

## **Synthesis and Antiviral activity of some novel diazabicyclo compounds**

**Chandra Shekhar Sharma<sup>1\*</sup>, Rajesh Kumar Nema<sup>1</sup>, V. K. Sharma<sup>2</sup> and S. N. Meyyanathan<sup>3</sup>**

**<sup>1</sup>Department of Pharmaceutical Chemistry, B. N. College of Pharmacy, Udaipur, (Rajasthan), India.**

**<sup>2</sup>Department of Chemistry, M. L. S. University, Udaipur, (Rajasthan), India.**

**<sup>3</sup>Department of Pharmaceutical Analysis, J. S. S. College of Pharmacy, Otacamund, (Tamilnadu), India.**

**\*E-mail: shekhar\_gurava@yahoo.co.in  
Fax: (91)(294)2413182; Tel: (91)9828173650**

**ABSTRACT:** 3,5-dinitro-benzoyl chloride was refluxed with different amino acids to form the amino acid derivatives that were reduced with zinc/formic acid, to form the products and the reduced products so formed were cyclized to give the title compounds with benzil in the presence of sodium ethoxide/ethanol. All the intermediates and title compounds were characterized by physical, chemical, analytical and spectral data. All the title compounds were screened for their *in-vitro* cytotoxic and antiviral activities.

**KEY WORDS:** antiviral activity, cytopathic effect inhibition and cytotoxicity assay.

### **INTRODUCTION**

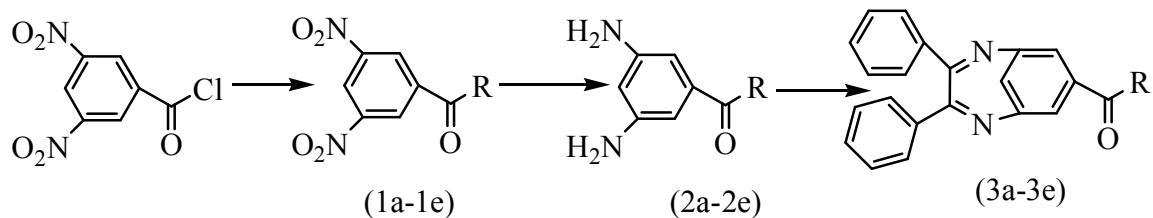
Infectious viral diseases remain an important world wide problem, because viruses have resisted prophylaxis or therapy longer than any other life form. Various bicyclo compounds have received the attention of medicinal chemist due to their wide range of biological activities, which include analgesic-anti inflammatory<sup>1-3</sup>, muscarinic receptor antagonist<sup>4</sup>, antibacterial<sup>5</sup>, antiviral<sup>6</sup>, antiprotozoal<sup>7</sup> and antispasmolytic<sup>8</sup>. In the present study it was envisaged that a molecule entity possessing such bicyclic systems could possess antiviral activities. The objective of the study was to develop potent antiviral agents incorporating amino acids linked to bicyclic system to enhance the hydrophilicity of the synthesized candidates, like bicyclo compounds may exhibit

completely the replication of any virus.

### **EXPERIMENTAL**

Melting points (m.p.) were determined in open glass capillary tubes and are uncorrected. Infrared (IR) spectra were recorded on an FT-IR Bruker Tensor 27 spectrometer and are expressed in  $\text{cm}^{-1}$ . The NMR spectra of the compounds were recorded on Bruker DRX-300 spectrometer. The chemical shifts were reported as parts per million ( $\delta$  ppm) using tetramethylsilane (TMS) as an internal standard. The LC mass spectra of the compounds were recorded on Shimadzu 8201PC spectrometer. The progress of the reaction was monitored on precoated silica gel 60 F254 plates (Merck) using different solvent systems.

### Scheme-1



- R = - NHCH<sub>2</sub>COOH
- NHCH(COOH)(CH<sub>2</sub>)<sub>2</sub>COOH
- N
 
 COOH
- NHCH(CH<sub>2</sub>SH) COOH
- NH(CH<sub>2</sub>)<sub>4</sub> CH (NH<sub>2</sub>)COOH

### *General procedure:*

### Synthesis of 2-(3, 5-dinitrobenzamido) acetic acid (1a):-

A solution of 3, 5-dinitrobenzoyl chloride (1 mole) in 1, 4-dioxan was added to glycine (1.2mole) in 0.1 N sodium hydroxide (10 ml) and refluxed for 6 hrs. The reaction mixture was allowed to cool and poured into 1N hydrochloric acid and crushed ice. The crude product was filtered, dried, recrystallized with methanol and column chromatographed on silica gel (60-120 mesh) eluting with methanol: ethyl acetate (8:2). Mol. Formula: C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>7</sub>. Yield 67%, m.p. 182°C.

All the other compounds **1b-1e** were prepared by the same procedure using the corresponding amino acid.

### ***General procedure:-***

## Synthesis of 2-(3,5-diamino-benzamido)acetic acid

**(2a):-** A suspension of 2-(3,5-dinitrobenzamido)acetic acid (1 mole) and zinc dust (2.5 mole) in methanol was stirred with 5 ml of 90% formic acid at room temperature for 5hrs. After completion of the reaction (monitored by TLC), the reaction mixture was filtered off. The organic layer was evaporated and the residue was dissolved in ether and washed with saturated sodium chloride solution (5 times) to remove of ammonium formate. Then the ethereal layer was evaporated to dryness<sup>9</sup>. The crude product was recrystallized with ethanol and purified by column chromatography on silica gel (60-120 mesh) eluting with chloroform: ethyl acetate (9:1). Mol. Formula: C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>, Yield 65%, m.p. 173°C.

All the other compounds **2b-2e** were prepared similarly.

### ***General procedure:-***

**Synthesis of 2-[(3, 4-diphenyl-2, 5-diaza-bicyclo [4.3.1] deca-1(9), 2, 4, 6(10), 7-pentaene-8-carbonyl) -amino]-pentanedioic acid (3a):-**

2-(3, 5-diamino-benzamido) acetic acid (1 mole) was dissolved in 0.1N sodium hydroxide solution. To this, a

mixture of benzil (1.1mole) and sodium ethoxide (2.3mole) in ethanol was dissolved with continuous stirring and refluxed for 55hrs. The reaction mixture was then allowed to cool and poured into 1N hydrochloric acid and crushed ice. The content was kept over night at room temperature, filtered, dried and recrystallized with methanol. The completion of the reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with methanol: ethyl acetate (8:2). Mol. Formula: C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>, Yield 48%, m.p. 83°C; IR (cm<sup>-1</sup>) : 3712(-OH), 3670(-NH<sub>2</sub>), 2858(-CH<sub>2</sub>), 1681(C=O), 2968(CH-Ar), 1530(C=N); <sup>1</sup>H NMR(DMSO) : δ 2.5 (d, 2H, CH<sub>2</sub>), 9.03 (t, 1H, NH), 8.86 (s, 1H, COOH), 7.60-7.93 (m, 13H, ArH) ; LCMS : m/z [M+1]<sup>+</sup>384.4, [M+2]<sup>+</sup>385.4.

**2-[(3, 4-Diphenyl-2, 5-diaza-bicyclo[4.3.1]deca-1(9), 2, 4, 6(10), 7-pentaene-8-carbonyl)-amino]-pentanedioic acid (3b)** : Mol. Formula: C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>, Yield 43%, m.p. 68°C; IR (cm<sup>-1</sup>): 3737(-OH), 3528(-NH<sub>2</sub>), 2888(-CH<sub>2</sub>), 1675, 1699(C=O), 3083(CH-Ar), 1588(C=N); <sup>1</sup>H NMR(DMSO) : δ 1.21 (s, 1H, CH), 8.66 (s, 1H, COOH), 2.5 (m, 4H, (CH<sub>2</sub>)<sub>2</sub>), 8.88 (t, 1H, NH), 9.10 (s, 1H, COOH), 7.53-7.93 (m, 13H, ArH) ; LCMS : m/z [M+1]<sup>+</sup> 456.

**1-(3, 4-Diphenyl-2, 5-diaza-bicyclo[4.3.1]deca-1(9), 2, 4, 6(10), 7-pentaene-8-carbonyl)-pyrrolidine-2-carboxylic acid (3c)** : Mol. Formula: C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>, Yield 47%, m.p. 55°C; IR (cm<sup>-1</sup>) : 3602(-OH), 2798(-CH<sub>2</sub>), 1707, 1546(C=O), 3098(CH-Ar), 1493(C=N), 1336(tert.N); <sup>1</sup>H NMR(DMSO) : δ 1.20 (t, 1H, CH), 3.75 (m, 4H, (CH<sub>2</sub>)<sub>2</sub> pyrrolidine ring), 2.5 (m, 2H, CH<sub>2</sub>), 9.0 (s, 1H, COOH), 7.23-8.02 (m, 13H, ArH); LCMS : m/z [M+1]<sup>+</sup> 424.4.

**2-[*(3, 4-Diphenyl-2, 5-diaza-bicyclo[4.3.1]deca-1(9), 2, 4, 6(10), 7-pentaene-8-carbonyl)-amino]-3-mercaptopropionic acid (3d)*** : Mol. Formula: C<sub>24</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>S. Yield

50%, m.p. 73°C; IR ( $\text{cm}^{-1}$ ) : 3506(-OH), 3399(-NH<sub>2</sub>), 2877(-CH<sub>2</sub>), 1711, 1626(C=O), 3084(CH-Ar), 1532(C=N), 2637(-SH) ; <sup>1</sup>H NMR(DMSO) :  $\delta$  1.20 (s, 1H, CH), 8.65 (s, 1H, COOH), 2.5 (d, 2H, CH<sub>2</sub>), 9.09 (t, 1H, NH), 8.87 (s, 1H, SH), 7.27-7.93 (m, 13H, ArH) ; LCMS : m/z [M-1]<sup>+</sup>428.3.

**3, 4-Diphenyl-2, 5-diaza-bicyclo[4.3.1]deca-1(9), 2, 4, 6(10), 7-pentaene-8-carboxylic acid (5-amino-6-hydroxy-6-oxo-hexyl)-amide (3e)** : Mol. Formula: C<sub>27</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>, Yield 42%, m.p. 77°C; IR ( $\text{cm}^{-1}$ ) : 3726(-OH), 3647(-NH<sub>2</sub>), 2864(-CH<sub>2</sub>), 1678(C=O), 2924(CH-Ar), 1569(C=N) ; <sup>1</sup>H NMR(DMSO) :  $\delta$  1.21 (t, 1H, CH), 8.66 (s, 1H, COOH), 2.5 (m, 2H, CH<sub>2</sub>), 9.09 (t, 1H, NH), 8.88 (d, 2H, NH<sub>2</sub>), 3.58 (m, 6H, CH<sub>2</sub>) 7.60-7.93 (m, 13H, ArH) ; LCMS : m/z [M+1]<sup>+</sup>455.4, [M-1]<sup>+</sup>452.8.

### Chemicals

3-(4,5-Dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide(MTT) and sulphorhodamine B (SRB) were obtained from Sigma Chemical Co., Mo, U.S.A., RPMI-1640 and antibiotics were obtained from Hi-media Ltd., Mumbai. Trichloro acetic acid (TCA) and tris buffer were obtained from SD-Fine Chemicals Pvt. Ltd., Boisar. Dimethyl sulphoxide (DMSO), glacial acetic acid and propanol were obtained from E. Merck Ltd., Mumbai.

### Cell Line and Culture Medium

Vero (Normal, African green monkey kidney) cell culture was obtained from Pasteur Institute of India, Coonoor, India and was cultured in RPMI-1640 and 10% heat activated New born calf serum with antibiotics (1000 I.U./ml Penicillin, 100  $\mu\text{g}/\text{ml}$  Streptomycin and 25  $\mu\text{g}/\text{ml}$  Amphotericin B). The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were subcultured twice a week. Herpes Simplex Virus type1 (HSV-1) was from the collection of the Christian Medical College and Hospital, Vellore. The virus was propagated in Vero cells and the infective titer of the stock solution was 10<sup>-7</sup> TCID<sub>50</sub>/ml (50% tissue culture infective dose).

### In-Vitro Cytotoxicity Screening

The cytotoxic assays were carried out using 0.1ml of cell suspension, containing 10,000 cells seeded in each well of a 96 well microtitre plate (Tarsons). Fresh medium containing different concentrations of the test compound added after 24 hrs seeding. Control cell were incubated with out test sample and with DMSO. The very little percentage of DMSO present in the wells (Maximal 0.2%) was proved not to affect the experiment. The microtitre plates were incubated at 37°C in 5% CO<sub>2</sub> atmosphere for a period of 72hrs. Sixteen wells were used for each concentration of the sample. The cells were observed at different time intervals after incubation in the presence or absence of the test solutions. Cellular viability was determined by using the standard MTT (Microculture Tetrazolium) and SRB (Sulphorhodamine B) assays. The percentage inhibition was plotted against concentration and CTC<sub>50</sub> (concentration required to reduce viability by 50%) value was calculated<sup>10-11</sup>.

### In-Vitro Antiviral Assay

Different nontoxic concentrations of test compounds i.e. lower than CTC<sub>50</sub> were checked for antiviral property by cytopathic (CPE) inhibition assay against challenge dose of 10 TCID<sub>50</sub>. Cells were seeded in a 96well microtitre plates with population 10,000 cells per well, incubated at 37°C in 5% CO<sub>2</sub> atmosphere for a period of 48hrs. The plates were washed with fresh RPMI-1640 medium and changed into maintenance medium containing virus (10 TCID<sub>50</sub>) and incubated at 37°C for 90min for adsorption of the virus. Then, the cultures were treated with different dilutions of the test compounds in fresh maintenance medium and incubated at 37°C for five days. Every 24hr the observation was made and cytopathic effects were recorded. Anti-HSV-1 activity was determined by the inhibition of the cytopathic effect compared with control, i.e. the protection offered by the test samples to the cells scored<sup>12</sup>.

## RESULTS AND DISCUSSION

All the five title compounds synthesized as per scheme-1 and were tested for *in-vitro* antiviral activity using CPE inhibition assay against Herpes Simplex Virus Type1. All the title compounds were initially tested for *in-vitro* cytotoxic activity against a normal vero cell culture. Two assays namely MTT assay and SRB assay were used for determining cytotoxicity and CTC<sub>50</sub> was calculated and the results are tabulated in Table 1. The cytotoxic range was 23 to 60.5 $\mu\text{g}/\text{ml}$  MTT assay and 29.5 to 64.25 $\mu\text{g}/\text{ml}$  in SRB assay respectively. The concentrations that were nontoxic to the vero cell culture were selected for antiviral activity screening.

Three concentrations as per the results obtained from cytotoxic study of the compound were selected for *in-vitro* antiviral screening against Herpes Simplex Virus Type1. The results are tabulated in Table 2 & Figure 1. Thus from the data it can be concluded that compound 3e, lysine derivative showed good antiviral activity at 40 and 50 $\mu\text{g}/\text{ml}$  concentration till 72hrs, when challenged with 10 TCID<sub>50</sub> viral suspension which is comparable to standard acyclovir used. The same compound at lower concentration of 30 $\mu\text{g}/\text{ml}$  protected the cell culture against infection till 24hrs only. Compound 3d, cysteine derivative protected only after 72hrs at 40 $\mu\text{g}/\text{ml}$  concentration. The remaining compounds did not show any significant protection against the challenge dose. The diazabicyclo compounds are gaining importance through their varied biological and pharmacological properties. In continuation of our work on various amino acids incorporated diazabicyclo compounds, we have now reported the synthesis, cytotoxicity and antiviral activity of some novel diazabicyclo compounds.

### ACKNOWLEDGEMENTS

Authors are thankful to J. S. S. College of Pharmacy, Ooty, Tamilnadu for providing necessary facilities to carry out research work.

**Table 1: Cytotoxic effect of test compounds on normal vero cell lines after 72hrs**

<b>Compound</b>	<b>MTT (<math>\mu</math>g / mL)</b>	<b>SRB (<math>\mu</math>g / mL)</b>	<b>Average (<math>\mu</math>g / mL)</b>
3a	36.50	37.50	37.000
3b	60.50	64.25	62.375
3c	32.00	34.75	33.375
3d	23.00	29.50	26.250
3e	35.00	37.50	36.250

**Table 2: Cytopathic effect inhibition (CPE) assay against *Herpes Simplex Virus* Type-1, 10 TCID<sub>50</sub>**

<b>Compound</b>	<b>Concentration (<math>\mu</math>g/ml)</b>	<b>Microscopic observation</b>			<b>%Protection at 72 hrs</b>
		<b>24 hrs</b>	<b>48 hrs</b>	<b>72 hrs</b>	
3a	50	+	+	+	25
	40	-	-	-	00
	30	-	-	-	00
3b	70	+	+	+	25
	60	-	-	-	00
	50	-	-	-	00
3c	50	+++	++	++	50
	40	+	+	+	25
	30	+	+	+	25
3d	40	+++	+++	/	90
	30	+++	++	++	50
	20	+++	++	++	50
3e	50	/	/	/	90
	40	/	/	/	90
	30	/	++	+++	75
Drug Control (Acyclovir)	25	/	/	/	90
Virus Control		+	++	-	00

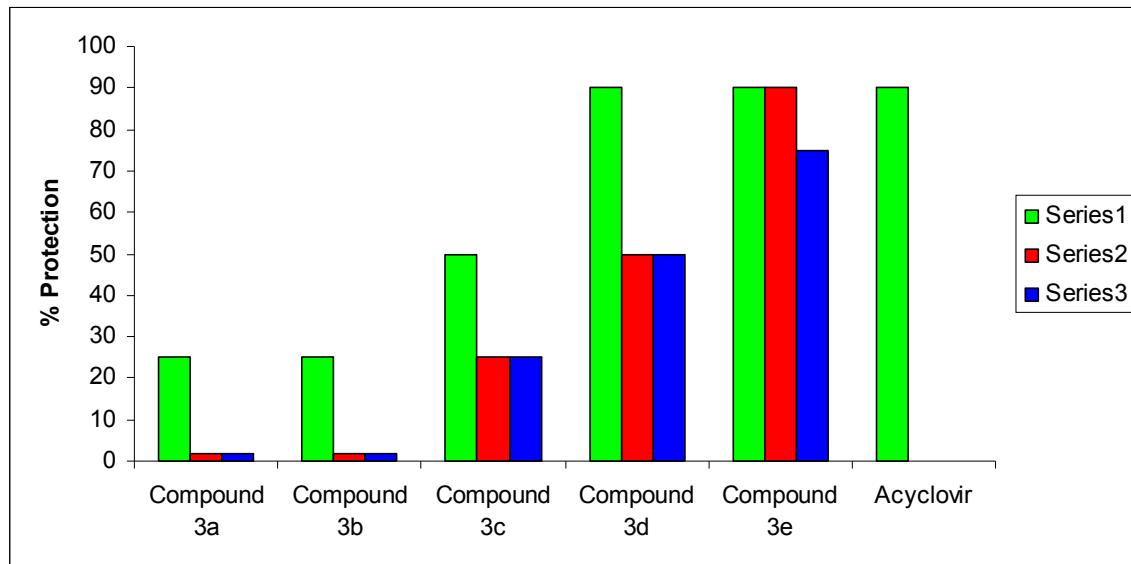
 $\checkmark$  = 90% Protection

+++ = 75% Protection

++ = 50% Protection

+ = 25% protection

- = 0 % Protection

**Figure 1: Graphical representation of % protection against *Herpes Simplex Virus Type-1, 10 TCID<sub>50</sub>*****REFERENCES**

- Peter D., Olsen G., Diazabicyclonane and decane derivatives and their use as opioid receptor ligands, European Patent, 2008, 1, 527, 075.
- Nagaev V.M., Dobryanskii V.S., Eleev A.F., Klimova T.A., Synthesis and analgesic activity of some 2-azabicycloheptanone derivatives, Pharm. Chem. J., 1999, 33, 137-140.
- Pinna G.A., Murrinedu G., Curzu M.M., Villa S., Synthesis, modeling and  $\mu$ -opioid receptor affinity of N-3(9)-arylpropenyl-3, 9-diaza bicyclo [3.3.1] nones, IL Farmaco, 2000, 55, 553-562.
- Myoung G.K., Erik T.K., Chen W., C (8) substituted 1-aza bicyclo [3.3.1] non-3-enes and C (8) substituted 1-aza bicyclo [3.3.1] nonane-4-ones: Novel muscarinic receptor antagonists, J. Med. Chem., 2003, 46, 2216-2226.
- Smirnova N.O., Plotnikov O.P., Vinogradova N.A., Sorokin, V.V., Synthesis and biological activity of substituted 7-aza-8-aza (oxa)-bicyclo [4.3.0]-6, 9-nonadienes, Pharm. Chem. J., 1995, 29, 49-50.
- Miller J.A., Ullah G.M., Welsh G.M., 8-Amino bicyclo [3.2.1] octanes: synthesis and antiviral activity, Tetrahedron Lett., 2001, 42, 7503-7507.
- Seeacher W., Schlappper C., Brun R., Kaiser, M., Antiprotozoal activity of new bicyclo [2.2.2] octane-2-imines and esters of bicyclo [2.2.2] octane-2-ols, Eur. J. Pharm. Sci., 2005, 24, 281-289.
- Rajdan B.K., Sharma A.K., Kumari K., Bodla R.B., Gupta B.L., Patnik G.K., Studies on aza bicyclo systems: synthesis and spasmolytic activity of analogues of 9-methyl-3, 9-diaza bicyclo [4.2.1] nonane and 10-methyl-3, 10-diaza bicyclo [4.3.1] decane, Eur. J. Med. Chem., 1987, 22, 573-577.
- Gowda D., Mahesh B., Shankare G., Zinc catalyzed ammonium formate reductions: reduction of nitro compounds, Ind. J. Chem., 2001, 40B, 75-77.
- Clemencia C.P., Determination of the cytotoxic effects of extracts, fractions or substances by means of the MTT test, 2006. ([www.iupac.org](http://www.iupac.org) accessed on 15.08.2008)
- Mehtap G., Semra U., Ningur N., Synthesis and in-vitro antimicrobial and cytotoxicity activities of 2-[(2-nitro-1-phenylalkyl) thio]benzoic acid derivatives, Turk. J. Chem., 2003, 29, 207-217.
- Meyyanathan S.N., Murli K.E., Chandrashekhar H.R., Godavarthi A., Dhanraj S.A., Suresh B., Synthesis of some amino acid incorporated 4(3H)- quinazolinones as possible antiherpes viral agents, Ind. Drugs, 2006, 43, 497-502.

\*\*\*\*\*