# SYNTHESIS AND SPECTRAL STUDIES OF NITROSOUREA DERIVATIVES OF 6-BROMO AND 6-CHLORO-2,3-DIHYDRO-1,4-BENZOTHIAZINES AS POSSIBLE ANTICANCER AGENTS

Dinesh Rai, Vandana Gupta and R.R. Gupta*
Department of Chemistry, University of Rajasthan, Jaipur – 302004, India

**Abstract** : The synthesis of nitrosourea derivatives of 6-bromo and 6-chloro-2,3-dihydro-1,4-benzothiazines by isocyanation and successive nitrosation is reported.

## Introduction :

Phenothiazines are well known heterocycles having a wide spectrum of biological activities. Their several derivatives are in clinical use. Phenothiazines also exhibit significant anticancer activities which have been assigned to their interaction with DNA. Biological activities of phenothiazines have been ascribed to their structural specificity of having a fold along nitrogen-sulfur axis (1). This structural specificity is also present in 1,4-benzothiazines (1,2) having an anticipation to patternize them for anticancer activities. 2,3-Dihydro-1,4-benzothiazines are less toxic and form an interesting class of heterocyclic drugs for anticancer activities.

Nitrosoureas are an important class of antitumour agents that have demonstrated activity against a wide range of human malignancies due to their unique chemical and biological characteristics. Several nitrosoureas are clinically significant. They interact with DNA by alkylation (3-7). However, their clinical use is limited, because of cumulative and delayed side effects exerted by these compounds. Therefore, it has been considered worthwhile to develop a series of nitrosoureas with minimum toxicity and side effects.

In 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines, heterocyclic nitrogen with a side chain at 4-position constitutes an N-nitrosourea linkage and as such these compounds contain both 1,4-benzothiazine nucleus and a nitrosourea moiety. They will interact with DNA by complexation as well as by alkylation and constitute a new class of bifunctional anticancer agents (8,9).

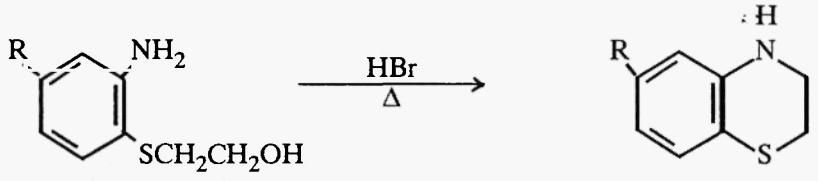
## Experimental :

Melting points of the synthesized compounds are uncorrected. The purity was checked by thin layer chromatography. Characterization of synthesized compounds was done by spectral studies. The infrared spectra were recorded on Perkin-Elmer spectrometer model 881 using potassium bromide

discs. NMR spectra were recorded on 90 MHz Jeol FX 90 Q FT NMR using TMS as internal standard. Mass spectra were scanned on Jeol JMSD-300 mass spectrometer at 70 ev with 100 $\mu$ amp ionising current. Physical data of newly synthesized compounds are summarized in Table-1.

**(i) Preparation of 6-bromo and 6-chloro-2,3-dihydro-1, 4-benzothiazines**

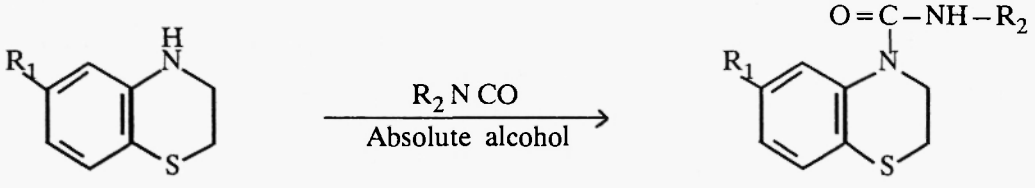
A mixture of 20 gms of freshly distilled 2-(2-aminophenylthio)ethanol **1** and 300 ml of 48% hydrobromic acid was refluxed for three days. The reaction mixture was cooled in ice bath, neutralized with sodium hydroxide and extracted several times with ether. The ether extracts were washed with water and extracted with 6N HCl. These acid extracts were cooled in ice and made basic to litmus with sodium hydroxide. The resulting oily compound 6-bromo and 6-chloro-2,3-dihydro-1,4-benzothiazines was extracted with ether. Again the combined ether extracts were washed with water and dried over magnesium sulfate. The ether was removed by vacuum rotatory evaporator and product was distilled under vacuum (Scheme-1).




**1** **Scheme-1** **2**

**(ii) Preparation of 6-Bromo/chloro-4-(N-alkyl/arylamido)-2,3-dihydro-1,4-benzothiazines :**

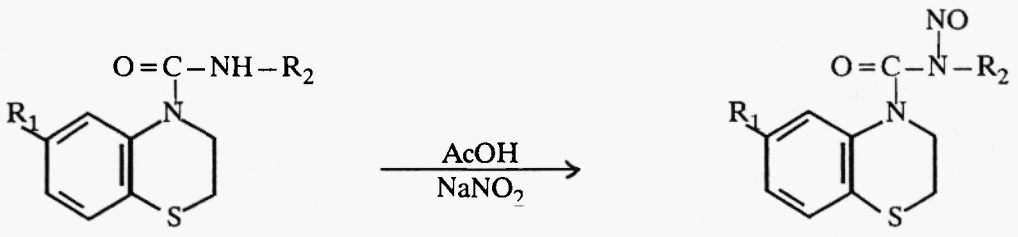
A mixture of 10 m moles of 6-bromo/chloro-2,3-dihydro-1,4-benzothiazines **2**, 10 ml of absolute alcohol and 10 m moles of alkyl/aryl isocyanate was warmed on a hot plate for two hours. Then the solvent was removed using a vacuum rotatory evaporator. The product 6-bromo/chloro-4-(N-alkyl/arylamido)-2,3-dihydro-1,4-benzothiazines was crystallized from ethanol (Scheme-2).




**2** **Scheme–2** **3**

**(iii) Preparation of 6-bromo/chloro-4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines :**

6-Bromo/chloro-4-(N-alkyl/arylamido)-2,3-dihydro-1,4-benzothiazines (3 m moles) was dissolved in 50 ml of acetic acid and sodium nitrite (5 m moles) was added portionwise with stirring. The mixture was stirred for 30 minutes at room temperature and after this for one hour at 50 °C. Acetic acid was evaporated under reduced pressure in vacuum rotatory evaporator. Residue was treated with water. The resulting precipitate of 6-bromo/chloro-4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1, 4-benzothiazineswas collected and crystallized from methanol (Scheme-3).




3 Scheme–3 4

| Compounds | $R_1$ | $R_2$ |
|---|---|---|
| a | Br | $-CH_2CH_2CH_3$ |
| b | Br | $-C_6H_4-Cl(m)$ |
| c | Br | $-C_6H_4-Cl(p)$ |
| d | Cl | $-CH_2CH_2CH_3$ |
| e | Cl | $-C_6H_4-Cl(m)$ |
| f | Cl | $-C_6H_4-Cl(p)$ |

**Results and discussion :**

The synthesis of 6-bromo/chloro-4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines is based on the synthesis of 2,3-dihydro-1,4-benzothiazines reported elsewhere (1). 2,3-Dihydro-1,4-benzothiazines are analogs of phenothiazines and like phenothiazines they bear a fold along nitrogen-sulfur axis which is considered responsible to impart them biological activities. Nitrosoureas, as already been mentioned in introduction, are also biologically active compounds. So it was designed to incorporate the activities of benzothiazines and nitrosoureas into one molecule i.e. nitrosourea derivatives of benzothiazines namely 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines. Benzothiazines are key compounds to synthesize the above mentioned compounds. Here the 2,3-dihydro-1,4-benzothiazines were allowed to undergo isocyanation at 4-position, thereby giving 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines. These were then let to undergo nitrosation with sodium nitrite in acetic acid. Some of the synthesized nitrosourea derivatives have shown good response to cure tumours with good therapeutic ratio and delayed tumour growth.

**Acknowledgement :**

Bhabha Atomic Research Center, Trombay, Bombay is gratefully acknowledged for the award of research fellowship. CDRI, Lucknow is also thanked for providing IR and Mass spectra.

**Table 1 : Physical data of substituted 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines**

| Compound | Molecular formula | M. Pt. °C | Yield % | % | | |
|---|---|---|---|---|---|---|
| | | | | C Found (Calcd) | H Found (Calcd) | H Found (Calcd) |
| a | $C_{12}H_{14}BrN_3O_2S$ | Viscous | 32 | 41.81 (41.87) | 4.19 (4.09) | 12.33 (12.20) |
| b | $C_{15}H_{11}BrClN_3O_2S$ | 135 | 46 | 43.60 (43.65) | 2.81 (2.68) | 10.27 (10.18) |
| c | $C_{15}H_{11}BrClN_3O_2S$ | Viscous | 50 | 43.74 (43.65) | 2.60 (2.68) | 10.13 (10.18) |
| d | $C_{12}H_{14}ClN_3O_2S$ | Viscous | 44 | 48.15 (48.07) | 4.86 (4.70) | 14.23 (14.01) |
| e | $C_{15}H_{11}Cl_2N_3O_2S$ | 80 | 63 | 48.97 (48.92) | 3.18 (3.10) | 11.56 (11.41) |
| f | $C_{15}H_{11}Cl_2N_3O_2S$ | Viscous | 52 | 48.73 (48.92) | 3.21 (3.10) | 11.49 (11.41) |

**Table 2 : Infrared spectral data of substituted 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines (in $cm^{-1}$)**

| Compound | Molecular Formula | C=O A | C–Cl B |
|---|---|---|---|
| a | $C_{12}H_{14}BrN_3O_2S$ | 1632 | – |
| b | $C_{15}H_{11}BrClN_3O_2S$ | 1598 | 760 |
| c | $C_{15}H_{11}BrClN_3O_2S$ | 1598 | 768 |
| d | $C_{12}H_{14}ClN_3O_2S$ | 1571 | 652 |
| e | $C_{15}H_{11}Cl_2N_3O_2S$ | 1596 | 764 |
| f | $C_{15}H_{11}Cl_2N_3O_2S$ | 1592 | 761 |

**Table 3 : NMR spectral data of substituted 4-(N-alkyl/aryl-N-nitrosoamido)-2,3-dihydro-1,4-benzothiazines**

| Compound | Solvent | δ (ppm) | Hydrogen | Multiplicity | Assignment |
|---|---|---|---|---|---|
| a | $CDCl_3$ | 7.41–6.91 | 3 | Multiplet | Aromatic Protons |
| | | 4.30–4.05 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.99–3.60 | 2 | Triplet | $CH_2$ Protons at $C_2$ |
| | | 3.42–3.07 | 2 | Triplet | $CH_2$ Protons at $C'_1$ |
| | | 2.47–2.06 | 2 | Multiplet | $CH_2$ Protons at $C'_2$ |
| | | 1.68–1.07 | 3 | Triplet | $CH_3$ Protons at $C'_3$ |
| b | $CDCl_3$ | 7.67–7.06 | 7 | Multiplet | Aromatic Protons |
| | | 4.18–4.02 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.55–3.29 | 2 | Triplet | $CH_2$ Protons at $C_2$ |
| c | $CDCl_3$ | 7.67–6.46 | 7 | Multiplet | Aromatic Protons |
| | | 3.74–3.58 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.10–2.94 | 2 | Triplet | $CH_2$ Protons at $C_2$ |
| d | $CDCl_3$ | 7.54–6.97 | 3 | Multiplet | Aromatic Protons |
| | | 4.21–4.02 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.98–3.36 | 2 | Triplet | $CH_2$ Protons at $C_2$ |
| | | 2.37–1.96 | 2 | Triplet | $CH_2$ Protons at $C'_1$ |
| | | 1.77–1.20 | 2 | Multiplet | $CH_2$ Protons at $C'_2$ |
| | | 0.82–0.62 | 3 | Triplet | $CH_3$ Protons at $C'_3$ |
| e | $CDCl_3$ | 7.41–6.46 | 7 | Multiplet | Aromatic Protons |
| | | 3.80–3.55 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.26–3.01 | 2 | Triplet | $CH_2$ Protons at $C_2$ |
| f | $CDCl_3$ | 7.44–6.49 | 7 | Multiplet | Aromatic Protons |
| | | 3.80–3.55 | 2 | Triplet | $CH_2$ Protons at $C_3$ |
| | | 3.17–3.01 | 2 | Triplet | $CH_2$ Protons at $C_2$ |

**References :**

1. R.R. Gupta (Ed.) "Phenothiazines and 1,4-Benzothiazines – Chemical and Biomedical Aspects", Elsevier, Amsterdam, 1988

2. H. Keyzer, G.M. Eckert, I.S. Forrest, R.R. Gupta, F. Gutmann, J. Molnar (Eds.), "Thiazines and Structurally Related Compounds" (Proceedings of Sixth International Conference on Phenothiazines and Structurally Related Psychotropic Compounds, Pasadena, California, Sep. 11-14 (1990). Krieger Publishing Company, Malabar, Florida, USA (1992)

3. A.W. Prestayko, S.T. Crooke, L.H. Baker et al (Eds.) "Nitrosoureas : Current status and new developments". Academic Press, New York 1981

4. B. Serrou, P.S. Schein, J.L. Imbach. (Eds.) "Nitrosoureas in Cancer Treatment". Elsevier, New York, 1981

5. J.A. Montgomery, T.P. Johnston. In D.E.V. Wilman (Ed.) "Chemistry of Antitumour Agents" Blackie Glasgow, 131-58, 1991

6. G. Eisenbrand, M.R. Berger, J. Fische et al, Anticancer Drug Design, **2**, 351 (1988)

7. D.J. Reed, "2-Chloroethylnitrosoureas". in G. Powis, R.A. Prough, (Eds.) "Metabolism and Action of Anti-cancer Agents.' London; Taylor and Francis 1-28, 1987

8. R.R. Gupta, P.K. Dev, M.L. Sharma, C.M. Rajoria, Archana Gupta and M. Nyati; Anti-Cancer Drugs **4**, 589-592 (1993)

9. Mukesh K. Nyati, C.M. Rajoria, R.R. Gupta and P.K. Dev; Anti-Cancer Drugs **6**, 693-96 (1995)

**Received November 7, 1996**