2-(2'-Pyridyl) Benzimidazole Analogs and their ß-Glucuronidase Inhibitory Activity

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Summary: Synthesis of 2-(2'-pyridyl) benzimidazole analogs **1-11** have been carried out and evaluated for *in vitro* ß-glucuronidase inhibitory potential. The compounds **4** (IC_{50} = 4.06 ± 0.34 μM), **5** (IC_{50} = 09.63 ± 0.81 μM), **1** (IC_{50} = 19.66 ± 0.44 μM), **7** (IC_{50} = 24.75 ± 0.25 μM), **6** (IC_{50} = 26.30 ± 1.37 μM), and **3** (IC_{50} = 32.11 ± 0.89 μM), showed ß-glucuronidase inhibitory activity superior to the standard D-saccharic acid 1,4-lactone, with (IC_{50} = 48.4 ± 1.25 μM). Based on structure-activity relationship, we discover a new class of potent ß-glucuronidase inhibitors.

Keywords: 2-(2'-Pyridyl) benzimidazole, ß-glucuronidase inhibition, glucuronosyl-*O*-bonds,

Introduction

Benzimidazole has been use as a carbon skeleton for *N*-heterocyclic carbenes. They are mostly used as ligands for metal complexes. They are often synthesized by the deprotonation with a base of *N*,*N*'-disubstituted benzimidazolium salt at 2-position [1-2]. The widely used method for this synthesis is the condensation of an *o*-arylenediamine with carbonyl compounds [3]. Many reports on the use of different reducing agents such as triethyl phosphite, triruthenium dodecacarbonyl and Se in the presence of carbonyl and $Na_2S_2O_4$ in both conventional conditions and on microwave irradiation and found that most of these methods required more amount of reducing agents than calculated by the stoichiometry of the reactions, use of carbon mono oxide and strong acidic conditions limit the reactions [4-9]. Later on, the synthesis of benzimidazole from *N*-substituted 2-nitroaniline on hydrogenation by using palladium catalyst was also reported [10]. Benzimidazoles are well known for their biological properties [11-12]. Compounds belonging to this class are commercially used as therapeutic agents for decades such as analgesic [13], anti-inflammatory [14], anticonvulsant [15], anticancer [16], antihepatitis [17], antihypertensive [18], antiviral [19], antifungal [20], antimicrobial [21-22], and antioxidant [23].

Glucuronidation is one of the main conjugation reactions responsible or converting lipophilic xenobiotics and endogenous compound into metabolites that are more water soluble and thus, more readily excreted in the urine or bile. Conjugation with glucuronic acid is catalyzed by UDP-glucoronosyl-transferases (UGTs) [24]. As a result of conjugation the activation of many compounds and is not limited to drug but also to environmentally toxic chemicals, carcinogens, steroid hormone, bile acids, and bilirubin [24]. Severe deficiency of this enzyme in accumulation of un-degraded glycosaminoglycans in the lysosomes and produces the disease mucopolysacchridosis type VII [25].

Our group is engaged in the search of new ß-glucuronidase inhibitors since a decade [26-27]. We recently reported also worked on benzimidazoles and concluded that this moiety posses excellent ß-glucuronidase inhibition potential [28]. In view of the biological significance of benzimidazoles it was of immense interest to synthesize and evaluate new derivatives of benzimidazoles against ß-glucuronidase enzyme. Herein, we describe a strategy towards convenient synthesis of some new derivatives of 2-(2'-pyridyl) benzimidazole (**1-11**). The structures of synthetic derivatives were elucidated by different spectroscopic techniques including, UV, IR, EI-MS and ^{1}H-NMR. All compounds gave satisfactory elemental analysis and the observed percentage of carbon and hydrogen atoms was found in good agreement with the

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calculated values [29]. The synthetic compounds were subjected for screening against β-glucuronidase enzyme; a structure-activity relationship is also established.

Experimental

Reagents were purchased from Sigma/Aldrich Chemical Company, USA. All the solvents were of analytical grade from E. Merck and were purified by a standard method. Thin layer chromatography was monitored by using pre-coated silica gel, GF-254. Spots were visualized under UV at (254 nm and 366 nm) or iodine vapors. All M.P were determined on GallenKamp apparatus and are uncorrected. UV spectra were recorded in methanol on Shimadzu UV/Visible 1601 spectrophotometer. Infrared (IR) spectra were carried out on Avatar 330 FTIR Thermo Nicolet Spectrometer. Mass spectra were determined under electron impact (EI) condition using Varian Massen spectrometer MAT 312, MAT 113D MASPEC system. The ^{1}H-NMR measurements were performed on a Bruker AM spectrometer operating at 400 MHz. The chemical shift values are displayed in ppm (δ), comparative to tetramethyl silane as an internal standard and in Hz coupling constant (J).

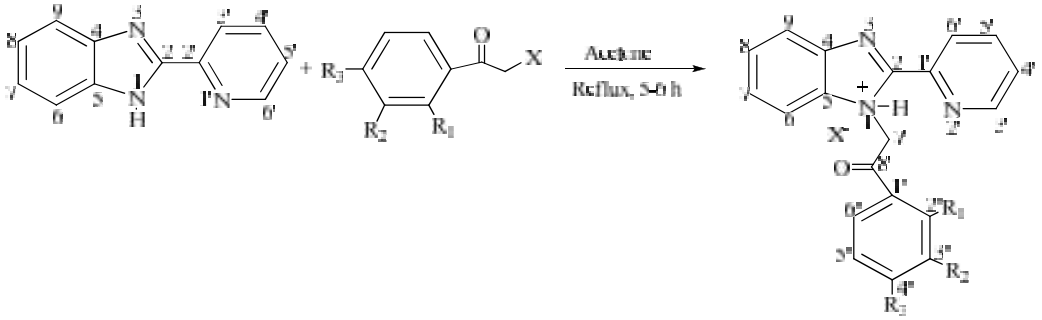
General Procedure for the Synthesis of Compounds **1-11**

2-(2'-Pyridyl) benzimidazole and the corresponding substituted phenacyl halide in equimolar quantities (0.01 mol) were dissolved separately in acetone (20 mL) and mixed together in a round-bottomed flask (Scheme-1). Reaction mixtures were refluxed on a water bath for about 5-6 h. The reaction progress was monitored through TLC in $CHCl_3$-MeOH (in varying proportions) solvent system. The precipitates were filtered after completion of reaction, and washed with warm acetone. The products thus obtained were re-crystallized from methanol. The pure compounds were dried in vacuum over anhydrous calcium sulphate. The structures of synthetic compounds **1-11** were determined through physical and spectral data [29].

Results and Discussion

Chemistry

2-(2'-Pyridyl) benzimidazole and the corresponding substituted phenacyl halide in equimolar quantities (0.01 mol) are separately dissolved in acetone (20 mL) and mixed together in a round-bottomed flask. Reaction mixtures were refluxed on a water bath for about 5-6 h. The reaction completion was monitored through TLC in solvent system chloroform: methanol (in varying proportions). Solid precipitate formation indicates the completion of reaction which was confirmed by TLC. The solid was washed with warm acetone and products thus obtained were re-crystallized from methanol Scheme-1. The pure compounds were dried in vacuum over anhydrous calcium sulphate. All the compounds **1-11** were deduced by using different spectroscopic techniques, including UV, IR, ^{1}H NMR and EI mass spectroscopy. All compounds gave acceptable CHN analysis [29].



Compound	R_1	R_2	R_3	X
1	**H**	**NO_2**	**H**	**Br**
2	**H**	**OH**	**OH**	**Cl**
3	**H**	**H**	**Cl**	**Br**
4	**NO_2**	**H**	**H**	**Br**
5	**H**	**H**	**NO_2**	**Br**
6	**H**	**H**	**F**	**Br**
7	**F**	**H**	**F**	**Br**
8	**H**	**H**	**H**	**Br**
9	**H**	**H**	**C_6H_5**	**Br**
10	**H**	**H**	**OCH_3**	**Br**
11	**OCH_3**	**H**	**OCH_3**	**Br**

Scheme-1: Synthesis of 2-(2'-pyridyl) benzimidazole derivatives **1-11**.

Biological Activity

β-Glucuronidase belong to the glycosidase family of enzymes which catalyzes the breakdown of complex molecules of carbohydrates. Enzyme inhibition is one of the most significant tools in pharmaceutical research as well as in the field of drugs discovery. During this study, we have synthesized eleven (11) 2-(2'-pyridyl) benzimidazole moieties and evaluated their *β*-glucuronidase enzyme inhibition activity. Six (6) compounds showed a potent inhibition superior to standard inhibitor of *β*-glucuronidase, while the remaining compounds showed good *β*-glucuronidase inhibition.

Compounds **1-11** exhibited a varying degree of *β*-glucuronidase inhibitory activity with IC_{50} values between 4.06 - 97.45 μM when compared with standard D-sacharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25). Compounds **4** (IC_{50} = 4.06 ± 0.34 μM), **5** (IC_{50} = 09.63 ± 0.81 μM), **1** (IC_{50} = 19.66 ± 0.44 μM), **7** (IC_{50} = 24.75 ± 0.25 μM), **6** (IC_{50} = 26.30 ± 1.37 μM), and **3** (IC_{50} = 32.11 ± 0.89 μM), showed excellent ⇦-glucuronidase inhibitory activity higher than the standard. Moreover compounds **2** (IC_{50} = 80.46 ± 0.26 μM), **8** (68.48 ± 0.21 μM), **9** (65.24 ± 0.32 μM), **10** (89.25 ± 0.28 μM), **11** (97.45 ± 0.41 μM) showed encouraging potential.

Limited SAR studies of the synthetic compounds revealed that the activity is markedly dependent on the mesomeric and inductive effects of various substituent presents in the phenacyl moiety (Table-1). The nitro group which has –I and -M effect showed very significant inhibitory potential against both the enzyme as in compounds **1** (IC_{50} = 19.66 ± 0.44 μM), **4** (IC_{50} = 4.06 ± 0.34 μM), and **5** (IC_{50} = 09.63 ± 0.81 μM), respectively, which demonstrated that ortho substitution of nitro group strongly influence the inhibitory activity of the compound **4**. The decrease in activity was observed by changing the position of nitro group phenyl ring of phenacyl moiety. Replacement of nitro group with fluorine demonstrated a decline in inhibitory activity due to –I but +M effect of the fluorine atom as in compounds **6** and **7**. Slight difference in the activities of compounds **6** (IC_{50} = 26.30 ± 1.37 μM) and **7** (IC_{50} = 24.75 ± 0.25 μM) may be due the number of fluorine atoms on phenyl ring of phenacyl residue. It was observed that inhibition was further declined by replacing flouro with chlorine as in compound **3** (IC_{50} = 32.11 ± 0.89 μM). It may due lesser electron withdrawing inductive ability of chlorine as compared to fluorine. The presence of phenyl group at *para* position of phenacyl group as in compound **9** (65.24 ± 0.32 μM) and its unsubstituted analogue **8** (68.48 ± 0.21 μM) showed moderate activities. This activity difference may be due to substitution effects. The activity is significantly reduced due to presence of hydroxyl and methoxy group which have strong +M effect as in compounds **2** (IC_{50} = 80.46 ± 0.26 μM), **10** (89.25 ± 0.28 μM), and **11** (97.45 ± 0.41 μM), respectively.

Table-1: In *vitro* *β*-glucuronidase activity of 2-(2'-pyridyl) benzimidazole derivatives.

Compound	IC_{50} ± [a]S.E.M (µM)
1	**19.66 ± 0.44**
2	**80.46 ± 0.26**
3	**32.11 ± 0.89**
4	**4.06 ± 0.34**
5	**9.63 ± 0.81**
6	**26.30 ± 1.37**
7	**24.75 ± 0.25**
8	**68.48 ± 0.21**
9	**65.24 ± 0.32**
10	**89.25 ± 0.28**
11	**97.45 ± 0.41**
D-Sacchric acid 1-4-lactone	**48.40 ± 1.25**

[a] S.E.M. = standard error of the mean

From the above data it is concluded that compounds **1, 3, 4, 5, 6** and **7** can serve as potential lead compound for further research in the field of *β*-glucuronidase enzyme inhibition.

Biological Assay

From *p*-nitrophenyl-*β*-D-glucuronide and bovine liver, G-0251) (N-1627) the ⇦-glucuronidase (E.C. 3.2.1.31, were buy from Sigma Chemical Co. (U.S.A.). Na_2CO_3 and other reagents of analytical grade were procured from E. Merck. EtOH and Anhydrous $CHCl_3$ were dried by using standard methods. All other solvents and reagents used are of reagent grad, the distilled benzoyl chloride was used.

Assay for β- Glucuronidase

⇦-D-Glucuronidase activity was determined through measuring the absorbance of *p*-nitrophenol at 405 nm which is formed from the substrate. 250 ↘L was the total reaction volume. The reaction mixture comprised of 185 ↘L of 0.1 M acetate buffer, 5 ↘L of test compound solution, and 10 ↘L of enzyme, was incubated at 37 ¯C for 30 min. The plates were read on a multiplate reader (SpectraMax plus 384, U. S. A.) at 405 nm after the addition of 50 ↘L of 0.4 mM *p*-nitrophenyl-*β*-D-glucuronide. All assays were carried out in triplicate [28].

Conclusions

Present study opens-up an avenue for further research on 2-(2'-pyridyl) benzimidazole derivatives for their evaluation against *β*-glucuronidase enzyme. Compounds **1, 3, 4, 5, 6** and **7** provide a solid base for using them as lead molecules.

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