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Original article

Synthesis, anticonvulsant and anti-inflammatory studies of new 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones

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ABSTRACT

The present work involves design and synthesis of new substituted 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones (**4a**–**s**, **5a**–**h**), starting from 4-hydroxybenzaldehyde. The final compounds were screened for their *in vivo* anticonvulsant activity by MES, scPTZ and 6 Hz methods, while their anti-inflammatory screening was performed by Carrageenan induced Paw Edema method. The results indicated that compounds carrying electron donating groups are anticonvulsant active, while most of the tested compounds exhibited significant anti-inflammatory activity. Compounds **4k**, **l**, **4p**–**s**, and **5c** showed rapid anti-inflammatory activity within 30 min and appeared as lead compounds. Further, Neurotoxicity study revealed that all the tested compounds are non-toxic up to 300 mg/kg doses. Selected compounds were also subjected to analgesic screening following Tail immersion method and they exhibited good activity.

1. Introduction

Epilepsy is a major neurological disorder that accounts for 1% of global burden of disease and affects more than 50 million people, all over the world [1]. Even though epilepsy is known to mankind for several thousands of years, rapid advances both in diagnosis and management have been made only in last few decades. Presently, there are many anticonvulsant drugs are available in the market that deals with control of disease. Nevertheless, about 30% of patients are pharmaco-resistant to available drugs and consequently, these patients experience uncontrolled seizures [2]. Moreover, present antiepileptic treatment demands continuous medication for years together that brings about many side effects [3]. According to literature, some of the available active anti-epileptic drugs do not link with any binding site of the receptor [4], where as many other drugs show their effect via different mechanism of actions [5]. As a result, identification of new therapeutic agents that are devoid of any side effects, with a known mechanism of action has become an active area of research, today in medicinal chemistry.

Dihydropyridines (DHPs) are the largest and most studied calcium channel blockers. In addition to their clinical utility as Ca^{2+} channel blockers, they are also used extensively as tools for study of voltage activated calcium channel structure and function [6]. It is well established that calcium is an important factor responsible for induction of convulsive seizures [7]. As a result, Ca^{2+} channel blockers are significant in controlling the convulsion [8]. In this context, many DHPs are well documented in literature as potential anticonvulsant agents [9,10]. Further, literature reports support that DHP derivatives are potent anti-inflammatory agents [11–13]. Interestingly, they were found to bind adenosine receptors (A_1, A_2, A_3) effectively in the brain and bring about therapeutic effects [14]. In fact, A_3 adenosine receptor antagonists are being sought as potential anti-inflammatory agents [15]. In our previous study [16] also, good anti-inflammatory activity was observed for new DHPs carrying amide pharmacophore. This clearly shows that DHP is a suitable heterocyclic scaffold for the development of new antiepileptic and anti-inflammatory agents.

Hydrazones containing azomethine (–NHN=CH) protons constitute a vital class of compounds for new drug development

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[17]. Several heterocyclic hydrazones were reported to possess various biological activities viz. anticonvulsant [18], anti-inflammatory [19,20], analgesic [21], anti-candida [22], anti-tubercular [23,24], antimicrobial [25], anticancer [26], anti-proliferative [27] and antiamoebic [28] activities. Particularly, hydrazone moiety attached to heterocyclic systems was shown to offer enhanced activity [29]. Interestingly, aryl hydrazones with terminal electron donating groups possess enhanced hydrogen bonding capabilities which influence their biological activity significantly [30]. Against this background, it has been planned to design and synthesize new dihydropyridine derivatives carrying aromatic hydrazone as an important pharmacophore at C_4 position of DHP through an aryl linker, and to investigate their anticonvulsant as well as anti-inflammatory properties. Further, most of the reported NSAIDs are exhibiting both anti-inflammatory and analgesic activities. Since their mode of actions are almost same, it has been also contemplated to screen selected target compounds of the present work for *in vivo* analgesic property.

A detailed literature reports on structure–activity relationship study of 1,4-DHP system revealed that unsubstituted free NH group in DHP ring is crucial for its better activity with respect to any medicinal property. Further, presence of methyl groups at 2nd and 6th positions, ester groups at 3rd and 5th positions and an aryl ring at 4th position are essential structural features for prominent biological effect [31,32]. Based on these observations, new dihydropyridine derivatives carrying azomethine group have been designed as shown in Fig. 1. Different aryl rings with various substituents have been incorporated in our new design in order to study the effect of substituents on their pharmacological activity.

2. Results and discussion

2.1. Chemistry

All the intermediates and target compounds **4a**–**s** and **5a**–**h** were synthesized according to Scheme 1. Required dihydropyridine derivative **1** was constructed following Hantzsch method, from 4-hydroxybenzaldehyde by refluxing it with two equivalents of ethyl acetoacetate and ammonium acetate under ethanol medium. The hydroxyl group was alkylated with ethyl chloroacetate in DMF medium under nitrogen atmosphere to obtain the product **2**. Under similar mild conditions, only phenolic OH group, but not NH group of DHP ring undergoes alkylation. The resulting ester **2** was converted to its hydrazide **3** through nucleophilic substitution reaction by refluxing it with hydrazine hydrate in ethanol for about 4 h. Interestingly, stable ester groups on DHP ring remained intact under this condition. The target hydrazones **4a**–**s** and **5a**–**h** were obtained by condensing hydrazide **3** with various aldehydes and ketones, in ethanol medium with trace of conc. sulphuric acid as catalyst.

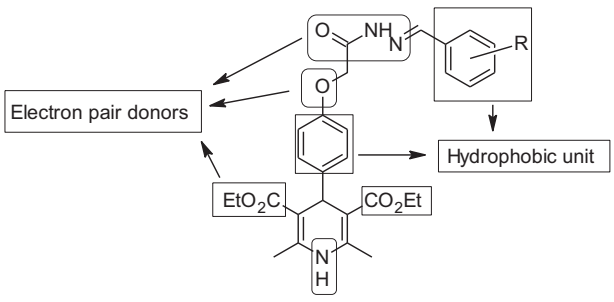

Fig. 1. Design of new dihydropyridine derivatives.

Newly synthesized target compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques followed by elemental analysis. Formation of DHP ring was confirmed by FTIR spectrum of **1**, where it showed prominent peaks at 3337 cm^{-1} and 1656 cm^{-1}, due to NH/OH and ester groups, respectively. This was further confirmed by its ^{1}H NMR spectrum, where it displayed singlets at δ 9.09 and δ 8.77 ppm, which are attributed to phenolic OH and NH proton of DHP ring, respectively. The presence of two ester groups were confirmed by its ^{1}H NMR spectrum, wherein it showed multiplet and triplet at δ 4.04 and δ 1.15 ppm, respectively for ester OCH_2CH_3 group. Appearance of a singlet at δ 4.74 ppm that corresponds to C_4 CH proton further confirms the proposed structure of the product. In ^{1}H NMR spectrum of compound **2**, disappearance of OH peak at δ 9.09 ppm clearly established that phenolic hydroxyl group was alkylated but not NH group of DHP ring. In FTIR spectrum of hydrazide **3**, shifting of carbonyl stretching frequency from 1739 cm^{-1} to lower frequency 1656 cm^{-1} indicated the formation of **3**. Further, its ^{1}H NMR spectrum displayed two singlets at δ 9.24 and δ 4.28 ppm confirming the presence of hydrazidic NH and NH_2 groups. Formation of various hydrazones **4a**–**s** and **5a**–**h** from hydrazide **3** was evidenced by their FTIR and ^{1}H NMR spectra. ^{1}H NMR spectrum of **4a** showed a new peak at δ 7.89 ppm which corresponds to –N=CH– proton, in the place of a singlet at δ 4.28 ppm confirming its structure. Further, in ^{1}H NMR spectrum of **5a** appearance of new peak at δ 2.31 ppm which corresponds to allylic methyl group, is the proof for its formation. Similar pattern of peaks were observed for remaining compounds of the series. Furthermore, structures of all the final compounds were confirmed by their ^{13}C NMR, mass spectral and elemental data.

2.2. Biological results

2.2.1. Anticonvulsant and toxicity studies

The preclinical discovery and development of a new bioactive chemical entity for the treatment of epilepsy depends heavily on the use of predictable animal models. The maximal electroshock (MES) [33] and subcutaneous pentylenetetrazole (scPTZ) [34] screening methods are the two important and routinely used *in vivo* animal models for the anticonvulsant studies [35]. They are claimed to detect new bioactive chemical entities affording protection to generalized tonic–clonic seizures and generalized absence seizures, respectively. Further, Rotarod method [36] is an effective route for detecting motor impairment of the new compounds. In this context, we also screened newly synthesized molecules following these methodologies. Amongst twenty seven new derivatives, only eight derivatives exhibited antiepileptic activity in MES method, while all the derivatives were found to be inactive in scPTZ method. The screening results of active target compounds are summarized in Table 1. Additionally, the MES active compounds were further screened by 6 Hz method [37] and these results are summarized in Table 2. These animal studies were performed in accordance with the ethical standards on animal experimentations.

The screening results clearly show that the hydrazone group carrying an electron rich aryl moiety is an essential structural feature for good anticonvulsant activities. The active compounds **4e**, **4i**–**k**, **4p**, **5c**, **5f** and **5h** exhibit their activities 4 h post i.p. injection of test samples, where as they are inactive at 0.5 h. Amongst arylhydroxy derivatives, compound **4e** containing 4-hydroxy substituent display prominent activity at 100 mg/kg dose. However, its ortho-hydroxyphenyl analogue **4f** is inactive even at high test dose of 300 mg/kg. It may be due to the steric hindrance offered by the ortho hydroxyl group on aryl ring, which can result in poor interaction of pharmacophore with the receptor. Similarly, 2,4-dihydroxyphenyl derivative **4q** does not show any activity. Based on these observations, it can be concluded that a p-hydroxyphenyl