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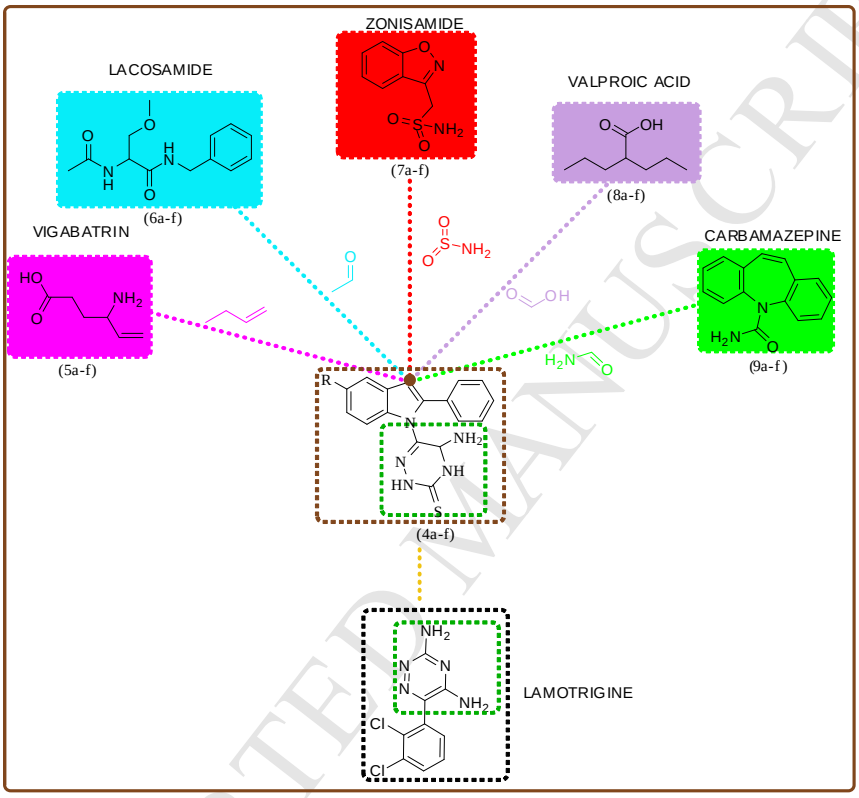

Indole C-3 substituted 5-amino-6-(5-substituted-2-phenyl-1*H*-indol-1-yl)-4,5-dihydro-1,2,4-triazine-3(2*H*)-thione **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** were synthesized considering the pharmacophoric elements and an array of functional groups necessary for anticonvulsant activity.

Anticonvulsant evaluation of clubbed indole-1,2,4-triazine derivatives: A Synthetic approach

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Abstract

A series of thirty indole C-3 substituted 5-amino-6-(5-substituted-2-phenyl-1*H*-indol-1-yl)-4,5-dihydro-1,2,4-triazine-3(2*H*)-thione **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** were synthesized to explore prospective anticonvulsant agents. The derivative 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1*H*-indol-3-yl)ethanone (**6b**) had significant activity in maximal electroshock test with minimal duration of limb extension (5.40 ± 0.61 sec) and quantitative median dose of 7 mg/kg. In subcutaneous pentylenetetrazole screen 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1*H*-indole-3-sulfonamide (**7b**) increased the seizure latency to onset of clonus and was effective at a median dose of 35 mg/kg. An *in vitro* radioligand binding assay on sodium channel and γ-amino butyric acid estimation was also performed on active compounds to perceive the mechanistic procedure responsible for it action.

Keywords: Triazine, Anticonvulsant, Quantification, Sodium channel, GABA

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1. Introduction

An idiopathic disorder of brain characterized by enduring predisposition resulting in sudden onset of seizures and episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness is defined as epilepsy [1,2]. In the International League against Epilepsy and International Bureau of Epilepsy it is depicted by the neurobiologic, cognitive, psychological, and social consequences of the condition [3]. Despite the vast therapeutic arsenal of several generations of antiepileptic drugs (AED's), about 30% of adults are not free from seizures. The condition gets worsened with infants having a series of encephalopathic syndromes [4-7]. Many AED's enlarge serious side effects that are increased when a lifelong medication is required [8]. Conversely the pharmacoresistant epilepsy [9], variable responses after epileptogenic brain insults [10], comorbid medical conditions [11, 12], refractory epileptic population [13], limits the attempts for treatment of antiepileptic patients. Moreover a major obstacle in the antiepileptic drug development is the insufficient knowledge of the pathophysiology of epilepsy and modes of action of existing drugs [14, 15]. It encourages the development of new antiepileptogenic [16] drugs for its use earlier in the treatment chain. The several approaches towards this global achievement in future has been the structural 'improvements' of already marketed drugs (anti-ictal) or on discovering AED's with novel mechanism of action (anti-epileptogenic) [17].

The indole ring has been found to be an integral nucleus in the past having diverse biological activities such as anticancer, antiepileptic, anthelminthic, antidepressant, anti-inflammatory, antihypertensive, carbonic anhydrase inhibitors etc [18-24]. Lamotrigine {3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine} is an antiepileptic drug associated with hypersensitivity reactions which are thought to be an immunological response to metabolically generated drug-protein adducts [25,26]. The *o*-dichlorophenyl moiety a metabolism-dependent hepatotoxicant, is a potential site for bioactivation in rat and human liver that is oxidized to electrophilic arene oxide that can be trapped by Glutathione. This epoxide is the primary cause for the hypersensitivity reactions of lamotrigine. The diaminotriazine substituent is the dominant site of biotransformation in most species (humans eliminate lamotrigine principally as *N*-glucuronides in urine) [27, 28]. The 5-amino group is however the obligatory parameter, hence the 1,2,4-triazine ring carrying the 5-amino group has been appended in our target compounds [29]. The different substituents at the C-3 position whereas phenyl ring at C-2 of indole ring were considered to have sufficient diverse electronic, steric and hydrophobic characters [30]. The C-3 vinyl, acetyl, sulphonamide, carboxylic and carboxamide substituents manifest the necessary structural features of the prevalent AED's [31-33]. They have also been ascertained as an essential substituent in many previously synthesized anticonvulsants [34-36]. These elements contribute as essential parameters to the pharmacophoric elements beside the lipophilic aryl ring, hydrogen bonding domain of the triazine and distal aryl ring responsible for antiepileptic activity (Figure 1) [37,38]. The structural considerations of the indole ring and the 5-amino-1,2,4-triazine ring of pharmacologically active AED lamotrigine gave impetus to synthesize their hybrid molecules. These molecules were evaluated for anticonvulsant activity using maximal

electroshock seizure (MES) test, subcutaneous pentylenetetrazole (scPTZ) test and minimal motor impairment using rotarod test. In accord, the study of potency of these new derivatives on two vital mechanisms such as γ-amino butyric acid (GABA) and sodium channel was also accomplished. The mechanistic approach together with the data derived from the animal models of seizures and epilepsy best describes the clinical efficacy profile of the drug.

2. Result and Discussion

2.1. Chemistry

The devised synthetic route for the preparation of the final compounds **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** is delineated as follows. With the aim of developing cleaner and more benign process, microwave assisted intramolecular electrophilic cyclization of substituted anilines and phenacyl bromide was carried out via solvent-free Bischler indole synthesis. This greener synthesis of compounds **1a-f** prohibited the use of protecting groups, metallic reagents and catalysts used in other synthetic methods of indoles. Further the drop-wise addition of chloroacetyl chloride in presence of anhydrous potassium carbonate liberated the hydrochloric acid to generate the compounds **2a-f**. The use of thiosemicarbazide cyclized the N-chloroacetylated indoles to the corresponding 3-thio-1,2,4-triazine derivatives **3a-f**. These derivatives were then added in small portions to a solution of potassium permanganate in dry liquid ammonia below 0 °C and purified to render the lead compound of our series **4a-f** (Scheme 1). The latter step was performed to inoculate the 5-amino group onto the triazine ring. This 5-substituted-2-phenyl-*N*-1,2,4-triazine indole derivatives **4a-f** were further isosterized at the indole 3-position to generate the target compounds. The presence of phenyl ring at the C-2 and the triazine ring at the N-1 position of indole sterically hinder this position, leading to the attack by these isosteric substituents at the C-3 position of π-excessive heteroarene indole ring [39]. The reaction with allyl bromide, acetic anhydride, sulfuryl chloride in ammonia, trichloroacetic acid anhydride followed by sodium hydroxide and further treatment of the corresponding carboxylic derivatives with thionyl chloride in aqueous ammonia yielded the target compounds **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** under their respective reaction conditions (Scheme 2). The compounds **5a-f** was synthesized using zinc-mediated barbier reaction while the compounds **6a-f** utilized the Chichibabin synthetic procedure for their synthesis. The structures of the compounds were confirmed by ^{1}HNMR, ^{13}CNMR, IR and mass spectral data.

2.2. Pharmacology

The initial evaluations for anticonvulsant activity were performed based on the Anticonvulsant drug development program, Epilepsy Branch Neurological Disorders Program, NINDS [40]. The maximal electroshock (MES) test was used as the experimental model to identify clinical candidates that prevent the spread of tonic-clonic seizures and of partial convulsions with or without secondary generalizations [41]. The subcutaneous pentylenetetrazole (scPTZ) test

provided the clinically effective compounds against absence seizures besides elevating seizure threshold [42]. The destitute from toxicity for the active compounds was established using the minimal motor impairment-rotarod test [43]. The compounds were administered intraperitoneally to mice at a dose of 30, 100 and 300 mg/kg at two pretreatment times (0.5 h and 4 h) prior to the test. The reference drugs phenytoin (for MES and rotarod test) and ethosuximide (for scPTZ test) were used as positive controls [44]. The negative control control group received 0.9 % saline (10 mL/kg).

In the preliminary screening all the newly synthesized compounds exhibited some degree of anti-MES activity. The protection offered by these compounds was indicative of their pharmacological ability against seizure spread at a certain dose level. The results obtained after investigating the anticonvulsant activity of the synthesized compounds **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** are summarized in table 1. An instill into these outcomes showed that compounds **6b**, **6d**, **7a**, **7b**, **8d** and **9c** were more effective at a dose of 30 mg/kg at both reported time intervals. It depicts the quick onset and prolonged anticonvulsant potential of these derivatives at minimum dose comparable to the reference drug phenytoin. It is explicit of the remarkable activity displayed by them at this preliminary level. A similar kind of rapid onset at low dose and long lasting effects (4 h) but at a higher dose of 100 mg/kg were shown by the compounds **5d**, **6a**, **7d**, **8c**, **8f** and **9b**. In the allyl substituted indole-3-derivatives **5a-f**, the electron withdrawing substituents trifluoromethyl (CF_3) and chloro were more effective than the electron donating substituents thiomethyl (SCH_3) and methoxy (OCH_3). The analysis of the results for electron withdrawing substituents is suggestive of the greater size of the CF_3 and Cl than the corresponding NO_2 and F, responsible for their anticonvulsant activity. An almost reverse of the activity of the compounds **5a-f** was depicted by the carboxamide derivatives **9a-f** with nitro derivative (**9c**) being the most effective at the minimum dose of 30 mg/kg at both the time intervals. This factual observation can be somewhat attributed to the negative inductive effect as well as resonance stabilization shown by the nitro group that helps it to effectively bind to the receptor site. Moreover compounds **6a-f** containing acetyl substitution at the indole C-3 showed that greater electronegative fluorine substituents (**6b**, **6d**) were more effective than the corresponding chloro derivatives. The cessation against electroshock protection was observed in nitro derivative (**6c**) at delayed absorption interval (4 h). On the other hand, among the sulphonamide substituted indole derivatives **7a-f**, halogens (Cl and F) and its hydrocarbon derivative (CF_3) indicated good protection than the corresponding electron donating substituents (OCH_3 and SCH_3) derivatives against electrically induced seizures. In these derivatives also the nitro derivative (**7c**) did not showed activity at 4 h. This can accord to the quick metabolism of the nitro derivative in presence of the acetyl and sulphonamide groups. Furthermore in the indole derivatives terminating with carboxylic acid **8a-f** at position three, the absorption by halogens (Cl and F) was at a higher dose following cessation of activity at longer duration. Amongst all the methoxy derivatives, the compounds **6e**, **7e**, **8e** and **9e** demonstrated anticonvulsant potential at a dose of 100 mg/kg at both the reported intervals irrespective of the substituent at the third

position of the indole ring. The methoxy substitution on the indole-3-allyl derivative (**5e**), however, was active at higher dose of 300 mg/kg followed by cessation of activity at 4 h duration. The CF_3 derivatives at indole 5-position showed high efficacy in conjunct with the strong electron withdrawing substituents ($COCH_3$ and COOH) followed by lesser electron withdrawing substituents (allyl and SO_2NH_2) at the indole 3-position. Replacement of electron withdrawing substituents by electron donating substituents ($CONH_2$) at the indole 3-position, leads to decline in the activity of CF_3 substituted derivatives.

The general anti-MES protective capability of the synthesized compounds can be demonstrated as:

5a-f: CF_3> Cl> SCH_3> NO_2> F ≡ OCH_3
6a-f: F ≡ CF_3> Cl> OCH_3> NO_2> SCH_3
7a-f: Cl ≡ F> CF_3> OCH_3> SCH_3> NO_2
8a-f: CF_3> NO_2 ≡ SCH_3> OCH_3> Cl ≡ F
9a-f: NO_2> F> OCH_3 ≡ SCH_3> Cl> CF_3

In the scPTZ screen, most of the compounds showed varying degree of protection and some derivatives were devoid of any activity. The strongest anticonvulsant potential was exhibited by the compounds **6b**, **7b** and **8c**. Furthermore, the compounds **5c**, **6c**, **7c**, **7f**, **8b** and **9b** had an equipotent effect to that of the reference drug ethosuximide. In the allyl substituted indole derivative **5a-f**, the tendency to protect chemically induced seizures was same as observed for the carboxamide derivatives **9a-f** in the MES test. On continuation of analyzing the outcomes of the acetyl derivatives **6a-f**, interestingly we found their activity trend similar to its MES protection except the interchange of the nitro with the trifluoromethyl derivative, former being more active than the latter. The CF_3 and SCH_3 substituted compounds showed equipotent activity in both the acetyl (**6d**, **6f**) and carboxylic acid (**8d**, **8f**) derivative. A drift in the activity of the thiomethyl substituted indole-3-sulphonamide derivative (**7f**) was seen compared to the acetyl derivatives making it equally efficacious to the corresponding nitro derivative (**7c**). Furthermore a likewise sequence of scPTZ protection was observed for indole-3-carboxylic acid derivative except the nitro substituent. The nitro derivative (**8c**) was found to be more effective than the electron withdrawing fluoro derivative (**8b**). This can be attributed to the additive resonance stabilization effect exhibited by this group compared to only –I effect of fluorine. In the indole-3-carboxamide terminal derivatives, the maximum efficacy was observed for the electron withdrawing substituents followed by the releasing groups. However, the chloro derivative (**9a**) lacked anticonvulsant activity against pentylenetetrazole induced seizures which can be envisaged to its lower electron affinity compared to other electron withdrawing groups.

We noticed that all the methoxy substituted derivatives **5e**, **6e**, **7e** and **8e** depicted the similar anticonvulsant potential at a dose of 300 mg/kg at both the intervals irrespective of the substitution at the indole 3-position. It corresponds to the similar behavior shown by the methoxy derivatives against electrically induced seizures in the MES test. The fluoro (**8b** and **9b**) and the thiomethyl derivatives (**8f** and **9f**) demonstrated alike activities in conjunct with carboxylic acid

and its amide at the third position of indole ring. Likewise the chloro derivative **6a**, **7a** and **8a** had same anti-scPTZ activity but at the maximum dose. On the other hand the nitro derivatives gave protection against the PTZ induced seizures at the same dose in the allyl (**5c**), acetyl (**6c**) and sulphonamide derivatives (**7c**) too. The chloro substitution was found to be ineffective whence in conjuction with the allyl (**5a**) and carboxamide (**9a**) moieties at indole 3-position. In all the synthesized compounds the greater electronegative fluoro derivatives were more effective than the corresponding chloro compounds as well as the sterically hindered trifluoromethyl substituted compounds.

The general anti-scPTZ protective capability of the synthesized compounds can be demonstrated as:

5a-f: $NO_2 > F \equiv OCH_3 > SCH_3 > Cl \equiv CF_3$

6a-f: $F > NO_2 > Cl \equiv OCH_3 > CF_3 \equiv SCH_3$

7a-f: $F > NO_2 \equiv SCH_3 > Cl \equiv OCH_3 > CF_3$

8a-f: $NO_2 > F > Cl \equiv OCH_3 > CF_3 \equiv SCH_3$

9a-f: $F > NO_2 \equiv CF_3 > SCH_3 > OCH_3 > Cl$

All the active compounds were found to be non-neurotoxic at the maximum dose at bith the reported time intervals.

The potent compounds in the preliminary anticonvulsant screen were further assessed against seizure challenges upon oral administration. The duration of limb extension formed the basis of MES screen [45] whereas seizure latency to onset of clonus [46] was defined as the parameter for the anti-scPTZ efficacy. The test compounds were administered at a dose of 100 mg/kg, 60 min before the injection of PTZ (85 mg/kg, sc) and MES test (150 mA, 0.2 s). The latency time was observed for 14 min in scPTZ test. Phenytoin (30 mg/kg, oral) and sodium valproate (200 mg/kg, p.o) [45,47] were used as reference standard for MES and scPTZ anticonvulsant evaluation. The test and the reference compounds were prepared in 0.5% sodium carboxymethylcellulose. The results were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's method and expressed as means ± SEM. The absence of hind limb tonic extension during electroshock induced seizures and non-mortality after PTZ administration was considered as protection. The record of the MES test suggested that compound **6b** was the most effective of the series. It significantly decreased the duration of limb extension compared to control ($p < 0.01$) besides manifesting 100% protection in the experimental group (Table 2). **7a** and **7b** caused a decrease in the duration of tonic extension but the % protection reduced to 66% and 83.3% respectively. The remaining examined derivatives offered moderate protection. In the scPTZ test the compounds **6b** and **7b** delayed the onset of myoclonic jerks of forelimbs with **7b** exceeding the latency period of the reference sodium valproate. The anti-scPTZ protection shown by them was however less than that of the standard. Conversely the compound **8c** had a non-significant impact on the latency interval in this experimental evaluation. All the efficacious compounds in this oral screen were non-neurotoxic

at the dose of administration. Hence the compound **6b** in MES test and **7b** in scPTZ test were crowned as the most effective of all the tested derivatives after oral administration.

The significantly active compounds in the primary MES and scPTZ test on i.p and oral administration crusaded in the need for quantitative studies [48]. Table 3 reports the median effective doses (ED_{50}) for both the screens and median doses for neurological impairment (TD_{50}) in mice after intraperitoneal administration. The pharmacological data of MES test revealed compound **6b** as the most potent with an ED_{50} of 7 mg/kg at the time of peak effect. However the TD_{50} of **6b** producing deficits in motor coordination in 50% of animals was found to be 290 mg/kg, much greater than the standard drug phenytoin (TD_{50} = 66 mg/kg) [49]. The compound **7b** though having equal ED_{50} to phenytoin, outplayed the median toxic dose to 225 mg/kg thereby increasing its protective index. The compound **7a** provided moderate efficacy. In the scPTZ test, **7b** was quantified as the most potent compound with nearly one-fourth the effective dose of the standard drug ethosuximide. Moreover the median toxic dose of **7b** (TD_{50} = 225 mg/kg) though less than standard (TD_{50} = 440.8 mg/kg) yet the overall protective index (TD_{50}/ED_{50}) of **7b** (PI = 6.43) exceeded that of the standard (PI = 3.39). **6b** gave nearly equal protection to the standard.

To infer the mechanistic approach of the synthesized derivatives, the active compounds were neurochemically investigated for their effect on GABA levels in whole rat brain. It was performed on a group of six rats each taking clobazam (30 mg/kg, p.o) as reference [50]. The test compounds were administered intraperitoneally in the first study to a set of animals at a dose of 100 mg/kg. The brains were removed after 2 h of drug administration and the levels of GABA were estimated in the whole brain. The values were expressed as means ± SEM. In the second set of animals the study was conducted for seven days. The test drugs were given orally for this period and the GABA levels were estimated on removal of brain after 4 h of seventh day. The findings are mentioned in the table 4. All the test compounds significantly increased the GABA levels in comparison to the control at both the reported time intervals. The values were compared using student's *t* test. It signifies their anticonvulsant potential as increase in GABA is inferred as a cardinal factor in the anticonvulsant drug discovery.

An insight into the physiological and biochemical events in clinical condition shadows the underlying molecular mechanisms in epilepsy. Many AED's like phenytoin, carbazepine and lamotrigine act via prolonging inactivation of voltage dependent sodium channels. Thus sodium channel is a potential site for pharmacological action of anticonvulsants. For a drug hunter, the valuable leads obtained via *in vivo* testing of compounds in MES test provided the basis for deducing *in vitro* binding affinity for neuronal sodium channels of these compounds. The displacement of [^{3}H]BTX from neurotoxin site 2 of voltage-gated sodium channels in rat forebrain tissue provides an indication of the selective pharmacological modulation by these compounds [51]. The outcomes of the three compounds **6b**, **7a** and **7b** are mentioned as IC_{50} (μM) and the % inhibition at a concentration of 100 μM in table 5. Amongst the three screened compounds, the compound **6b** blocked the [3H]BTX-B binding on statistically significant

fraction of sodium channel receptor site by 50.40% at 100 μM. It was found to behave similarly to the standard drug phenytoin with 49.61% inhibition, used as a positive control. The IC_{50} values of **6b**, **7a** and **7b** are 108 ± 5 μM, 300 ± 2 μM and 220 ± 6 μM compared to 126 ± 7 μM for phenytoin (Figure 2). Hence the radioligand binding result of **6b** boosts the confidence in rational design of the targeted compound as neuronal sodium channel blocker.

3. Conclusion

A series of thirty 5-amino-6-(3,5-disubstituted-2-phenyl-1*H*-indol-1-yl)-4,5-dihydro-1,2,4-triazine-3(2*H*)-thione **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** were synthesized by us envisaged as potential anticonvulsants. The rationale employed behind these derivatives was the addition of the structural elements of the established drugs to the indole-*N*-1,2,4-triazine nucleus such as to improve the anticonvulsant potential. To derive a mechanistic procedure responsible for its action the study on sodium and GABA channel was also carried out based on its structural resemblance to the reference AED's. After the preliminary studies in both the MES and scPTZ models on intraperitoneal and oral administration, the active compounds were challenged against the quantitative evaluation and GABA estimation in rat whole brain. *In vitro* screening for sodium channel activity by [^{3}H]BTX radioligand assay was also performed for the compounds having activity in MES test. The compound **6b** was the most active of the series possessing a minimal ED_{50} and a very high TD_{50} values, reducing acute mortality and duration of limb extension, while increasing the GABA level as well as the % inhibition of sodium channel. The compound **7b** had significant activity in the scPTZ screen decreasing the frequency of seizures besides increasing the latency to spontaneous recurrent seizures. The outcomes of this viable schematic strategy experiments stimulated the molecular modeling and quantitative structure activity relationship tools to be applied on these compounds. They are underway and will be reported in due course.

4. Experimental

4.1. Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Thin layer Chromatography (TLC) was performed with Silica gel 60 F_{254} TLC aluminium sheet (Merck, KGaA, Darmstadt, Germany) using Toluene: Ethyl acetate: Formic acid (5:4:1) as eluents. Ashless Whatmann No. 1 filter paper was used for vacuum filtration. Melting points were determined by using open capillary tubes in a Hicon melting point apparatus and are uncorrected. The purity of the compounds was determined through elemental analysis. Elemental data of C, H and N was found in accordance with ± 0.4% of the theoretical value respectively as determined by Vario EL III CHNS Elementar. The Infrared Spectra of compound was recorded on Bio-rad FTS-135 spectrophotometer using KBr pellets. The abbreviated forms mentioned in the IR interpretation are as follows Ar, aryl; br, broad; str, stretching. ^{1}HNMR and ^{13}CNMR

spectra in DMSO-d_6/$CDCl_3$ solutions were respectively recorded at 400 and 100 MHz with Bruker 400 Ultra shield TM NMR spectrometer (400 MHz) using TMS [$(CH_3)_4Si$] as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; dd, double doublet. Chemical shifts (δ) values are given in parts per million (ppm). *J* values are given in Hz. The Mass Spectra were recorded on a Waters Micro-mass ZQ 2000 Spectrophotometer.

*4.1.1. General procedure for the preparation of compound (**1a-f**)*

Phenacyl bromide (1 mmol) was stirred with para-substituted aniline (2 mmol) at room temperature without any base to neutralize the liberated hydrogen bromide. The mixture was kept at room temperature with occasional stirring for 3 h. To the solid mixture, containing *N*-phenacyl aniline and anilinium hydrobromide, was added 3-4 drops of dimethyl formamide and the mixture was irradiated in a microwave oven at 600 W for 1 min for the cyclization. After completion of the reaction, the mixture was treated as below to give the pure 2-arylindoles. After completion of the reaction the mixture was loaded onto a silica gel column and pure 2-arylindoles were obtained by chromatography, eluting with a gradient starting from 9:1 petroleum ether-ethyl acetate. Alternatively, the mixture could also be extracted with ethyl acetate, washed with water, dried and evaporated before chromatography.

*4.1.2. General procedure for the preparation of compound (**2a-f**)*

The indole derivatives **1a-f** (0.05 mol) and anhydrous potassium carbonate (0.05 mol) were dissolved in 100 mL absolute toluene. To this refluxing solution, there was slowly added drop-wise chloroacetylchloride (6.4 mL, 0.08 mol) dissolved in toluene (20 mL), after which the reaction mixture was refluxed for another hour. The residue was re-crystallized from ethanol after distillation of the solvent and the excess of chloroacetylchloride.

*4.1.3. General procedure for the preparation of compound (**3a-f**)*

A mixture of the chloroacetylated derivatives **2a-f** (0.01 mol) and thiosemicarbazide (0.01 mol) in 10% ethanolic sodium hydroxide solution (20 mL) was refluxed for 8 h. The reaction mixture was cooled and poured onto ice/cold water acidified with few drops of hydrochloric acid. The formed precipitate was filtered off, dried and re-cystallized from chloroform.

*4.1.4. General procedure for the preparation of compound (**4a-f**)*

3-thio-1,2,4-triazine derivatives **3a-f** (10 mmol) was added in small portions to a solution of potassium permanganate (2.0 g) in dry liquid ammonia (100 mL) below 0 °C and stirred. The mixture was kept at this condition for 15 minutes. Ammonia was then evaporated and the residue was extracted with hot isopropanol (50 mL). Removal of the solvent and re-crystallization from ethanol gives the pure product.

*4.1.5. General procedure for the preparation of compound (**5a-f**)*

A mixture of indoles **4a-f** (5 mmol), allyl bromide (10 mmol), zinc powder (10 mmol) in Tetrahydrofuran (10 mL) was stirred at room temperature for 5-6 h. After completion of the reaction as indicated by TLC, the reaction mixture was quenched with saturated ammonium chloride (15 mL) and extracted with ethyl acetate (2×15 mL). Evaporation of the solvent followed by purification on silica gel using ethyl acetate-hexane (9:5) as solvent afforded pure allyl indole derivatives **5a-f**.

*4.1.5.1. 6-(3-allyl-5-chloro-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5a**)*

Yield: 85%; m.p. 185-188 ºC; IR (KBr) υ (cm^{-1}): 3095 (C-H Ar str), 2940 (C-H str), 1615 (N-C=N str), 1596, 1475 (C=C str), 1550 (Het. Triazine str), 1518 (C=C Ar str), 1040 (C-Cl str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.12 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.6 Hz), 3.10 (d, 2H, CH_2, *J* = 6.7 Hz), 3.97 (s, 1H, 5”CH), 4.23 (brs, 2H, 5”NH_2, D_2O exchangeable), 5.05 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.6 Hz), 5.34 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.8 Hz, *J* = 1.6 Hz, *J* = 6.7 Hz), 7.09 (dd, 1H, 6CH, *J* = 2.5 Hz, *J* = 8.5 Hz), 7.22-7.39 (m, 3H, 3’,4’,5’CH, *J* = 4.8 Hz, *J* = 8.5 Hz), 7.71 (d, 2H, 2’,6’CH, *J* = 7.28 Hz), 7.76 (d, 1H, 7CH, *J* = 8.5 Hz), 8.21 (s, 1H, 4CH), 9.03 (brs, 1H, 4”NH, D_2O exchangeable), 12.21 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 35.2 (CH_2), 66.4 (5”CH), 109.1 (C-3), 110.4 (C-7), 114.7 (CH=$\underline{CH_2}$), 119.3 (C-4), 121.0 (C-6), 124.2 (2’,6’CH), 126.1 (C-5), 127.2 (4’CH), 127.7 (CH-$\underline{C}$ (q) fused carbon), 128.5 (3’,5’CH), 130.8 (C-2), 133.4 (1’CH), 134.5 ($\underline{C}$-N (q) fused carbon), 135.1 ($\underline{CH}$=CH_2), 160.9 (6”CH), 185.2 (C=S); MS (70 ev): m/z = 396.35 (M+1); Anal. Calcd. for $C_{20}H_{18}ClN_5S$ (395.91): C, 60.67; H, 4.58; N, 17.69, Found, C, 60.74; H, 4.52; N, 17.60.

*4.1.5.2. 6-(3-allyl-5-fluoro-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5b**)*

Yield: 88%; m.p. 178-183 ºC; IR (KBr) υ (cm^{-1}): 3090 (C-H Ar str), 2945 (C-H str), 1623 (N-C=N str), 1598, 1471 (C=C str), 1551 (Het. Triazine str), 1525 (C=C Ar str), 1154 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.14 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.3 Hz), 3.13 (d, 2H, CH_2, *J* = 6.5 Hz), 3.99 (s, 1H, 5”CH), 4.22 (brs, 2H, 5”NH_2, D_2O exchangeable), 5.08 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.6 Hz), 5.32 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.9 Hz, *J* = 1.6 Hz, *J* = 6.5 Hz), 7.12 (dd, 1H, 6CH, *J* = 2.1 Hz, *J* = 8.4 Hz), 7.23-7.37 (m, 3H, 3’,4’,5’CH, *J* = 4.8 Hz, *J* = 8.7 Hz), 7.68 (d, 2H, 2’,6’CH, *J* = 7.28 Hz), 7.84 (d, 1H, 7CH, *J* = 8.0 Hz), 8.40 (s, 1H, 4CH), 9.05 (brs, 1H, 4”NH, D_2O exchangeable), 12.25 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 35.5 (CH_2), 66.1 (5”CH), 109.5 (C-3), 110.7 (C-7), 111.3 (C-4), 114.4 (CH=$\underline{CH_2}$), 117.2 (C-6), 124.6 (2’,6’CH), 127.0 (4’CH), 127.8 (CH-$\underline{C}$ (q) fused carbon), 128.1 (3’,5’CH), 130.3 (C-2), 133.2 (1’CH), 134.7 ($\underline{C}$-N (q) fused carbon), 135.6 ($\underline{CH}$=CH_2), 160.6 (6”CH), 161.5 (C-5), 184.8 (C=S); MS (70 ev): m/z = 380.10 (M+1); Anal. Calcd. for $C_{20}H_{18}FN_5S$ (379.45): C, 63.31; H, 4.78; N, 18.46, Found, C, 63.39; H, 4.72; N, 18.39.

*4.1.5.3. 6-(3-allyl-5-nitro-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5c**)*

Yield: 74%; m.p. 198-202 ºC; IR (KBr) υ (cm^{-1}): 3096 (C-H Ar str), 2948 (C-H str), 1630 (N-C=N str), 1600, 1470 (C=C str), 1563 (Het. Triazine str), 1531 (C=C Ar str), 1325 (NO_2 str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.17 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.6 Hz), 3.11 (d, 2H, CH_2, *J* = 6.5 Hz), 3.97 (s, 1H, 5"CH), 4.19 (brs, 2H, 5"NH_2, D_2O exchangeable), 5.06 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.8 Hz), 5.35 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.8 Hz, *J* = 1.8 Hz, *J* = 6.6 Hz), 7.15 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.2 Hz), 7.27-7.38 (m, 3H, 3',4',5'CH, *J* = 4.9 Hz, *J* = 8.9 Hz), 7.70 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.95 (d, 1H, 7CH, *J* = 8.4 Hz), 8.71 (s, 1H, 4CH), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 35.1 (CH_2), 66.3 (5"CH), 108.5 (C-7), 109.2 (C-3), 114.7 (CH=$\underline{CH_2}$), 116.7 (C-6), 124.5 (2',6'CH), 127.1 (4'CH), 127.3 (C-4), 127.8 (CH-$\underline{C}$ (q) fused carbon), 128.4 (3',5'CH), 130.6 (C-2), 132.4 (C-5), 133.8 (1'CH), 134.9 ($\underline{CH}$=CH_2), 140.5 ($\underline{C}$-N (q) fused carbon), 161.0 (6"CH), 185.2 (C=S); MS (70 ev): m/z = 407.00 (M+1); Anal. Calcd. for $C_{20}H_{18}N_6O_2S$ (406.46): C, 59.10; H, 4.46; N, 20.68, Found, C, 59.15; H, 4.52; N, 20.62.

*4.1.5.4. 6-(3-allyl-2-phenyl-5-(trifluoromethyl)-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5d**)*

Yield: 79%; m.p. 210-215 ºC; IR (KBr) υ (cm^{-1}): 3099 (C-H Ar str), 2941 (C-H str), 1620 (N-C=N str), 1602, 1475 (C=C str), 1546 (Het. Triazine str), 1535 (C=C Ar str), 1164 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.11 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.4 Hz), 3.08 (d, 2H, CH_2, *J* = 6.8 Hz), 3.95 (s, 1H, 5"CH), 4.25 (brs, 2H, 5"NH_2, D_2O exchangeable), 5.06 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.7 Hz), 5.30 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.6 Hz, *J* = 1.7 Hz, *J* = 6.7 Hz), 7.22-7.35 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.6 Hz), 7.54 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.0 Hz), 7.68 (d, 2H, 2',6'CH, *J* = 7.26 Hz), 7.87 (d, 1H, 7CH, *J* = 8.1 Hz), 8.86 (s, 1H, 4CH), 9.04 (brs, 1H, 4"NH, D_2O exchangeable), 12.20 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 35.3 (CH_2), 66.4 (5"CH), 109.4 (C-3), 111.5 (C-7), 114.8 (CH=$\underline{CH_2}$), 115.1 (C-6), 117.4 (C-4), 124.0 (2',6'CH), 124.4 (CF_3), 125.5 (C-5), 127.7 (4'CH), 128.0 (CH-$\underline{C}$ (q) fused carbon), 128.4 (3',5'CH), 130.5 (C-2), 133.1 (1'CH), 134.6 ($\underline{C}$-N (q) fused carbon), 135.3 ($\underline{CH}$=CH_2), 161.0 (6"CH), 185.5 (C=S); MS (70 ev): m/z = 430.09 (M+1); Anal. Calcd. for $C_{21}H_{18}F_3N_5S$ (429.46): C, 58.73; H, 4.22; N, 16.31, Found, C, 58.78; H, 4.17; N, 16.35.

*4.1.5.5. 6-(3-allyl-5-methoxy-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5e**)*

Yield: 76%; m.p. 203-208 ºC; IR (KBr) υ (cm^{-1}): 3092 (C-H Ar str), 2955 (C-H str), 1618 (N-C=N str), 1592, 1475 (C=C str), 1560 (Het. Triazine str), 1533 (C=C Ar str), 1252 (C-O-C str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.10 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.5 Hz), 3.12 (d, 2H, CH_2, *J* = 6.6 Hz), 3.68 (s, 3H, O$\underline{CH_3}$), 3.99 (s, 1H, 5"CH), 4.21 (brs, 2H, 5"NH_2, D_2O exchangeable), 5.07 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.5 Hz), 5.34 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.7 Hz, *J* = 1.8 Hz, *J* = 6.5 Hz), 7.07 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.3 Hz), 7.26-7.37 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.4 Hz), 7.69 (s,

1H, 4CH), 7.75 (d, 2H, 2',6'CH, *J* = 7.27 Hz), 7.82 (d, 1H, 7CH, *J* = 8.4 Hz), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.26 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 14.6 (SCH_3), 35.5 (CH_2), 55.6 (OCH_3), 66.2 (5"CH), 101.4 (C-4), 109.8 (C-3), 110.3 (C-7), 112.2 (C-6), 114.8 (CH=$\underline{CH_2}$), 124.1 (2',6'CH), 127.3 (4'CH), 127.8 (CH-$\underline{C}$ (q) fused carbon), 128.5 (3',5'CH), 130.3 (C-2), 133.6 (1'CH), 134.2 ($\underline{C}$-N (q) fused carbon), 135.5 ($\underline{CH}$=CH_2), 149.0 (C-5), 160.8 (6"CH), 185.0 (C=S); MS (70 ev): m/z = 392.12 (M+1); Anal. Calcd. for $C_{21}H_{21}N_5OS$ (391.49): C, 64.43; H, 5.41; N, 17.89, Found, C, 64.50; H, 5.38; N, 17.93.

*4.1.5.6. 6-(3-allyl-5-(methylthio)-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5f**)*

Yield: 78%; m.p. 207-211 ºC; IR (KBr) υ (cm^{-1}): 3092 (C-H Ar str), 2950 (C-H str), 1624 (N-C=N str), 1605, 1470 (C=C str), 1550 (Het. Triazine str), 1527 (C=C Ar str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.10 (m, 1H, $\underline{CH}$=CH_2, *J* = 1.7 Hz), 2.35 (s, 3H, $S\underline{CH_3}$), 3.14 (d, 2H, CH_2, *J* = 6.7 Hz), 4.00 (s, 1H, 5"CH), 4.17 (brs, 2H, 5"NH_2, D_2O exchangeable), 5.08 (d, 1H, H_A=$\underline{CH_2}$, *J* = 1.7 Hz), 5.31 (dd, 1H, H_B=$\underline{CH_2}$, *J* = 0.8 Hz, *J* = 1.8 Hz, *J* = 6.7 Hz), 7.10 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.0 Hz), 7.28-7.41 (m, 3H, 3',4',5'CH, *J* = 4.6 Hz, *J* = 8.6 Hz), 7.66 (s, 1H, 4CH), 7.72 (d, 2H, 2',6'CH, *J* = 7.29 Hz), 7.79 (d, 1H, 7CH, *J* = 8.0 Hz), 9.04 (brs, 1H, 4"NH, D_2O exchangeable), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 35.2 (CH_2), 66.6 (5"CH), 109.3 (C-3), 111.4 (C-7), 112.0 (C-6), 114.6 (CH=$\underline{CH_2}$), 119.5 (C-4), 124.4 (2',6'CH), 127.2 (4'CH), 127.7 (CH-$\underline{C}$ (q) fused carbon), 128.4 (3',5'CH), 130.6 (C-2), 132.1 (C-5), 133.3 (1'CH), 134.4 ($\underline{C}$-N (q) fused carbon), 135.0 ($\underline{CH}$=CH_2), 161.2 (6"CH), 185.5 (C=S); MS (70 ev): m/z = 408.10 (M+1); MS (70 ev): m/z = 408.23 (M+1); Anal. Calcd. for $C_{21}H_{21}N_5S_2$ (407.55): C, 61.89; H, 5.19; N, 17.18, Found, C, 61.83; H, 5.22; N, 17.23.

*4.1.6. General procedure for the preparation of compound (**6a-f**)*

To a stirring solution of indoles **4a-f** (10 mmol) in dichloromethane (20 mL) in inert condition at 0 °C was added $SnCl_4$ (12 mmol) in a single portion via syringe. After the ice bath was removed, the mixture was stirred at room temperature for 30 min, and then acetic anhydride (10 mmol) was added in small portions to the suspension, followed by nitromethane (15 mL). The mixture was stirred for 2 h at room temperature. After being quenched with ice and water (30 mL), the mixture was filtered to remove inorganic precipitates, and the organic material was extracted with ethyl acetate (50 mL). The organic phase was dried over anhydrous sodium sulphate and concentrated at reduced pressure to give the product as a crystalline solid.

*4.1.6.1. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-chloro-2-phenyl-1H-indol-3-yl)ethanone (**6a**)*

Yield: 71%; m.p. 205-208 ºC; IR (KBr) υ (cm^{-1}): 3091 (C-H Ar str), 2948 (C-H str), 1682 (C=O str), 1627 (N-C=N str), 1547 (Het. Triazine str), 1529 (C=C Ar str), 1054 (C-Cl str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.65 (s, 3H, $COCH_3$), 3.95 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O

exchangeable), 7.07 (dd, 1H, 6CH, *J* = 2.5 Hz, *J* = 8.2 Hz), 7.25-7.40 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.7 Hz), 7.70 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.75 (d, 1H, 7CH, *J* = 8.2 Hz), 8.24 (s, 1H, 4CH), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.25 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 28.5 (CH_3), 66.6 (5"CH), 109.7 (C-3), 110.5 (C-7), 119.1 (C-4), 121.4 (C-6), 124.2 (2',6'CH), 126.3 (C-5), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.4 (3',5'CH), 130.9 (C-2), 133.5 (1'CH), 134.2 (C-N (q) fused carbon), 161.1 (6"CH), 185.0 (C=S), 199.9 (C=O); MS (70 ev): m/z = 398.45 (M+1); Anal. Calcd. for $C_{19}H_{16}ClN_5OS$ (397.88): C, 57.35; H, 4.05; N, 17.60, Found, C, 57.40; H, 4.12; N, 17.55.

*4.1.6.2. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indol-3-yl)ethanone (**6b**)*

Yield: 73%; m.p. 210-214 ºC; IR (KBr) υ (cm^{-1}): 3098 (C-H Ar str), 2954 (C-H str), 1690 (C=O str), 1632 (N-C=N str), 1549 (Het. Triazine str), 1535 (C=C Ar str), 1178 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.63 (s, 3H, $COCH_3$), 3.96 (s, 1H, 5"CH), 4.22 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.10 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.4 Hz), 7.23-7.35 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.9 Hz), 7.68 (d, 2H, 2',6'CH, *J* = 7.31 Hz), 7.85 (d, 1H, 7CH, *J* = 8.3 Hz), 8.43 (s, 1H, 4CH), 9.00 (brs, 1H, 4"NH, D_2O exchangeable), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 28.2 (CH_3), 66.3 (5"CH), 110.0 (C-3), 110.8 (C-7), 111.5 (C-4), 117.1 (C-6), 124.5 (2',6'CH), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.1 (3',5'CH), 130.4 (C-2), 133.0 (1'CH), 134.4 (C-N (q) fused carbon), 160.6 (6"CH), 161.3 (C-5), 185.0 (C=S), 200.2 (C=O); MS (70 ev): m/z = 382.19 (M+1); Anal. Calcd. for $C_{19}H_{16}FN_5OS$ (381.43): C, 59.83; H, 4.23; N, 18.36, Found, C, 59.80; H, 4.29; N, 18.39.

*4.1.6.3. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indol-3-yl)ethanone (**6c**)*

Yield: 79%; m.p. 219-221 ºC; IR (KBr) υ (cm^{-1}): 3097 (C-H Ar str), 2958 (C-H str), 1688 (C=O str), 1650 (N-C=N str), 1555 (Het. Triazine str), 1530 (C=C Ar str), 1345 (NO_2 str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.62 (s, 3H, $COCH_3$), 3.95 (s, 1H, 5"CH), 4.21 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.17 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.1 Hz), 7.25-7.37 (m, 3H, 3',4',5'CH, *J* = 4.9 Hz, *J* = 8.7 Hz), 7.72 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.93 (d, 1H, 7CH, *J* = 8.1 Hz), 8.69 (s, 1H, 4CH), 9.05 (brs, 1H, 4"NH, D_2O exchangeable), 12.24 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 28.3 (CH_3), 66.5 (5"CH), 108.8 (C-7), 110.2 (C-3), 116.6 (C-6), 124.7 (2',6'CH), 127.1 (4'CH), 127.5 (C-4), 127.9 (CH-C (q) fused carbon), 128.4 (3',5'CH), 130.5 (C-2), 132.8 (C-5), 133.8 (1'CH), 140.4 (C-N (q) fused carbon), 160.9 (6"CH), 185.4 (C=S), 200.0 (C=O); MS (70 ev): m/z = 409.05 (M+1); Anal. Calcd. for $C_{19}H_{16}N_6O_3S$ (408.43): C, 55.87; H, 3.95; N, 20.58, Found, C, 55.93; H, 3.90; N, 20.50.

*4.1.6.4. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indol-3-yl)ethanone (**6d**)*

Yield: 72%; m.p. 225-228 ºC; IR (KBr) υ (cm^{-1}): 3090 (C-H Ar str), 2950 (C-H str), 1685 (C=O str), 1631 (N-C=N str), 1551 (Het. Triazine str), 1525 (C=C Ar str), 1190 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.60 (s, 3H, $COCH_3$), 3.93 (s, 1H, 5"CH), 4.22 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.22-7.38 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.5 Hz), 7.50 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 7.8 Hz), 7.70 (d, 2H, 2',6'CH, *J* = 7.26 Hz), 7.85 (d, 1H, 7CH, *J* = 8.0 Hz), 8.84 (s, 1H, 4CH), 9.01 (brs, 1H, 4"NH, D_2O exchangeable), 12.25 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 28.6 (CH_3), 66.5 (5"CH), 110.2 (C-3), 111.7 (C-7), 114.9 (C-6), 117.5 (C-4), 124.1 (2',6'CH), 124.6 (CF_3), 125.4 (C-5), 127.5 (4'CH), 128.0 (CH-C (q) fused carbon), 128.6 (3',5'CH), 130.5 (C-2), 133.1 (1'CH), 134.8 (C-N (q) fused carbon), 161.2 (6"CH), 185.3 (C=S), 200.5 (C=O); MS (70 ev): m/z = 432.39 (M+1); Anal. Calcd. for $C_{20}H_{16}F_3N_5OS$ (431.43): C, 55.68; H, 3.74; N, 16.23, Found, C, 55.73; H, 3.68; N, 16.29.

*4.1.6.5. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indol-3-yl)ethanone (**6e**)*

Yield: 77%; m.p. 198-201 ºC; IR (KBr) υ (cm^{-1}): 3095 (C-H Ar str), 2952 (C-H str), 1686 (C=O str), 1634 (N-C=N str), 1549 (Het. Triazine str), 1531 (C=C Ar str), 1248 (C-O-C str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.66 (s, 3H, $COCH_3$), 3.70 (s, 3H, OCH_3), 3.98 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.09 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.2 Hz), 7.26-7.39 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.6 Hz), 7.65 (s, 1H, 4CH), 7.70 (d, 2H, 2',6'CH, *J* = 7.27 Hz), 7.80 (d, 1H, 7CH, *J* = 8.4 Hz), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.25 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 28.4 (CH_3), 55.5 (OCH_3), 66.9 (5"CH), 101.5 (C-4), 110.1 (C-3), 110.5 (C-7), 112.4 (C-6), 124.1 (2',6'CH), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.4 (3',5'CH), 130.3 (C-2), 133.5 (1'CH), 134.0 (C-N (q) fused carbon), 149.1 (C-5), 160.6 (6"CH), 185.0 (C=S), 200.4 (C=O); MS (70 ev): m/z = 394.27 (M+1); Anal. Calcd. for $C_{20}H_{19}N_5O_2S$ (393.46): C, 61.05; H, 4.87; N, 17.80, Found, C, 61.12; H, 4.81; N, 17.85.

*4.1.6.6. 1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-(methylthio)-2-phenyl-1H-indol-3-yl)ethanone (**6f**)*

Yield: 82%; m.p. 192-195 ºC; IR (KBr) υ (cm^{-1}): 3098 (C-H Ar str), 2958 (C-H str), 1690 (C=O str), 1625 (N-C=N str), 1546 (Het. Triazine str), 1533 (C=C Ar str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3H, $COCH_3$), 2.62 (s, 3H, SCH_3), 3.97 (s, 1H, 5"CH), 4.24 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.27-7.42 (m, 3H, 3',4',5'CH, *J* = 4.5 Hz, *J* = 8.2 Hz), 7.45 (dd, 1H, 6CH, *J* = 1.9 Hz, *J* = 7.9 Hz), 7.69 (d, 2H, 2',6'CH, *J* = 7.29 Hz), 7.85 (d, 1H, 7CH, *J* = 8.1 Hz), 7.90 (s, 1H, 4CH), 9.05 (brs, 1H, 4"NH, D_2O exchangeable), 12.20 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 15.2 (SCH_3), 28.8 (CH_3), 66.4 (5"CH), 110.1 (C-3), 111.5 (C-7), 112.2 (C-6), 119.4 (C-4), 124.5 (2',6'CH), 127.1 (4'CH), 127.8 (CH-C (q) fused carbon), 128.2 (3',5'CH), 130.9 (C-2), 132.5 (C-5), 133.1 (1'CH), 134.0 (C-N (q) fused carbon), 161.0 (6"CH), 185.2 (C=S), 200.7 (C=O); MS (70 ev): m/z = 410.25 (M+1); Anal. Calcd. for $C_{20}H_{19}N_5OS_2$ (409.53): C, 58.66; H, 4.68; N, 17.10, Found, C, 58.71; H, 4.72; N, 17.05.

*4.1.7. General procedure for the preparation of compound (**7a-f**)*

The indole-triazine derivatives **4a-f** was N-protected by 9-fluorenylmethoxycarbonyl (Fmoc) and converted smoothly to sulfonyl chloride via treatment with sulfuryl chloride in dimethylformamide and refluxed for 6 h. Sulfonyl chloride was then reacted with ammonia in a parallel fashion to generate sulfonamide represented by general structure **7a-f**. Finally, concomitant deprotection of the Fmoc group at the triazine N-2 and N-4 was executed after completion of the reaction by adding 4-(aminomethyl)piperidine (0.5-2 mL) with continuous stirring to provide the sulfonamide derivative that was purified by crystallization in petroleum ether: dichloromethane (4:1) to give pure compounds **7a-f**.

*4.1.7.1. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-chloro-2-phenyl-1H-indole-3-sulfonamide (**7a**)*

Yield: 80%; m.p. 172-175 ºC; IR (KBr) υ (cm^{-1}): 3360 (N-H str, br), 3096 (C-H Ar str), 1634 (N-C=N str), 1545 (Het. Triazine str), 1533 (C=C Ar str), 1326, 1143 (S=O str), 1054 (C-Cl str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.97 (s, 1H, 5”CH), 4.20 (brs, 2H, 5”NH_2, D_2O exchangeable), 7.05 (dd, 1H, 6CH, *J* = 2.4 Hz, *J* = 8.2 Hz), 7.25-7.38 (m, 3H, 3’,4’,5’CH, *J* = 4.6 Hz, *J* = 8.5 Hz), 7.65 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.72 (d, 2H, 2’,6’CH, *J* = 7.31 Hz), 7.77 (d, 1H, 7CH, *J* = 8.1 Hz), 8.34 (s, 1H, 4CH), 9.04 (brs, 1H, 4”NH, D_2O exchangeable), 12.21 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.5 (5”CH), 110.2 (C-7), 114.8 (C-3), 119.5 (C-4), 120.9 (C-6), 124.0 (2’,6’CH), 126.4 (C-5), 127.1 (4’CH), 127.5 (CH-<u>C</u> (q) fused carbon), 128.5 (3’,5’CH), 130.7 (C-2), 133.2 (1’CH), 134.7 (<u>C</u>-N (q) fused carbon), 161.4 (6”CH), 185.2 (C=S); MS (70 ev): m/z = 435.70 (M+1); Anal. Calcd. for $C_{17}H_{15}ClN_6O_2S_2$ (434.92): C, 46.95; H, 3.48; N, 19.32, Found, C, 47.00; H, 3.42; N, 19.27.

*4.1.7.2. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-sulfonamide (**7b**)*

Yield: 86%; m.p. 177-179 ºC; IR (KBr) υ (cm^{-1}): 3369 (N-H str, br), 3090 (C-H Ar str), 1631 (N-C=N str), 1550 (Het. Triazine str), 1528 (C=C Ar str), 1328, 1140 (S=O str), 1170 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.94 (s, 1H, 5”CH), 4.21 (brs, 2H, 5”NH_2, D_2O exchangeable), 7.09 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.3 Hz), 7.24-7.37 (m, 3H, 3’,4’,5’CH, *J* = 4.5 Hz, *J* = 8.8 Hz), 7.60 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.71 (d, 2H, 2’,6’CH, *J* = 7.30 Hz), 7.83 (d, 1H, 7CH, *J* = 8.4 Hz), 8.47 (s, 1H, 4CH), 9.01 (brs, 1H, 4”NH, D_2O exchangeable), 12.19 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.5 (5”CH), 110.8 (C-7), 111.4 (C-4), 114.5 (C-3), 117.5 (C-6), 124.3 (2’,6’CH), 127.1 (4’CH), 127.8 (CH-<u>C</u> (q) fused carbon), 128.2 (3’,5’CH), 130.2 (C-2), 133.5 (1’CH), 134.5 (<u>C</u>-N (q) fused carbon), 160.5 (6”CH), 161.3 (C-5), 184.9 (C=S); MS (70 ev): m/z = 419.11 (M+1); Anal. Calcd. for $C_{17}H_{15}FN_6O_2S_2$ (418.47): C, 48.79; H, 3.61; N, 20.08, Found, C, 48.85; H, 3.57; N, 20.01.

*4.1.7.3. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indole-3-sulfonamide (**7c**)*

Yield: 70%; m.p. 215-218 ºC; IR (KBr) υ (cm^{-1}): 3373 (N-H str, br), 3099 (C-H Ar str), 1630 (N-C=N str), 1552 (Het. Triazine str), 1530 (C=C Ar str), 1340 (NO_2 str), 1325, 1140 (S=O str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.96 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.18 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.1 Hz), 7.25-7.34 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.7 Hz), 7.75 (d, 2H, 2',6'CH, *J* = 7.28 Hz), 7.84 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.92 (d, 1H, 7CH, *J* = 8.2 Hz), 8.71 (s, 1H, 4CH), 9.05 (brs, 1H, 4"NH, D_2O exchangeable), 12.23 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.2 (5"CH), 108.3 (C-7), 114.5 (C-3), 116.4 (C-6), 124.3 (2',6'CH), 126.9 (4'CH), 127.2 (C-4), 127.6 (CH-C (q) fused carbon), 128.6 (3',5'CH), 130.4 (C-2), 132.3 (C-5), 133.8 (1'CH), 140.4 (C-N (q) fused carbon), 161.2 (6"CH), 185.1 (C=S); MS (70 ev): m/z = 446.33 (M+1); Anal. Calcd. for $C_{17}H_{15}N_7O_4S_2$ (445.48): C, 45.83; H, 3.39; N, 22.01, Found, C, 45.90; H, 3.32; N, 21.95.

*4.1.7.4. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indole-3-sulfonamide (**7d**)*

Yield: 73%; m.p. 191-194 ºC; IR (KBr) υ (cm^{-1}): 3374 (N-H str, br), 3093 (C-H Ar str), 1625 (N-C=N str), 1554 (Het. Triazine str), 1532 (C=C Ar str), 1327, 1142 (S=O str), 1199 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.94 (s, 1H, 5"CH), 4.23 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.22-7.38 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.5 Hz), 7.52 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.1 Hz), 7.71 (d, 2H, 2',6'CH, *J* = 7.26 Hz), 7.80 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.88 (d, 1H, 7CH, *J* = 8.2 Hz), 8.85 (s, 1H, 4CH), 9.03 (brs, 1H, 4"NH, D_2O exchangeable), 12.20 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.6 (5"CH), 111.5 (C-7), 114.7 (C-3), 115.3 (C-6), 117.2 (C-4), 124.0 (2',6'CH), 124.5 (CF_3), 125.3 (C-5), 127.6 (4'CH), 128.2 (CH-C (q) fused carbon), 128.4 (3',5'CH), 130.3 (C-2), 133.0 (1'CH), 134.7 (C-N (q) fused carbon), 161.2 (6"CH), 185.3 (C=S); MS (70 ev): m/z = 469.34 (M+1); Anal. Calcd. for $C_{18}H_{15}F_3N_6O_2S_2$ (468.48): C, 46.15; H, 3.23; N, 17.94, Found, C, 46.20; H, 3.20; N, 17.89.

*4.1.7.5. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indole-3-sulfonamide (**7e**)*

Yield: 81%; m.p. 182-184 ºC; IR (KBr) υ (cm^{-1}): 3365 (N-H str, br), 3088 (C-H Ar str), 2958 (C-H str), 1636 (N-C=N str), 1557 (Het. Triazine str), 1528 (C=C Ar str), 1332, 1141 (S=O str), 1250 (C-O-C str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.72 (s, 3H, $O\underline{CH_3}$), 3.99 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.11 (dd, 1H, 6CH, *J* = 2.1 Hz, *J* = 8.3 Hz), 7.26-7.41 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.4 Hz), 7.68 (s, 1H, 4CH), 7.74 (d, 2H, 2',6'CH, *J* = 7.27 Hz), 7.69 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.81 (d, 1H, 7CH, *J* = 8.4 Hz), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.24 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 55.7 (OCH_3), 66.5 (5"CH), 101.6 (C-4), 110.5 (C-7), 112.3 (C-6), 114.6 (C-3), 124.0 (2',6'CH), 127.4 (4'CH), 127.5 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.4 (C-2), 133.5 (1'CH), 134.3 (C-N (q) fused carbon), 149.2 (C-5), 160.8 (6"CH), 185.1 (C=S); MS (70

ev): m/z = 431.09 (M+1); Anal. Calcd. for $C_{18}H_{18}N_6O_3S_2$ (430.50): C, 50.22; H, 4.21; N, 19.52, Found, C, 50.28; H, 4.17; N, 19.47.

*4.1.7.6. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-(methylthio)-2-phenyl-1H-indole-3-sulfonamide (**7f**)*

Yield: 77%; m.p. 217-220 ºC; IR (KBr) υ (cm^{-1}): 3372 (N-H str, br), 3095 (C-H Ar str), 2960 (C-H str), 1626 (N-C=N str), 1554 (Het. Triazine str), 1529 (C=C Ar str), 1325, 1145 (S=O str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.38 (s, 3H, S$\underline{CH_3}$), 3.95 (s, 1H, 5”CH), 4.22 (brs, 2H, 5”NH_2, D_2O exchangeable), 7.25-7.40 (m, 3H, 3’,4’,5’CH, *J* = 4.5 Hz, *J* = 8.2 Hz), 7.43 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 7.9 Hz), 7.61 (brs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 7.72 (d, 2H, 2’,6’CH, *J* = 7.28 Hz), 7.83 (d, 1H, 7CH, *J* = 8.1 Hz), 7.95 (s, 1H, 4CH), 9.05 (brs, 1H, 4”NH, D_2O exchangeable), 12.26 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 16.0 (SCH_3), 66.7 (5”CH), 111.3 (C-7), 112.3 (C-6), 114.8 (C-3), 119.3 (C-4), 124.5 (2’,6’CH), 127.0 (4’CH), 127.6 (CH-$\underline{C}$ (q) fused carbon), 128.4 (3’,5’CH), 130.7 (C-2), 132.2 (C-5), 133.5 (1’CH), 134.5 ($\underline{C}$-N (q) fused carbon), 161.0 (6”CH), 185.0 (C=S); MS (70 ev): m/z = 447.26 (M+1); Anal. Calcd. for $C_{18}H_{18}N_6O_2S_3$ (446.57): C, 48.41; H, 4.06; N, 18.82, Found, C, 48.45; H, 4.01; N, 18.75.

*4.1.8. General procedure for the preparation of compound (**8a-f**)*

To a solution of compounds **4a-f** (22.3 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL), trichloroacetic acid anhydride (33.5 mmol) was added and the mixture was stirred for 6 hours at 0 °C. The saturated sodium bicarbonate solution was added to the mixture and the resultant mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate and then concentrated in vacuum. To a solution of the residue in tetrahydrofuran (135 mL), 4M sodium hydroxide solution (65.2 mmol) was added and stirred at room temperature for 6 hours. The mixture was diluted with diethyl ether and 1M sodium hydroxide solution. The aqueous layer was washed with diethyl ether and neutralized with 1M hydrochloric acid. The precipitate was separated from the resultant aqueous solution and dried in vacuum to give compounds **8a-f**.

*4.1.8.1. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-chloro-2-phenyl-1H-indole-3-carboxylic acid (**8a**)*

Yield: 85%; m.p. 164-168 ºC; IR (KBr) υ (cm^{-1}): 3350 (OH str, br), 3092 (C-H Ar str), 1715 (C=O str), 1627 (N-C=N str), 1555 (Het. Triazine str), 1530 (C=C Ar str), 1055 (C-Cl str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.90 (s, 1H, 5”CH), 4.25 (brs, 2H, 5”NH_2, D_2O exchangeable), 7.05 (dd, 1H, 6CH, *J* = 1.9 Hz, *J* = 8.4 Hz), 7.21-7.37 (m, 3H, 3’,4’,5’CH, *J* = 4.8 Hz, *J* = 8.5 Hz), 7.72 (d, 2H, 2’,6’CH, *J* = 7.29 Hz), 7.78 (d, 1H, 7CH, *J* = 8.3 Hz), 8.21 (s, 1H, 4CH), 9.06 (brs, 1H, 4”NH, D_2O exchangeable), 11.05 (brs, 1H, CO$\underline{OH}$), 12.24 (brs, 1H, 2”NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.5 (5”CH), 110.2 (C-7), 110.6 (C-3), 119.0 (C-4), 121.2 (C-6), 124.2 (2’,6’CH), 126.0 (C-5), 127.1 (4’CH), 127.6 (CH-$\underline{C}$ (q) fused

carbon), 128.5 (3',5'CH), 130.8 (C-2), 133.5 (1'CH), 134.8 (C-N (q) fused carbon), 161.0 (6"CH), 170.5 (C=O), 185.5 (C=S); MS (70 ev): m/z = 400.65 (M+1); Anal. Calcd. for $C_{18}H_{14}ClN_5O_2S$ (399.85): C, 54.07; H, 3.53; N, 17.51, Found, C, 54.13; H, 3.47; N, 17.56.

*4.1.8.2. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-carboxylic acid (**8b**)*

Yield: 90%; m.p. 172-174 ºC; IR (KBr) υ (cm^{-1}): 3356 (OH str, br), 3091 (C-H Ar str), 1718 (C=O str), 1625 (N-C=N str), 1558 (Het. Triazine str), 1535 (C=C Ar str), 1168 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.92 (s, 1H, 5"CH), 4.23 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.08 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.3 Hz), 7.23-7.38 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.7 Hz), 7.68 (d, 2H, 2',6'CH, *J* = 7.33 Hz), 7.85 (d, 1H, 7CH, *J* = 8.5 Hz), 8.44 (s, 1H, 4CH), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 11.02 (brs, 1H, COOH), 12.25 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.1 (5"CH), 110.3 (C-3), 110.9 (C-7), 111.5 (C-4), 117.0 (C-6), 124.6 (2',6'CH), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.4 (3',5'CH), 130.1 (C-2), 133.0 (1'CH), 134.4 (C-N (q) fused carbon), 160.6 (6"CH), 161.4 (C-5), 170.8 (C=O), 185.1 (C=S); MS (70 ev): m/z = 384.02 (M+1); Anal. Calcd. for $C_{18}H_{14}FN_5O_2S$ (383.40): C, 56.39; H, 3.68; N, 18.27, Found, C, 56.45; H, 3.72; N, 18.21.

*4.1.8.3. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indole-3-carboxylic acid (**8c**)*

Yield: 83%; m.p. 181-183 ºC; IR (KBr) υ (cm^{-1}): 3351 (OH str, br), 3098 (C-H Ar str), 1709 (C=O str), 1634 (N-C=N str), 1547 (Het. Triazine str), 1528 (C=C Ar str), 1335 (NO_2 str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.95 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.18 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.1 Hz), 7.25-7.35 (m, 3H, 3',4',5'CH, *J* = 4.9 Hz, *J* = 8.5 Hz), 7.74 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.93 (d, 1H, 7CH, *J* = 8.2 Hz), 8.68 (s, 1H, 4CH), 9.04 (brs, 1H, 4"NH, D_2O exchangeable), 11.04 (brs, 1H, COOH), 12.23 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.3 (5"CH), 108.6 (C-7), 110.2 (C-3), 116.8 (C-6), 124.3 (2',6'CH), 127.0 (4'CH), 127.5 (C-4), 127.8 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.8 (C-2), 132.6 (C-5), 133.8 (1'CH), 140.3 (C-N (q) fused carbon), 161.0 (6"CH), 170.6 (C=O), 185.2 (C=S); MS (70 ev): m/z = 411.17 (M+1); Anal. Calcd. for $C_{18}H_{14}N_6O_4S$ (410.41): C, 52.68; H, 3.44; N, 20.48, Found, C, 52.72; H, 3.37; N, 20.40.

*4.1.8.4. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indole-3-carboxylic acid (**8d**)*

Yield: 78%; m.p. 188-190 ºC; IR (KBr) υ (cm^{-1}): 3358 (OH str, br), 3090 (C-H Ar str), 1718 (C=O str), 1625 (N-C=N str), 1548 (Het. Triazine str), 1530 (C=C Ar str), 1209 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.91 (s, 1H, 5"CH), 4.22 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.22-7.36 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.2 Hz), 7.53 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.0 Hz), 7.75 (d, 2H, 2',6'CH, *J* = 7.25 Hz), 7.88 (d, 1H, 7CH, *J* = 8.1 Hz), 8.87 (s, 1H, 4CH), 9.04 (brs, 1H, 4"NH, D_2O exchangeable), 11.02 (brs, 1H, COOH), 12.25 (brs, 1H, 2"NH, D_2O

exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.3 (5"CH), 110.5 (C-3), 111.8 (C-7), 115.2 (C-6), 117.1 (C-4), 124.1 (2',6'CH), 124.4 (CF_3), 125.7 (C-5), 127.7 (4'CH), 128.1 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.2 (C-2), 133.4 (1'CH), 134.7 (C-N (q) fused carbon), 161.0 (6"CH), 170.8 (C=O), 184.9 (C=S); MS (70 ev): m/z = 434.20 (M+1); Anal. Calcd. for $C_{19}H_{14}F_3N_5O_2S$ (433.41): C, 52.65; H, 3.26; N, 16.16, Found, C, 52.60; H, 3.22; N, 16.21.

*4.1.8.5. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indole-3-carboxylic acid (**8e**)*

Yield: 82%; m.p. 166-169 ºC; IR (KBr) υ (cm^{-1}): 3360 (OH str, br), 3098 (C-H Ar str), 2965 (C-H str), 1726 (C=O str), 1625 (N-C=N str), 1560 (Het. Triazine str), 1536 (C=C Ar str), 1255 (C-O-C str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3), 3.97 (s, 1H, 5"CH), 4.18 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.08 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.4 Hz), 7.25-7.40 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.5 Hz), 7.63 (s, 1H, 4CH), 7.71 (d, 2H, 2',6'CH, *J* = 7.27 Hz), 7.81 (d, 1H, 7CH, *J* = 8.4 Hz), 9.00 (brs, 1H, 4"NH, D_2O exchangeable), 11.05 (brs, 1H, CO**O**H), 12.21 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 55.6 (OCH_3), 66.7 (5"CH), 101.2 (C-4), 110.4 (C-3), 110.7 (C-7), 112.1 (C-6), 124.3 (2',6'CH), 127.5 (4'CH), 127.6 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.2 (C-2), 133.4 (1'CH), 134.0 (C-N (q) fused carbon), 149.1 (C-5), 160.6 (6"CH), 170.4 (C=O), 185.0 (C=S); MS (70 ev): m/z = 396.22 (M+1); Anal. Calcd. for $C_{19}H_{17}N_5O_3S$ (395.43): C, 57.71; H, 4.33; N, 17.71, Found, C, 57.79; H, 4.40; N, 17.66.

*4.1.8.6. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-(methylthio)-2-phenyl-1H-indole-3-carboxylic acid (**8f**)*

Yield: 87%; m.p. 185-190 ºC; IR (KBr) υ (cm^{-1}): 3365 (OH str, br), 3100 (C-H Ar str), 2968 (C-H str), 1730 (C=O str), 1622 (N-C=N str), 1558 (Het. Triazine str), 1532 (C=C Ar str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.39 (s, 3H, SCH_3), 3.99 (s, 1H, 5"CH), 4.22 (brs, 2H, 5"NH_2, D_2O exchangeable), 7.27-7.38 (m, 3H, 3',4',5'CH, *J* = 4.5 Hz, *J* = 8.2 Hz), 7.44 (dd, 1H, 6CH, *J* = 1.9 Hz, *J* = 8.1 Hz), 7.67 (d, 2H, 2',6'CH, *J* = 7.29 Hz), 7.82 (d, 1H, 7CH, *J* = 8.0 Hz), 7.91 (s, 1H, 4CH), 9.03 (brs, 1H, 4"NH, D_2O exchangeable), 11.00 (brs, 1H, CO**O**H), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 15.5 (SCH_3), 66.5 (5"CH), 110.3 (C-3), 111.7 (C-7), 112.2 (C-6), 119.6 (C-4), 124.3 (2',6'CH), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.8 (C-2), 132.0 (C-5), 133.3 (1'CH), 134.8 (C-N (q) fused carbon), 161.2 (6"CH), 173.0 (C=O), 185.3 (C=S); MS (70 ev): m/z = 412.28 (M+1); Anal. Calcd. for $C_{19}H_{17}N_5O_2S_2$ (411.50): C, 55.46; H, 4.16; N, 17.02, Found, C, 55.50; H, 4.22; N, 16.97.

*4.1.9. General procedure for the preparation of compound (**9a-f**)*

A mixture of carboxylic derivatives **8a-f** (0.1 mol), thionyl chloride (10 mL) and dimethyl formamide (0.1 mL) was refluxed for 12 h. The solution was evaporated and the residue triturated with toluene (10 mL) and re-evaporated. The resulting solid was stirred at 25 °C with

concentrated aqueous ammonia for 24 h. The carboxamide derivatives **9a-f** crystallized from water as colourless needles.

*4.1.9.1. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-chloro-2-phenyl-1H-indole-3-carboxamide (**9a**)*

Yield: 71%; m.p. 158-161 ºC; IR (KBr) υ (cm^{-1}): 3305 (NH str, br), 3092 (C-H Ar str), 1660 (C=O str), 1628 (N-C=N str), 1565 (Het. Triazine str), 1529 (C=C Ar str), 1060 (C-Cl str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.99 (s, 1H, 5"CH), 4.18 (brs, 2H, 5"NH_2, D_2O exchangeable), 4.70 (brs, 2H, CO$\underline{NH_2}$, D_2O exchangeable), 7.05 (dd, 1H, 6CH, *J* = 2.3 Hz, *J* = 8.5 Hz), 7.22-7.35 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.5 Hz), 7.72 (d, 2H, 2',6'CH, *J* = 7.29 Hz), 7.78 (d, 1H, 7CH, *J* = 8.5 Hz), 8.20 (s, 1H, 4CH), 9.02 (brs, 1H, 4"NH, D_2O exchangeable), 12.25 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.7 (5"CH), 110.0 (C-7), 110.6 (C-3), 119.3 (C-4), 121.1 (C-6), 124.0 (2',6'CH), 126.3 (C-5), 127.2 (4'CH), 127.7 (CH-$\underline{C}$ (q) fused carbon), 128.3 (3',5'CH), 131.0 (C-2), 133.1 (1'CH), 134.2 ($\underline{C}$-N (q) fused carbon), 160.7 (6"CH), 172.4 (C=O), 185.3 (C=S); MS (70 ev): m/z = 399.74 (M+1); Anal. Calcd. for $C_{18}H_{15}ClN_6OS$ (398.87): C, 54.20; H, 3.79; N, 21.07, Found, C, 54.25; H, 3.72; N, 21.00.

*4.1.9.2. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-carboxamide (**9b**)*

Yield: 78%; m.p. 173-176 ºC; IR (KBr) υ (cm^{-1}): 3309 (NH str, br), 3090 (C-H Ar str), 1665 (C=O str), 1633 (N-C=N str), 1562 (Het. Triazine str), 1527 (C=C Ar str), 1135 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.95 (s, 1H, 5"CH), 4.23 (brs, 2H, 5"NH_2, D_2O exchangeable), 4.73 (brs, 2H, CO$\underline{NH_2}$, D_2O exchangeable), 7.07 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.4 Hz), 7.21-7.38 (m, 3H, 3',4',5'CH, *J* = 4.8 Hz, *J* = 8.7 Hz), 7.68 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.85 (d, 1H, 7CH, *J* = 8.4 Hz), 8.46 (s, 1H, 4CH), 9.04 (brs, 1H, 4"NH, D_2O exchangeable), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.4 (5"CH), 110.2 (C-7), 110.5 (C-3), 111.4 (C-4), 116.9 (C-6), 124.5 (2',6'CH), 127.3 (4'CH), 127.9 (CH-$\underline{C}$ (q) fused carbon), 128.2 (3',5'CH), 130.0 (C-2), 133.1 (1'CH), 134.5 ($\underline{C}$-N (q) fused carbon), 160.6 (6"CH), 161.2 (C-5), 172.7 (C=O), 185.2 (C=S); MS (70 ev): m/z = 383.11 (M+1); Anal. Calcd. for $C_{18}H_{15}FN_6OS$ (382.41): C, 56.53; H, 3.95; N, 21.98, Found, C, 56.60; H, 3.90; N, 21.92.

*4.1.9.3. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indole-3-carboxamide (**9c**)*

Yield: 82%; m.p. 180-184 ºC; IR (KBr) υ (cm^{-1}): 3310 (NH str, br), 3094 (C-H Ar str), 1667 (C=O str), 1625 (N-C=N str), 1568 (Het. Triazine str), 1535 (C=C Ar str), 1325 (NO_2 str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.98 (s, 1H, 5"CH), 4.21 (brs, 2H, 5"NH_2, D_2O exchangeable), 4.82 (brs, 2H, CO$\underline{NH_2}$, D_2O exchangeable), 7.20 (dd, 1H, 6CH, *J* = 2.2 Hz, *J* = 8.1 Hz), 7.25-7.37 (m, 3H, 3',4',5'CH, *J* = 4.9 Hz, *J* = 8.5 Hz), 7.72 (d, 2H, 2',6'CH, *J* = 7.29 Hz), 7.93 (d, 1H, 7CH, *J* = 8.4 Hz), 8.69 (s, 1H, 4CH), 9.01 (brs, 1H, 4"NH, D_2O exchangeable), 12.24 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.4 (5"CH), 108.7 (C-7),

110.6 (C-3), 116.6 (C-6), 124.2 (2',6'CH), 127.0 (4'CH), 127.4 (C-4), 128.0 (CH-C (q) fused carbon), 128.5 (3',5'CH), 130.4 (C-2), 132.6 (C-5), 133.5 (1'CH), 140.2 (C-N (q) fused carbon), 161.0 (6"CH), 172.4 (C=O), 184.9 (C=S); MS (70 ev): m/z = 410.25 (M+1); Anal. Calcd. for $C_{18}H_{15}N_7O_3S$ (409.42): C, 52.80; H, 3.69; N, 23.95, Found, C, 52.87; H, 3.60; N, 24.01.

*4.1.9.4. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indole-3-carboxamide (**9d**)*

Yield: 76%; m.p. 195-199 ºC; IR (KBr) υ (cm^{-1}): 3311 (NH str, br), 3097 (C-H Ar str), 1661 (C=O str), 1630 (N-C=N str), 1560 (Het. Triazine str), 1526 (C=C Ar str), 1174 (C-F str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.95 (s, 1H, 5"CH), 4.25 (brs, 2H, 5"NH_2, D_2O exchangeable), 4.75 (brs, 2H, CONH_2, D_2O exchangeable), 7.22-7.38 (m, 3H, 3',4',5'CH, *J* = 4.7 Hz, *J* = 8.4 Hz), 7.51 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 7.9 Hz), 7.73 (d, 2H, 2',6'CH, *J* = 7.24 Hz), 7.85 (d, 1H, 7CH, *J* = 8.0 Hz), 8.84 (s, 1H, 4CH), 9.05 (brs, 1H, 4"NH, D_2O exchangeable), 12.22 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 66.7 (5"CH), 110.7 (C-3), 111.4 (C-7), 115.0 (C-6), 117.4 (C-4), 124.0 (2',6'CH), 124.6 (CF_3), 125.3 (C-5), 127.5 (4'CH), 128.4 (CH-C (q) fused carbon), 128.7 (3',5'CH), 130.5 (C-2), 133.0 (1'CH), 134.7 (C-N (q) fused carbon), 161.3 (6"CH), 172.3 (C=O), 185.2 (C=S); MS (70 ev): m/z = 433.03 (M+1); Anal. Calcd. for $C_{19}H_{15}F_3N_6OS$ (432.42): C, 52.77; H, 3.50; N, 19.43, Found, C, 52.83; H, 3.42; N, 19.39.

*4.1.9.5. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indole-3-carboxamide (**9e**)*

Yield: 80%; m.p. 177-179 ºC; IR (KBr) υ (cm^{-1}): 3309 (NH str, br), 3090 (C-H Ar str), 2971 (C-H str), 1668 (C=O str), 1623 (N-C=N str), 1569 (Het. Triazine str), 1533 (C=C Ar str), 1255 (C-O-C str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.70 (s, 3H, OCH_3), 4.00 (s, 1H, 5"CH), 4.20 (brs, 2H, 5"NH_2, D_2O exchangeable), 4.97 (brs, 2H, CONH_2, D_2O exchangeable), 7.08 (dd, 1H, 6CH, *J* = 2.0 Hz, *J* = 8.4 Hz), 7.24-7.38 (m, 3H, 3',4',5'CH, *J* = 4.6 Hz, *J* = 8.5 Hz), 7.64 (s, 1H, 4CH), 7.68 (d, 2H, 2',6'CH, *J* = 7.25 Hz), 7.82 (d, 1H, 7CH, *J* = 8.5 Hz), 9.03 (brs, 1H, 4"NH, D_2O exchangeable), 12.23 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 55.4 (OCH_3), 66.3 (5"CH), 101.8 (C-4), 110.3 (C-7), 110.7 (C-3), 112.3 (C-6), 124.1 (2',6'CH), 127.2 (4'CH), 127.6 (CH-C (q) fused carbon), 128.2 (3',5'CH), 130.5 (C-2), 133.5 (1'CH), 134.3 (C-N (q) fused carbon), 149.3 (C-5), 161.0 (6"CH), 172.9 (C=O), 185.3 (C=S); MS (70 ev): m/z = 395.32 (M+1); Anal. Calcd. for $C_{19}H_{18}N_6O_2S$ (394.45): C, 57.85; H, 4.60; N, 21.31, Found, C, 57.90; H, 4.66; N, 21.37.

*4.1.9.6. 1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-(methylthio)-2-phenyl-1H-indole-3-carboxamide (**9f**)*

Yield: 84%; m.p. 194-198 ºC; IR (KBr) υ (cm^{-1}): 3315 (NH str, br), 3096 (C-H Ar str), 2966 (C-H str), 1665 (C=O str), 1628 (N-C=N str), 1572 (Het. Triazine str), 1528 (C=C Ar str); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.42 (s, 3H, SCH_3), 3.97 (s, 1H, 5"CH), 4.22 (brs, 2H, 5"NH_2, D_2O

exchangeable), 5.00 (brs, 2H, CO$\underline{NH_2}$, D_2O exchangeable), 7.27-7.40 (m, 3H, 3',4',5'CH, *J* = 4.5 Hz, *J* = 8.3 Hz), 7.48 (dd, 1H, 6CH, *J* = 1.9 Hz, *J* = 7.9 Hz), 7.73 (d, 2H, 2',6'CH, *J* = 7.30 Hz), 7.82 (d, 1H, 7CH, *J* = 8.3 Hz), 7.91 (s, 1H, 4CH), 9.03 (brs, 1H, 4"NH, D_2O exchangeable), 12.24 (brs, 1H, 2"NH, D_2O exchangeable); ^{13}CNMR δ (ppm) (DMSO-d_6, TMS): 15.8 (SCH_3), 66.8 (5"CH), 110.4 (C-3), 111.6 (C-7), 112.3 (C-6), 119.6 (C-4), 124.7 (2',6'CH), 127.1 (4'CH), 127.8 (CH-$\underline{C}$ (q) fused carbon), 128.3 (3',5'CH), 130.6 (C-2), 132.4 (C-5), 133.6 (1'CH), 134.3 ($\underline{C}$-N (q) fused carbon), 161.1 (6"CH), 172.6 (C=O), 185.5 (C=S); MS (70 ev): m/z = 411.08 (M+1); Anal. Calcd. for $C_{19}H_{18}N_6OS_2$ (410.52): C, 55.59; H, 4.42; N, 20.47, Found, C, 55.66; H, 4.37; N, 20.40.

4.2. Pharmacology

4.2.1. Anticonvulsant screening

In these studies adult male albino mice weighing (20 ± 2 g) and adult wistar rats (180 ± 10 g) (obtained from the Central Animal House Facility, (173/CPCSEA, 28 Jan., 2000) Hamdard University, New Delhi-110062) were used throughout. Animals were housed in groups of 4-5 and were allowed free access to food pellets and water except for the short time that animals were removed from their cages for testing. They were allowed to acclimatize with for a 24 h period before testing. All behavioural experiments were conducted during the period between 10:00 and 13:00 with normal room light (12 h regular light/dark cycle) and temperature (26 ± 2 $^{\circ}$C). Animals housing and rearing had been followed optimum standard rules. Unnecessary disturbance of animals was avoided. Animals were treated gently; squeezing, pressure and tough manoeuvre is avoided. Instruments used for drug preparation and animal injection were previously sterilized. All the experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC) for animal care and use as follows:

4.2.1.1. Preliminary anticonvulsant screening

All the synthesized compounds were screened for their potential *in vivo* anticonvulsant activity. The preliminary pharmacological screening involves MES and scPTZ assays. The Electro-convulsometer with ear clip electrode was used for the assessment of anticonvulsant potential against electroshock induced seizures. Neurotoxicity was ascertained by Minimal motor impairment test using rotarod. Four animals (swiss albino mice) were selected for each treatment and standard group. Phenytoin and ethosuximide were used as standards during the intraperitoneal administration of the test compounds [44]. The active compounds used for oral investigation (100 mg/kg) and the reference drug phenytoin (30 mg/kg, MES test) and sodium valproate (200 mg/kg, scPTZ test) [45,47] suspended in a vehicle (0.5% sodium carboxymethylcellulose) were administered to a group of six mice each 60 min before the injection of PTZ and MES test. The control animals were administered the vehicle. The

procedures were carried out as described. One way analysis of variance (ANOVA) followed by Dunnett's method with the aid of Graph pad Prism software, version 5 (inc., San Diego, USA) was used for statistical analysis.

4.2.1.1.1. Maximal Electroshock test (MES test)

In MES test [41] mice were pre-screened 24 hour before by delivering maximal electroshock (50mA; 60Hz & 0.2s duration) by means of corneal electrodes. A drop of 0.9% Sodium chloride was instilled in each eye prior to the application of electrodes in order to prevent death of the animal. The test compounds were suspended in 0.5% sodium carboxymethylcellulose-water mixture. The test solutions of each compound at three doses (30, 100, and 300 mg/kg body mass) were administered intraperitoneally and the anticonvulsant activity was assessed after 0.5 h and 4.0 h intervals respectively. Abolition of hind limb tonic extensor component of the seizure in half or more of the animals was defined as protection. For the oral evaluation of the active compounds maximal electric shock of 150 mA current for 0.2 s was applied through corneal electrodes to induce convulsions and duration of hind limb tonic extension was noted as the outcome [45] (Table 2). The abolition or reduction of the duration of hind limb tonic extension of compounds was expressed as the mean ± S.E.M. for each group.

4.2.1.1.2. sub-cutaneous Pentylenetetrazole test (scPTZ test)

In sc PTZ test [42] a dose of pentylenetetrazol 70 mg/Kg was utilized. This produced clonic seizure in >95% of animals lasting for a period of atleast 5 seconds. The test compounds were administered at the three graded doses viz. 30, 100 and 300 mg/Kg intraperitoneally. At the anticipated time the convulsant was administered as 0.5% solution subcutaneously in the posterior midline. Animals were observed over a 30 min period. Absence of clonic spasm in half or more of the animals in the observed time period indicates a compounds ability to abolish the effect of pentylenetetrazol on seizure threshold. During the oral evaluation of the active compounds against PTZ (85 mg/Kg, sc) induced seizures, each animal was placed into an individual plastic cage for observation over a period of 20 min. Seizure latency was defined as the time elapsed from the injection of pentylenetetrazole to the first two myoclonic jerks of the forelimbs. Animals devoid of generalized convulsions were considered to be protected and the results were represented as percentage protection [46] (Table 2).

4.2.1.1.3. Minimal motor impairment test (Rotarod test)

The minimal motor impairment is measured in mice by rotarod test [43]. The mice were trained to stay on an accelerating rotarod of diameter 3.2 cm that rotates at 10 rpm. The animals were injected intraperitoneally with the test compounds at a dose of 30, 100 and 300 mg/kg in a group of four mice each. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for atleast one minute in each of the three trials. The dose at which 50% of the animals enabled to balance themselves and fall off the rotating rod was determined. The

active candidates were given orally (100 mg/kg) in the mice for neurotoxicity investigation following the similar evaluation procedure as mentioned above.

4.2.1.2. Quantitative anticonvulsant study

The quantification study of the selected compounds for the determination of median effective dose (ED_{50}) and median neurotoxic dose (TD_{50}) was done using the literary procedure. For the determination of ED_{50} and TD_{50} values, groups of six mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plot of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of the computer program [48].

4.2.2. Isolation of Rat Brain Regions and GABA Assay

The GABA assay was performed in brain tissue extracts enzymatically. Adult Wistar rats were divided into three groups of six animals each. After 2 h of drug administration (100 mg/kg, i.p), the animals were decapitated and the brains were dropped into separate vials containing 4-6 mL of ice-cold 80% ethanol and processed further as described in reference [50]. A chronic study was also carried out after p.o administration of the test compounds (30 mg/kg) for 7 days.

4.2.3. Sodium Channel Binding Assay

The procedure was similar to reported methods [51]. Synaptoneurosomes were prepared from rat cerebral cortex as follows: cerebral cortex (approximately 1-g weight) was homogenized in 2 mL of buffer containing 130 mM choline chloride, 50 mM HEPES [adjusted to pH 4 with tris-(hydroxymethyl)aminomethane, approximately 23 mM Tris base], 5.5 mM glucose, 0.8 mM $MgSO_4$ and 5.4 mM KCl. The tissue was homogenized with 10-12 strokes of a glass-glass homogenizer. The final volume was adjusted to 6 mL and the preparation centrifuged at 1000 g for 15 min at 4 °C. The pellet was resuspended in a total volume of 20 mL of HEPES buffer for binding studies. Incubations were carried out for 40 min at 25 °C in a total volume of 320 µL containing 10 nM [^{3}H]BTX-B, 50 µg/mL of scorpion venom, approximately 980 µg of the particulate vesicular protein, and varying concentrations of added test compound (from 50 mM stock solutions in 50% MeOH/H,O). The MeOH concentration was in all cases less than 1 %. Incubations were terminated by dilution of the reaction mixture with 3 mL of ice-cold wash buffer and filtration through a Whatman GF/C filter paper. Filters were washed with wash buffer (3 x 3 mL). The wash buffer contained the following: 163 mM choline chloride, 5 mM HEPES (adjusted to pH 7.4 with Tris base), 1.8 mM $CaCl_2$, and 0.8 mM $MgSO_4$. Filters were counted in a Beckman scintillation counter using 10 mL of 3270B counting cocktail. Specific binding was determined by subtracting the nonspecific binding, measured in the presence of 250 µM veratridine, from the total binding of [^{3}H]BTX-B. Specific binding was about 80% of total binding. All experiments were performed in triplicate. IC_{50}**,** values (concentration of compound required to inhibit 50% of specific neurotoxin binding) were determined from a dose-response

curve generated by plotting the log of anticonvulsant concentration versus percent of specifically bound [^{3}H]BTX-B.

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Captions

Scheme 1 Synthetic procedure for the preparation of intermediate compounds **4a-f**

Scheme 2 Synthetic procedure for the preparation of titled compounds **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f**

Figure 1 Pharmacophoric representation of designed molecules

Figure 2 IC_{50} (μM) and the % inhibition of active compounds at a concentration of 100 μM in *in vitro* sodium channel binding by [^{3}H]BTX radioligand assay

Table 1 Preliminary evaluation of synthesized compounds **5a-f**, **6a-f**, **7a-f**, **8a-f** and **9a-f** for Anticonvulsant activity and Neurotoxicity

Compd. No.	MES Screen		scPTZ Screen		Minimal motor impairment test	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
5a	100	100	-	-	X	X
5b	300	-	300	300	300	100
5c	300	300	100	300	300	300
5d	30	100	-	-	300	300
5e	300	-	300	300	300	100
5f	100	300	-	300	X	X
6a	30	100	300	300	300	300
6b	30	30	100	100	300	300
6c	100	-	100	300	300	100
6d	30	30	-	300	300	300
6e	100	100	300	300	300	100
6f	300	300	-	300	X	X
7a	30	30	300	300	300	300
7b	30	30	100	100	300	300
7c	300	-	100	300	300	-
7d	30	100	300	-	300	-
7e	100	100	300	300	X	X
7f	300	300	100	300	300	100
8a	300	-	300	300	300	300
8b	300	-	100	300	300	100
8c	30	100	100	100	300	300
8d	30	30	300	-	300	300
8e	100	100	300	300	300	100
8f	30	100	300	-	300	300
9a	100	-	-	-	X	X
9b	30	100	100	300	300	300
9c	30	30	300	300	300	300
9d	300	-	300	300	300	100
9e	100	100	-	300	X	X
9f	100	100	300	-	X	X
PHY[c)]	30	30	x	x	100	100
ETX[c)]	-	-	100	300	-	-

[a)]number of animals protected/number of animals tested, the number of mice is four.
[b)]number of animals toxic/number of animals tested, the number of mice is four.
(-) indicates devoid of activity at the maximum dose administered (300 mg/kg).
(x) indicates not tested.
[c)] Data taken from reference [48] and dose in mg/Kg

Table 2 Anticonvulsant activity and neurotoxicity (NT) of selected active compounds administered orally to mice (100 mg/kg)

Compd. No.	MES[a]		scPTZ[a]		NT[b]
	Duration of limb extension (sec)	Protection (%)	Seizure latency to onset of clonus (min)	Protection (%)	
6b	5.40 ± 0.61**	100.0	9.80 ± 0.54**	83.3	NNT
6d	13.50 ± 0.80^{ns}	33.3	ND	-	-
7a	9.80 ± 0.90**	66.6	-	-	NNT
7b	6.60 ± 0.40**	83.3	> 14**	83.3	NNT
8c	-	-	4.54 ± 0.75^{ns}	66.6	-
8d	14.00 ± 0.91^{ns}	33.3	-	-	-
9c	12.70 ± 0.87*	50.0	-	-	NNT
Control	16.20 ± 0.50	-	1.54 ± 0.90	-	-
PHY[c]	NIL	100.0	-	-	NNT
SVAL[d]	-	-	12.60 ± 1.81**	100.0	NNT

Values represent the mean ± S.E.M. for each group *p<0.05; **p<0.01; ns = not significant (n=6). One way analysis of variance (ANOVA) followed by Dunnett's method was applied for statistical analysis

[a]The test compounds were administered 60 min before the injection of PTZ (85 mg/kg, sc) and MES test (150 mA, 0.2 s)

[b]Percentage of animals that fail to maintain equilibrium on the accelerating rod for 60 sec. NNT indicates non-neurotoxic and a dash (-) indicates Not Determined

Control animals were administered 0.5% sodium carboxymethylcellulose.

[c]Reference standard: Phenytoin (30 mg/kg, oral), data taken from Ref. [49].

[d]Reference standard: Sodium valproate (200 mg/kg, p.o), data taken from Ref. [51].

Latency time was observed for 14 min in scPTZ.

Table 3 Quantitative evaluation of selected active compounds for Anticonvulsant activity and Neurotoxicity in mice after intraperitoneal administration

Compd. No.	Phase II, mice (time of peak effect)		
	MES, ED_{50} [a)]	Tox, TD_{50} [b)]	scPTZ, ED_{50} [a)]
6b	7	290	68
6d	40	95	-
7a	15	185	-
7b	9.5	225	35
8c	-	180	110
8d	30	100	-
9c	24	125	-
PHY [c)]	9.50	66	-
ETX [c)]	> 1000	440.8	130

[a)] ED_{50} – median effective dose eliciting anticonvulsant protection in 50% animals.
[b)] TD_{50} median toxic dose eliciting minimal neurological toxicity in 50% animals.
[c)] Data taken from reference [53] and dose in mg/kg (n=6).

Table 4 Effect of Selected Compounds in the GABA System

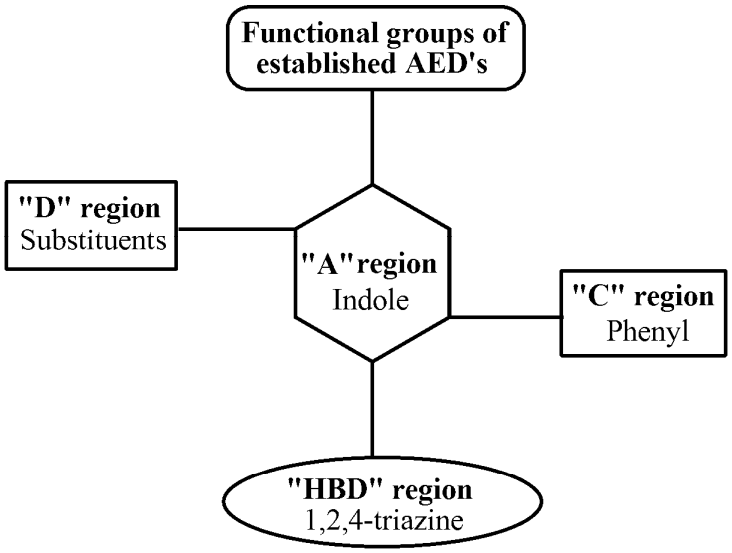
	GABA level in rat whole brain concentration (μg/100 mg if tissue)[b)]	
Compd. No.[a)]	**2 h post-treatment[c)]**	**7 days post-treatment[d)]**
6b	93.5 ± 5.32***	110 ± 1.58***
6d	75.4 ± 6.04*	79.1 ± 5.95**
7a	88.7 ± 4.80***	100.6 ± 2.50***
7b	90.4 ± 8.20**	104.8 ± 4.30***
8c	77.1 ± 4.50**	79.5 ± 5.48***
8d	72.4 ± 5.02*	74.1 ± 6.20**
9c	85.4 ± 6.14**	88.4 ± 6.22***
Control	46.8 ± 6.03	49.2 ± 3.01
Clobazam	101 ± 6.77	110 ± 4.83

[a]The compounds were tested at a dose of 100 mg/kg (ip) and clobazam (30 mg/kg).
[b]The data indicate the minimum concentration whereby at least 50% inhibition was demonstrated in one or more time points.
[c]Each value represents the mean (SEM of six rats, significantly different from the control at ***, $p < 0.001$; **, $p < 0.005$ and *, $p < 0.01$ (Student's *t* test).
[d]The compounds were tested at a dose of 30 mg/kg (po) for 7 days.

Table 5 In vitro screening for sodium channel activity by [^{3}H]BTX radioligand assay

Compd. No.	BTX, IC_{50}[a] (μM)	Inhibition (%)[a]
6b	108 ± 5	50.40
7a	300 ± 2	18.25
7b	220 ± 6	38.43
PHY	126 ± 7	49.61

[a])% inhibition at 100 μM



"A" Hydrophobic domain; "HBD" Hydrogen bonding domain; "C" Distal hydrophobic domain; "D" Electron donor/acceptor moiety

Figure 1

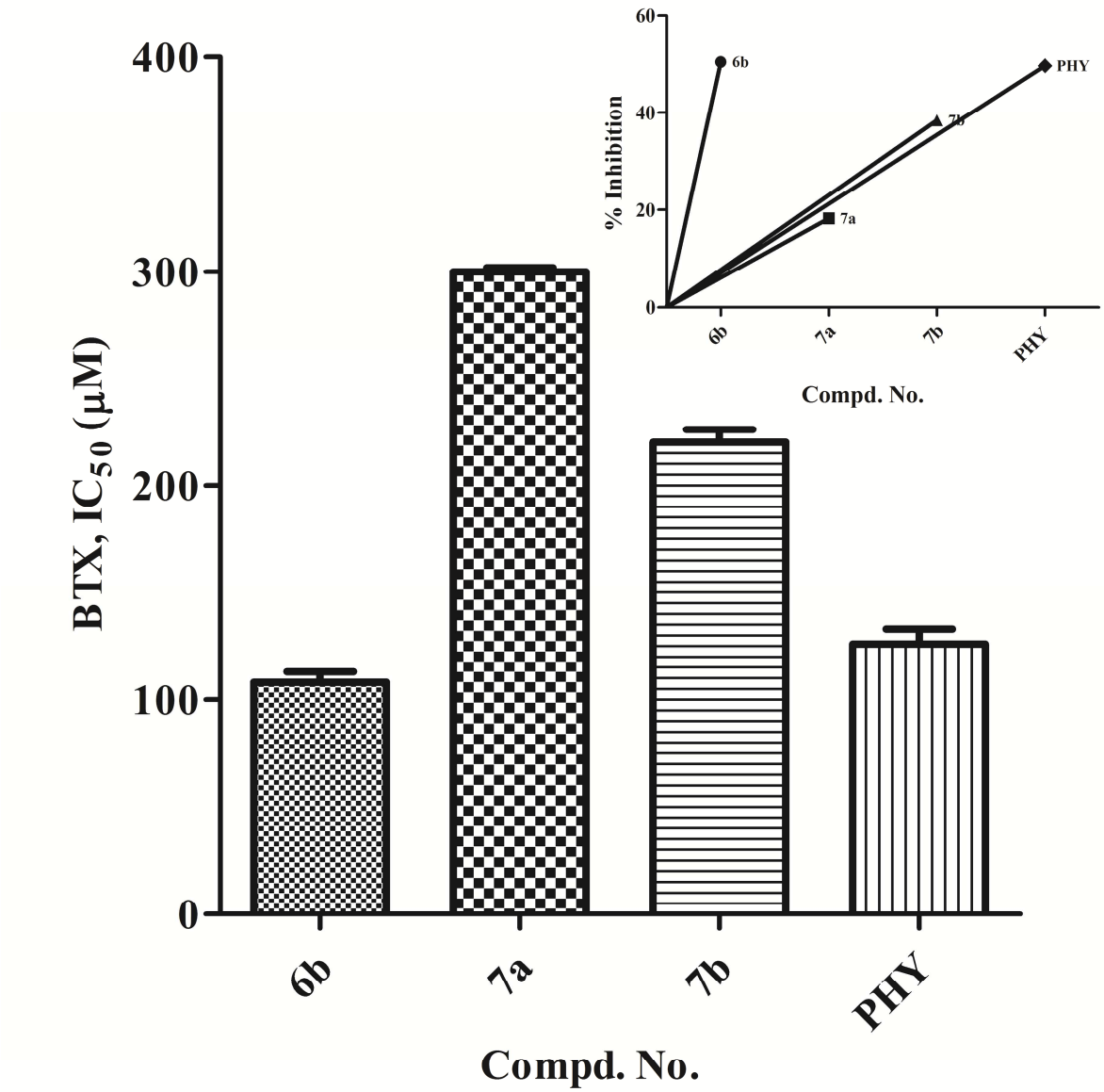
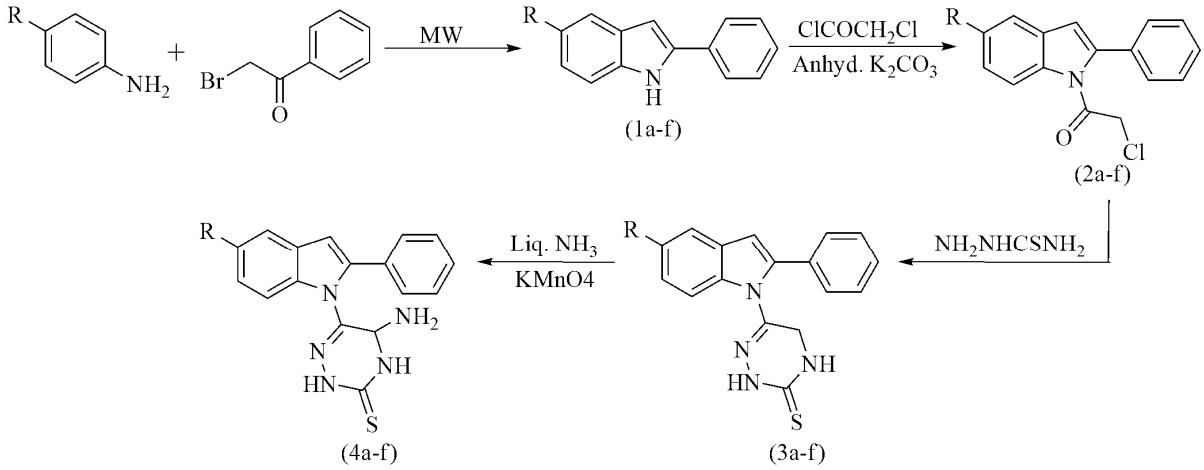
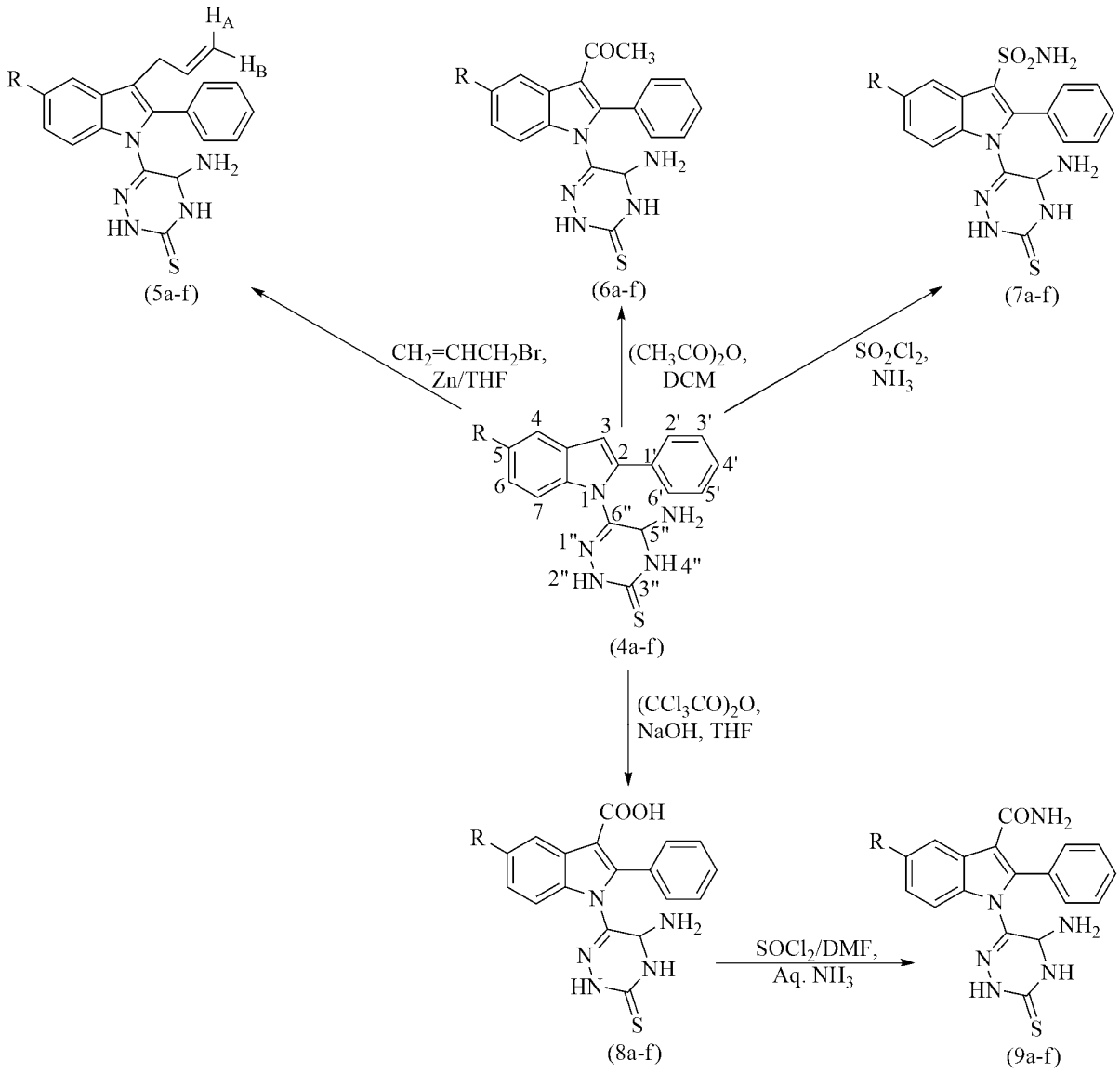

400
300
200
100
0
BTX, IC50 (µM)
6b
7a
7b
PHY
Compd. No.
60
40
20
0
% Inhibition
6b
7a
7b
PHY
Compd. No.

Figure 2



Compd.	R
a	Cl
b	F
c	NO_2
d	CF_3
e	OCH_3
f	SCH_3

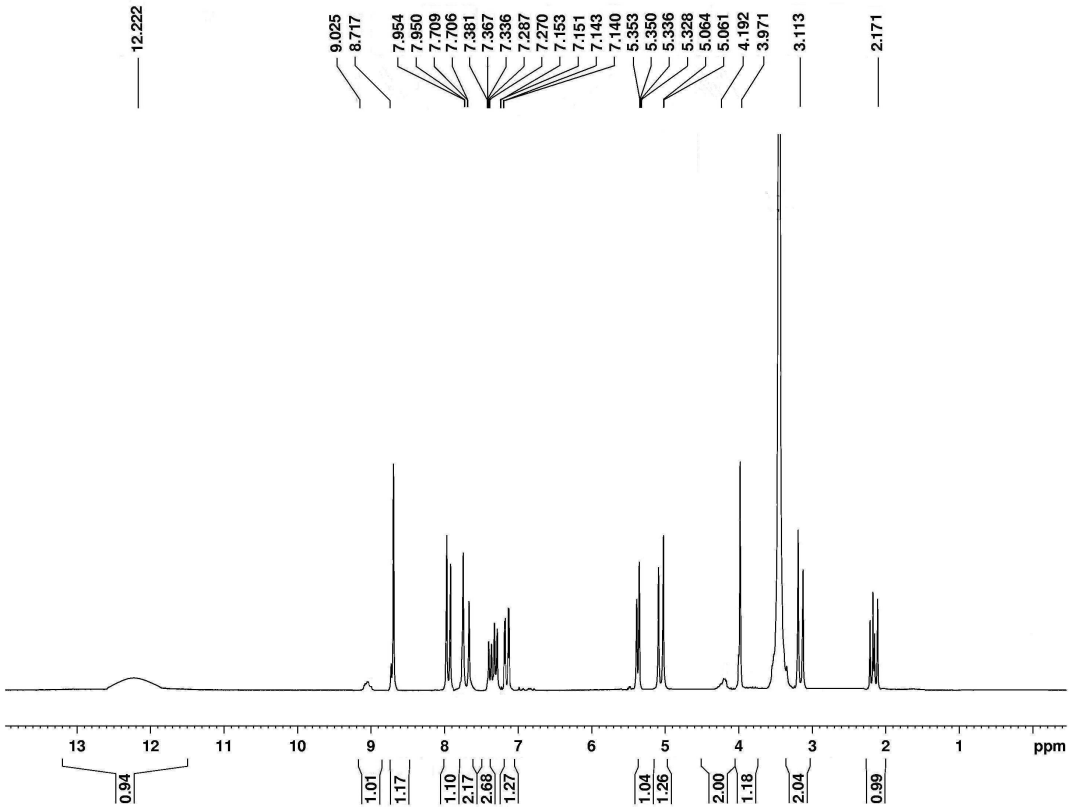
Scheme 1


(5a-f)
(6a-f)
(7a-f)
CH2=CHCH2Br, Zn/THF
(CH3CO)2O, DCM
SO2Cl2, NH3
(4a-f)
(CCl3CO)2O, NaOH, THF
(8a-f)
SOCl2/DMF, Aq. NH3
(9a-f)

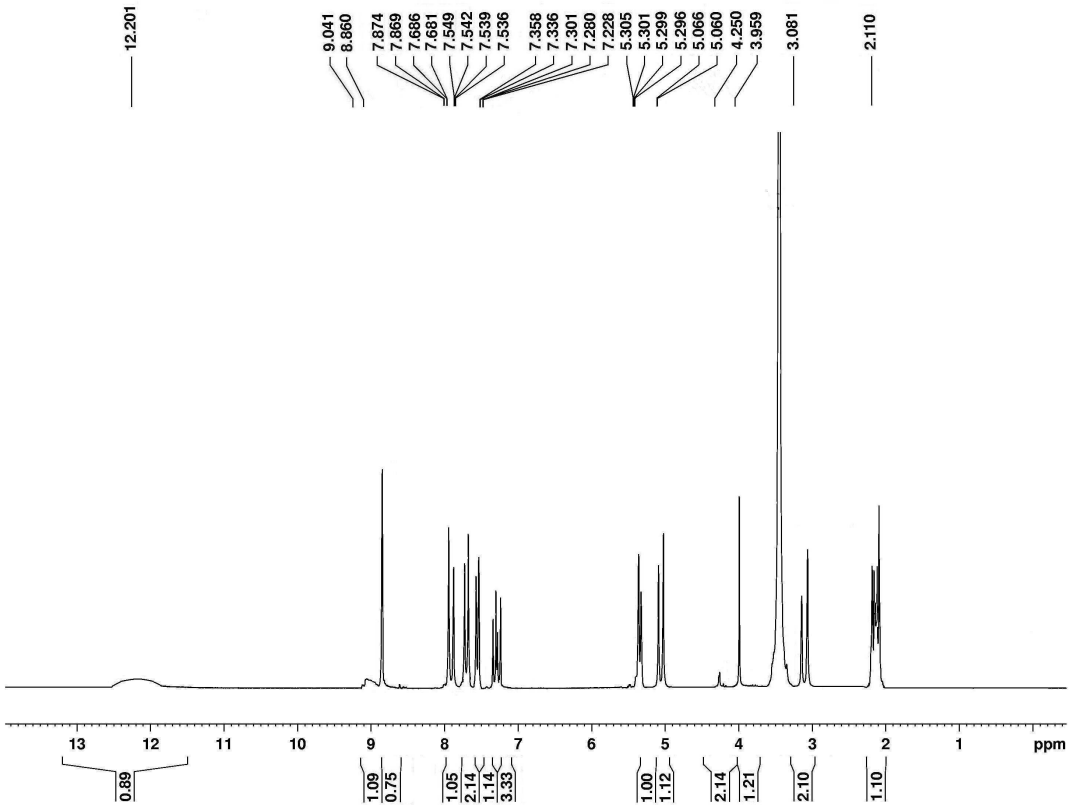
Scheme 2

Highlights:

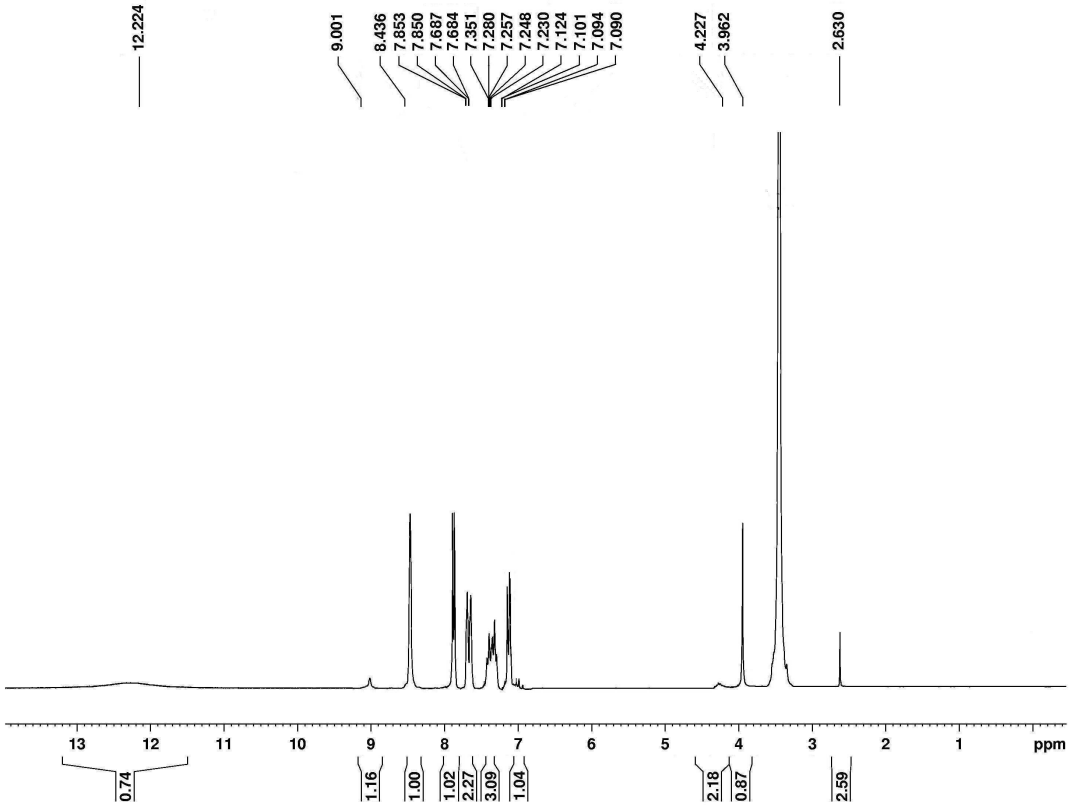
- Indole-1,2,4-triazine derivatives were reported as potential anticonvulsant agents.
- **6b** exhibited minimal duration of limb extension as 5.40 ± 0.61 sec in MES test.
- **7b** gave the seizure latency to onset of clonus > 14 min in scPTZ test.
- ED_{50} of **6b** in MES test is 7 mg/kg and of **7b** in scPTZ test is 35 mg/kg.
- *In vitro* sodium channel assay and GABA estimation showed significant outcomes.



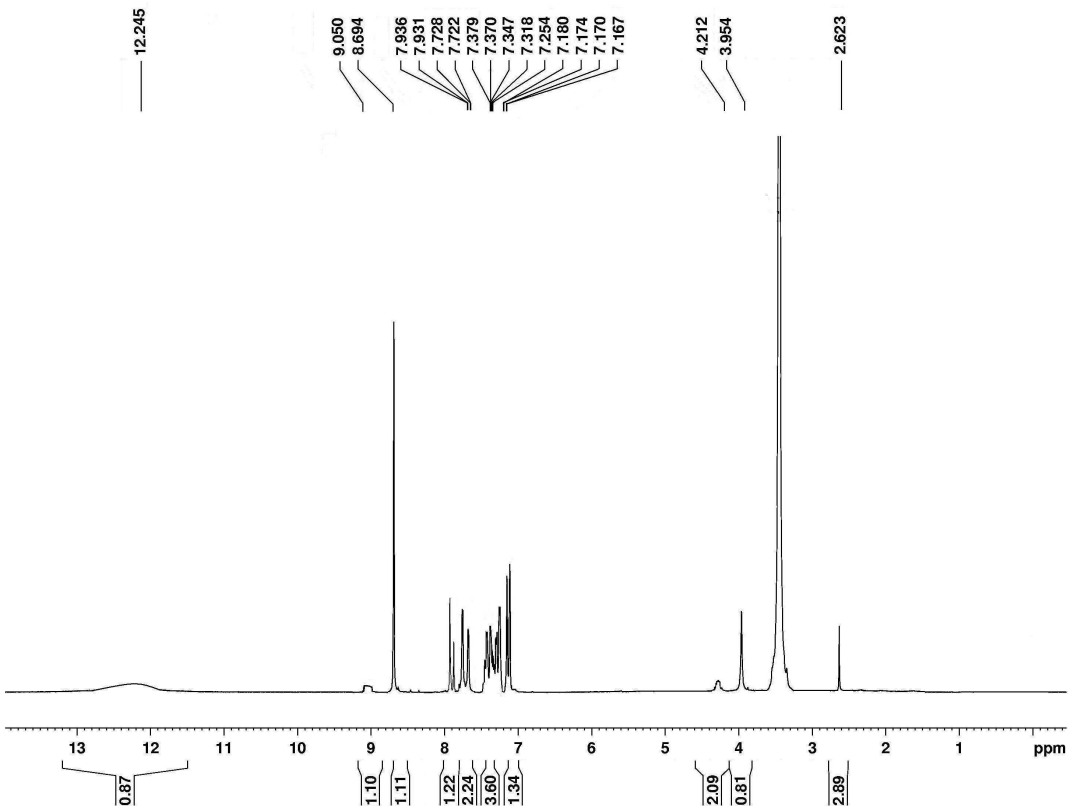
*6-(3-allyl-5-nitro-2-phenyl-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5c**)*



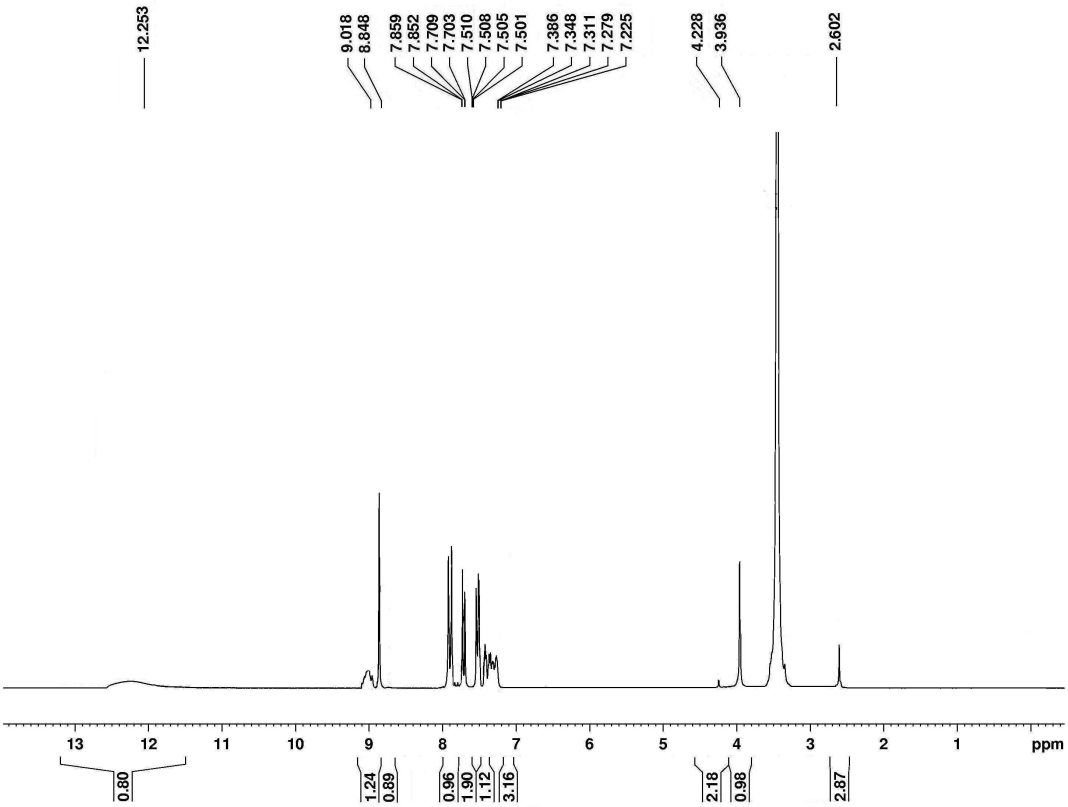
*6-(3-allyl-2-phenyl-5-(trifluoromethyl)-1H-indol-1-yl)-5-amino-4,5-dihydro-1,2,4-triazine-3(2H)-thione (**5d**)*



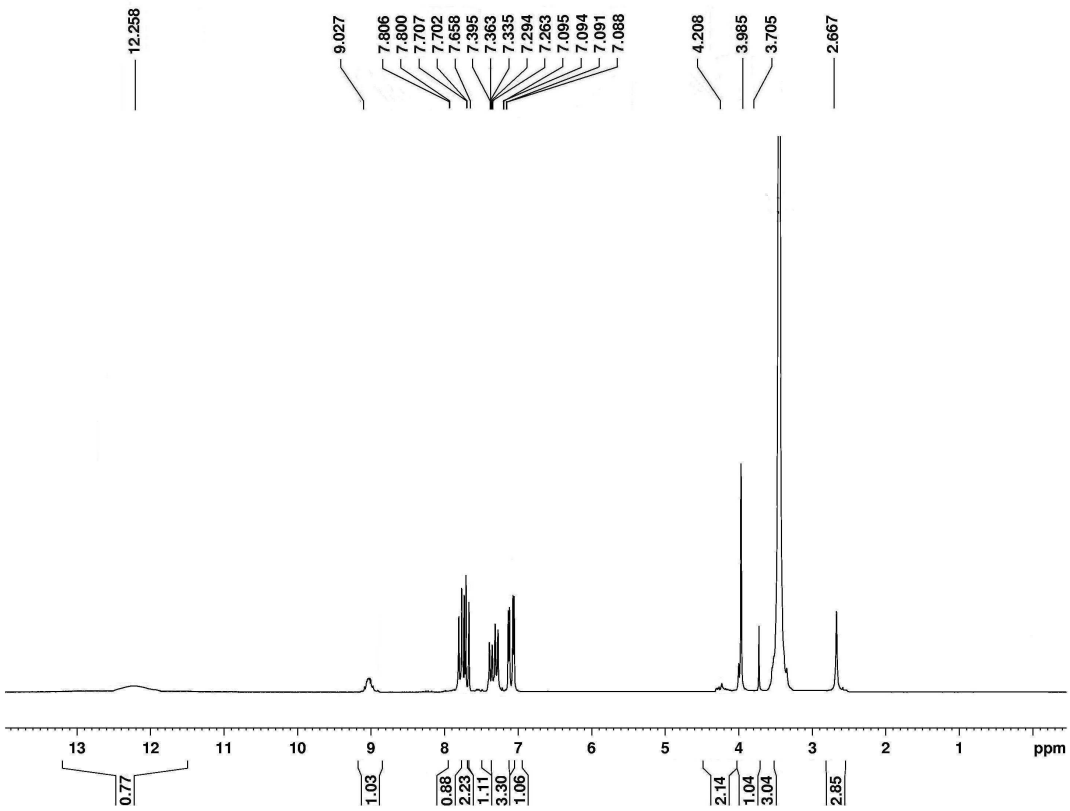
*1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indol-3-yl)ethanone (**6b**)*



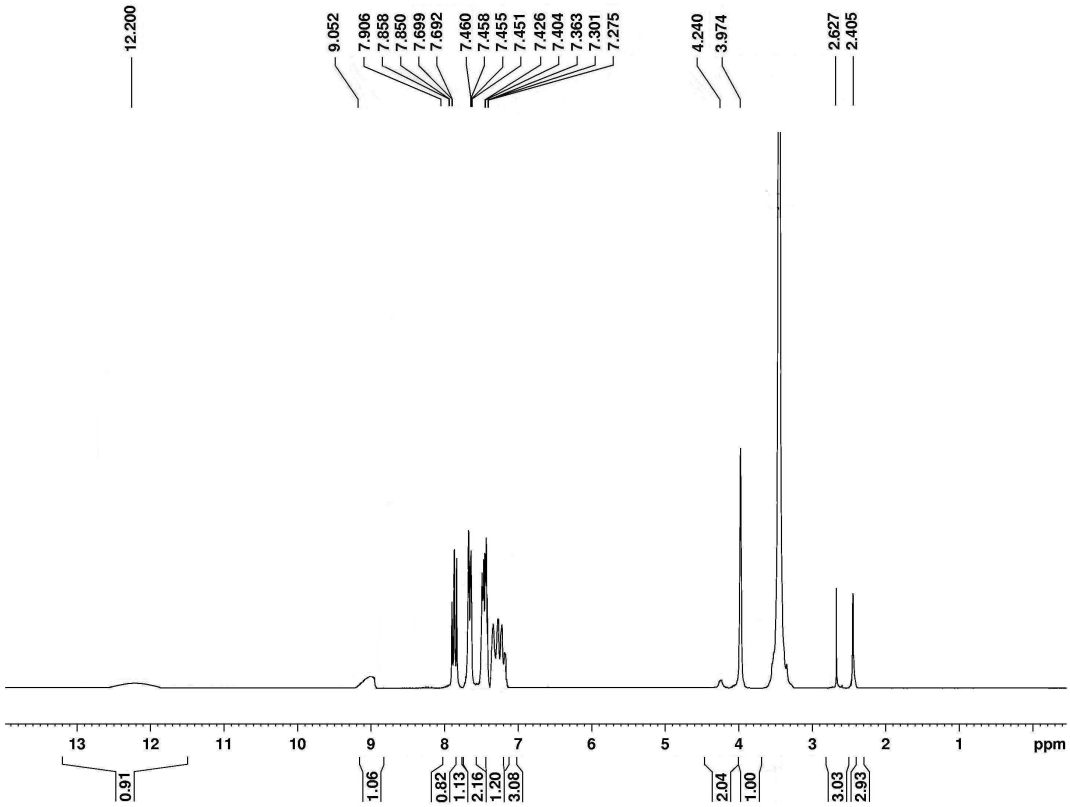
*1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indol-3-yl)ethanone (**6c**)*



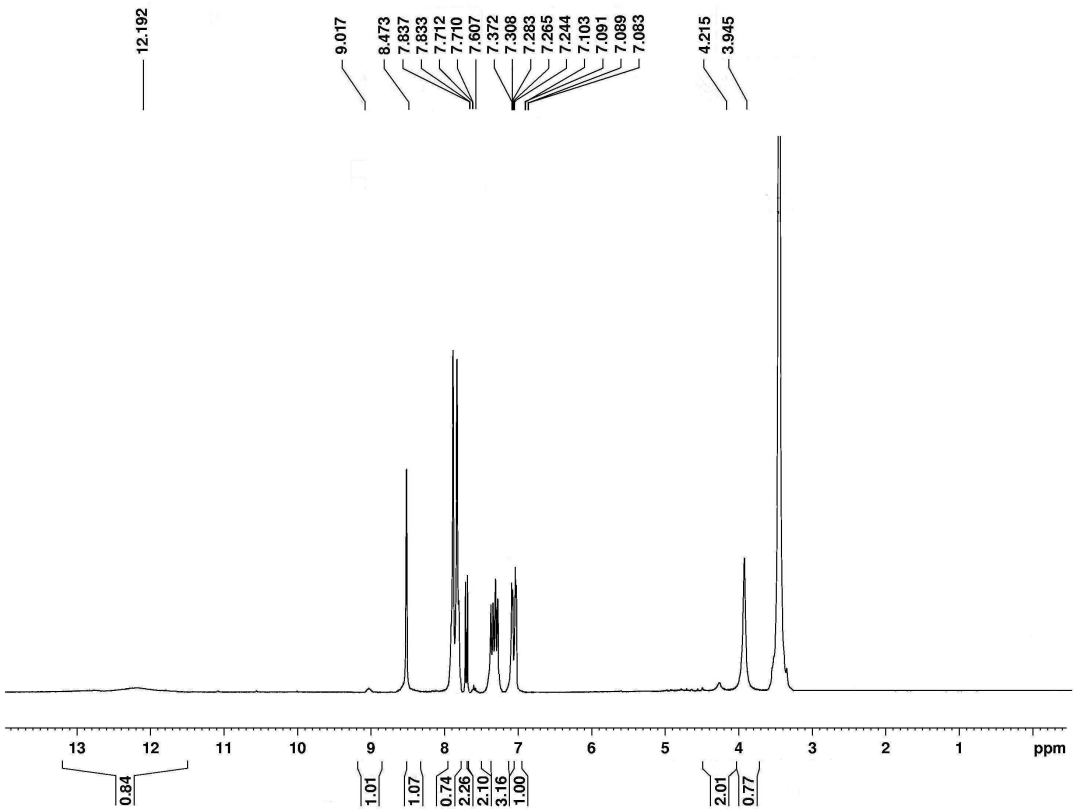
*1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indol-3-yl)ethanone (**6d**)*



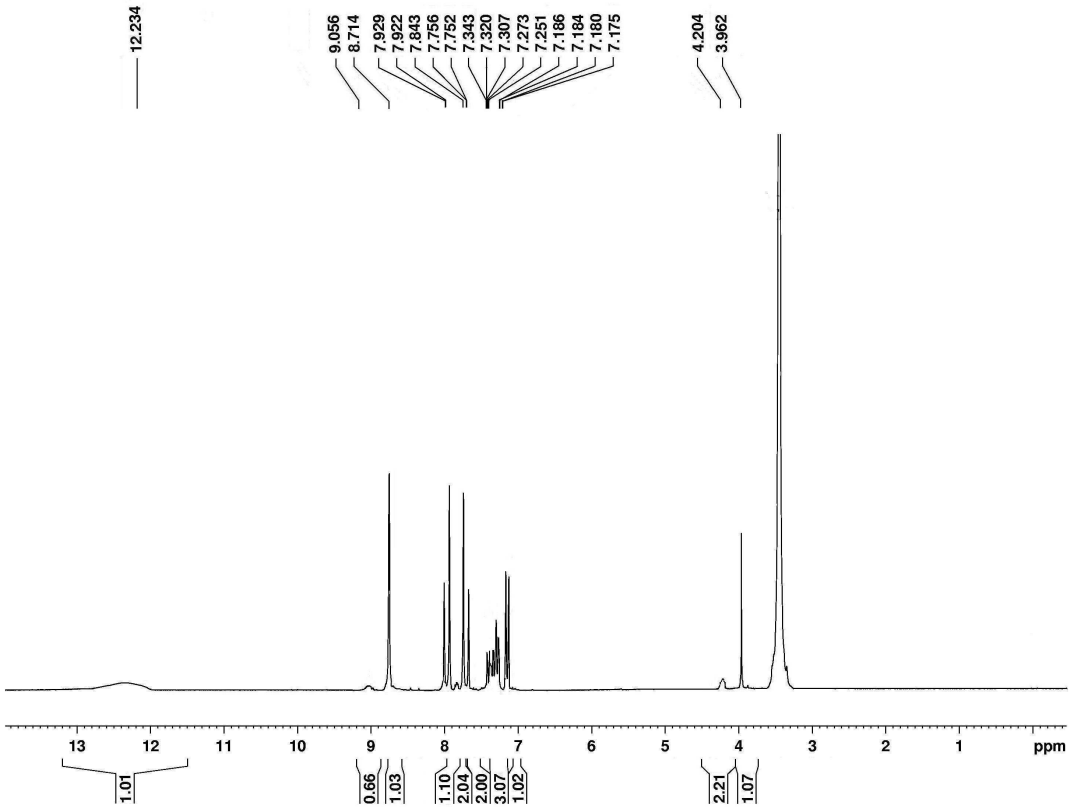
*1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indol-3-yl)ethanone (**6e**)*



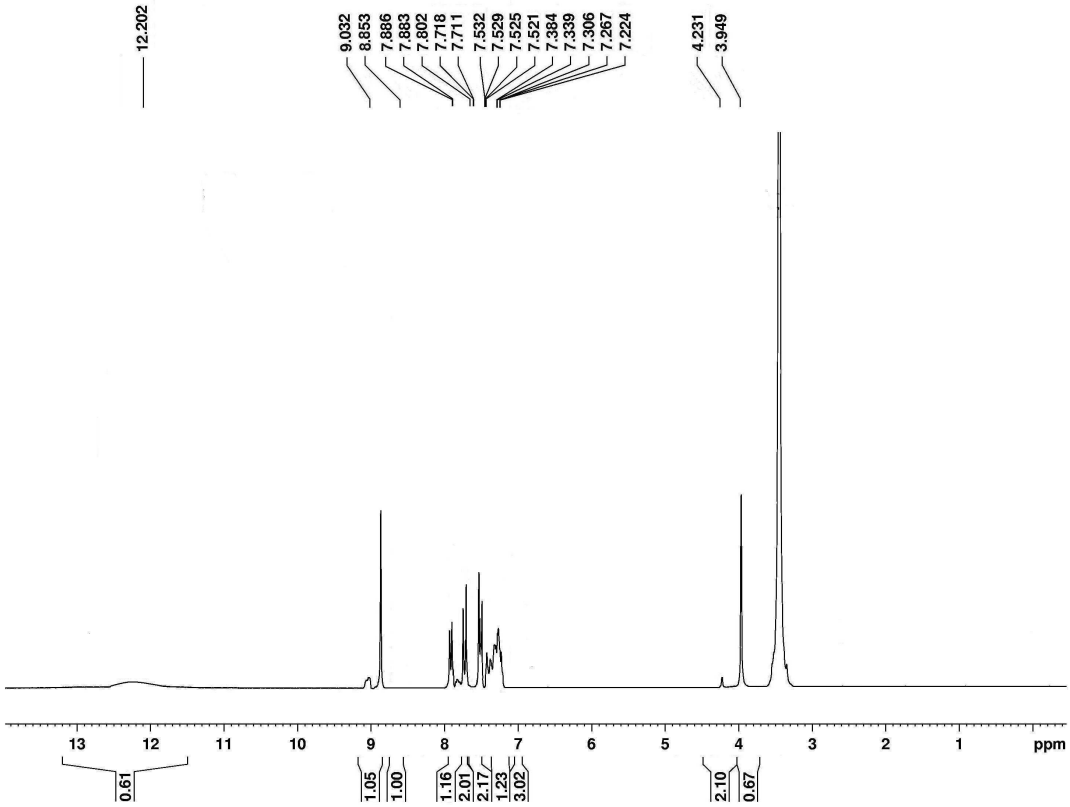
*1-(1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-(methylthio)-2-phenyl-1H-indol-3-yl)ethanone (**6f**)*



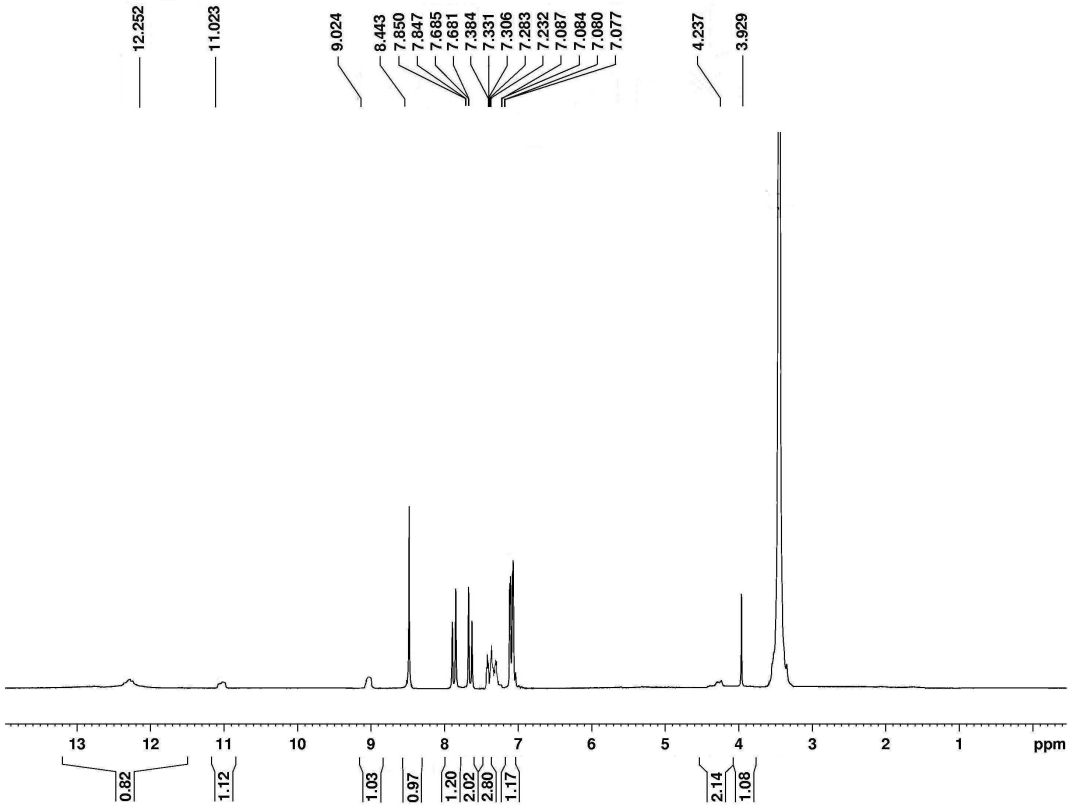
*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-sulfonamide (**7b**)*



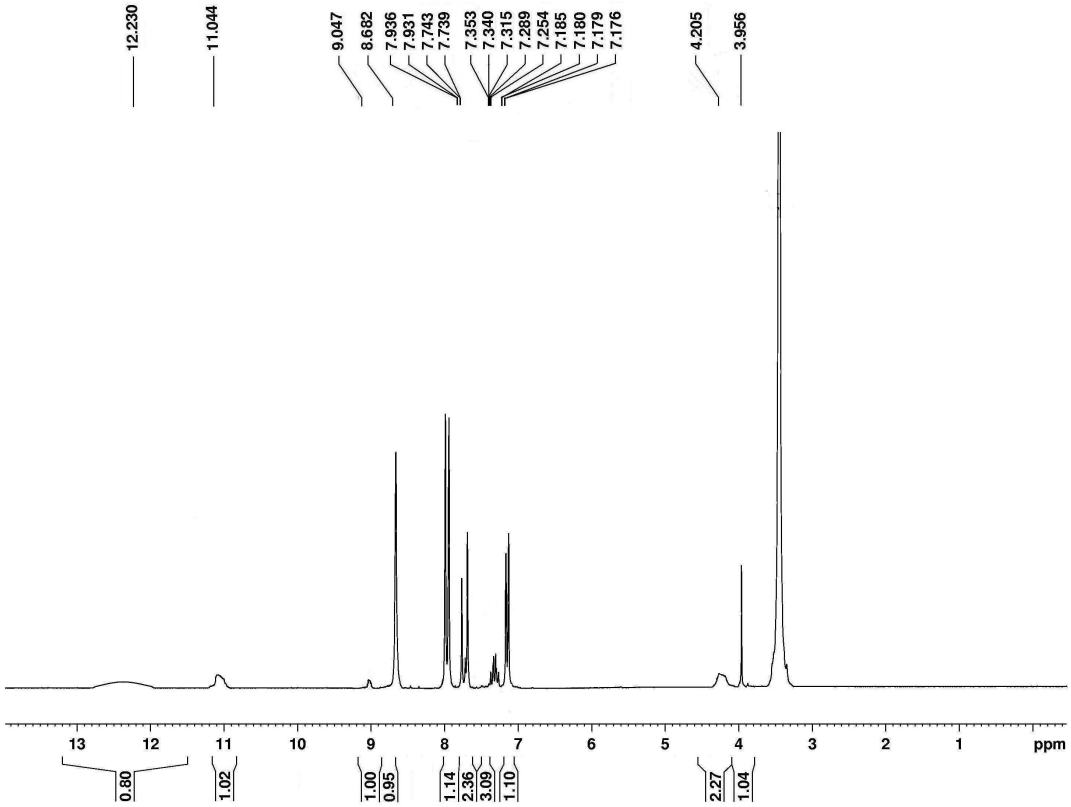
*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indole-3-sulfonamide (**7c**)*



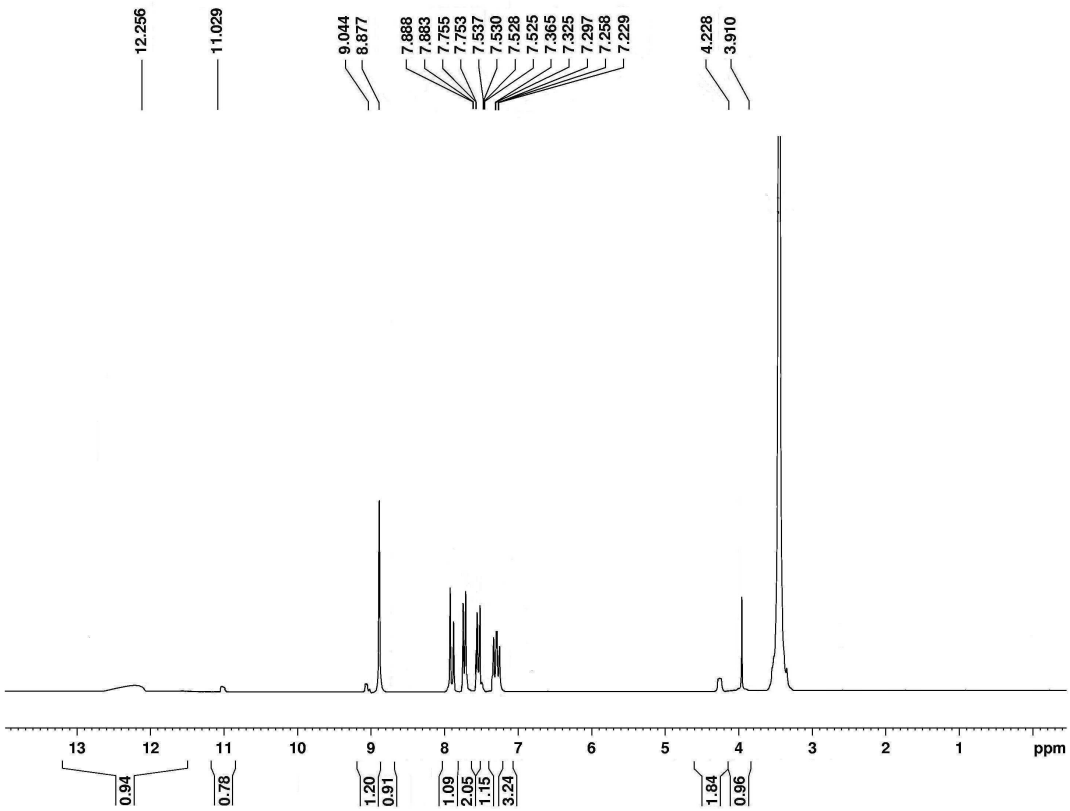
*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indole-3-sulfonamide (**7d**)*



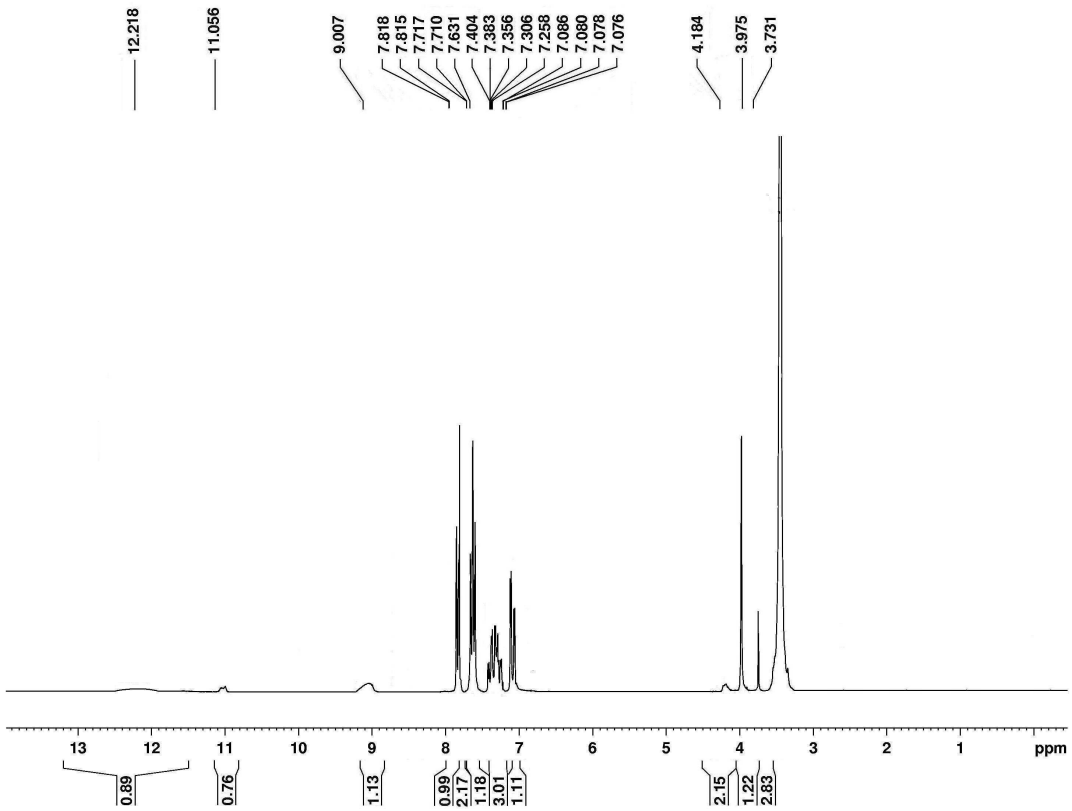
*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-carboxylic acid (**8b**)*



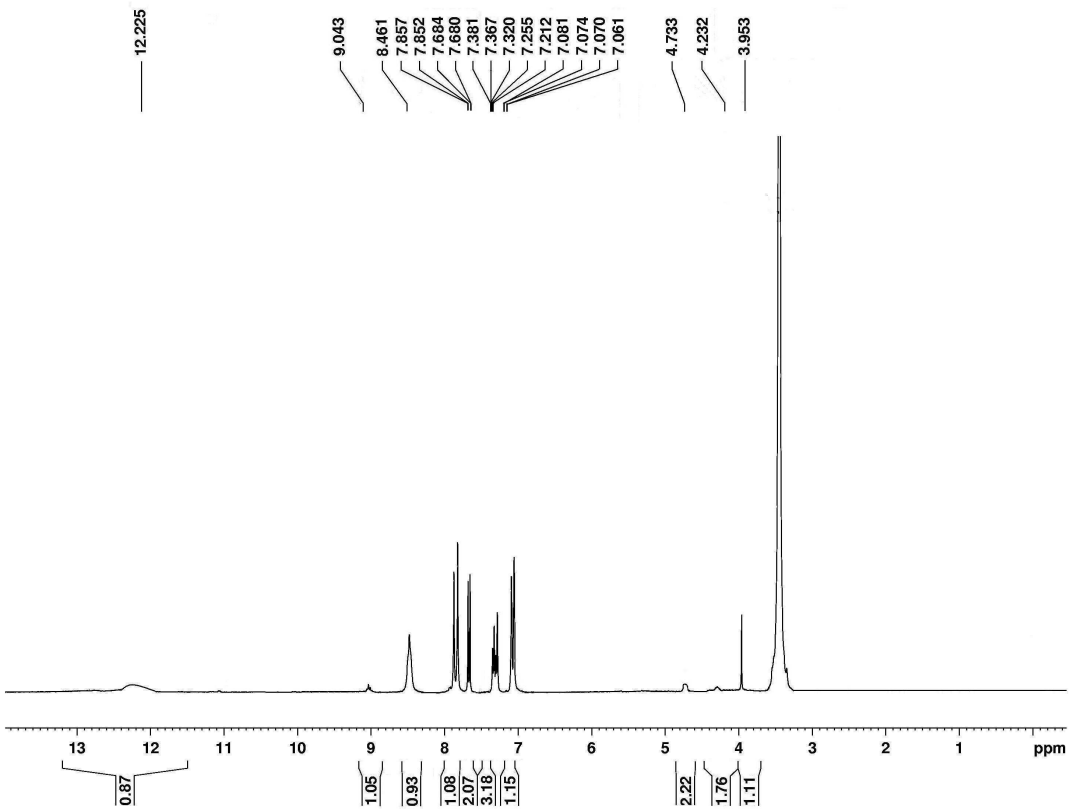
*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-nitro-2-phenyl-1H-indole-3-carboxylic acid (**8c**)*



*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-2-phenyl-5-(trifluoromethyl)-1H-indole-3-carboxylic acid (**8d**)*



*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-methoxy-2-phenyl-1H-indole-3-carboxylic acid (**8e**)*



*1-(5-amino-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-5-fluoro-2-phenyl-1H-indole-3-carboxamide (**9b**)*