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Synthesis of 6-chloro-2-Aryl-1H-imidazo[4,5-b]pyridine derivatives: Antidiabetic, antioxidant, β-glucuronidase inhibiton and their molecular docking studies

Muhammad Taha [a,b,*], Nor Hadiani Ismail [a,b], Syahrul Imran [a,b], Hesham Rashwan [c], Waqas Jamil [d], Sajjad Ali [e], Syed Muhammad Kashif [d], Fazal Rahim [g], Uzma Salar [f], Khalid Mohammed Khan [f]

[a] Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[b] Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia
[c] Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam 42300, Selangor, Malaysia
[d] Institute of Advance Research Studies in Chemical Sciences, University of Sindh Jamshoro, Pakistan
[e] Department of Chemistry, Karakoram International University, Gilgit-Baltistan, Gilgit 15100, Pakistan
[f] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[g] Department of Chemistry, Hazara University, Mansehra 21120, Pakistan

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ABSTRACT

6-Chloro-2-Aryl-1H-imidazo[4,5-b]pyridine derivatives **1**–**26** were synthesized and characterized by various spectroscopic techniques. All these derivatives were evaluated for their antiglycation, antioxidant and β-glucuronidase potential followed their docking studies. In antiglycation assay, compound **2** (IC_{50} = 240.10 ± 2.50 μM) and **4** (IC_{50} = 240.30 ± 2.90 μM) was found to be most active compound of this series, while compounds **3** (IC_{50} = 260.10 ± 2.50 μM), **6** (IC_{50} = 290.60 ± 3.60 μM), **13** (IC_{50} = 288.20 ± 3.00 μM) and **26** (IC_{50} = 292.10 ± 3.20 μM) also showed better activities than the standard rutin (IC_{50} = 294.50 ± 1.50 μM). In antioxidant assay, compound **1** (IC_{50} = 69.45 ± 0.25 μM), **2** (IC_{50} = 58.10 ± 2.50 μM), **3** (IC_{50} = 74.25 ± 1.10 μM), and **4** (IC_{50} = 72.50 ± 3.30 μM) showed good activities. In β-glucuronidase activity, compounds **3** (IC_{50} = 29.25 ± 0.50 μM), compound **1** (IC_{50} = 30.10 ± 0.60 μM) and compound **4** (IC_{50} = 46.10 ± 1.10 μM) showed a significant activity as compared to than standard D-Saccharic acid 1,4-lactonec (IC_{50} = 48.50 ± 1.25 μM) and their interaction with the enzyme was confirm by docking studies.

1. Introduction

Latest study shows that occurrence of type 2 diabetes which represents 90% of all diabetes cases is rapidly raising throughout the world. International Diabetes Federation estimates an increase in number of diabetic patient from 366 million to 552 million by 2030 [1]. Type-2 diabetes causes harmful effects such as production of advanced glycation end products (AGEPs) [2]. Considering this fact, study on new drugs which could reduce production of advanced glycation end products (AGEs) to treat diabetic patient is extremely crucial. Currently, number of efficient antiglycation agent is very few and effort of finding novel inhibitors is still unsuccessful [3]. Plenty of research had been carried out to reverse glycation process using potential new antiglycation agents [4]. It was found that some inhibitors function by splitting AGEPs cross-links which provide the opportunity of reversing diabetic complications [5]. Besides that, AGEPs bind to receptor and induces production of reactive oxygen species (ROS). It causes pleiotropic transcription factor nuclear factor NF-κB to be activated and causes various pathological alterations in gene expression [6]. Besides involving in glycation process, free radicals from hydroxyl, hydrogen peroxide, superoxide anions, peroxyl and lipid peroxides play a significant role in initiating various diseases like respiratory diseases, cardiovascular diseases, gout, cancer, aging, and atherosclerosis [7,8]. Observation also suggests that oxidation is involved in development of diabetic complications. It was found that metabolic abnormalities related to diabetes results in overproduction of mitochondrial superoxide. The increased amount of superoxide anion induces tissue damage. Superoxide anions are also involved in activating various pathways which leads to

* Corresponding author at: Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.
E-mail addresses: taha_hej@yahoo.com, muhamm9000@puncakalam.uitm.edu.my (M. Taha).

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pathogenesis complications and inactivation of antiatherosclerotic enzymes, prostacyclin synthase and eNOS [9].

6-chloro-2-Aryl-1H-imidazo[4,5-b]pyridine and its analogues consist of a versatile heterocyclic pharmacophore which is extremely important in organic chemistry due to their diverse biological activities [10]. Several benzimidazole derivatives had been proven to possess bioactivities such as antiviral [11], antihypertensive [12], vasodilator [13], anticancer [14] antimicrobial [15,16], antioxidant and antiglycation [17]. Benzimidazoles are also found to be potent inhibitors for numerous protein such as urease [18], carbonic anhydrase [19] and tyrosine phosphatase [20].

We have been working on heterocyclic compounds [21–24]. In this communication we are reporting the multiple activity of 6-c hloro-2-Aryl-1H-imidazo[4,5-b]pyridine derivatives **1**–**26**.

2. Result and discussion

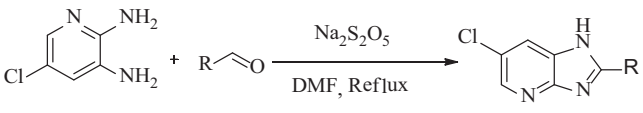
2.1. Chemistry

6-chloro-2-Aryl-1H-imidazo[4,5-b]pyridine **1**–**26** were obtained by reacting together with commercially available 5-chloropyridine-2,3-diamine and different aldehydes in *N*,*N*-dimethylformamide. The products were appeared in significant yields (Scheme 1). In this study, the reaction was carried out by mixing sodium metabisulfite ($Na_2S_2O_5$) with mixture of 5-chloropyridine-2,3-diamine (3.12 mmol) and different substituted aromatic aldehydes (3.16 mmol) in *N*,*N*-dimethylformamide. Reaction mixture was refluxed for 2 h. Upon completion, water (30 mL) was added to the crude reaction mixture to afford solid mass as precipitates, after filtration the solid 6-chloro-2-Aryl-1H-imidazo[4,5-b]pyridine derivatives **1**–**26** were obtained in high yields. The structures of synthetic were confirmed by spectroscopic techniques including ^{1}H NMR and EI MS.

2.2. Antiglycation study

All the synthesized compounds **1**–**26** were evaluated for their antiglycation properties and compared with the standard Rutin (IC_{50} = 294.46 ± 1.50 μM) respectively.

In antiglycation assay, all –OH group containing derivative were found to be active and marked as efficient antiglycating agents. Compound **2** (IC_{50} = 240.10 ± 2.40 μM) and **4** (IC_{50} = 240.30 ± 2.90 μM) was found to be most active compound of this series, while compounds **3** (IC_{50} = 260.10 ± 2.50 μM), **6** (IC_{50} = 290.60 ± 3.60 μM), **13** (IC_{50} = 288.20 ± 3.00 μM) and **26** (IC_{50} = 292.10 ± 3.20 μM) also showed excellent activity. All of these derivatives were found to be better active than the standard Rutin (IC_{50} = 294.50 ± 1.50 μM). Mostly *di*-OH group containing derivatives found be more active than *mono* –OH derivatives but surprisingly 3,4-di –OH compound **1** (IC_{50} = 320.20 ± 3.20 μM) was found to be less active than *di* as well as *mono* –OH group containing derivatives. It was further observed that all compounds which showed better activity than the standard bear at least one *ortho* –OH group, although compound **26** have –OH group at position 4 (*para*) was found to be slight better active than the standard. So the *ortho* position of –OH group is also matter for better antiglycation activity. So, the active compounds have competent capability to bind protein or glucose and inhibit the further progression of glycation. All other derivatives were found to be inactive.



Scheme 1. Synthesis of 6-chloro-2-Aryl-1H-imidazo[4,5-b]pyridine **1**–**26** derivatives.

2.3. Antioxidant potential

In anti-oxidant assay, all the hydroxyl derivatives showed good reducing capability. Among hydroxyl group containing derivatives, compound **1** (IC_{50} = 69.45 ± 0.25 μM), **2** (IC_{50} = 58.10 ± 2.50 μM), **3** (IC_{50} = 74.25 ± 1.10 μM), and **4** (IC_{50} = 72.50 ± 3.30 μM) showed better activity than other molecules. It was observed that all of these most active compounds containing more than hydroxyl group, so the activity is directly related with numbers of hydroxyl group. On comparing these active derivatives, it was found that compound **2**, a 2,4-dihydroxy derivative is most active compound of this series, whereas compound **1**, **3** and **4** also exhibited good anti-oxidant properties. A decline in anti-oxidant activity was observed when one of the hydroxyl was replaced by –H or OCH_3 group, as in case of compound numbers **6** (IC_{50} = 98.10 ± 0.30 μM), **8** (IC_{50} = 145.50 ± 1.90 μM), **13** (IC_{50} = 120.80 ± 1.60 μM), **19** (IC_{50} = 130.30 ± 1.50 μM), and **26** (IC_{50} = 146.20 ± 2.20 μM). It was also seen that OCH_3 group produced more suppressing effect on activity than –H. All of these compound were found be less active than the standard but slight modification in structure may result better anti-oxidant compounds. All other derivatives **5**, **7**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **20**, **21**, **22**, **23**, **24**, **25** were showed no activities (Table 1).

2.4. β-Glucuronidase activity

All the synthesized compounds **1**–**26** were evaluated for β-glucuronidase and compared with standard D-Saccharic acid 1,4-lactone (IC_{50} = 48.50 ± 1.25 μM). In this activity compounds **3** (IC_{50} = 29.25 ± 0.50 μM), compound **1** (IC_{50} = 30.10 ± 0.60 μM) and compound **4** (IC_{50} = 46.10 ± 1.10 μM) showed excellent activity. It was observed that all of these most active compounds contain more than one hydroxyl group, so the activity is directly related with numbers of hydroxyl group. Compound **3**, having 2,3-dihydroxy, compound **1**, having 3,4-dihydroxy and compound **4**, having 2,5-dihydroxy derivatives are most active compounds of this series. Other compound **2** (IC_{50} = 49.60 ± 1.20 μM) and compound **26** (IC_{50} = 52.60 ± 1.60 μM) also showed good activity as when compared with standard D-Saccharic acid 1,4-lactonec. In this series compound **8**, **13** and **19** showed good to moderate β-glucuronidase activity. All other derivatives **5**, **6**, **7**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **20**, **21**, **22**, **23**, **24** and **25** were found to be inactive against β-glucuronidase.

2.5. Docking studies

In this study, molecular docking studies were performed using Human β-D-glucuronidase protein structure to predict favorable binding mode of imidazo[4,5-*b*]pyridine derivatives (**1**–**26**) [25]. Docking studies were done to obtain more insight on how imidazo[4,5-*b*]pyridine derivatives could bind within the active site of β-D-glucuronidase and to validate the experimental results. In this study, X-ray crystal structure of human β-glucuronidase enzyme at 2.6 Å resolution (PDB ID: 1BHG) was employed to further identify binding modes involved in the inhibition activity [25].

Based on previous docking studies on human β-D-glucuronidase, the modeled substrate-bound structure suggests that the glycoside bond of *p*-nitrophenyl β-D-glucuronide is oriented towards the catalytic residues Glu451, Glu540, and Tyr504 (Fig. 1). It has been proposed for human β-D-glucuronidase that during catalysis, Glu451 acts as the acid/base catalyst while Glu540 serves as the nucleophilic residue [25], while Tyr504 was