9N_3O_2$: C 63.43, H 3.99, N 18.49. Found: C 63.39, H 3.94, N 18.45.

5.2.1.47. 8,9-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7g). Yield: 74%; white solid; mp 278–281 °C; IR (KBr): 3332, 2947, 1652, 1586, 1338, 754 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.76 (s, 1H, pyridine ring), 8.36 (d, 1H, J = 7.75 Hz, pyridine ring), 7.74 (d, 1H, J = 7.98 Hz, pyridine ring), 7.32 (t, 1H, J = 7.15 Hz, pyridine ring), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.79 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.8, 43.8, 105.3, 114.9, 121.2, 124.8, 125.4, 129.1, 142.1, 147.8, 148.9, 157.8, 173.1. MS (ESI) m/z 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.68, H 4.57, N 17.38.

5.2.1.48. 8-Cyclohexyl-9-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7h). Yield: 72%; white solid; mp 231–233 °C; IR (KBr): 3342, 2938, 1663, 1579, 1451, 1335, 747 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.72 (s, 1H, pyridine ring), 8.25 (d, 1H, J = 7.96 Hz, pyridine ring), 7.69 (d, 1H, J = 7.65 Hz, pyridine ring), 7.35 (t, 1H, J = 7.35 Hz, pyridine ring), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.74–2.72 (m, 1H, cyclohexyl ring), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.7, 27.2, 28.9, 34.1, 41.8, 102.3, 114.8, 121.7, 123.6, 125.4, 129.3, 142.1, 148.6, 148.9, 161.3, 173.2. MS (ESI) m/z 310 $[M+1]^+$; Anal. Calcd for $C_{18}H_{19}N_3O_2$: C 69.88, H 6.19, N 13.58. Found: C 69.85, H 6.14, N 13.54.

5.2.1.49. 9-Methyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7i). Yield: 75%; white solid; mp >290 °C; IR (KBr): 3343, 2947, 1667, 1625, 1456, 1336, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.65 (s, 1H, pyridine ring), 8.39 (d, 1H, J = 7.80 Hz, pyridine ring), 8.10 (d, 1H, J = 7.27 Hz, pyridine ring), 7.73–7.56 (m, 5H, benzene ring), 7.32 (t, 1H, J = 7.38 Hz, pyridine ring), 3.68 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.1, 101.7, 114.3, 120.5, 123.6, 125.4, 126.9, 127.8, 128.4, 129.3, 139.6, 142.1, 147.5, 148.9, 156.8, 173.2. MS (ESI) m/z 304 $[M+1]^+$; Anal. Calcd for $C_{18}H_{13}N_3O_2$: C 71.28, H 4.32, N 13.85. Found: C 71.23, H 4.29, N 13.83.

5.2.1.50. 8-Benzyl-9-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7j). Yield: 73%; white solid; mp >290 °C; IR (KBr): 3342, 2938, 1658, 1617, 1458, 1338, 746 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.94 (s, 1H, pyridine ring), 8.36 (d, 1H, J = 7.81 Hz, pyridine ring), 8.03 (d, 1H, J = 7.36 Hz, pyridine ring), 7.31 (t, 1H, J = 7.43 Hz, pyridine ring), 7.26–7.18 (m, 5H, benzene ring), 4.27 (s, 2H, benzyl –CH_2), 3.68 (s, 3H, pyrrole ring N–CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 37.6, 102.3, 114.8, 120.7, 123.6, 125.4, 127.3, 127.8, 129.8, 129.1, 137.6, 142.1, 147.6, 149.3, 157.2, 172.9. MS (ESI) m/z 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.89, H 4.73, N 13.21.

5.2.1.51. 3-Methyl-9*H*-1,7,9-Triaza-fluorene-6-carboxylic acid (7k). Yield: 74%; white solid; mp 268–270 °C; IR (KBr): 3247, 2942, 1657, 1626, 1321, 746 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.01–11.99 (bs, 1H, pyrrole ring –*NH*), 8.94 (s, 1H, pyridine ring), 8.91 (s, 1H, pyridine ring), 8.39 (s, 1H, pyridine ring), 7.61 (s, 1H, pyridine ring), 2.35 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.6, 105.3, 119.9, 124.4, 124.8, 125.4, 129.3, 143.2, 148.4, 148.7, 149.3, 173.2. MS (ESI) m/z 228 $[M+1]^+$; Anal. Calcd for $C_{12}H_9N_3O_2$: C 63.43, H 3.99, N 18.49. Found: C 63.41, H 3.97, N 18.51.

5.2.1.52. 3,8-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7l). Yield: 75%; white solid; mp >290 °C; IR (KBr): 3336, 2943, 1663, 1622, 1334, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.98–11.95 (bs, 1H, pyrrole ring –*NH*), 8.74 (s, 1H, pyridine ring), 8.28 (s, 1H, pyridine ring), 7.63 (s, 1H, pyridine ring), 2.79 (s, 3H, –C(CH_3)=N), 2.29 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.9, 24.6, 103.3, 120.4, 121.5, 124.8, 125.2, 129.0, 143.6, 148.2, 148.9, 158.1, 173.2. MS (ESI) m/z 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.68, H 4.57, N 17.39.

5.2.1.53. 8-Cyclohexyl-3-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7m). Yield: 71%; white solid; mp 274–276 °C(dec.); IR (KBr): 3296, 2947, 1648, 1625, 1452, 1336, 743 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.03–12.0 (bs, 1H, pyrrole ring –*NH*), 8.67 (s,

1H, pyridine ring), 8.29 (s, 1H, pyridine ring), 7.61 (s, 1H, pyridine ring), 2.76–2.75 (m, 1H, cyclohexyl ring), 2.31 (s, 3H, $-CH_3$), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.3, 23.7, 27.3, 28.7, 34.2, 102.3, 123.6, 124.8, 125.4, 129.3, 142.1, 147.6, 148.9, 161.8, 172.9. MS (ESI) *m*/*z* 310 $[M+1]^+$; Anal. Calcd for $C_{18}H_{19}N_3O_2$: C 69.88, H 6.19, N 13.58. Found: C 69.85, H 6.17, N 13.56.

5.2.1.54. 3-Methyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7n). Yield: 72%; white solid; mp >290 °C; IR (KBr): 3328, 2949, 1654, 1625, 1458, 1337, 749 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.97–11.95 (bs, 1H, pyrrole ring –*NH*), 8.87 (s, 1H, pyridine ring), 8.42 (s, 1H, pyridine ring), 8.02 (s, 1H, pyridine ring), 7.73–7.57 (m, 5H, benzene ring), 2.32 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.6, 102.1, 121.3, 121.7, 123.2, 125.2, 125.4, 127.8, 128.4, 129.3, 137.3, 142.1, 147.6, 147.6, 156.8, 173.2. MS (ESI) *m*/*z* 304 $[M+1]^+$; Anal. Calcd for $C_{18}H_{13}N_3O_2$: C 71.28, H 4.32, N 13.85. Found: C 71.23, H 4.29, N 13.83.

5.2.1.55. 8-Benzyl-3-methyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7o). Yield: 74%; white solid; mp 212–214 °C; IR (KBr): 3289, 2948, 1667, 1594, 1438, 1336, 744 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11–12.08 (bs, 1H, pyrrole ring –*NH*), 8.87 (s, 1H, pyridine ring), 8.28 (s, 1H, pyridine ring), 8.01 (s, 1H, pyridine ring), 7.26–7.17 (m, 5H, benzene ring), 4.32 (s, 2H, benzyl $-CH_2$), 2.31 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.9, 38.4, 102.3, 121.9, 123.4, 123.8, 125.4, 127.3, 127.8, 128.6, 129.3, 137.6, 142.1, 147.6, 148.9, 156.5, 174.1. MS (ESI) *m*/*z* 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.90, H 4.71, N 13.23.

5.2.1.56. 3,9-Dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7p). Yield: 70%; white solid; mp 234–236 °C; IR (KBr): 2948, 1658, 1603, 1438, 1343, 1329, 754 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.96 (s, 1H, pyridine ring), 8.91 (s, 1H, pyridine ring), 8.31 (s, 1H, pyridine ring), 7.72 (s, 1H, pyridine ring), 3.93 (s, 3H, pyrrole ring N–CH_3), 2.30 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.7, 45.2, 100.3, 121.3, 124.6, 124.8, 129.2, 129.9, 139.8, 147.2, 149.2, 150.1, 172.8. MS (ESI) *m*/*z* 242 $[M+1]^+$; Anal. Calcd for $C_{13}H_{11}N_3O_2$: C 64.72, H 4.60, N 17.42. Found: C 64.68, H 4.58, N 17.40.

5.2.1.57. 3,8,9-Trimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7q). Yield: 71%; white solid; mp 283–285 °C; IR (KBr): 2937, 1649, 1629, 1348, 1336, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (s, 1H, pyridine ring), 8.35 (s, 1H, pyridine ring), 7.67 (s, 1H, pyridine ring), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.81 (s, 3H, –C(CH_3)=N), 2.28 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.7, 21.6, 43.8, 103.3, 120.2, 121.7, 124.8, 129.4, 129.0, 142.1, 146.3, 148.9, 157.6, 172.8. MS (ESI) *m*/*z* 256 $[M+1]^+$; Anal. Calcd for $C_{14}H_{13}N_3O_2$: C 65.87, H 5.13, N 16.46. Found: C 65.83, H 5.11, N 16.42.

5.2.1.58. 8-Cyclohexyl-3,9-dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7r). Yield: 71%; white solid; mp 243–245 °C; IR (KBr): 2936, 1667, 1628, 1451, 1352, 1334, 747 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, pyridine ring), 8.38 (s, 1H, pyridine ring), 7.71 (s, 1H, pyridine ring), 3.65 (s, 3H, pyrrole ring N–CH_3), 2.69–2.68 (m, 1H, cyclohexyl ring), 2.28 (s, 3H, $-CH_3$), 1.28–1.19 (m, 10H, cyclohexyl ring). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.8, 23.4, 27.2, 28.9, 34.2, 41.7, 102.1, 120.7, 123.6, 125.3, 128.4, 129.3, 143.1, 147.6, 147.9, 159.8, 173.7. MS (ESI) *m*/*z* 324 $[M+1]^+$; Anal. Calcd for $C_{19}H_{21}N_3O_2$: C 70.57, H 6.55, N 12.99. Found: C 70.51, H 6.53, N 12.97.

5.2.1.59. 3,9-Dimethyl-8-phenyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7s). Yield: 73%; white solid; mp >290 °C; IR (KBr): 2952, 1658, 1567, 1456, 1343, 1332, 748 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.57 (s, 1H, pyridine ring), 8.32 (s, 1H, pyridine ring), 7.74 (s, 1H, pyridine ring), 7.71–7.57 (m, 5H, benzene ring), 3.67 (s, 3H, pyrrole ring N–CH_3), 2.31 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.7, 43.2, 101.8, 120.3, 122.1, 124.1, 126.9, 127.6, 128.4, 129.3, 129.3, 139.6, 142.1, 147.6, 148.7, 155.2, 172.9. MS (ESI) *m*/*z* 318 $[M+1]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_2$: C 71.91, H 4.76, N 13.24. Found: C 71.88, H 4.75, N 13.19.

5.2.1.60. 8-Benzyl-3,9-dimethyl-9*H*-1,7,9-triaza-fluorene-6-carboxylic acid (7t). Yield: 75%; white solid; mp >290 °C; IR (KBr): 2939, 1664, 1589, 1452, 1338, 741 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.86 (s, 1H, pyridine ring), 8.32 (s, 1H, pyridine ring), 7.78 (s, 1H, pyridine ring), 7.25–7.17 (m, 5H, benzene ring), 4.24 (s, 2H, benzyl $-CH_2$), 3.69 (s, 3H, pyrrole ring N–CH_3), 2.28 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.9, 37.3, 102.3, 120.5, 121.7, 124.5, 125.4, 127.1, 127.8, 129.8, 129.6, 129.1, 137.2, 143.1, 147.6, 149.6, 158.1, 174.0. MS (ESI) *m*/*z* 332 $[M+1]^+$; Anal. Calcd for $C_{20}H_{17}N_3O_2$: C 72.49, H 5.17, N 12.68. Found: C 72.47, H 5.15, N 12.66.

5.3. In vitro glucose–dependent insulin secretion (RIN5F cell assay screening protocol)

RIN5F (Rat Insulinoma) cells were cultured in RPMI 1640 medium supplemented with sodium pyruvate (1 mM), HEPES and glucose (4.5 g/L), in a humidified incubator (5% CO_2), at 37 °C. After trypsinization, RIN5F cells were seeded at a density of 0.2×10^6 cells per well, in 12-well plates. The cells were grown overnight to 80% confluence and the insulin secretion experiments were performed as follows.[29,30]

Cells were washed once with phosphate-buffered saline (PBS) solution, followed by 40 min incubation in fresh Krebs–Ringer Balanced Buffer, containing NaCl (115 mmol/L), KCl (4.7 mmol/L), $CaCl_2$ (1.28 mmol/L), $MgSO_4.7H_2O$ (1.2 mmol/L), KH_2PO_4 (1.2 mmol/L), $NaHCO_3$ (10 mmol/L), and HEPES (25 mmol/L), containing glucose (1.1 mM) and Bovain Serum Albumin (BSA; 0.5 %), pH 7.4. The buffer was replaced after 40 min and the cells were incubated with the test and the standard compounds in presence or absence of glucose load (16.7 mM), at different concentrations, for 30 min, at 37 °C. The supernatant was collected and

the insulin amount was measured by ultra sensitive Rat insulin ELISA kit (Crystal Chem, IL). The protein was estimated in the supernatant, using Bicinchoninic Acid kit, according to the manufacturer's protocol (Sigma Aldrich, MO). The total insulin content obtained in Picogram (pg) was divided by the total protein (μg) in order to normalize the differences in cell density between wells.

5.4. In vitro radioligand α_2-Adrenoreceptors (ARs) binding assays

Affinity for α_2-ARs in the rat brain was assessed by measuring the ability of the test compounds to displace [^{3}H]clonidine from these receptors.[31] In this assay, the cerebral cortex of rat brain was homogenized in 20 volumes of Tris buffer (50 mM, pH 7.4) and 5 mM EDTA with a 30 s burst from a PT10 polytron homogenizer set at 6. The homogenate was centrifuged at 500*g* for 10 min. The supernatant obtained was then centrifuged at 65,000*g* for 25 min and the resulting pellet was washed twice with Tris–HCl (50 mM) without EDTA. The final pellet was resuspended in the same buffer and stored at −80 °C until required. Competition binding assays were performed by incubating washed rat cerebral membrane (200 μg of protein) with 5 nM [^{3}H]clonidine (NEN, 60–63 Ci/mmol) in the absence or presence of test compounds in a total volume of 400 μL of Tris assay buffer (50 mM Tris–HCl, pH 7.4). Non-specific binding was defined as the concentration of bound ligand in the presence of 10 μM phentolamine. Specific binding represented about 75% of the total binding at 5 nM [^{3}H]clonidine. Following equilibrium (45 min at 25 °C), bound radioactivity was separated from free by filtration through a GF/B filter with a Brandel cell harvester. Bound radioactivity on the glass fiber filter was determined by liquid scintillation counting. Each point was performed in triplicate.

5.5. In vitro radioligand I_1-imidazoline-binding site (IBS) assays

Bovine adrenal medulla plasma membranes were prepared as described by Molderings et al.[32] These membranes (0.8 mg protein/mL) were incubated for 40 min with 7 nM [^{3}H]clonidine at 22 °C in binding buffer (PBS, 0.5 mM EGTA, 0.5 mM $MgCl_2$, 0.5% ascorbic acid, pH 7.5) and increasing concentrations of test compounds, in the presence of 1 μM of RX821002 to mask α_2-adrenoreceptors. To stop the incubation, samples were filtered very quickly through GF/B glass fiber filters, filters were washed with cold buffer, and the radioactivity retained on the dried filters was determined. Non-specific binding was defined as [^{3}H]clonidine binding in the presence of 1μM of S22687-1 ([5-(2-methylphenoxy-methyl)-1,3-oxazolin-2-yl]amine; high affinity I_1 competing drug, $K_i = 4.98 \times 10^{-9}$ M).[33]

5.6. In vitro radioligand I_2-imidazoline-binding site (IBS) assays

Rabbit kidney membrane preparation and determination of affinities of compounds were performed as described in the literature.[31] Rabbit kidney was homogenized in 10 volumes of Tris–HCl buffer (50 mM, pH 7.4) and 250 mM sucrose, and centrifuged at 500*g* for 10 min. The supernatant was centrifuged at 28,000*g* for 30 min and the resulting pellet was washed twice with the same buffer without sucrose. The final pellet was resuspended in Tris–HCl buffer (50 mM, pH 7.4) and stored at −80 °C until use. Rabbit kidney membranes (200 μg of protein) were incubated with 5-nM [^{3}H]idazoxan (Amersham, 43 Ci/mmol) in the absence or presence of different concentrations of test compounds in a total volume of 400 μL of assay buffer. To mask adrenoreceptors, 10 μM (−)-norepinephrine (in the presence of 0.005% ascorbic acid) was added to all tubes. Non-specific binding was determined with 10 μM of cirazoline. Specific binding represented about 90% of the total binding at 5 nM [^{3}H]idazoxan. Following equilibrium (45 min at 25 °C) bound radioactivity was separated from free by filtration as described above. Each point was performed in triplicate. K_i values were calculated by the equation of Cheng and Prusoff.[34] Each curve was repeated at least three times and the results are given as means ± SEM.

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