Benzimidazoles: A New Class of Carbonic Anhydrase Inhibitors

[1]KHALID MOHAMMED KHAN, [1,3]MOMIN KHAN, [1]MUHAMMAD SALEEM, [1,4]MUHAMMAD TAHA, [2]SHAHNAZ PERVEEN AND [1,5]MOHAMMAD. IQBAL CHOUDHARY

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University Karachi, Karachi-75270, Pakistan

[2]PCSIR Laboratories Complex Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan

[3]Department of Chemistry, Abdul Wali Khan University, Mardan 23200, Pakistan

[4]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

[5]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah Saudi Arabia

khalid.khan@iccs.edu*

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Summary: Carbonic anhydrase inhibitory activity of benzimidazole derivatives **1-24** has been evaluated. Compounds **22** (IC_{50} = 7.47 ± 0.39 μM), **21** (IC_{50} = 10.31 ± 0.11 μM), **20** (IC_{50} = 23.1 ± 1.78 μM), **12** (IC_{50} = 12.16 ± 0.10 μM), **11** (IC_{50} = 12. 81 ± 0.29 μM), and **15** (IC_{50} = 13.18 ± 0.37 μM) showed carbonic anhydrase inhibitory activity, and compared with the standard acetozolamide (IC_{50} = 0.31 ± 0.0006 μM). All the compounds were characterized by spectroscopic techniques.

Keywords: Benzimidazole, carbonic anhydrase inhibition, phenylenediamine, aromatic aldehydes

Introduction

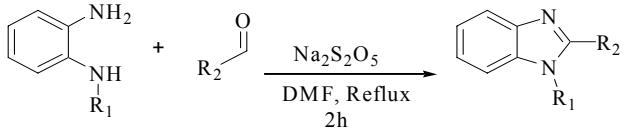
In recent years modified benzimidazoles are reported to exhibit a broad spectrum of biological activities including antifungal and anticancer activities [1, 2]. Benzimidazole derivatives have also been found to possess various biological activities such as antibacterial [3-7], antihelmintic [8, 9], epileptic, antdiabetic, anti-fertility [10, 11], antiviral [12-15], antiinflammatory [16], antihistaminic [17], antioxidant [18-21], antihypertensive [22], anticoagulant [23], proton pump inhibitory [24, 25], antileukaemic [26], and antiulcer properties.

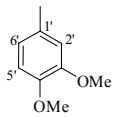
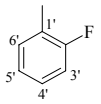
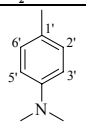
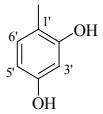
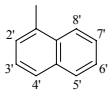
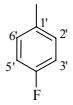
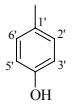
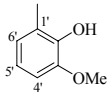
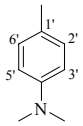
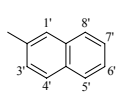
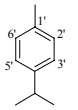
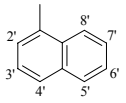
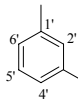
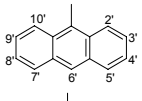
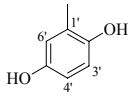
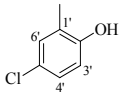
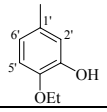
Previously benzimidazole-based zinc complexes were used as structural carbonic anhydrase models. Furthermore, benzimidazole and indapamide-like benzenesulfonamides have been reported as Rho kinase and carbonic anhydrases I, II, VII, and VIII, respectively [27]. We present here the *in vitro* carbonic anhydrase inhibitory activity of a series of benzimidazole derivatives **1-24**. Carbonic anhydrase (EC 4.2.1.1) is a Zn^{2+} containing enzyme and composed of a single polypeptide bonds, and a tightly bound Zn^{2+} ion is required for activity of this enzyme, it catalyzes the interconversion of carbon dioxide and water to bicarbonate and proton and thus maintain acid-base balance in blood and other tissues. It plays a key role in transportation of carbon dioxide out of the tissues. These are the known physiological function of carbonic anhydrase; it also affects on the diverse processes, such as physiological pH control, gas balance, and calcification. Since last three decades carbonic anhydrase inhibitors have been used in the treatment of glaucoma. Sulfonamide, a carbonic anhydrase inhibitor, has been used in the treatment of glaucoma [28], whilst, acetazolamide is found to potent carbonic anhydrase inhibitor, useful in the management of glaucoma [29-32], which opened new avenues for the medicinal chemists to work on other carbonic anhydrase inhibitors. Currently used carbonic anhydrase inhibitors are administered systemically, include acetazolamide, dichlorophenamide, ethoxzolamide and methazolamide. Each of these drugs possesses a free sulfamoyl group, attached to an aromatic heterocyclic ring and inhibits the enzyme *in vitro* with potency in the nanomolar range [31]. Intraocular pressure is decreased by reduction in humor formation stemming from the inhibition of carbonic anhydrase, present in the ciliary epithelium. A number of carbonic anhydrase inhibitors have been reported to lower the intraocular pressure when instilled topically in animals [33]. In the present study, benzimidazoles were randomly screened for *in vitro* inhibition of bovine carbonic anhydrase. The carbonic anhydrase inhibitory activity exhibited by compound **22** is 24 fold lower than the standard (IC_{50} = 0.31 ± 0.0006 μM) Table-1. This represents the potential of this class of compounds as carbonic anhydrase inhibitors for possible treatment of associated diseases.

Chemistry

Benzimidazoles **1-24** were synthesized by reacting together with commercially available 2-phenylenediamine and different aromatic aldehydes in *N*,*N*-dimethylformamide (DMF). The products were appeared in significant yields (Scheme-1). In a typical reaction, sodium metabisulfite ($Na_2S_2O_5$) was added to the stirring mixture of 2-phenylenediamine

(3.12 mmol) and different substituted aromatic aldehydes (3.16 mmol) in *N*,*N*-dimethylformamide. Reaction mixture was refluxed for 2 h. The progress of reaction was monitored by TLC and after completion of reaction; mixture was allowed to cool to room temperature. Water (30 mL) was added to the crude reaction mixture to afford solid mass as precipitates, after filtration the solid benzimidazole derivatives **1-24** were obtained in high yields. Re-crystallization from methanol afforded pure products and the structures of synthetic compounds **1-24** were evaluated by spectroscopic techniques including ^{1}H-NMR, and EI MS [34].



Comp. No.	R_1	R_2	Comp. No.	R_1	R_2	Comp. No.	R_1	R_2
1	**H**		**9**	**H**		**17**	**H**	
	H		**10**	**H**		**18**	**H**	
	H		**11**	**H**		**19**	**Ph**	
	H		**12**	**H**		**20**	**Ph**	
	H		**13**	**H**		**21**	**Ph**	
	H		**14**	**H**		**22**	**Ph**	
	H		**15**	**H**		**23**	**H**	
	H		**16**	**H**		**24**	**H**	

Scheme-1: Synthetic benzimidazole derivatives **1-24.**

Table-1: *In vitro* carbonic anhydrase activity of compounds **1-24.**

S. No.	% Inhibition	IC_{50} ± SEM[a] (*µ*/M)	S. No.	% Inhibition	IC_{50} ± SEM[a] (*µ*/M)
1	**15.9**	**NA[b]**	**13**	**5.9**	**NA[b]**
2	**17.1**	**NA[b]**	**14**	**- 4.9**	**NA[b]**
3	**13.5**	**NA[b]**	**15**	**62.2**	**13.18 ± 0.37**
4	**Precipitated**	**NA[b]**	**16**	**Precipitated**	**NA[b]**
5	**29.1**	**NA[b]**	**17**	**6.4**	**NA[b]**
6	**Precipitated**	**NA[b]**	**18**	**40.9**	**NA[b]**
7	**41.2**	**NA[b]**	**19**	**Precipitated**	**NA[b]**
8	**21.3**	**NA[b]**	**20**	**62.3**	**23.1 ± 1.78**
9	**26.3**	**NA[b]**	**21**	**83.1**	**10.31 ± 0.11**
10	**21.4**	**NA[b]**	**22**	**79.7**	**7.47 ± 0.39**
11	**61.1**	**12. 81 ± 0.29**	**23**	**34.8**	**NA[b]**
12	**78.2**	**12.16 ± 0.10**	**24**	**- 3.2**	**NA[b]**
Acetozolamide (Standard)		**0.31 ± 0.002**			

SEM[a]: is the standard error of the mean, NA[b]: Not active Acetozolamide; standard inhibitor of Carbonic anhydrase.

Bioactivity

Twenty four (24) **s**ynthetic benzimidazole derivatives were screened for their carbonic anhydrase inhibitory potential. Six of them showed above 50% inhibition, we therefore screened them for their IC_{50} values. Compounds **22** (IC_{50} = 7.47 ± 0.39 μM), **21** (IC_{50} = 10.31 ± 0.11 μM), **20** (IC_{50} = 23.1 ± 1.78 μM), **12** (IC_{50} = 12.16 ± 0.10 μM), **11** (IC_{50} = 12. 81 ± 0.29 μM), and **15** (IC_{50} = 13.18 ± 0.37 μM) showed good carbonic anhydrase inhibitory activity. Acetozolamide, (IC_{50} = 0.31 ± 0.0006 μM) was used as standard in the assay.

Limited SAR studies revealed that the carbonic anhydrase inhibitory activity is dependent on the nature of R_1 and R_2 substitutions. The highest inhibition was demonstrated by the compound **22** (IC_{50} = 7.47 ± 0.39 μM), which has a phenyl group as R_1 and 3',4'-dimethoxy phenyl as R_2 on benzimidazole skeleton. Interestingly, compound **1** was found to be completely inactive, though it has same R_2 substituents on benzimidazole ring. The comparison of activity of compounds **1** and **22** indicate that the activity is largely due to R_1 phenyl substituents. Compounds **20**-**22** contain R_1 as phenyl substituents have shown diverse level of activities. Compound **21** showed an IC_{50} value 10.31 ± 0.11 μM as the second most active compound of the series. Likewise its analogous compound **13** with same R_2 showed inactivity. Compound **12** contain an isopropyl group at phenyl as R_2 and H as R_1 showed an activity with an IC_{50} value 10.31 ± 0.11 μM. Compound **11** with an IC_{50} value 12. 81 ± 0.29 μM was found to be fourth most active compound of the series which contains 2-hydroxy and 3-methoxy at phenyl as R_2. Compound **15** with an IC_{50} value 13.18 ± 0.37 μ/M contains 3,4-dichloro phenyl as R_2. In addition, compound **20** (IC_{50} = 23.1 ± 1.78 μM) having a 1-naphthyl as R_2 and phenyl as R_1, but its analogous compound **10** found to be completely inactive. This further indicates the importance of R_1 as phenyl as well right substituents on R_2. This SAR is by no means conclusive and needs evaluation of a larger library of benzimidazoles.

This study, however, demonstrates that compound **22** can be an initial point for further study hence its chemical modification may lead to more potent carbonic anhydrase inhibitors.

Material and Methods:

The carbonic anhydrase was purchased from Sigma Aldrich (Cat. No. C-3934), 4-nitrophenyl acetate was purchased from MP Bio (Cat. No. 160888). HEPES buffer was purchase from DOJINDO (Cat. No. 346-01373), *tris*-(hydroxymethyl)-aminomethane (reagent grade) was purchase from Scharlau (Cat. No. TR0423). DMSO and ethanol of reagent grade were used in the experiment. The experiment was performed in triplicate. IC_{50} values were calculated by using EZ-fit enzyme kinetics software (USA)

Carbonic Anhydrase Inhibition Assay

In this assay, colorless 4-nitrophenyl acetate (4-NPA), is hydrolyzed to 4-nitrophenol and CO_2 and reaction is followed by measuring the formation of 4-nitrophenol, a yellow colored compound.

The experiment was performed in buffer containing HEPES and *Tris* at a total concentration of 20 mM and pH was 7.4. For each sample the reaction tube contained 140 μL of the *HEPES-Tris* solution, 20 μL of freshly prepared aqueous solution of purified bovine erythrocyte carbonic anhydrase II (0.1 mg/mL of deionized water for 96-well), 20 μL of test compound dissolve in DMSO (10% final concentration), 20 μL of substrate 4-PNA at concentration of 0.7 mM diluted in ethanol.

The reaction was initiated by addition of 4-PNA after 15 min incubation of test compounds. The compounds were tested in triplicate at different concentration. In this assay the reaction was performed in 96-well plate. *SPECTRA-max* 340 spectrophotometer, Molecular Devices (USA) was used to monitor the reaction and the amount of product formed was monitored at 1 min interval for 30 min at 400 nm [35, 36].

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