Original article

Synthesis and anticonvulsant properties of 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea derivatives

Nadeem Siddiqui [a,*], M. Shamsher Alam [a], James P. Stables [b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Hamdard Nagar, New Delhi 110062, India
[b] Epilepsy Branch, National Institute of Neurological Disorders and Stroke, NIH, Rockville, MD 20892, USA

ARTICLE INFO

ABSTRACT

Various 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (5a—p) were designed keeping in view the structural requirements suggested in the pharmacophore model for anticonvulsant activity. Their in vivo anticonvulsant screenings were performed by two most adopted seizure models, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ). Compound 5f was found active in MES screening while compounds 5h, 5i, 5k and 5l showed significant anticonvulsant activity in both the screenings and were devoid of any neurotoxicity. Compound 5h and 5i showed marked protection at 300 mg/kg against MES and scPTZ screening. Compound 5i also showed protection against MES screening at the dose of 100 mg/kg. In 6 Hz screening these two compounds showed significant protection and emerged as lead compounds for future investigations.

1. Introduction

Epilepsy is a collective term given to a group of syndromes that involve spontaneous, intermittent, abnormal electrical activity in the brain, which manifest as seizures. It has been estimated that about 20% of the patients with epilepsy using the first generation of anti-epileptic drugs (Phenobarbital, Phenytoin, Carbamazepine, Sodium valproate and Diazepam) were not able to acquire adequate control of seizure [1]. In fact, epilepsy is the third most frequent neurological disorder encountered in the elderly after cerebrovascular disease and dementia [2]. There is continuing demand for new anticonvulsant agents as several of the currently available antiepileptic drugs (AED) have been associated with severe side effects and fail to control seizures in about 30% of epileptic patients [3—5]. The search for antiepileptic agents with more selectivity and lower toxicity continues to be an area of investigation in medicinal chemistry.

Study revealed that Isatin is a privileged lead molecule for designing potential bioactive agents, and its derivatives have been shown to possess a broad spectrum of bioactivity as many of which were assessed anti-HIV, [6] antiviral, [7] anti-tumor, [8—10] antifungal, [11,12] anti-angiogenic, [13] anticonvulsants, [14] anti-Parkinson's disease therapeutic, [15] and effective SARS coronavirus

3CL protease inhibitor. [16] These interesting properties prompted many efforts toward the synthesis and pharmacological screening of isatin derivatives.

Considering extensive applications of Isatin moiety in medicinal chemistry and in continuation of our ongoing project biologically active heterocycles [17—21], an attempt has been made to synthesize amino-N-arylmethanethio urea derivatives containing Isatin moiety and evaluate for their in vivo screening.

2. Chemistry

The synthesis of the titled compounds (5a—p) was carried out as presented in Scheme 1.

5-Nitroindoline-2, 3-dione (1) has been prepared by the re-fluxing of indoline-2, 3-dione with a mixture of 95—100% sulfuric acid and 70% nitric acid in water bath at 60 °C for 1 h. Compounds (2a,b) were synthesized by stirring a mixture of compound (1), anhydrous potassium carbonate in acetone and substituted benzyl chloride. Compounds (2a,b) get reduced to 5-amino-1-substituted benzylindoline-2, 3-dione (3a,b) on heating with a mixture of iron powder and hydrochloric acid. Compounds (3a,b) on stirring with sodium cyanate and glacial acetic acid, get converted to its urea derivatives (4a,b). Finally titled compounds 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (5a—p) were prepared on refluxing different substituted phenyl-isothiocyanates with compounds (4a,b). The physicochemical parameters of the synthesized compounds are presented in Table 1.

* Corresponding author. Tel.: +91 11 26059688x5639; fax: +91 11 26059688x5307.
E-mail addresses: nadeems_03@yahoo.co.in, nadeems_03@rediffmail.com
(N. Siddiqui).
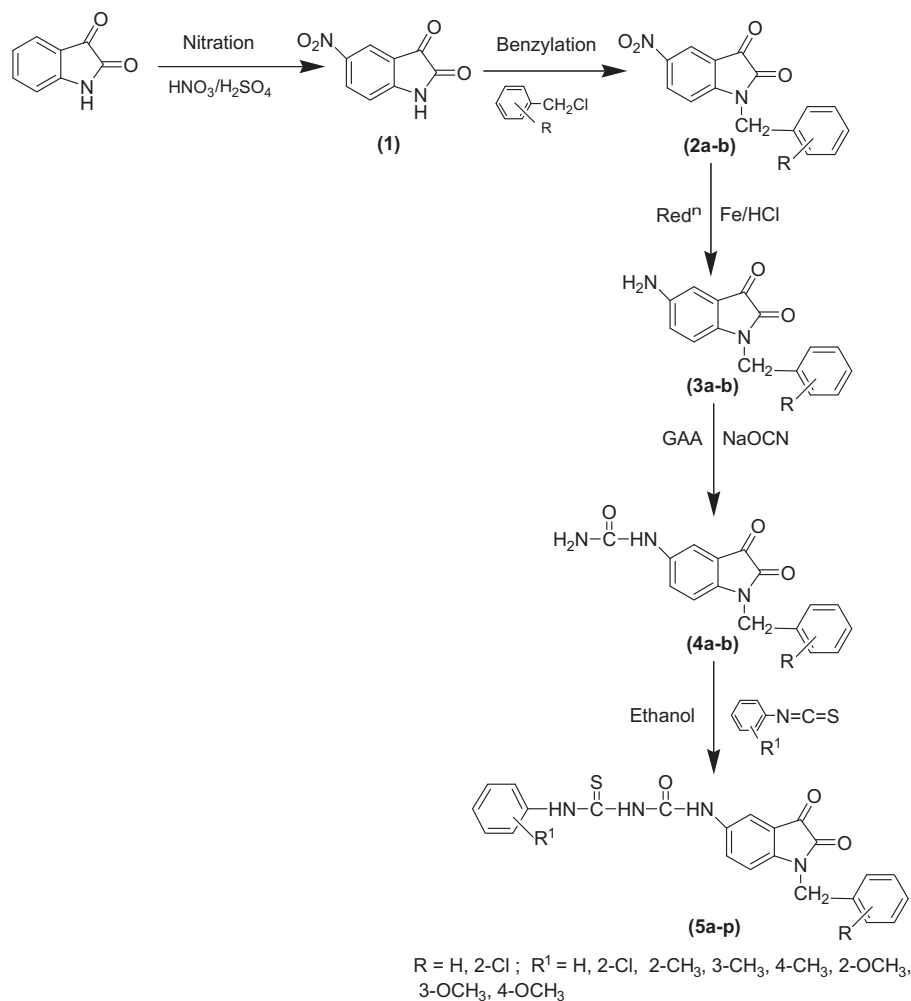
Scheme 1. Synthetic route to the synthesized compounds (**5a–p**).

Table 1
Physicochemical properties of titled compounds: (**5a–p**).

Compd. No.	R	R^1	Mol. Formula[a]	Log P[b] found (calculated)	R_f[c] (R_m)[d]	Elemental analysis (%)[e]			
						C	H	N	S
5a	H	H	$C_{23} H_{18} N_4 O_3 S$	2.48 (2.57)	0.94 (−1.19)	64.17	4.21	13.01	7.45
5b	H	2Cl	$C_{23} H_{17} Cl N_4 O_3 S$	3.01 (3.29)	0.83 (−0.68)	59.42	3.69	12.05	6.90
5c	H	2CH$_3$	$C_{24} H_{20} N_4 O_3 S$	2.98 (3.07)	0.77 (−0.52)	64.85	4.54	12.60	7.21
5d	H	3CH$_3$	$C_{24} H_{20} N_4 O_3 S$	2.92 (3.07)	0.94 (−1.19)	64.85	4.54	12.60	7.21
5e	H	4CH$_3$	$C_{24} H_{20} N_4 O_3 S$	2.96 (3.07)	0.77 (−0.52)	64.85	4.54	12.60	7.21
5f	H	2OCH$_3$	$C_{24} H_{20} N_4 O_4 S$	2.38 (2.49)	0.83 (−0.68)	62.60	4.38	12.17	6.96
5g	H	3OCH$_3$	$C_{24} H_{20} N_4 O_4 S$	2.40 (2.49)	0.94 (−1.19)	62.60	4.38	12.17	6.96
5h	H	4OCH$_3$	$C_{24} H_{20} N_4 O_4 S$	2.39 (2.49)	0.94 (−1.19)	62.60	4.38	12.17	6.96
5i	2Cl	H	$C_{23} H_{17} Cl N_4 O_3 S$	3.18 (3.29)	0.78 (−0.54)	59.42	3.69	12.05	6.90
5j	2Cl	2Cl	$C_{23} H_{16} Cl_2 N_4 O_3 S$	3.98 (4.00)	0.76 (−0.50)	55.32	3.23	11.22	6.42
5k	2Cl	2CH$_3$	$C_{24} H_{19} Cl N_4 O_3 S$	3.70 (3.79)	0.90 (−0.95)	60.18	4.00	11.70	6.70
5l	2Cl	3CH$_3$	$C_{24} H_{19} Cl N_4 O_3 S$	3.72 (3.79)	0.91 (−1.00)	60.18	4.00	11.70	6.70
5m	2Cl	4CH$_3$	$C_{24} H_{19} Cl N_4 O_3 S$	3.73 (3.79)	0.94 (−1.19)	60.18	4.00	11.70	6.70
5n	2Cl	2OCH$_3$	$C_{24} H_{19} Cl N_4 O_4 S$	3.18 (3.21)	0.79 (−0.57)	58.24	3.87	11.32	6.48
5o	2Cl	3OCH$_3$	$C_{24} H_{19} Cl N_4 O_4 S$	3.19 (3.21)	0.82 (−0.65)	58.24	3.87	11.32	6.48
5p	2Cl	4OCH$_3$	$C_{24} H_{19} Cl N_4 O_4 S$	3.24 (3.21)	0.80 (−0.60)	58.24	3.87	11.32	6.48

A logarithmic function of R_f-value was also calculated.
[a] Solvent of crystallization—Ethanol.
[b] Log P was determined by octanol: phosphate buffer method; CLog P was calculated using software ChemOffice 6.0.
[c] Solvent system — Toluene: Ethyl acetate: Formic acid (5:4:1).
[d] $R_m = \log(1 − 1/R_f)$.
[e] Experimental value of Elemental analysis for C, H, N and S were within ±0.4% of the theoretical value.

The structures and purity of the final compounds were confirmed on the basis of spectral and elemental analyses and the data were within ±0.4% of the theoretical values.

3. Pharmacology

The pharmacological testing of all the final compounds have been performed by National Institute of Neurological Disorders and Stroke (NINDS), USA under Anticonvulsant screening program (ASP), following the protocol adopted by the Antiepileptic Drug Development (ADD) program [22]. All the compounds prepared herein were screened for their potential in vivo biological activities such as anticonvulsant and minimal motor impairment test. The Phase I pharmacological screening comprised of MES, scPTZ and neurotoxicity. Further, some of the selected compounds were tested for their activity at 6 Hz model.

4. Results and discussion

4.1. Anticonvulsant activity

Phase I studies of the investigated compounds (**5a**–**p**) involved three tests: maximal electroshock seizure (MES), subcutaneous Pentylenetetrazole (scPTZ), and rotorod test for neurological toxicity. For the MES assay, experimental compound is administered to sets of one, three and one animals at three respective doses (30, 100, and 300 mg/kg) at both 0.5 h and 4.0 h time periods. The similar standard for dosing and time is used for the scPTZ testing. All animals were observed for potential neurological deficit at each dose and time period.

For the identification of anticonvulsant activity in mice, test compounds were administered intraperitoneally and challenged by maximal electroshock and subcutaneous Pentylenetetrazole. Compounds found to be effective in these seizure challenges are generally regarded to be potentially useful candidates in treatment of partial, generalized and even absence seizures. Neurotoxicity of the test compounds was determined using the rotorod toxicity test (TOX). The results of the in vivo tests are summarized in Table 2.

Titled compounds 1-(amino-*N*-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (**5a**–**p**) showed some protection against MES and scPTZ seizures in mice in Phase 1

Table 3
Anticonvulsant screening project (ASP): Test 7 results in mice; 6 Hz assay (Dose 100 mg/kg).

Compd.	Test	Time (h)				
		0.25	0.5	1.0	2.0	4.0
5h	6Hz[a]	1/4	1/4	0/4	0/4	0/4
	TOX[b]	0/4	0/4	0/4	0/4	0/4
5i	6Hz[a]	3/4	2/4	2/4	0/4	0/4
	TOX[b]	0/4	0/4	0/4	0/4	0/4

[a] Qualitative 6 Hz assay (number of animal protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested) endpoint: unable to maintain balance on rotorod.

screening. The most potent anticonvulsant compounds found to be were **5f**, **5h**, **5i**, **5k** and **5l** and interestingly all these compounds were devoid of any neurotoxicity. In MES test, compound **5i** was active at dose of 100 mg//kg (1/3 animal protected) 4.0 h after administration; interestingly compound **5i** was also active at dose of 300 mg/kg (1/1 & 1/1 animals protected) after 0.5 h & 4.0 h. In 300 mg/kg dose, compounds **5f** and **5h** were also found to be active (1/1 and 1/1 animals protected) 0.5 h and (1/1 and 1/1 animals protected) 4.0 h after administration. In 300 mg/kg dose, compounds **5k** and **5l** were active (1/1 and 1/1 animals protected) 4.0 h after administration.

In scPTZ screening compounds **5h** and **5i** were active at dose of 300 mg//kg (1/5 and 2/5 animals protected) 0.5 h after administration. Compounds **5k** and **5l** also showed protection at dose of 300 mg/kg (1/1 and 1/1 animals protected) 4.0 h after administration.

In the neurotoxicity screening, compounds **5f**, **5h**, **5i**, **5k** and **5l** that were found to be potent were devoid of minimal motor impairment at any doses. Rest all the compounds, although were not potent showed lack of any neurotoxicity.

The compounds 5**h** and **5i** that displayed a marked MES as well as scPTZ activity in mice at the dose of 100 & 300 mg/kg i.p. were evaluated for 6 Hz assay in mice, Test 7 results (Table 3). Compound **5h** showed protection in the Minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p. dose) (1/4 & 1/4 animals protected) 0.25 & 0.5 h after administration. In 6 Hz test compound **5i** showed protection at 100 mg/kg (i.p. dose) (3/4, 2/4 & 2/4 animals protected) 0.25, 0.5 & 1.0 h after administration. Interestingly it lacks any toxicity in given dose in any time intervals.

Table 2
Anticonvulsant screening project (ASP): Phase 1- test results in mice

Compd.	Test 1 results – Intraperitoneal injection in mice																	
	MES screening						scPTZ screening						Minimal motor impairment screening					
	30 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg	
	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)	0.5 h (N/F)	4.0 h (N/F)
5a	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5b	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5c	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5d	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5e	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5f	0/1	0/1	0/3	0/3	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5g	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5h	0/1	0/1	0/3	0/3	1/1	1/1	0/1	0/1	0/1	0/1	1/5	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5i	0/1	0/1	0/3	1/3	1/1	1/1	0/1	0/1	0/1	0/1	2/5	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5j	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5k	0/1	0/1	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1	0/4	0/2	0/8	0/4	0/4	0/2
5l	0/1	0/1	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1	0/4	0/2	0/8	0/4	0/4	0/2
5m	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5n	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5o	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
5p	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2

N/F = Number of animals active or toxic over the number tested.
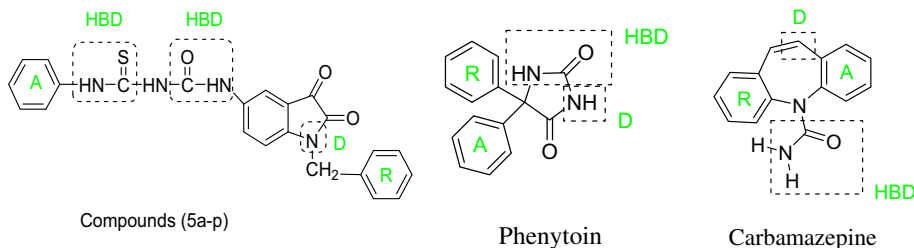
Fig. 1. Anticonvulsant agents showing the required pharmacophoric elements.

These results suggest that thioamide and amide as two hydrogen bonding domains (HBD), Nitrogen of Isatin as electron donar (*D*), unsubstitution or 2-chloro substitution as electron withdrawing agent in hydrophobic domain (*A*) and unsubstitution or methoxy/methyl substitution as electron donating agent in distal hydrophobic domain (*R*) made the compound potent in the MES screen at 100 & 300 mg/kg doses, scPTZ screen at 100 & 300 mg/kg (i.p. dose) in mice as well as minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p. dose) in mice. Anticonvulsant agents having the above required pharmacophoric elements have been shown in Fig. 1.

4.2. Log P determination

The dependence of biological activity in the set of congeneric agents or lipophilic character has been shown in many types of drug action in particular, the reports by Lien and co-workers indicate that the anticonvulsant activity of different types of compounds were correlated with lipophilicity [23]. However, it has been observed that the maximum potency of the drugs that act on the central nervous system (CNS) is obtained with congeners having an optimum lipophilicity (log Po). In this study, we attempted to correlate the anticonvulsant activity of synthesized compounds with their log *P* value. The experimental log *P* values were determined using octanol-phosphate buffer method and the calculated log *P* values were taken from the software ChemOffice 6.0 and the results are shown in Table 1. Most of the synthesized compounds met the criteria for lipophilicty and showed good anticonvulsant activity with lesser neurotoxicity. Due to their higher lipophilicity they are expected to have rapid onset and shorter duration of action.

5. Conclusions

Pharmacophore model for anticonvulsant 1-(amino-*N*-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea has been established on the basis of results of pharmacological, physicochemical, distance mapping and three-dimensional structural investigations. In this model, the presence of thioamide and amide (HBD) fragment is essential for activity. Beside this, all of the compounds contained two hydrophobic units (A & R aryl rings) as a distal binding site and Isatin as electron donar (*D*). Five compounds showed better anticonvulsant activity and among these compounds **5h** and **5i** emerged as a lead compounds in the series when they were subjected to preliminary anticonvulsant screenings. They also showed marked lower neurotoxicity and, therefore, a higher protective index. These can be regarded as strong candidates for future investigations.

6. Experimental protocols

6.1. Chemistry

The chemicals used were obtained from various chemical units E. Merck India Ltd., CDH, and s.d. Fine Chem. and Qualigens. These solvents and reagents were of LR grade and purified before their use. The silica gel G (160–120 mesh) used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Two solvent systems were used Benzene: Acetone (8:2) and (6:4), Toluene: Ethyl acetate: Formic acid (5:4:1). All the melting points were determined in open glass capillary using Kjeldahl flask containing paraffin and are uncorrected. Both ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 400 Ultra shield™ (400 MHz) NMR spectrometer in DMSO-d_6/CDCl$_3$ using tetramethylsilane [(CH$_3$)$_4$Si] as internal standard. Chemical shift (δ) are expressed in ppm. All the Fourier transform infra-red (FT-IR) spectra were recorded in KBr pellets on a Jasco FT/IR 410 spectrometer. Elemental analyses were realized on a Perkin–Elmer model 240c analyzer and were within ±0.4% of the theoretical values.

6.1.1. General procedure for the synthesis of titled compounds (5a–p)

6.1.1.1. 5-Nitroindoline-2, 3-dione (1).
Compound indoline-2, 3-dione (48.5 g, 0.33 mol) was added into a mixture of 50 g (35 mL, 0.5 mol) of conc. nitric acid and 74 g (40 mL, 0.75 mol) conc. sulfuric acid with frequent shaking in 500-mL round bottomed flask, keeping the mixture cool by immersing the flask in ice cold water. After adding all indoline-2, 3-dione, flask was fitted with reflux condenser and the mixture was heated on water bath maintaining the temperature at 60 °C for 1 h to obtain the desired compound 5-nitroindoline-2, 3-dione (**1**). Content was then poured into a beaker containing 500 mL cold water, stirred in order to wash out as much acid from the desired compound. When compound (**1**) completely settled to the bottom, the upper acid liquor was completely poured off from the mixture. Then the residual liquid was transferred to the separating funnel and shaked vigorously with about 50 mL of water. Bottom layer was collected, dried with anhydrous calcium chloride and finally filtered to obtain the pure compound (**1**). Yield 62%, mp 230 °C. IR (KBr) cm^{-1}: 3306 (NH$_{str.}$), 3015 (Ar-CH$_{str.}$), 1725 (C=O, Isatin), 1563 (NO$_{str.}$-sym.), 1325 (NO$_{str.}$-asym.), ^{1}H NMR (CDCl$_3$) δ (ppm): 7.12–7.89 (m, 3H, Ar-H), 9.53 (s, 1H, NH-Isatin); ^{13}C NMR (DMSO-d_6) δ (ppm): 107.34, 114.46, 127.24, 129.83, 144.24, 152.73, 160.26, 178.45.

6.1.1.2. 5-Nitro-1-substituted benzylindoline-2, 3-dione (2a,b).
To a solution of compound **1** (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in acetone (30 ml), substituted benzyl chloride (0.01 mol) was added drop wise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over sodium sulfate anhydrous and concentrated under vacuum to give the pure compound. Desired compound **2a,b** was finally recrystallized with ethanol.

6.1.1.2.1. 5-Nitro-1-benzylindoline-2, 3-dione (2a).
Yield 60%, mp 260 °C. IR (KBr) cm^{-1}: 3063 (Ar-CH$_{str.}$), 2825 (CH$_{str.}$), 1723 (C=O, Isatin), 1548 (NO$_{str.}$-sym.), 1352 (NO$_{str.}$-asym.), ^{1}H NMR (CDCl$_3$) δ (ppm): 4.23 (s, 2H, CH$_2$-Ar), 7.01–7.78 (m, 8H, Ar-H), ^{13}C NMR (DMSO-d_6) δ (ppm): 48.32, 120.43, 122.78, 124.26, 124.28, 125.10, 127.25, 129.23, 129.54, 130.13, 138.52, 143.27, 156.38, 158.34, 183.35.

*6.1.1.2.2. 1-(2-Chlorobenzyl)-5-nitroindoline-2, 3-dione (**2b**).* Yield 62%, mp 275 °C. IR (KBr) cm^{-1}: 3037 (Ar-CH$_{str.}$), 2818 (CH$_{str.}$), 1690 (C=O, Isatin), 1540 (NO$_{str.}$-sym.), 1348 (NO$_{str.}$-asym.), 725 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 4.15 (s, 2H, CH$_2$-Ar), 7.23–7.85 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 47.45, 122.48, 123.36, 124.24, 126.48, 127.12, 129.37, 129.01, 131.27, 132.37, 137.72, 142.67, 157.38, 158.92, 183.35.

*6.1.1.3. 5-Amino-1-substituted benzylindoline-2, 3-dione (**3a,b**).* Compound **2a,b** (0.01 mol) and iron powder (0.01 mol) was placed in round bottomed flask containing 200 mL ethanol. The mixture was heated on oil bath till temperature attained to 80–85 °C. Then 4 mL of HCl (1.2 M) was added and content was further stirred for 4 h while maintaining the temperature. Content was then filtered at reduced pressure and the filtrate was adjusted to pH 7–8 using NaHCO$_3$ to get the precipitate of compound **3a,b**. It was then recrystallized with ethanol.

*6.1.1.3.1. 5-Amino-1-benzylindoline-2, 3-dione (**3a**).* Yield 71%, mp 265 °C. IR (KBr) cm^{-1}: 3265 (NH$_{str.}$), 3125 (Ar-CH$_{str.}$), 2848 (CH$_{str.}$), 1764 (C=O, Isatin); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.76 (s, 1H, NH), 4.15 (s, 2H, CH$_2$-Ar), 7.21–7.89 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 46.27, 121.46, 123.59, 124.38, 124.75, 125.79, 126.28, 129.38, 129.41, 130.27, 138.56, 144.28, 157.48, 159.14, 182.65.

*6.1.1.3.2. 1-(2-Chlorobenzyl)-5-aminoindoline-2,3-dione (**3b**).* Yield 68%, mp 272 °C. IR (KBr) cm^{-1}: 3356 (NH$_{str.}$), 3234 (Ar-CH$_{str.}$), 2858 (CH$_{str.}$), 1726 (C=O, Isatin), 738 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.65 (s, 1H, NH), 4.02 (s, 2H, CH$_2$-Ar), 6.84–7.85 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 45.48, 121.26, 123.35, 124.32, 124.59, 125.24, 126.29, 129.47, 129.46, 130.28, 138.18, 144.29, 157.27, 159.17, 184.15.

*6.1.1.4. 1-(1-Substituted benzyl-2, 3-dioxoindolin-5-yl) urea (**4a,b**).* Compound **3a,b** (0.01 mol) was dissolved in 10 mL glacial acetic acid and was diluted to 100 mL in a conical flask. To it a solution of NaOCN (0.01 mol) and 50 mL warm water were added with continuous shaking. Content was allowed to stand for 30 min, and then cooled in ice, allowed to stand for further 30 min, filtered at the pump, washed with water and dried at 100 °C. The desired compound **4a,b** were formed and recrystallized with ethanol.

*6.1.1.4.1. 1-(1-Benzyl-2, 3-dioxoindolin-5-yl) urea (**4a**).* Yield 74%, mp 282 °C. IR (KBr) cm^{-1}: 3325 (NH$_{str.}$), 3137 (Ar-CH$_{str.}$), 2839 (CH$_{str.}$), 1785 (C=O, Isatin), 1625 (C=O, Urea); ^{1}H NMR (CDCl$_3$) δ (ppm): 4.26 (s, 1H, NH-Urea, D$_2$O exchangeable), 4.85 (s, 2H, CH$_2$-Ar), 6.57–7.83 (m, 8H, Ar-H), 8.42 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 49.25, 121.28, 122.38, 124.27, 124.36, 125.48, 127.38, 129.25, 130.42, 132.21, 137.52, 144.47, 156.43, 157.44, 162.18, 184.15.

*6.1.1.4.2. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]urea (**4b**).* Yield 78%, mp 295 °C. IR (KBr) cm^{-1}: 3364 (NH$_{str.}$), 3128 (Ar-CH$_{str.}$), 2848 (CH$_{str.}$), 1728 (C=O, Isatin), 1612 (C=O, Urea), 710 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 4.12 (s, 1H, NH-Urea, D$_2$O exchangeable), 4.63 (s, 2H, CH$_2$-Ar), 6.78–7.84 (m, 7H, Ar-H), 8.37 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 52.67, 120.46, 123.78, 124.25, 124.94, 125.28, 127.17, 129.37, 130.51, 134.61, 138.92, 146.42, 156.74, 159.47, 168.37, 186.35.

*6.1.1.5. 1-(Amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (**5a–p**).* A mixture of compound **4a,b** (0.01 mol) and substituted phenylisothiocyanates (0.01 mol) in 20 mL of absolute ethanol was refluxed for 5–6 h. After completion of the reaction, mixture was concentrated and kept overnight at room temperature. The needle shaped crystals thus obtained were purified by repeated washing with petroleum ether. Desired titled compounds **5a–p** were recrystallized with ethanol.

*6.1.1.5.1. 1-(Amino-N-phenylmethanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5a**).* Yield 67%, mp 300 °C. IR (KBr) cm^{-1}: 3505 (NH$_{str.}$), 2999 (NH$_{str.}$), 2955 (NH$_{str.}$), 2918 (Ar-CH$_{str.}$), 2850 (CH$_{str.}$), 1758 (C=O, Isatin), 1458 (C=O, amide), 1427 (C=S$_{str.}$); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.85 (s, 2H, CH$_2$-Ar), 7.00–7.89 (m, 10H, Ar-H), 6.97–7.96 (m, 3H, ArH-Isatin), 7.98 (s, 1H, NH, D$_2$O exchangeable), 9.08 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.72 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 46.21, 109.51, 115.82, 119.54, 121.56, 123.62, 124.21, 124.28, 126.24, 126.47, 128.02, 128.21, 128.34, 129.71, 129.73, 134.72, 137.21, 141.32, 149.51, 152.32, 158.84, 182.36, 183.67; MS: m/z 429 $(M - 1)$.

*6.1.1.5.2. 1-{Amino-N-(2-chlorophenyl) methanethio}-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5b**).* Yield 59%, mp 230 °C. IR (KBr) cm^{-1}: 3525 (NH$_{str.}$), 3095 (NH$_{str.}$), 3055 (NH$_{str.}$), 2958 (Ar-CH$_{str.}$), 2950 (CH$_{str.}$), 1752 (C=O, Isatin), 1658 (C=O, amide), 1627 (C=S$_{str.}$), 665 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.82 (s, 2H, CH$_2$-Ar), 7.25–7.88 (m, 9H, Ar-H), 6.93–7.68 (m, 3H, ArH-Isatin), 8.96 (s, 1H, NH, D$_2$O exchangeable), 9.78 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.89 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 45.25, 108.32, 114.72, 118.57, 120.45, 122.67, 123.27, 124.51, 126.32, 126.48, 127.14, 128.16, 128.21, 129.32, 129.58, 133.74, 136.46, 140.27, 148.12, 151.37, 157.59, 181.46, 182.64.

*6.1.1.5.3. 1-(Amino-N-o-tolylmethanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5c**).* Yield 77%, mp 120 °C. IR (KBr) cm^{-1}: Yield 59%, mp 230 °C. IR (KBr) cm^{-1}: 3386 (NH$_{str.}$), 3268 (NH$_{str.}$), 3071 (NH$_{str.}$), 2924 (Ar-CH$_{str.}$), 2854 (CH$_{str.}$), 1707 (C=O, Isatin), 1640 (C=O, amide), 1606 (C=S$_{str.}$); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.27 (s, 3H, CH$_3$), 3.80 (s, 2H, CH$_2$-Ar), 7.01–7.80 (m, 9H, Ar-H), 6.98–7.93 (m, 3H, ArH-Isatin), 8.83 (s, 1H, NH, D$_2$O exchangeable), 9.09 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.88 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.68, 46.54, 107.24, 115.47, 118.32, 121.52, 123.52, 123.87, 124.42, 125.24, 125.24, 126.84, 127.01, 127.54, 128.34, 129.28, 132.34, 135.67, 141.74, 148.12, 150.23, 157.59, 181.14, 181.54.

*6.1.1.5.4. 1-(Amino-N-m-tolylmethanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5d**).* Yield 47%, mp 110 °C. IR (KBr) cm^{-1}: 3356 (NH$_{str.}$), 3224 (NH$_{str.}$), 3053 (NH$_{str.}$), 2901 (Ar-CH$_{str.}$), 2824 (CH$_{str.}$), 1704 (C=O, Isatin), 1612 (C=O, amide), 1604 (C=S$_{str.}$); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.22 (s, 3H, CH$_3$), 3.76 (s, 2H, CH$_2$-Ar), 7.23–7.89 (m, 9H, Ar-H), 6.35–7.98 (m, 3H, ArH-Isatin), 8.87 (s, 1H, NH, D$_2$O exchangeable), 9.64 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.69 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 16.54, 45.64, 106.24, 114.26, 117.56, 122.24, 122.42, 123.48, 123.84, 124.34, 125.12, 125.23, 128.45, 128.63, 128.85, 129.02, 131.34, 134.37, 142.14, 147.15, 151.22, 156.57, 182.17, 183.54.

*6.1.1.5.5. 1-(Amino-N-p-tolylmethanethio)-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5e**).* Yield 63%, mp 57 °C. IR (KBr) cm^{-1}: 3352 (NH$_{str.}$), 3202 (NH$_{str.}$), 3053 (NH$_{str.}$), 2942 (Ar-CH$_{str.}$), 2814 (CH$_{str.}$), 1715 (C=O, Isatin), 1614 (C=O, amide), 1602 (C=S$_{str.}$); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.12 (s, 3H, CH$_3$), 3.89 (s, 2H, CH$_2$-Ar), 7.23–7.78 (m, 9H, Ar-H), 6.89–7.69 (m, 3H, ArH-Isatin), 8.89 (s, 1H, NH, D$_2$O exchangeable), 9.35 (s, 1H, NH-Ar, D$_2$O exchangeable), 9.97 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 16.24, 44.14, 105.27, 113.54, 118.03, 121.84, 122.64, 123.21, 123.87, 125.24, 125.42, 126.24, 127.26, 128.74, 128.87, 129.46, 130.41, 133.37, 143.14, 146.54, 151.84, 156.14, 183.74, 184.34.

*6.1.1.5.6. 1-[Amino-N-(2-methoxyphenyl)methanethio]-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (**5f**).* Yield 87%, mp 132 °C. IR (KBr) cm^{-1}: 3390 (NH$_{str.}$), 2924 (NH$_{str.}$), 2853 (NH$_{str.}$), 2828 (Ar-CH$_{str.}$), 2815 (CH$_{str.}$), 1710 (C=O, Isatin), 1692 (C=O, amide), 1639 (C=S$_{str.}$), 1115 (OCH$_3$); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.49 (s, 3H, OCH$_3$), 3.36 (s, 2H, CH$_2$-Ar), 6.73–7.82 (m, 9H, Ar-H), 6.70–7.97 (m, 3H, ArH-Isatin), 7.98 (s, 1H, NH, D$_2$O exchangeable), 9.01 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.59 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 42.56, 56.42, 108.24, 114.34, 120.05, 122.74, 122.81, 123.01, 123.86, 125.27, 125.84, 126.22, 127.24, 128.07, 128.14, 129.01, 130.37, 135.31, 145.04, 148.53, 150.54, 159.17, 182.72, 186.37.

6.1.1.5.7. 1-[Amino-N-(3-methoxyphenyl)methanethio]-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (5g). Yield 68%, mp 205 °C. IR (KBr) cm^{-1}: 3385 (NH$_{str.}$), 2945 (NH$_{str.}$), 2885 (NH$_{str.}$), 2824 (Ar-CH$_{str.}$), 2815 (CH$_{str.}$), 1701 (C=O, Isatin), 1654 (C=O, amide), 1621 (C=S$_{str.}$), 1118 (OCH$_3$); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.12 (s, 3H, OCH$_3$), 3.57 (s, 2H, CH$_2$-Ar), 6.58–7.87 (m, 9H, Ar-H), 6.86–7.69 (m, 3H, ArH-Isatin), 7.69 (s, 1H, NH, D$_2$O exchangeable), 9.38 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.48 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 40.25, 54.34, 106.45, 116.37, 121.95, 122.34, 122.94, 123.45, 123.83, 125.28, 125.35, 126.25, 127.54, 127.64, 128.54, 129.21, 130.34, 134.62, 144.03, 146.54, 148.24, 158.13, 181.77, 185.34.

6.1.1.5.8. 1-[Amino-N-(4-methoxyphenyl)methanethio]-3-(1-benzyl-2, 3-dioxoindolin-5-yl) urea (5h). Yield 55%, mp 206 °C. IR (KBr) cm^{-1}: 3384 (NH$_{str.}$), 2936 (NH$_{str.}$), 2895 (NH$_{str.}$), 2858 (Ar-CH$_{str.}$), 2814 (CH$_{str.}$), 1712 (C=O, Isatin), 1643 (C=O, amide), 1612 (C=S$_{str.}$), 1165 (OCH$_3$); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.56 (s, 3H, OCH$_3$), 3.89 (s, 2H, CH$_2$-Ar), 6.45–7.89 (m, 9H, Ar-H), 6.58–7.76 (m, 3H, ArH-Isatin), 7.57 (s, 1H, NH, D$_2$O exchangeable), 9.54 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.37 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.24, 56.38, 104.46, 117.32, 121.04, 122.32, 122.74, 123.36, 123.82, 125.11, 125.34, 126.26, 127.01, 127.65, 128.24, 129.45, 130.34, 134.14, 142.01, 145.53, 146.24, 156.37, 182.74, 183.35.

6.1.1.5.9. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-phenylmethanethio) urea (5i). Yield 68%, mp 185 °C. IR (KBr) cm^{-1}: 3451 (NH$_{str.}$), 3259 (NH$_{str.}$), 3086 (NH$_{str.}$), 2924 (Ar-CH$_{str.}$), 2950 (CH$_{str.}$), 1753 (C=O, Isatin), 1631 (C=O, amide), 1527 (C=S$_{str.}$), 646 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.37 (s, 2H, CH$_2$-Ar), 6.50–7.97 (m, 9H, Ar-H), 6.63–7.96 (m, 3H, ArH-Isatin), 9.85 (s, 1H, NH, D$_2$O exchangeable), 10.39 (s, 1H, NH-Ar, D$_2$O exchangeable), 10.67 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 51.37, 102.64, 115.56, 120.57, 121.24, 122.54, 123.76, 123.87, 124.31, 125.45, 126.37, 127.41, 127.85, 128.31, 129.01, 130.24, 133.42, 141.24, 144.57, 145.38, 154.08, 180.77, 186.65; MS: *m/z* 463(*M* − 1).

6.1.1.5.10. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-[amino-N-(2-chlorophenyl)methanethio] urea (5j). Yield 68%, mp 185 °C. IR (KBr) cm^{-1}: 3456 (NH$_{str.}$), 3245 (NH$_{str.}$), 3062 (NH$_{str.}$), 2936 (Ar-CH$_{str.}$), 2912 (CH$_{str.}$), 1723 (C=O, Isatin), 1648 (C=O, amide), 1623 (C=S$_{str.}$), 684 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 3.59 (s, 2H, CH$_2$-Ar), 6.54–7.59 (m, 8H, Ar-H), 6.59–7.48 (m, 3H, ArH-Isatin), 9.39 (s, 1H, NH, D$_2$O exchangeable), 10.48 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.67 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 52.24, 98.54, 110.25, 118.53, 120.53, 121.14, 123.06, 123.32, 124.14, 125.27, 126.19, 127.38, 127.48, 128.51, 129.34, 130.25, 133.40, 142.27, 143.25, 145.37, 151.24, 182.37, 188.39; MS: *m/z* 463(*M* − 1).

6.1.1.5.11. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-o-tolylmethanethio) urea (5k). Yield 82%, mp 215 °C. IR (KBr) cm^{-1}: 3456 (NH$_{str.}$), 3259 (NH$_{str.}$), 3090 (NH$_{str.}$), 2925 (Ar-CH$_{str.}$), 2728 (CH$_{str.}$), 1707 (C=O, Isatin), 1633 (C=O, amide), 1534 (C=S$_{str.}$), 644 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.50 (s, 3H, CH$_3$), 3.31 (s, 2H, CH$_2$-Ar), 7.16–8.20 (m, 8H, Ar-H), 7.16–8.23 (m, 3H, ArH-Isatin), 8.76 (s, 1H, NH, D$_2$O exchangeable), 10.42 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.52 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.54, 55.32, 96.46, 108.12, 117.34, 121.34, 122.39, 123.01, 123.34, 124.44, 125.02, 126.67, 127.23, 127.65, 128.24, 129.03, 130.54, 132.41, 143.24, 143.85, 145.24, 152.65, 183.87, 189.34.

6.1.1.5.12. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-m-tolylmethanethio) urea (5l). Yield 58%, mp 300 °C. IR (KBr) cm^{-1}: 3452 (NH$_{str.}$), 3212 (NH$_{str.}$), 3035 (NH$_{str.}$), 2985 (Ar-CH$_{str.}$), 2941 (CH$_{str.}$), 1732 (C=O, Isatin), 1645 (C=O, amide), 1627 (C=S$_{str.}$), 656 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.69 (s, 3H, CH$_3$), 3.78 (s, 2H, CH$_2$-Ar), 7.34–8.53 (m, 8H, Ar-H), 7.25–8.57 (m, 3H, ArH-Isatin), 8.69 (s, 1H, NH, D$_2$O exchangeable), 10.38 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.12 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.32, 54.75, 92.32, 105.14, 116.94, 120.54, 121.32, 123.23, 123.54, 124.14, 125.24, 126.64, 127.24, 127.65, 128.24, 129.34, 130.57, 133.71, 143.64, 144.25, 145.74, 150.64, 185.37, 188.32.

6.1.1.5.13. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-(amino-N-p-tolylmethanethio) urea (5m). Yield 57%, mp 260 °C. IR (KBr) cm^{-1}: 3453 (NH$_{str.}$), 3254 (NH$_{str.}$), 3068 (NH$_{str.}$), 2941 (Ar-CH$_{str.}$), 2935 (CH$_{str.}$), 1782 (C=O, Isatin), 1635 (C=O, amide), 1595 (C=S$_{str.}$), 638 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.35 (s, 3H, CH$_3$), 3.58 (s, 2H, CH$_2$-Ar), 7.34–8.68 (m, 8H, Ar-H), 7.24–8.58 (m, 3H, ArH-Isatin), 8.79 (s, 1H, NH, D$_2$O exchangeable), 10.48 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.27 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 16.22, 55.73, 96.56, 111.15, 117.34, 121.24, 122.33, 123.53, 123.64, 124.04, 125.45, 126.85, 127.32, 127.58, 128.21, 129.02, 130.37, 132.51, 144.14, 144.27, 145.64, 151.65, 186.17, 189.31.

6.1.1.5.14. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-[amino-N-(2-methoxyphenyl)methanethio] urea (5n). Yield 75%, mp 123 °C. IR (KBr) cm^{-1}: 3454 (NH$_{str.}$), 3394 (NH$_{str.}$), 3080 (NH$_{str.}$), 2924 (Ar-CH$_{str.}$), 2912 (CH$_{str.}$), 1712 (C=O, Isatin), 1692 (C=O, amide), 1633 (C=S$_{str.}$), 1175 (OCH$_3$), 709 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.50 (s, 3H, OCH$_3$), 3.40 (s, 2H, CH$_2$-Ar), 7.22–8.36 (m, 8H, Ar-H), 6.58–8.76 (m, 3H, ArH-Isatin), 8.82 (s, 1H, NH, D$_2$O exchangeable), 9.19 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.11 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.85, 54.73, 94.31, 110.14, 116.42, 120.27, 122.34, 123.25, 123.84, 124.21, 125.34, 126.51, 127.33, 127.57, 128.64, 129.31, 130.35, 133.54, 144.24, 144.28, 145.24, 153.65, 184.13, 188.41.

6.1.1.5.15. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-[amino-N-(3-methoxyphenyl)methanethio] urea (5o). Yield 52%, mp 240 °C. IR (KBr) cm^{-1}: 3456 (NH$_{str.}$), 3342 (NH$_{str.}$), 3036 (NH$_{str.}$), 2985 (Ar-CH$_{str.}$), 2957 (CH$_{str.}$), 1732 (C=O, Isatin), 1684 (C=O, amide), 1612 (C=S$_{str.}$), 1123 (OCH$_3$), 704 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.46 (s, 3H, OCH$_3$), 3.57 (s, 2H, CH$_2$-Ar), 7.56–8.79 (m,8H, Ar-H), 6.23–8.69 (m, 3H, ArH-Isatin), 8.58 (s, 1H, NH, D$_2$O exchangeable), 9.48 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.35 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 43.32, 56.53, 96.41, 112.16, 115.52, 121.67, 122.44, 123.45, 123.49, 124.20, 125.34, 126.21, 127.51, 127.58, 128.21, 129.34, 130.75, 132.64, 143.44, 144.35, 145.28, 154.67, 189.33, 189.47.

6.1.1.5.16. 1-[1-(2-Chlorobenzyl)-2,3-dioxoindolin-5-yl]-3-[amino-N-(4-methoxyphenyl)methanethio] urea (5p). Yield 58%, mp 197 °C. IR (KBr) cm^{-1}: 3484 (NH$_{str.}$), 3336 (NH$_{str.}$), 3085 (NH$_{str.}$), 2942 (Ar-CH$_{str.}$), 2910 (CH$_{str.}$), 1714 (C=O, Isatin), 1658 (C=O, amide), 1623 (C=S$_{str.}$), 1141 (OCH$_3$), 712 (C–Cl); ^{1}H NMR (CDCl$_3$) δ (ppm): 2.78 (s, 3H, OCH$_3$), 3.58 (s, 2H, CH$_2$-Ar), 7.45–8.69 (m, 8H, Ar-H), 6.12–8.49 (m, 3H, ArH-Isatin), 8.37 (s, 1H, NH, D$_2$O exchangeable), 9.57 (s, 1H, NH-Ar, D$_2$O exchangeable), 11.58 (s, 1H, NH-Isatin, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.72, 55.43, 99.31, 113.56, 114.58, 122.68, 123.47, 123.65, 123.75, 124.32, 125.44, 126.31, 127.57, 127.59, 128.01, 129.44, 131.74, 132.74, 143.74, 144.39, 148.18, 154.68, 188.53, 189.45.

6.2. Pharmacology

Anticonvulsant screening at the ASP includes the maximal electroshock test (MES), the subcutaneous Pentylenetetrazole test (scPTZ), and evaluations of toxicity (TOX). The data of each condition is presented as *N/F*, where *N* equals to the number of animals protected and *F* equals to the number of animals tested. For test of toxicity (TOX), *N* equals the number of animals displaying toxic effects and *F* equals the number of animals tested.

6.2.1. Anticonvulsant screening

6.2.1.1. Maximal electroshock test (MES test). The MES test is a model, for example, generalized tonic–clonic seizures and identifies compounds that prevent seizure spread and phenytoin is considered as the prototypical in this model. For all tests based on MES convulsions, 60 Hz of alternating current (50 mA in mice, 150

in rats) is delivered for 0.2 s by corneal electrodes which have been primed with an electrolyte solution containing an anesthetic agent (0.5% tetracaine HCl). For Test 1, mice are tested at various intervals following doses of 30, 100 and 300 mg/kg of test compound given by i.p. injection of a volume of 0.01 mL/g. In Test 2, rats are tested after a dose of 30 mg/kg (p.o.) in a volume of 0.04 mL/10 g. In test 7, mice are tested after a dose of 100 mg/kg in qualitative 6 Hz assay. An animal is considered 'protected' from MES-induced seizures upon abolition of the hindlimb tonic extensor component of the seizure. [24,25].

6.2.1.2. Subcutaneous pentylenetetrazole threshold test (scPTZ). The scPTZ test detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals are pretreated with various doses of the test compound (in the similar manner to the MES test, although a dose of 50 mg/kg (p.o.) is the standard for Test 2 scPTZ). At the previously determined TPE of the test compound, the dose of Pentylenetetrazole which induce convulsions in 97% of animals (CD$_{97}$: 85 mg/kg mice; 70 mg/kg rats) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress [26] and observed for the next 30 min for the presence or absence of the seizure. An episode of clonic spasms, approximately 3–5 s, of the forte and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected.

6.2.1.3. Minimal clonic seizure (6 Hz) test. Some clinically useful AED are ineffective in the standard MES and scPTZ tests but still have anticonvulsant activities in vivo. In order to identify potential AED with this profile, some compounds were tested in the minimal clonic seizure (6 Hz or psychomotor) test. Like the maximal electroshock (MES) test, the minimal clonic seizure (6 Hz) test [27] was used to assess compound's efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3 s). Test compounds were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% of animals (32 mA for 3 s). The untreated mice would display seizures characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors, described originally as being similar to the aura of human patients with partial seizures. Animals not displaying this behavior were considered to be protected.

6.2.2. Acute toxicity-minimal motor impairment

In mice, the rotorod [28] procedure is used to disclose minimal muscular or motor impairment. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered toxic if it falls of this rotating rod three times during a 1-min period. In rats, minimal motor deficit is indicated by ataxia, which is manifested by an abnormal, uncoordinated gait. Rats used for evaluating toxicity are examined before the test drug is administered, since individual animals may have peculiarities in gait, equilibrium, placing response, etc., which might be attributed erroneously to the test substance.

6.3. Log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature (29 °C) using a procedure described elsewhere [29]. Ten milliliter of octanol and 10 mL phosphate buffer (pH = 7.4) were taken in a glass stoppered graduated tube and 5 mg of accurately weighed drug was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature. The mixture was then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy.

Partition coefficient was calculated by:

PC = (Ct − Ca)/Ca. PC = Partition coefficient, Ct = Concentration of total drug, Ca = Concentration of the drug in aqueous phase.

References

[1] R.H. Mattson, in: R. Levy, R.H. Mattson, R. Meldrum (Eds.), Antiepileptic Drugs, Raven Press, New York, 1995, pp. 123–135.
[2] G. Kramer, Epilepsia 42 (Suppl. 3) (2001) 55–59.
[3] O.J. Mc Namara, in: L.L. Brunton, J.S. Lazo, K.L. Parker (Eds.), The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 2006, pp. 501–526.
[4] P. Kwan, M.J. Brodie, N. Engl. J. Med. 342 (2000) 314–319.
[5] B.B. Spear, Epilepsia 42 (2001) 31–34.
[6] T.R. Bal, B. Anand, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. 15 (2005) 4451–4455.
[7] T. Jiang, K.L. Kuhen, K. Wolff, H. Yin, K. Bieza, J. Caldwell, B. Bursulaya, T. Tuntland, K. Zhang, D. Karanewsky, Y. He, Bioorg. Med. Chem. Lett. 16 (2006) 2109–2112.
[8] R. Tripathy, A. Reiboldt, P.A. Messina, M. Iqbal, J. Singh, E.R. Bacon, T.S. Angeles, S.X. Yang, M.S. Albom, C. Robinson, H. Chang, B.A. Ruggeri, J.P. Mallamo, Bioorg. Med. Chem. Lett. 16 (2006) 2158–2162.
[9] A. Cane, M.C. Tournaire, D. Barritault, A.M. Crumeyrolle, Biochem. Biophys. Res. Commun. 276 (2000) 379–384.
[10] V.C. Silveira, J.S. Luz, C.C. Oliveira, I. Graziani, M.R. Ciriolo, A.M.C. Ferreira, J. Inorg. Biochem. 102 (2008) 1090–1103.
[11] A.A. Raj, R. Raghunathan, M.R. Sridevikumaria, N. Raman, Bioorg. Med. Chem. 11 (2003) 407–419.
[12] M.C.R. Arguelles, S.M. Vazaquez, P.T. Touceda, J.S. Matalobos, A.M.G. Deibe, M.B. Ferraris, G. Pelosi, C. Pelizzi, F.J. Zani, J. Inorg. Biochem. 101 (2007) 138–147.
[13] L. Maskell, E.A. Blanche, M.A. Colucci, J.L. Whatmore, C.J. Moody, Bioorg. Med. Chem. Lett. 17 (2007) 1575–1578.
[14] M. Verma, S.N. Pandeya, K.N. Singh, J.P. Stables, Acta Pharm. 54 (2004) 49–56.
[15] N. Igosheva, C. Lorz, E.O. Conner, V. Glover, H. Mehmet, Neurochem. Int. 47 (2005) 216–224.
[16] L.R. Chen, Y.C. Wang, Y.W. Lin, S.Y. Chou, S.F. Chen, L.T. Liu, Y.T. Wu, C.J. Kuo, T.S.S. Chen, S.H. Juang, Bioorg. Med. Chem. Lett. 15 (2005) 3058–3062.
[17] N. Siddiqui, S.N. Pandeya, S.A. Khan, J.P. Stables, A. Rana, M. Alam, M.F. Arshad, M.A. Bhat, Bioorg. Med. Chem. Lett. 17 (2007) 255–259.
[18] N. Siddiqui, W. Ahsan, Eur. J. Med. Chem. 45 (2010) 1536–1543.
[19] N. Siddiqui, A. Rana, S.A. Khan, M.A. Bhat, S.E. Haque, Bioorg. Med. Chem. Lett. 17 (2007) 4178–4182.
[20] N. Siddiqui, A. Rana, S.A. Khan, S.E. Haque, M.S. Alam, M.F. Arshad, W. Ahsan, Acta. Chim. Slov. 56 (2009) 462–469.
[21] S.N. Pandeya, S. Kohli, N. Siddiqui, Pol. J. Pharmacol. 55 (2003) 565–571.
[22] Anticonvulsant Screening Project, Antiepileptic Drug Development Program. National Institute of Health, DHEW Publ (NIH) (US), 1978, pp. 78–1093.
[23] E.J. Lien, R.C.H. Liao, H.G. Shinouda, J. Pharm. Sci. 68 (1979) 463–465.
[24] E.A. Swinyard, J.H. Woodhead, H.S. White, M.R. Franklin, General principles: experimental selection, quantification, and evaluation of anticonvulsants, Antiepileptic Drugs. Raven Press, New York, 1989, pp. 85–102.
[25] H.S. White, M. Johnson, H.H. Wolf, H.J. Kupferberg, Ital. J. Neurol. Sci. 16 (1995) 73–77.
[26] E.A. Swinyard, L.D. Clark, J.T. Miyahara, H.H. Wolf, J. Physiol. 132 (1961) 97–102.
[27] M.E. Barton, B.D. Klein, H.H. Wolf, H.S. White, Epilepsy. Res. 47 (2001) 217–227.
[28] M.S. Dunham, T.A. Miya, J. Am. Pharm. Assoc. Sci. 46 (1975) 208–209.
[29] V.A. Farrar, M. Ciechanowicz-Rutkowska, J. Grochowski, P. Serda, T. Pilati, G. Filippini, C.N. Hinko, A. El-Assadi, J.A. Moore, I.O. Edafiogho, C.W. Andrews, M. Cory, J.M. Nicholson, K.R. Scott, J. Med. Chem. 36 (1993) 3517–3525.