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Synthesis, *in vitro* β-glucuronidase inhibitory activity and *in silico* studies of novel (*E*)-4-Aryl-2-(2-(pyren-1-ylmethylene)hydrazinyl)thiazoles

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ABSTRACT

Current research is based on the synthesis of novel (*E*)-4-aryl-2-(2-(pyren-1-ylmethylene)hydrazinyl)thiazole derivatives (**3**–**15**) by adopting two steps route. First step was the condensation between the pyrene-1-carbaldehyde (**1**) with the thiosemicarbazide to afford pyrene-1-thiosemicarbazone intermediate (**2**). While in second step, cyclization between the intermediate (**2**) and phenacyl bromide derivatives or 2-bromo ethyl acetate was carried out. Synthetic derivatives were structurally characterized by spectroscopic techniques such as EI-MS, ^{1}H NMR and ^{13}C NMR. Stereochemistry of the iminic double bond was confirmed by NOESY analysis. All pure compounds **2**–**15** were subjected for *in vitro* β-glucuronidase inhibitory activity. All molecules were exhibited excellent inhibition in the range of IC_{50} = 3.10 ± 0.10–40.10 ± 0.90 μM and found to be even more potent than the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 μM). Molecular docking studies were carried out to verify the structure-activity relationship. A good correlation was perceived between the docking study and biological evaluation of active compounds.

1. Introduction

Pyrene is a symmetrical, tetracyclic aromatic hydrocarbon having 16 π electrons. It is usually obtained by the combustion of organic compounds [1,2]. Pyrene has attracted considerable attention due to its fluorescent behavior as well as its ability to monitor numerous functions and processes on a microscopic level [3]. Fluorescent behavior of pyrene usually utilized for the preparation of imaging agents and sensors [4–6]. Pyrene has unique properties such as comparatively long lifetime and well-defined vibronic emission bands which is broadly used as a fluorescent probe [7,8]. Pyrene nucleus incorporated with the heterocycle and sugar moieties, were reported to have antiviral activities against HIV-1 and HSV-1 [9]. Specifically, benzo[*a*]pyrene (BaP) is reported to be a carcinogen present in the environment such as in food and the workplaces [10]. However, reports regarding the enzyme inhibition activities of pyrene based compounds are difficult to find.

Compounds containing atoms like sulfur and nitrogen are mainly used in the medical applications for the treatment of gastric ulcer, cancer and microbial infections, etc. [11,12]. Sulfur atom has capability to interact with the receptors which results in higher efficiency against various diseases [13,14]. The thiazole core are present in several biologically active pharmacophores and natural products, like thiamine (Vitamin-B). It is also a part of antibiotics such as micrococcins and penicillins [15]. Some of the active drugs available in the market bearing the thiazole moiety like ritonavir [16,17], fanetizole and meloxicam [18,19]. Some of the chiral thiazole based motif act as histone acetyltransferase inhibitors [20]. Thiazoles are continuously receiving interest due to various pharmacological activities such as antiinflammatory, antibacterial, antifungal, analgesic, anthelmintic, antitubercular, anticancer and central nervous system (CNS) stimulate [21–30]. More recently, thiazoles also been found to be of interest in materials sciences [31].

β-Glucuronidase enzyme (EC 3.2.1.31, exoglycosidase) catalyzes the breakage of glucuronosyl-*O*-bonds and found in anaerobic *Escherichia*, *Bacteroides*, *Clostridia* and *Peptostreptococcus* genera. This enzyme belongs to hydrolases class and present in lysosomes

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and microsomes. It is found in many human organs and body fluids such as bile, kidney, spleen, lungs, muscle, serum, gastric juice and urine [32,33]. Activity of β-glucuronidase increases in some pathologies such as cancer, inflammation in joints, hepatic diseases, renal diseases, urinary tract infection, AIDS, epilepsy, transplantation rejection, neoplasm of bladder, breast, larynx and testes [34,35]. Therefore, it is an upmost task to inhibit this enzyme in order to cure several pathological condition.

Our research group had already reported many heterocycles such as thiadiazole, thiazole, oxadiazole, benzimidazole, benzothiazole, pyrimidone, *bis*-indole and coumarin [33–41] as potential leads for β-glucuronidase inhibitory activity (Fig. 1).

In the light of our previous work regarding the search for potential leads for β-glucuronidase inhibition (Fig. 1), we intend to merge the active pharmacophore such as thiazole with the molecule which is infrequently reported for enzyme inhibitory activity. So, by keeping in mind some reported biological potential of pyrene nucleus, we tried to install the thiazole moiety which has frequently reported for the β-glucuronidase inhibition. Thus, this article describes the synthesis of novel scaffold (hybrid of pyrene and thiazole ring) **2**–**15** and their β-glucuronidase inhibitory activities. To the best of our knowledge, all synthetic compounds were never reported before structurally as well as for β-glucuronidase inhibitory activity (Fig. 2).

2. Results and discussion

2.1. Chemistry

Synthesis of (*E*)-4-aryl-2-(2-(pyren-1-ylmethylene)hydrazinyl) thiazole derivatives was carried out by the condensation of pyrene-1-carbaldehyde (**1**) with thiosemicarbazide in the presence of glacial acetic acid as catalyst to afford thiosemicarbazone derivative **2**. Than thiosemicarbazone derivative **2** undergo cyclization reaction with a variety of phenacyl bromide in the presence of triethylamine to afford thiazole ring with different R groups (Scheme 1). Completion of reaction was monitored by periodic TLC. All newly synthesized compounds **2**–**15** were structurally confirmed by EI-MS, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

2.2. Characteristic spectral feature of representative compound **8**

^{1}H- and ^{13}C NMR spectra of compound **8** were recorded in deuterated DMSO-d_6 on a 400 MHz machine. In ^{1}H NMR spectrum, acidic NH proton was resonated as the most downfield signal at δ_H 12.55 and appeared as broad singlet. Downfield signal was due to the extensive conjugation of lone pair of nitrogen. Characteristic signal of methine proton HC═N— was resonated at δ_H 9.06 as a sharp singlet. Another characteristic signal of H-5′ was resonated at δ_H 7.74 and appeared as singlet in the aromatic region which confirmed the formation of the desired product. Protons of the pyrene ring and aryl ring (R) were appeared in the aromatic region δ_H 7.75–8.77 (Fig. 3).

^{13}C NMR broad-band decoupled spectrum (100 MHz, DMSO-d_6) showed total 26 signals of carbon including eleven quarternary carbon and fifteen methine carbons. The quaternary carbon C-2′ was appeared at δ_C 171.6 as the most downfield signal due to adjacent electronegative atoms (N, N ans S). Quaternary C-4′ was appeared as deshielded signal at δ_C 150.3 due to directly attached nitrogen atom. Similarly, iminic carbon (C═N) was resonated at δ_C 143.4 as the deshield signal due to doubly bonded nitrogen atom. Carbons of pyrene ring and aryl ring (R) were appeared in the usual aromatic region of δ_C 122.6–148.5. (*E*) stereochemistry of the imine group (C═N) was confirmed by observing the NOESY interaction between the NH and iminic proton (H-C═N) (Fig. 4).

The EI-MS of compound **8** showed the molecular ion peak $[M]^+$ at *m*/*z* 448 which corresponds to the molecular weight of the desired compound. However, the fragmentation observed in the spectrum was deduced by cleavage of molecular ion in three ways (Fig. 5).

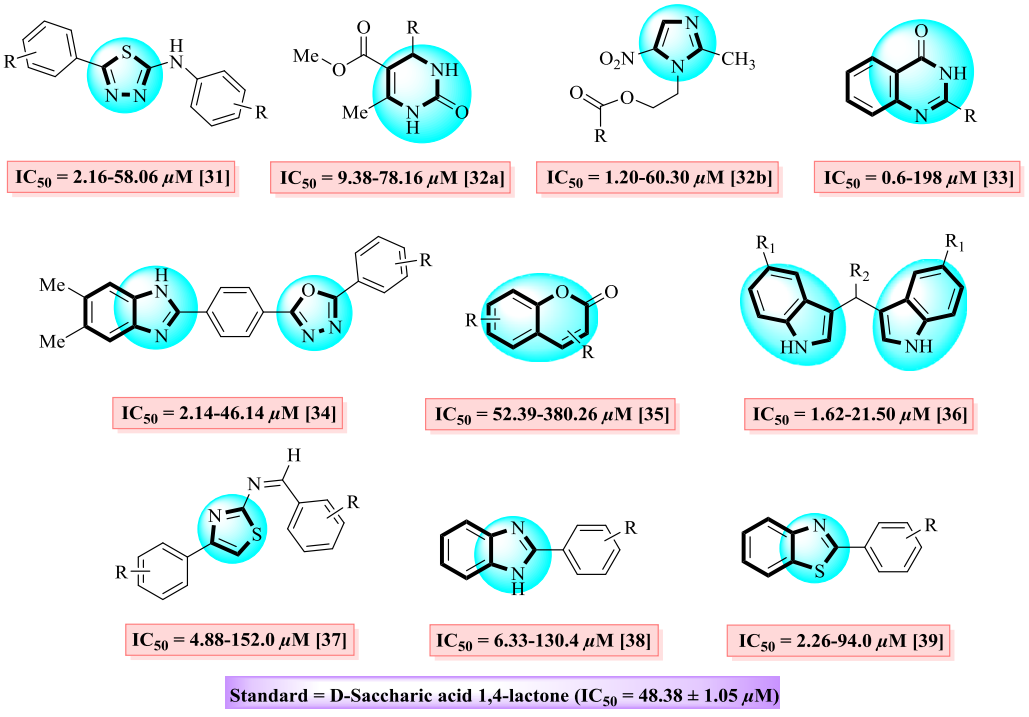

Fig. 1. Identified leads for β-glucuronidase inhibition.