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Original article

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

Priya Ahuja, Nadeem Siddiqui*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

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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 s) 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.

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 [7,11], refractory epileptic population [12], 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 [13,14]. It encourages the development of new antiepileptogenic [15] 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) [16].

The indole ring has been found to be an integral nucleus in the past having diverse biological activities such as anticancer, anti-epileptic, anthelminthic, antidepressant, anti-inflammatory, anti-hypertensive, carbonic anhydrase inhibitors etc [14,17–22]. 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 [23,24]. 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) [25,26]. 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 [27]. 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 [28]. The C-3 vinyl, acetyl, sulphonamide, carboxylic and carboxamide substituents manifest the necessary structural features of the prevalent AED's [29–31]. They have also been ascertained as an essential substituent in many previously synthesized

* Corresponding author.
E-mail address: nadeems_03@rediffmail.com (N. Siddiqui).

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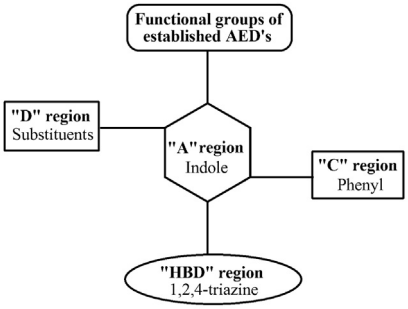
anticonvulsants [32–34]. These elements contribute as essential parameters to the pharmacophoric elements beside the lipophilic aryl ring, hydrogen bonding domain of the triazine and distal aryl ring responsible for antiepileptic activity (Fig. 1) [35,36]. The structural considerations of the indole 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 chloroacetylchloride 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 [37]. 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}H NMR, ^{13}C NMR, IR and mass spectral data.



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

Fig. 1. Pharmacophoric representation of designed molecules.

2.2. Pharmacology

The initial evaluations for anticonvulsant activity were performed based on the Anticonvulsant drug development program, Epilepsy Branch Neurological Disorders Program, NINDS [38]. 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 [39]. The subcutaneous pentylenetetrazole (scPTZ) test provided the clinically effective compounds against absence seizures besides elevating seizure threshold [40]. The destitute from toxicity for the active compounds was established using the minimal motor impairment-rotarod test [41]. 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 [42]. The negative 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