International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

# Design and Synthesis Of 2-(2-{3-Chloro-4[1-(Arylamido/Imido-Alkyl)-1h-Benzimidazol-2-Yl] Phenyl} Ethyl) Isoindol-1, 3-Diones for Studying their Anti-Japanese Encephalitis Virus (Jev) Activity

**Dr. Amar Singh[1], V.K. Pandey[2]**

[1, 2]Department of Chemistry, University of Lucknow, Lucknow-226007, India

**Abstract:** ***Phthalic anhydride on heating with β-ethanolamine (cholamine) at 210°C furnished 2-(2-hydroxyethyl)-isoindol-1,3-dione (1) which reacted with 2-chlorobenzoic acid in $H_2SO_4$ to give 2-chloro-4-[2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl) ethyl] benzoic acid (2). Reaction of 2 with o-phenylenediamine in dry pyridine afforded 2-{2-[4-(lH-benzimidazol-2-yl)-3-chlorophenyl] ethyl} isoindol-l,3-dione (3), which on condensation with amido/imidoalcanols resulted in 2-(2-{3-chloro-4-[1-(arylamidol/ imidoalkyl)-l Hbenzimidazol-2-yl] phenyl} ethyl)-isoindol-l,3-diones (4). Compounds 4 were evaluated for their antiviral activity against Japanese Encephalitis Virus (JEV), a highly human pathogenic virus.***

**Keywords:** Benzimidazole derivative, Enviroxime, Minimum Essential Medium.

## 1. Introduction

The first genuine benzimidazole derivative to find clinical application was 2-(hydroxybenzyl)-benzimidazole (HBB). This is the selective inhibitor of RNA containing enteroviruses, although lymphocytic choriomeningitis virus and Aren virus are also inhibited to a certain degree[2]. Another benzimidazole compound to find FDA approval is enviroxime, which is generally used against common cold. It has antiviral effect against rhino, Coxsackie, echo, and polioviruses. It prevents viraluncoating and inhibits viral RNA polymerase from acting on late phase replication[3], Another significant benzimidazole compound in this class of chemicals is 5,6-dichloro-l-β-ritJofuranosyl benzimidazole which is inhibitory in vitro to both RNA and DNA viruses[4]. It is very interesting that this compound is an enhancer of interferon inductions[5]. A modified structure of HBS viz; 1,2-bis-(5-methoxy-2-benzimidazole-2-yl) l,2-ethane diol has been shown to have antiviral activity in vivo and in vitro; the animal test being a rhinovirus infection experimentally induced in chimpanzees[6-7].

Benzimidazoles as antiviral agents are effective mainly against RNA viruses and inhibit the formation of virus induced RNA polymerase, thereby preventing or retarding RNA synthesis[8]. The mode of action of benzimidazoles is rather more interesting. It is thought that the benzimidazoles readily take part in intermolecular hydrogen bonding[9]. These compounds form H-bonding structures linked by either HO-----OH bonds or N----H-O bonds and they also form copper chelates[10]. This suggests that the highly specific H-bonding or possible metal chelation involving a benzimidazole compound and a macromolecule (either viral DNA itself or a specific enzyme required in its formation) might result in inhibition of RNA synthesis[11]. These valid observations prompted the authors to undertake the synthesis of 2-(2- {3-chloro-4-[l-( aryl-amido/ inidoalkyl)-lH-benzimidazoi-2-yl] phenyl} ethyl) isoindol-1,3-diones as target compounds for assaying their anti-viral activity against ***Japanese Encephalitis Virus*** (JEV) that is a highly human pathogenic virus.

Heating an equimolar mixture of phthalic anhydride and β-ethanolamine (cholamine) at 210°C for 30 minutes yielded 2-(2- hydroxyethyl)-isoindol-l,3-dione **(1)** in quantitative yield. The intermediate **2** was synthesized by the interaction of **1** and 2-chforobenzoic acid in concentrated sulphuric acid.

This is an C-amidoalkylation reaction as suggested by Tscherniac[12]. The reaction of **3** with o-phenylenediamine in dry pyridine solvent afforded a benzimidazole compound **3** which underwent a Mannich type reaction with arylamido/ imidoalcanols to furnish **4**. All the intermediates and the target compounds **4** were characterized on the basis of their elemental analysis, IR, 1 $^{1}$HNMR, $^{13}$CNMR and mass spectral data.

***Antiviral activity***

All the four compounds (4a - 4d) were bioevaluated for their antiviral activity against Japanese Encephalitis Virus (JEV), an RNA virus of greater pathogenecity *in vitro.* Cytotoxicity and antiviral assay of the compounds were performed by the standard method of Sidwell and Huffmann[13]. For carrying out cytotoxic studies, confluent monolayer of vero cells were loaded with two fold serial dilutions of the investigational compound and a dose of 500 - 4 µg/ml/ well was tested for each compound. The solution of each compound was made in Foetal Bovine Serum (FBS), pH 7.2. The treated cultures were incubated for a period of24 hr at 37°C and after incubation the plates were examined for the evidence of cytotoxicity such as distortion, swelling, and sloughing of cells. In order to study the antiviral activity in vitro, 0.1 ml of virus ($10TCID_{50}$ $ml^{-1}$) was allowed to adsorb for 90

minutes in cell monolayers at 37°C.

The unadsorbed virus was removed by decanting the micro-titre plates and washing each well with 0.1 ml Minimum Essential Medium (MEM). Subsequently, 0.1 ml of MEM with 2.5 FBS was added to each well. Non-toxic dilutions varying from 125 - 8 µg/ ml/ well of the compounds to be investigated were added to appropriate wells. Each dilution was tested in duplicate and each panel included virus control (containing MEM with 2.5% serum, which had already been adsorbed for 90 minutes) and cell control (containing only MEM with 2.5% serum). The microplates were incubated at 37°C for 72 hr and examined microscopically for the evidence of Cytopathic Effect (CPE) caused by the viruses and their inhibition by the examined compound as compared to untreated virus control well which showed about 100% CPE within 72 hr at 37°C.

Two benzimidazole compounds viz; 4a and 4d showed measurable level of antiviral activity in vitro. Compound 4a showed 50% CPE inhibition while compound 4d displayed a slightly low order of percent CPE inhibition (30%). Other two compounds of the series (4b and 4c) were found less significant with the anti-JEV activity point of view since these compounds were found unable to provoke any observable level of antiviral activity against JEV. It is very interesting to observe here that phthalimido methyl (4a) and phthalimido ethyl (4d) substituted compounds are more appropriate in eliciting the antiviral activity than the benzamido and nicotinamido substituted compounds.

## 2. Experimental

Melting points were determined in open. capillary tubes in an electrical Toshniwal melting point apparatus and the values reported herein are uncorrected. The purity of compounds was checked by the TLC using silica gel-G (Acme). IR spectra were recorded on an FT-IR Perkin Elmer (model); spectrophotometer in KBr discs ($cm^{-1}$), The $^{1}$HNMR and $^{13}$CNMR spectra were recorded on a DPX-200 Bruker FT-NMR spectrometer at 300 MHz and 50 MHz, respectively. Chemical shifts are expressed in δ-scale downfield from TMS (internal standard). The FAB mass spectra were recorded on JEOL SX 102/ DA-600 mass spectrophotometer data system using Argon Xenon (6 KV 10 mA) as the FAB gas.

2-(2-Hydroxy ethyl)-isoindol-l ,3-dione(**1**),2-chloro-4-[2-(l ,3-dioxo-1,3-dihydro-isoindol-2-yl)ethyl]-benzoic acid (2) and arylamido imidoalcanols were synthesized following the literature methods[14-18].

**2-{2-[4-(1H-BENZIMIDAZOL-2-YL) 3-CHLOROPHENYL] ETHYL} ISOINDOL-l,3-DIONE (3)**

A solution of 2-chloro-4-[2-(l ,3-dioxo-1,3-dihydro-isoindol-2-yl) ethyl] benzoic acid **(2)** (0.02 mole) and o-phenylenediamine (0.02 mole) in anhydrous pyridine (50 ml) was heated under reflux for 6 hr under anhydrous conditions of reaction.

The solution was allowed to cool at room temperature and poured into dil. Hcl (100 ml). Solidification occurred and the resulting solid was filtered off. It was washed with cold water in order to remove the adhered pyridine. The crude benzimidazole compound thus obtained was dried under vacuum and recrystallization from dil. ethanol gave a light brown compound in the crystalline form, It melted at 210°C; yield, 75%.

**IR(KBr):** 3100 and 3400 (sec. NH-str.), 1695 (imide C=O str.), 1640 (C=N str.), 714 (C-Cl str.). **$^{1}$HNMR ($CDCl_3$):** 6.75- 7.90 (m, 11H, ArH) , 3.65 (t, 2H, N-$CH_2$), 3.50 (t, 2H, C-$CH_2$). **$^{13}$CNMR ($CDCl_3$):** 55.50, 70.25, 117.50, 119.50, 121.50, 125.l8, 127.52, 129.09, 130.40, 139.71, 144.14, 168.50, 175.25.
**Mass (FAB) (m/ 2):** 401 ($M^+$), 403 ($M^{+2}$) 105 (Base Peak).

**2-(2-{3-CHLORO-4-[1-(ARYL-AMIDO/IMIDOALKYL)- 1H-BENZIMIDA ZOL-2-YL] PHENYL} ETHYL) ISOINDOL-l,3- DIONES (4)**

Synthesis of **(4)** involved the formation of a new carbon-nitrogen bond. A mixture consisting of 2-{2-[4-(lH-benzimidazol-2-yl)-3- chlorophenyl] ethyl} isoindol-Li-dione **(3)** (0.01 mole) and an arylamido/ imidoalcanol (0.01 mole) was dissolved in cone, $H_2SO_4$ (50 ml) by stirring carefully and cautiously. The contents were allowed to cool occasionally during the course of the dissolution of the contents. Subsequently, the resultant dark acidic solution was stirred mechanically for one hour and refrigerated overnight. Pouring into the ice-cold water resulted in the separation of a solid, which was allowed to settle down. It was filtered off and washed with cold water. Drying was done in vacuo and the benzimidazole derivative was recrystallized from ethanol. Benzimidazoles thus synthesized are recorded in Table-1.

**Table 1:** Characterization and antiviral activity data of 2-(2-{3-chloro-4-[1-(arylamido/ imidoalkyl)-1H-benzimidazol-2-yl] phenyl} ethyl) isoindol-1,3-diones (4)

| Compd. No. | R | n | m.p. ($^0$C) | Yield (%) | Anti-JEV Activity | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | CT50 (µg/ ml) | $EC_{50}$ (µg/ ml) | TI | % CPE In. |
| 4a | Phthalimido | 1 | 273 | 70 | 500 | 125 | 04 | 50 |
| 4b | Nicotinamido | 1 | 273 -274 | 65 | 500 | - | - | - |
| 4c | Benzamido | 1 | 280 | 68 | 500 | - | - | - |
| 4d | Phthalimido | 2 | 260 -261 | 70 | 500 | 250 | 02 | 30 |

All compounds gave correct elemental analysis.

***IR (KBr) ($cm^{-1}$):** 1640 (C=N), 1712 (imide C=O), 714(C-Cl), 1425 (Ar-$CH_2$).
**$^{1}$HNMR ($CDCl_3$) (δ ppm):** 6.75 - 7.85(m, 15H, ArH), 3.82(s, 2H, N-$CH_2$- N)~ 3.65(t, N-$CH_2$, J = 6.3) 3.50(t, C-$CH_2$, J = 6.3)
**$^{13}$CNMR ($CDCl_3$) (δ ppm):** 76.56, 77.43, 83.54, 115.32, 119.10, 121.50, 125.18, 127.52, 129.09, 130.40, 131.71, 144.14, 166.95, 169.05

**Mass (FAB) (m/ 2):** 560 ($M^+$), 562 ($M^{+2}$) Base Peak appeared at m/2 160 due to phthalimidomethyl [$C_6H_4(CO)_2NCH^+_2$] ion.
**$CT_{50}$** = Cytotoxic concentration (50%), **$EC_{50}$** = Effective concentration (50%), **TI** = Therapeutic index, **CPE** = Cytopathic effect.

## 3. Acknowledgement

Authors express their sincere thanks to The Head, Department of chemistry, University of Lucknow, Lucknow for providing necessary laboratory facilities. Thanks are also due to The Director, Central Drug Research Institute (CDRI), Lucknow for providing elemental, spectral, biological activity data and **Council of Science and Technology (**CST.**UP)** to providing financial support **under young scientist scheme to one of the author (Dr.Amar Singh Yadav).**

## References

[1] Tamm, I. and Caliguiri, L.A., "HBB and related compounds in "Chemotherapy of viral Diseases", Vol I (International Encyclopedia of Pharmacology and Therapeutics), D. J. Bauer, Ed., Pergamon Press, Oxford, 1972, 115.
[2] Caliguiri, L.A. and Tamm, I., Guanidine and 2- (a-hydroxybenzyl) benzimidazole (HBB) "Selective Inhibitor of Picorna Virus Multiplication", W. A. Carter, Ed. Cl'C Press, Cleveland, 1973,257.
[3] Phillipotts, R., *Antimicro. Agents Chemother.*, 1987, 23, 671.
[4] Sehgal, P.B., Tamm, I. and Villula, *J., Virology,* 1976, 70, 532.
[5] Stella, J.P., Michaeleon, S.L.D., Morgan, J.H. and Pface, G.J., *Antimicrob. Agents Chemother.*, 1974,6, 754.
[6] Roderick, W.R., Nordeen, C.W., VonEsch, A.M. and Appel, R.N., *J. Med. Chem.*, 1972, 15, 655.
[7] Shipkowitz, M.L., Bower, R.R., Schleicher, J.B., Aquino, F., Appel, R.N. and Roderick, R.R., *Appl. Microbiol.*, 1972, 23, 117.
[8] Tamrn, I., *J BioI. Med.*, 1956, 29, 33.
[9] O'Sullivan, D.G. and Saddler, P.W., *Nature,* 1961, 192, 341.
[10] Durechinsky, R., *US* Pat., 2, 207,186; *Chem. Abstr.*, 1956, 50, 5766.
[11] Goldber, M.G. and Fehr, H.H., *US* Pat., 2, 602, 086; *Chern. Abstr.*, 1953, 47, 69874.
[12] Tscherniac, J., *Ger. Pat.* 134979; *Chem. Abstr.*, 1902, 2, 1084.
[13] Sidwell, R.A. and Huffman, T.H., *Appl. Microbiol.*, 1971, 22, 791-801.
[14] Hirwe, N.W, and Rana, K.N., *Ber.*, 1939, 72, 1346.
[15] Garg, P., In: "Synthesis and Bioassay of Potential Anthelmintic Comopunds", A Ph. D. Thesis, Chemistry Department, Lucknow University, Lucknow. 1986, 121.
[16] Buc, S.R., *J. Am. Chem.Soc.,* 1947, 69, 254-256.
[17] Sckiya, M, and Ito, *K., Chem. Pharm. Bull* (Tokyo). 1963, 11, 888-891.
[18] Zhdanovich, E.S., Chekrnareva, I.B., Bauline, G.A. and Kaplina, L.I., *Med. Prom. SSSR,* 1962, 16, 25.