Design, Synthesis of Mannich Bases Derivatives of Thiosemicarbazide and their Evaluation for Anticancer Activity using Potato Disk Bioassay Method

Geetanjali Sandip Patil a and Sachin Ashok Pishawikar b*

a Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College Pharmacy, Chitri Nagar, Kolhapur, Maharashtra, Pin-416013, India.
b Department of Pharmaceutical Chemistry, Anandi College of Pharmacy, Kale Kolhapur, Pin-416205, India.

ABSTRACT

Mannich bases and thiosemicarbazide individually are known to show variety of pharmacological activities such as anti-inflammatory, anticancer, antifilarial, antibacterial, antifungal, anticonvulsant, anthelmintic, antitubercular, analgesic, anti-HIV, antimalarial, antipsychotic, antiviral and so forth. As novel attempt in present work in first step synthesis of verity of mannich bases is done using structurally different types of aldehyde, ketones and amines. The condensation of synthesized mannich bases is done with thiosemicarbazide to form a novel class of compounds called mannich bases of thiosemicarbazide. Use of bioassay is one of the ways of carrying out preliminary investigation of activity. A. tumefaciens induced potato disc tumor assay has been used to investigate anticancer activity of synthesized compounds. The compounds B2, B4, B25, B26, B28, B29 and B30 have shown same or better inhibitory activity compared to Gemcitabine used as standard.

*Corresponding author: E-mail: sachin_pishawikar@rediffmail.com;
Keywords: Mannich bases; thiosemicarbazide; bioassay; potato disc; anticancer; heterocyclic.

1. INTRODUCTION

There are number of life-threatening diseases prevalent globally and slowly cancer has become a big threat to human beings. Newer types of cancer are coming into picture. Through various reports on cancer cases it is estimated that 19.3 million new cancer cases (18.1 million excluding nonmelanoma skin cancer) and almost 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) occurred globally in 2020 [1].

In India, category wise i.e. the total number of new cases in males will increased from 0.589 million in 2011 to 0.934 million by the year 2026. In females the new cases of cancer increased from 0.603 to 0.935 million [1-2].

Cancer has become second largest disease after cardiovascular disorders responsible for maximum mortality with around 0.3 million deaths per year in India [1-2]. This has led to the continuous increase in demand for new antitumor drugs with different mechanism of action [3-8].

As per the CPCAC guidelines 3 Rs likewise replacement, reduction and refinement have led restricted use of animal in research. There is increase emphasis on replacement of animal methods by non-animal methods. The refinement of experimental method emphasizes on reducing the pain and suffering of animals used [2-3]. Most convenient and inexpensive alternative to animal studies are plant tumor assay methods that can be used for screening new anticancer drugs [9-10].

Bioassay methods offer special advantages in establishing the biological activities like antitumor, antibacterial, antioxidant and phytotoxic properties and are becoming a preliminary step in drug discovery. For preliminary investigation of anticancer activity Potato disc assay has become most useful method. The bioassay is based on inhibition of tumour caused by Agrobacterium tumefaciens in potato disc. The mechanism of initiating tumour in plant tissues, is similar to tumour generated by carcinogenic agents in humans which validates the use of assay in screening new anti-cancer drugs. In fact, Kempf, et al., have confirmed that Bartonella henselae, a bacterium causing tumour in human shows a similar pathogenic strategy as shown by plant pathogen A. tumefaciens. The similarities include the use of common toxins, secretion system, adhesion mechanism, invasion and regulation. The antitumor potato disc assay is considered to be sensitive for screening chemical compounds having different modes of action for interfering with cell cycle whereby they can show anticancer activity [9-11].

Mannich base and thiosemicarbazide individually are known to show different types of pharmacological activities as well as act as important pharmacophores or lead structures used in synthesis of various potential agents with high medicinal value [12,4].

The aim of present work was to combine two pharmacologically active entities in synthesizing thiosemicarbazide derivatives of mannich bases as novelty with expectation that each derivative would show good anticancer activity.

2. MATERIALS AND METHOD

2.1 Synthesis of Mannich Bases of Thiosemicarbazide

Step One

Synthesis of mannich bases is done using aldehyde, ketone and amines having aliphatic, aromatic, cyclic and heterocyclic nature in proportion 1.00 molecular equivalent of carbonyl compound (ketone), 1.05-1.10 molecular equivalent of amine in the form of hydrochloride salt and 1.5-2.0 molecular equivalence of aldehyde. The reaction conditions had to be optimized individually and the time of reaction varied from 45 minutes to 12-14 hr.

Step Two

The synthesized mannich bases were condensed with thiosemicarbazide in one is to one proportion to get mannich bases of thiosemicarbazide.

2.2 Characterization of Synthesized Compounds

Identification and Characterization of Synthesized Compounds

All the synthesized compounds were identified and characterized by following methods:
Scheme 1. Scheme for Synthesis

- Physicochemical characterization
- Qualitative chemical analysis
- Thin layer chromatography
- Infra-red spectroscopy
- UV-Visible spectroscopy
- Nuclear magnetic resonance spectroscopy

Results are mentioned in Table 9

2.3 Preliminary Evaluation of Anticancer activity Using Bioassay

Potato disk bioassay

The inhibition of A. tumefaciens-induced tumors (or Crown Gall) in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor effects.

Procedure

Fresh Russet potatoes were collected from a local grocery store. Sterilized in laboratory using a 20% bleaching solution. The potato discs of dimension 1 cm x 1 cm x 0.5 cm were cut. Five discs were placed in 1.5% agar media in petri plates and allowed to submerged up to 2/3. The experiment was performed in three groups; Test group (Solutions of different synthesized compounds), control group (DMSO with Sterile water) and Standard group (Gemcitabine). 10 ml of solution of each synthesized manich bases of thiosemicarbazide having concentrations of 100 PPM and 10PPM was prepared using DMSO in disposable culture tubes. Further inoculums were prepared as follows:

1) 1.5 ml sterile water, 2.5 ml 48 hrs incubated bacterial culture and 5 ml sample were added in DMSO.

2) Controls were prepared by replacing extract with only DMSO with Sterile water.

3) Same procedure was followed for standard anticancer drug Gemcitabine.

From different groups, 0.05 ml sample were added on five potato discs in respective labeled Petri plates (test with respective compounds, standard and control). Plates were covered, tape the lids using cello tape (to minimize moisture loss), and incubate under dry conditions at room temperature for 7 –12 days. After 7 –12 days incubation, the potatoes discs were analyzed using a colony counter magnifying glass after staining with Lugol’s Solution and experiment was repeated in triplicate. The tumors lack of starch will turn orange to black in the presence of the stain while the potato discs will turn dark blue. Potato discs inoculated with the control solutions should average 10-30 tumors. Finally calculation of the percentage inhibition of crown gall tumors was done using following formula:

\[
\% \text{ inhibition of tumor} = \frac{\text{Average number of tumors in test}}{\text{Average number of tumors in control}} \times 100
\]

3. RESULT

Synthesis of manich bases of thiosemicarbazide was done using two step reactions. In first step manich base were synthesized using manich reaction. In second step synthesized manich bases were condensed with thiosemicarbazide to get manich bases of thiosemicarbazide.
List of Thiosemicarbazide derivatives of mannich bases synthesized is given in following tables.

**Fig. 1. Potato Disk Bioassay**

**Table 1. Aromatic - Ketone, Aldehyde, Amine**

| Compound | R       | R1      | R2 | R3       |
|----------|---------|---------|----|---------|
| B1       | -C₆H₅   | -C₆H₅   | -H | -C₆H₅   |
| B2       | -C₆H₄NO₂ | -C₆H₅   | -H | -C₆H₅   |
| B3       | -C₆H₅   | -C₆H₅   | -H | -C₁₀H₉  |
| B4       | -C₆H₅   | -C₆H₅   | -H | -C₆H₄F  |

**Table 2. Aromatic - Ketone, Amine and Aliphatic- Aldehyde**

| Compound | R       | R1      | R2 | R3       |
|----------|---------|---------|----|---------|
| B5       | -C₆H₅   | -CH₃    | -H | -C₆H₅   |
| B6       | -C₆H₄NO₂ | -CH₃    | -H | -C₆H₅   |
| B7       | -C₆H₄NH₂ | -CH₃    | -H | -C₁₀H₉  |
| B8       | -C₆H₄OH  | -CH₃    | -H | -C₁₀H₉  |
| B9       | -C₆H₄OH  | -CH₃    | -H | -C₆H₄F  |
| B10      | -C₆H₄O₂  | -H      | -H | -C₁₀H₉  |

**Table 3. Aromatic - Ketone, Aldehyde and Aliphatic- Amine**

| Compound | R       | R1      | R2 | R3       |
|----------|---------|---------|----|---------|
| B11      | -C₆H₅   | -C₆H₅   | -C₂H₅ | -C₂H₅   |
| B12      | -C₆H₄NO₂ | -C₆H₅   | -C₂H₅ | -C₂H₅   |
| B13      | -C₆H₄NO₂ | -CH₃    | -H  | -C₆H₁₃  |
| B14      | -C₇H₈    | -CH₃    | -H  | -C₆H₁₃  |
| B15      | -C₆H₅    | -C₆H₅   | -H  | -C₆H₄NO|
Physicochemical Characterization of the synthesized compounds was done by determination of melting point, TLC

| Compound | Molecular Formula | Mol. Wt.(gm) | Color            | Solubility | M. P. (°C) | %Yield | Rf value |
|----------|------------------|--------------|------------------|------------|------------|---------|----------|
| B1       | C_{20}H_{20}N_{10}S | 374.50       | Green            | EtOH       | 120°-122°C | 68%     | 0.5      |
| B2       | C_{20}H_{20}N_{10}O_{12}S | 419.49    | Light Green      | EtOH       | 128°-130°C | 72%     | 0.6      |
| B3       | C_{20}H_{20}N_{10}O_{12}S | 424.56    | Mahogany         | EtOH       | 124°-126°C | 67%     | 0.5      |
| B4       | C_{20}H_{20}N_{10}O_{12}S | 392.49    | Maroon           | EtOH       | 208°-210°C | 62%     | 0.6      |
| B5       | C_{17}H_{20}N_{10}O_{12}S | 312.43    | FlatteryBrown    | EtOH       | 116°-118°C | 69%     | 0.6      |
| B6       | C_{17}H_{20}N_{10}O_{12}S | 357.43    | Drab             | EtOH       | 84°-86°C   | 78%     | 0.5      |
| B7       | C_{21}H_{20}N_{10}O_{12}S | 377.75    | Arsenic          | EtOH       | 190°-192°C | 70%     | 0.7      |
| B8       | C_{21}H_{20}N_{10}O_{12}S | 378.49    | Orange           | EtOH       | 198°-200°C | 79%     | 0.6      |
| B9       | C_{21}H_{20}N_{10}O_{12}S | 464.53    | Bistre Brown     | EtOH       | 200°-202°C | 77%     | 0.6      |
| B10      | C_{17}H_{19}N_{10}O_{12}S | 346.42    | Orange           | EtOH       | 120°-124°C | 80%     | 0.7      |
| B11      | C_{20}H_{20}N_{10}O_{12}S | 354.51    | Light Orange     | EtOH       | 180°C-     | 79%     | 0.8      |
Table 10. IR interpretations of few synthesized compounds

| Sr. No. | Compound | IR cm⁻¹ |
|---------|----------|---------|
| 1       | B2       | C – H stretching at 2886.15 cm⁻¹, N – H stretching at 3294.79 cm⁻¹, C = S stretching at 1294 cm⁻¹, C = N stretching at 1560.13 cm⁻¹, CH₂ – CH₂ at 2992.7 cm⁻¹ |
| 2       | B4       | C – H stretching at 2881.13 cm⁻¹, N – H stretching at 3398.92 cm⁻¹, C = S stretching at 1293.04 cm⁻¹, C = N stretching at 1556.27 cm⁻¹, CH₂ – CH₂ at 2931.93 cm⁻¹ |
| 3       | B16      | C – H stretching at 2880.17 cm⁻¹, N – H stretching at 3339.14 cm⁻¹, C = S stretching at 1294.89 cm⁻¹, C = N stretching at 1557.24 cm⁻¹, CH₂ – CH₂ at 2931.93 cm⁻¹ |

Table 11. NMR interpretations of few synthesized compounds

| Sr. No. | Compound | NMR(ppm) |
|---------|----------|----------|
| 1       | B24      | 7.15 – 7.62(Ar-H), 1.5-5 (-NH₂, Proton on saturated carbon attached to heteroatom), 1.2-1.4(Secondary alkyl), 1.5 (Tertiary alkyl), 8.7-9.2 CH=N |
| 2       | B4       | 1.5-4 (NH₃), 1.2-1.4(Secondary alkyl), 6.35 – 8.16(Ar-H), 2.2-3 (Ar-CH) |
Table 12. % Inhibition of tumor shown by various compound in Potato Disk Bioassay

| Sr. No. | Compound | Conc. PPM | % Inhibition of Tumor | Sr. No. | Compound | Conc. PPM | % Inhibition of Tumor |
|---------|----------|-----------|-----------------------|---------|----------|-----------|-----------------------|
| 1       | B1       | 10        | 75                    | 19      | B19      | 10        | 80                    |
|         |          | 100       | 78                    |         |          | 100       | 76                    |
| 2       | B2       | 10        | 85                    | 20      | B20      | 10        | 71                    |
|         |          | 100       | 88                    |         |          | 100       | 69                    |
| 3       | B3       | 10        | 72                    | 21      | B21      | 10        | 68                    |
|         |          | 100       | 76                    |         |          | 100       | 62                    |
| 4       | B4       | 10        | 84                    | 22      | B22      | 10        | 62                    |
|         |          | 100       | 86                    |         |          | 100       | 70                    |
| 5       | B5       | 10        | 62                    | 23      | B23      | 10        | 75                    |
|         |          | 100       | 62                    |         |          | 100       | 84                    |
| 6       | B6       | 10        | 66                    | 24      | B24      | 10        | 64                    |
|         |          | 100       | 60                    |         |          | 100       | 78                    |
| 7       | B7       | 10        | 57                    | 25      | B25      | 10        | 82                    |
|         |          | 100       | 63                    |         |          | 100       | 87                    |
| 8       | B8       | 10        | 63                    | 26      | B26      | 10        | 92                    |
|         |          | 100       | 62                    |         |          | 100       | 95                    |
| 9       | B9       | 10        | 38                    | 27      | B27      | 10        | 76                    |
|         |          | 100       | 42                    |         |          | 100       | 80                    |
| 10      | B10      | 10        | 75                    | 28      | B28      | 10        | 84                    |
|         |          | 100       | 73                    |         |          | 100       | 88                    |
| 11      | B11      | 10        | 63                    | 29      | B29      | 10        | 84                    |
|         |          | 100       | 63                    |         |          | 100       | 85                    |
| 12      | B12      | 10        | 54                    | 30      | B30      | 10        | 80                    |
|         |          | 100       | 65                    |         |          | 100       | 84                    |
| 13      | B13      | 10        | 48                    | 31      | B31      | 10        | 63                    |
|         |          | 100       | 58                    |         |          | 100       | 64                    |
| 14      | B14      | 10        | 69                    | 32      | B32      | 10        | 70                    |
|         |          | 100       | 65                    |         |          | 100       | 76                    |
| 15      | B15      | 10        | 60                    | 33      | B33      | 10        | 74                    |
|         |          | 100       | 64                    |         |          | 100       | 70                    |
| 16      | B16      | 10        | 48                    | 34      | B34      | 10        | 72                    |
|         |          | 100       | 51                    |         |          | 100       | 68                    |
| 17      | B17      | 10        | 82                    | 35      | Control  | -         | 11                    |
|         |          | 100       | 78                    |         |          | -         | 16                    |
| 18      | B18      | 10        | 79                    | 36      | Gemcitabine | 10  | 82                    |
|         |          | 100       | 82                    |         |          | 100       | 84                    |

Fig. 2. IR of compound B4
Fig. 3. IR of compound B24

Fig. 4. IR of compound B29

Fig. 5. NMR of compound B24
Preliminary anticancer bioassay

Potato disk bioassay is based on inhibition of A. tumefaciens-induced tumors in potato disc tissue. In this bioassay, as the % inhibition of tumors increases, there is increase in cell growth retardation. Synthesized compounds (B2, B4, B25, B26, B28, B29 and B30) with concentration of 10 and 100 PPM have shown promising results of % tumor inhibition compared with standard Gemcitabine.

4. DISCUSSION

Compounds like B2 and B4 in which all components used for synthesis of manich base are aromatic in nature have shown comparable activity to standard. The compound with nitro substituted aromatic ketone like B25 have shown good activity.

Compound having combination of Aromatic – Aldehyde, Aliphatic- Ketone and Heterocyclic: Amine like compound B26 has shown highest activity.

5. CONCLUSION

From bioassay results it can be concluded that thiosemicarbazide derivatives of manich base as new chemical entity have shown promising results. By carrying out further In-vitro and in-vivo screening studies confirmation of the potential of developed manich bases of thiosemicarbazide as anticancer drugs as well as new chemical entity needs to be done.
COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. Hyuna Sung, Jacques Ferlay, Rebecca L Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, Freddie Bray Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA Cancer J Clin. 2021;71(3):209-249.
2. D’Souza ND, Murthy NS, Projection of burden of cancer mortality for India, 2011-2026., Aras RY. Asian Pac J Cancer Prev. 2013;14(7):4387-92.
3. Carl Mannich, Krösche, W. Ueber ein Kondensationsprodukt aus Formaldehyd, Ammoniak und Antipyrin. Archiv der Pharmazie (in German). 1912;250(1):647–667.
4. Blicke FF. The Mannich Reaction. Organic Reactions. 2011;1(10):303–341.
5. Pishawikar SA, More HN. Synthesis of Mannish Bases of Thiosemicarbazide as DNA polymerase Inhibitors and Novel Antibacterial Agents. Int. J. Pharm. Bio. Sci. 2013;4:549 – 556.
6. Plech T, Wujec M, Siwek A, Kosikowska U, Malm A. Synthesis and antimicrobial activity of thiosemicarbazides, s-triazoles and their Mannich bases bearing 3-chlorophenyl moiety. Eur. J. Med. Chem. 2011;46:241-248.
7. Rajendran and Priyadarshini M. Synthesis and characterization of a novel ionic liquid (TBA-AMPS) and its applications in Mannich condensation reactions under solvent free conditions. Afr. J. Pure Appl. Chem. 2010;4:183-187.
8. Rajveer Ch, Stephenrathinaraj B, Sudharshini S, Kumaraswamy D, Bhupendra S, Choudhury PK. Synthesis of some mannich base cyclohexanone derivatives and their pharmacological activities. RJPBCS. 2010;1:100-107.
9. Srirama R, Ramesha G, Ravikanth RUS, Ganeshaiah KN. Are plants with anticancer activity resistant to crown gall? A test of hypothesis. Current Science. 2007;95,10-25.
10. Turker, A.U. and Camper, N.D. Biological Activity of Common Mullein, a Medicinal Plant. Journal of Ethnopharmacology, 2002;82:117-125.
11. Galisky AG, Wilsey JP, Powell RG. Crown gall tumor disc bioassay: a possible aid in the detection of compounds with antitumor activity. Plant Physiology. 1980;65:184-185.
12. Ferigni NR, Putnam JE, Anderson B, et al. Modification and evaluation of the potato disc assay and antitumor screening of Euphorbiaceae Seads, J. Nat. Prod. 1982;45(6):679–686.

© 2022 Patil and Pishawikar: This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/83427