Original Research Article

Design and Synthesis of Mannich Bases derivatives of Thiosemicarbazide and its evaluation of Anticancer Activity of by Potato Disk Bioassay Method

ABSTRACT

Introduction:

In present work a successful novel attempt has been done to carry out synthesis of verity of mannich bases and condense them with thiosemicarbazide to form a novel class of compounds called mannich bases of thiosemicarbazide. As Mannich bases and thiosemicarbazide individually are known to have different types of pharmacological activities like anti-inflammatory, anticancer, antifilarial, antibacterial, antifungal, anticonvulsant, anthelmintic, antitubercular, analgesic, anti-HIV, antimalarial, antipsychotic, antiviral and so forth the designing of synthetic scheme was done.

Material and Method:

In step one aldehyde, ketones and amines with different structural features like aliphatic, cyclic, aromatic and heterocyclic are used for synthesis of mannich bases. While in step 2 the synthesized mannich bases were condensed with thiosemicarbazide to form thiosemicarbazide derivatives of mannich base.

Potato Disk Bioassay for Preliminary Evaluation of Anticancer Activity

As per the CPCAC guidelines 3 Rs likewise replacement, reduction and refinement have led restricted use of animal in research. Hence preliminary evaluation for anticancer activity of synthesized compounds was carried out on *A. tumefaciens* induced potato disc tumor. Assay is considered as effective indicator of antitumor activity regardless of the mechanism of drug action.

Result:

The compounds B2, B4, B25, B26, B28, B29 and B30 have shown same or better inhibitory activity compared to Gemcitabine used as standard.

Discussion:

Compounds like B2 and B4 in which all components used for synthesis of mannich base are aromatic in nature have shown comparable activity to standard.

The nitro substituted aromatic ketone containing compound like B25 have also shown good activity.

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

Key Words: Mannich bases, thiosemicarbazide, Bioassay, potato disc, anticancer, Heterocyclic

1. INTRODUCTION:

There are number of lifethreatening diseases prevalent globally and slowly cancer has become a big threat to human beings. Newer types of cancer are coming into picture. It is rough estimate that by 2021 the world population suffering from cancer would be 7.5 billion or more, with a prediction that on an average 15.0 million new cancer cases will be diagnosed, with about 12.0 million deaths every year. 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.

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.[3-4]

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.[3-5]

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. [6-8]

Hence in present work a novel attempt has been done in combining mannich bases with thiosemicarbazide to synthesize thisemicarbazide derivatives of mannich bases and carry out preliminary investigation for anticancer activity using potato disk bioassay.

2. MATERIALS AND METHOD:

2.1 Synthesis of Mannich Bases of Thiosemicarbazide

Step One

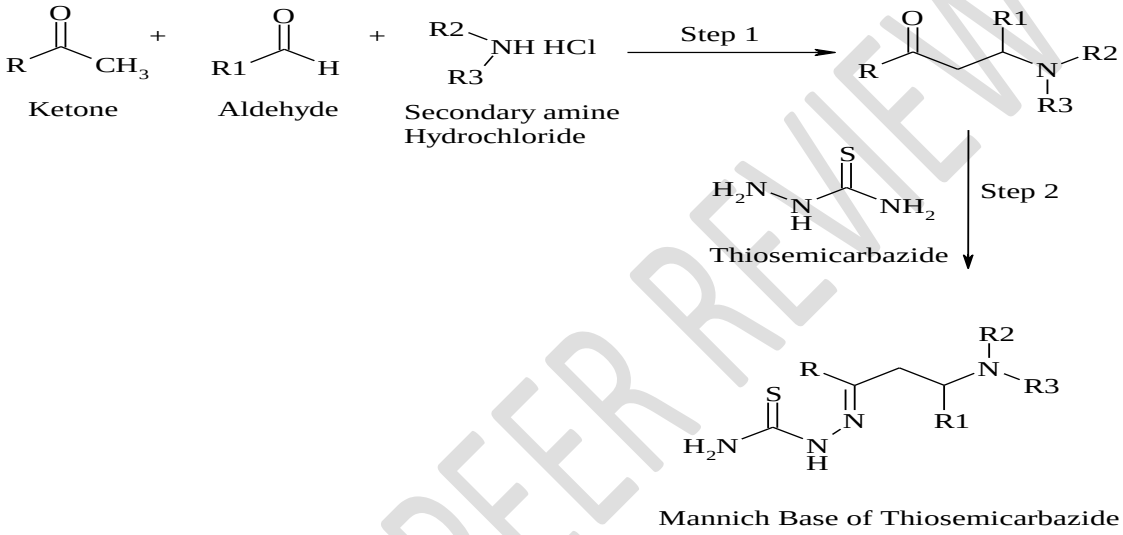
Synthesis of mannich bases is done using aldehyde, ketone and amines having aliphatic, aromatic, cyclic and heterocyclic nature in proportion 1.00 molecular equivalent of carbonyl compound (ketone), 1.05-1.10 molecular equivalent of amine in the form of hydrochloride salt

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.

Scheme 1: Scheme for Synthesis:



2.2 Characterization of Synthesized Compounds:

Identification and Characterization of Synthesized Compounds

All the synthesized compounds were identified and characterized by following methods:

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

Results are mentioned in Table No. 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 mannich 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.

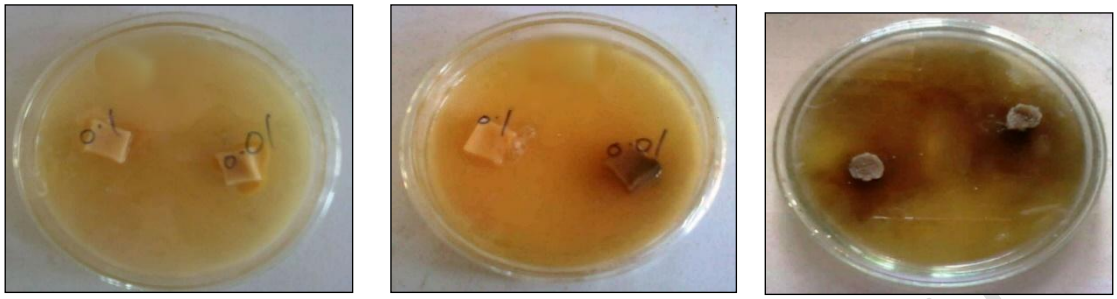
2) Controls were prepared by replacing extract with only DMSO with Sterile water.

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

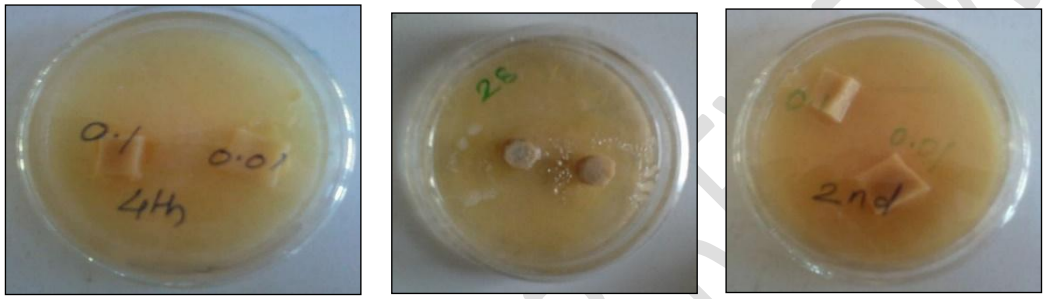
From different groups, 0.05 ml sample were added on five potato discs in respective labeled Petri plates (test with respective compounds, standard and control). Plates were covered, tape the lids using cello tape (to minimize moisture loss), and incubate under dry conditions at room temperature for 7 –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:

Average number of tumors in test

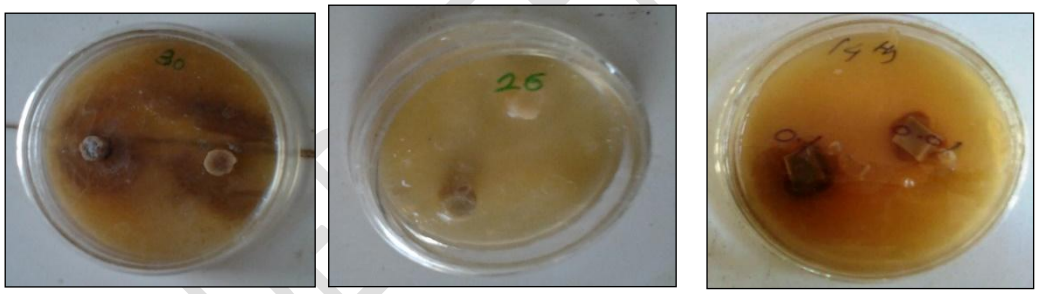
% inhibition of tumor = $1 - \frac{\text{Average number of tumors in control}}{\text{Average number of tumors in control}} \times 100$



Control Standard B2



B4 B14 B26



B28 B29 B30

Figure 1: Potato Disk Bioassay

3. RESULT

Synthesis of mannich bases of thiosemicarbazide was done using two step reactions. In first step mannich base were synthesized using mannich reaction. In second step synthesized mannich bases were condensed with thiosemicarbazide to get mannich bases of thiosemicarbazide.

List of Thiosemicarbazide derivatives of mannich bases synthesized is given in following tables:

Table 1: Aromatic - Ketone, Aldehyde, Amine

Compound	R	R1	R2	R3
B1	$-C_6H_5$	$-C_6H_5$	-H	$-C_6H_5$
B2	$-C_6H_4NO_2$	$-C_6H_5$	-H	$-C_6H_5$
B3	$-C_6H_5$	$-C_6H_5$	-H	$-C_{10}H_9$
B4	$-C_6H_5$	$-C_6H_5$	-H	$-C_6H_4F$

Table 2: Aromatic - Ketone, Amine and Aliphatic- Aldehyde

Compound	R	R1	R2	R3
B5	$-C_6H_5$	$-CH_3$	-H	$-C_6H_5$
B6	$-C_6H_4NO_2$	$-CH_3$	-H	$-C_6H_5$
B7	$-C_6H_4NH_2$	$-CH_3$	-H	$-C_{10}H_9$
B8	$-C_6H_4OH$	$-CH_3$	-H	$-C_{10}H_9$
B9	$-C_6H_4OH$	$-CH_3$	-H	$-C_6H_4F$
B10	$-C_8H_8O_2$	-H	-H	$-C_{10}H_9$

Table 3: Aromatic - Ketone, Aldehyde and Aliphatic- Amine

Compound	R	R1	R2	R3
B11	$-C_6H_5$	$-C_6H_5$	$-C_2H_5$	$-C_2H_5$
B12	$-C_6H_4NO_2$	$-C_6H_5$	$-C_2H_5$	$-C_2H_5$
B13	$-C_6H_4NO_2$	$-CH_3$	-H	$-C_6H_{13}$
B14	$-C_7H_8$	$-CH_3$	-H	$-C_6H_{13}$
B15	$-C_6H_5$	$-C_6H_5$	-H	$-C_4H_8NO$

Table 4: Aromatic – Ketone and Aliphatic and heterocyclic Amine, aliphatic Aldehyde

Compound	R	R1	R2	R3
B16	$-C_6H_5$	$-CH_3$	$-C_2H_5$	$-C_2H_5$
B17	$-C_6H_4NO_2$	$-CH_3$	$-C_2H_5$	$-C_2H_5$
B18	$-C_{10}H_{12}O3$	-H	-H	$-C_6H_{13}$
B19	$-C_6H_4NO_2$	-H	-H	$-CH_3$
B20	$-C_6H_4Cl$	-H	-H	$-CH_3$
B21	$-C_6H_5$	-H	-H	$-CH_3$
B22	$-C_6H_5$	-H	-H	$-C_4H_8NO$
B23	$-C_6H_5$	$-CH_3$	-H	$-C_4H_8NO$
B24	$-C_6H_5$	-H	-H	$-C_5H_{10}N$
B25	$-C_6H_4NO_2$	-H	-H	$-C_5H_{10}N$

Table 5: Aromatic – Aldehyde and Aliphatic- Ketone, Heterocyclic: Amine,

Compound	R	R1	R2	R3
B26	$-C_6H_9O$	$-C_6H_5$	-H	$-C_4H_8NO$
B27	$-CH_3$	-H	-H	$-C_4H_8NO$

Table 6: Aromatic – Amine and Aliphatic - Ketone, Aldehyde

Compound	R	R1	R2	R3
B28	$-C_6H_9O$	-H	-H	$-C_6H_4F$
B29	$-C_6H_9O$	-H	-H	$-C_6H_5CH_2$

Table 7: Aliphatic and heterocyclic- Amine and aliphatic Ketone, Aldehyde

Compound	R	R1	R2	R3
B30	$-C_6H_9O$	-H	$-C_2H_5$	$-C_2H_5$

B31	$-C_6H_9O$	-H	-H	$-C_4H_8NO$
B32	$-CH_3$	-H	-H	$-C_4H_8NO$

Table 8: Aromatic- Amine, Ketone and Aliphatic- Aldehyde

Compound	**R**	**R1**	**R2**	**R3**
B33	$-C_6H_5$	-H	-H	$-C_6H_5$
B34	$-C_6H_5$	$-CH_3$	-H	$-C_6H_5CH_2$

Physicochemical Characterization of the synthesized compounds was done by determination of melting point, TLC

Table 9: Physicochemical Data

Comp -ound	**Molecular Formula**	**Mol. Wt.(gm)**	**Color**	**Solubility**	**M. P. (°C)**	**% Yield**	**R_f value**
B1	$C_{22}H_{22}N_4S$	374.50	Green	EtOH	120^0-122^0C	68%	0.5
B2	$C_{22}H_{21}N_5O_2S$	419.49	Light Green	EtOH	128^0-130^0C	72%	0.6
B3	$C_{26}H_{24}N_4S$	424.56	Mahogany	EtOH	124^0-126^0C	67%	0.5
B4	$C_{22}H_{21}N_4S$	392.49	Maroon	EtOH	208^0-210^0C	62%	0.6
B5	$C_{17}H_{20}N_4S$	312.43	Flattery	EtOH	116^0-	69%	0.6

			Brown		118^{0}C		
B6	$C_{17}H_{19}N_5O_2S$	357.43	Drab	EtOH	84^0-86^{0}C	78%	0.5
B7	$C_{21}H_{23}N_5S$	377.75	Arsenic	EtOH	190^0-192^{0}C	70%	0.7
B8	$C_{21}H_{22}N_4OS$	378.49	Orange	EtOH	198^0-200^{0}C	79%	0.6
B9	$C_{24}H_{24}N_4O_4S$	464.53	Bistre Brown	EtOH	200^0-202^{0}C	77%	0.6
B10	$C_{17}H_{19}FN_4OS$	346.42	Orange	EtOH	120^0-124^{0}C	80%	0.7
B11	$C_{20}H_{26}N_4S$	354.51	Light Orange	EtOH	180^{0}C-182^{0}C	79%	0.8
B12	$C_{20}H_{25}N_5O_2S$	399.50	Maroon	EtOH	186^0-188^{0}C	83%	0.5
B13	$C_{23}H_{32}N_4S$	396.59	Light red	EtOH	180^0-182^{0}C	69%	0.7
B14	$C_{22}H_{30}N_4S$	382.56	Light red	EtOH	186^0-188^{0}C	74%	0.6
B15	$C_{20}H_{23}N_5O_3S$	413.49	Light Orange	EtOH	180^0-182^{0}C	86%	0.9

B16	$C_{15}H_{24}N_4S$	292.44	Earth yellow	EtOH	140^0-142^0C	84%	0.6
B17	$C_{15}H_{23}N_5O_2S$	337.44	Drab	EtOH	138^0-140^0C	88%	0.7
B18	$C_{20}H_{30}N_4O_4S$	422.54	Dark Orange	EtOH	120^0-122^0C	65%	0.7
B19	$C_{11}H_{15}N_5O_2S$	281.33	Light Orange	EtOH	118^0-120^0C	60%	0.6
B20	$C_{11}H_{15}ClN_4S$	270.78	Crimson	EtOH	110^0-112^0C	66%	0.8
B21	$C_{11}H_{16}N_4S$	236.33	Dark Orange	EtOH	116^0-118^0C	59%	0.7
B22	$C_{14}H_{21}N_5O_2S$	323.41	Light Brown	EtOH	120^0-122^0C	64%	0.8
B23	$C_{14}H_{20}N_4OS$	292.39	Orange	EtOH	128^0-130^0C	76%	0.7
B24	$C_{15}H_{22}N_4OS$	306.42	Brown	EtOH	102^0-104^0C	80%	0.6
B25	$C_{15}H_{22}N_4S$	290.42	Dark Brown	EtOH	118^0-120^0C	71%	0.6

B26	$C_{15}H_{22}N_4S$	290.42	Chrome yellow	EtOH	110^0-112^0C	78%	0.7
B27	$C_{15}H_{21}N_5O_2S$	335.42	Cinnamon	EtOH	116^0-118^0C	87%	0.8
B28	$C_9H_{18}N_4OS$	230.33	Orange	EtOH	110^0-112^0C	83%	0.7
B29	$C_{18}H_{26}N_4OS$	346.49	Light red	EtOH	126^0-128^0C	79%	0.7
B30	$C_{14}H_{19}FN_4S$	294.39	Yellow	EtOH	120^0-122^0C	83%	0.7
B31	$C_{15}H_{22}N_4S$	290.42	Dark Brown	EtOH	124^0-126^0C	82%	0.6
B32	$C_{12}H_{24}N_4S$	256.41	Yellow	EtOH	130^0-132^0C	75%	0.8
B33	$C_{13}H_{22}N_4S$	268.42	Orange	EtOH	108^0-110^0C	78%	0.6
B34	$C_{10}H_{20}N_4S$	228.35	Dark Yellow	EtOH	116^0-118^0C	74%	0.7

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 3294.79cm^{-1}, C = S stretching at 1294 cm^{-1}, C = N stretching at 1560.13 cm^{-1}, CH_2 – CH_2 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}, CH_2 – CH_2 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}1516.74 cm^{-1} (Ar-NO_2)
4	**B29**	C – H stretching at 2806.92 cm^{-1}, N – H stretching at 3218.61cm^{-1} C = S stretching at 1281. 47 cm^{-1}, C = N stretching at 1525.42 cm^{-1}, CH_2 – CH_2 at 2938.98cm^{-1}
5	**B26**	C – H stretching at 2863.59 cm^{-1}, N – H stretching at 3146.29cm^{-1} C = S stretching at 1275.68 cm^{-1}, C = N stretching at 1556.27 cm^{-1}, CH_2 – CH_2 at 2949.59cm^{-1}
6	**B31**	C – H stretching at 2806.92cm^{-1}, N – H stretching at 3218.61cm^{-1}C = S stretching at 1281.47 cm^{-1}, C = N stretching at 1525.42 cm^{-1}
7	**B24**	C – H stretching at 2924.44 cm, N – H stretching at 3239.85 cm C = S stretching at 1239. 47 cm^{-1}, C = N stretching at 1597.67 cm^{-1}

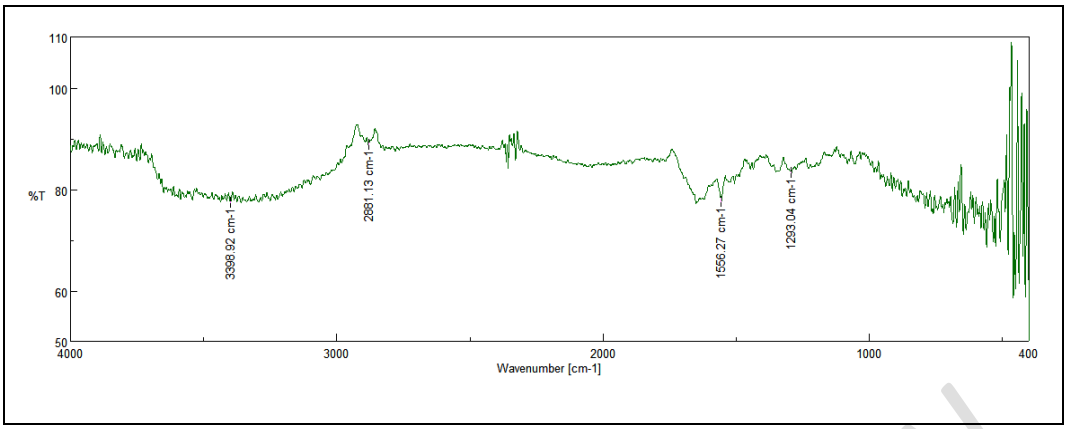


Figure 2: IR of compound B4

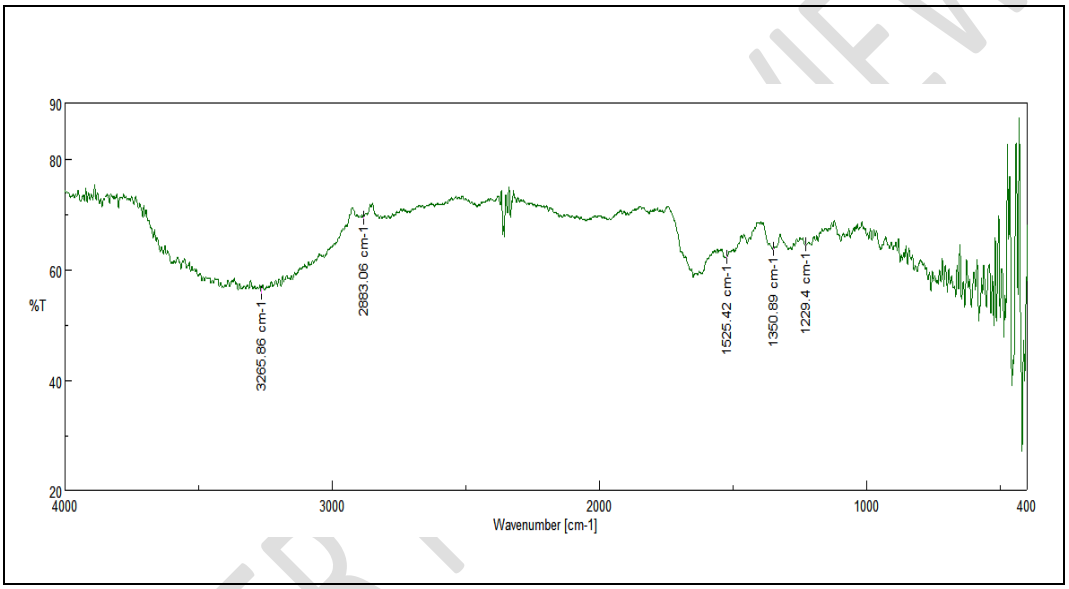


Figure 3: IR of compound B24

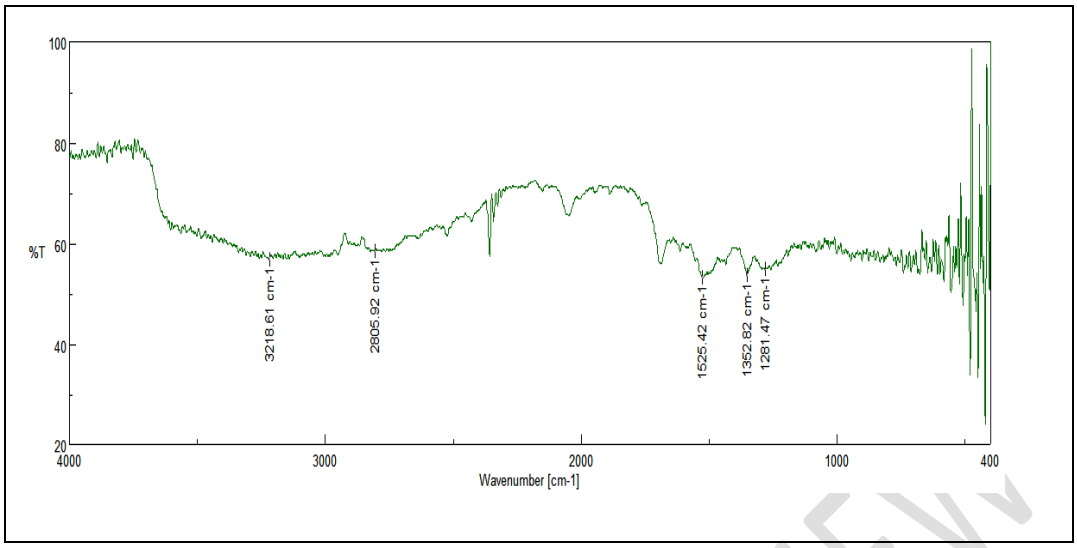


Figure 4: IR of compound B29

Table 11: NMR interpretations of few synthesized compounds

Sr. No.	Compound	NMR(ppm)
1	B24	7.15 – 7.62(Ar-H), 1-5 (-NH_2, 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 (NH_2),1.2-1.4(Secondary alkyl), 6.35 – 8.16(Ar-H), 2.2-3 (Ar-CH)

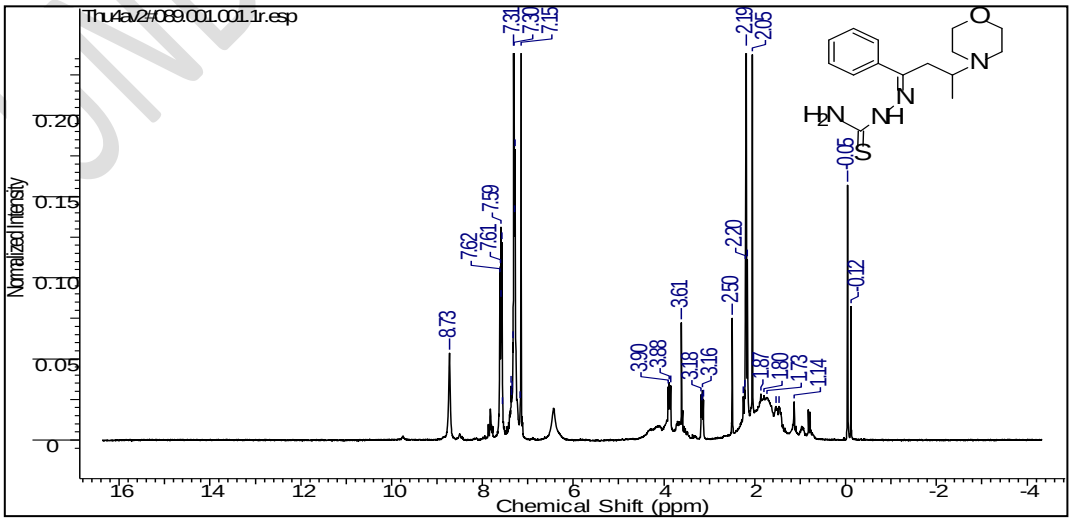


Figure 5: NMR of compound B24

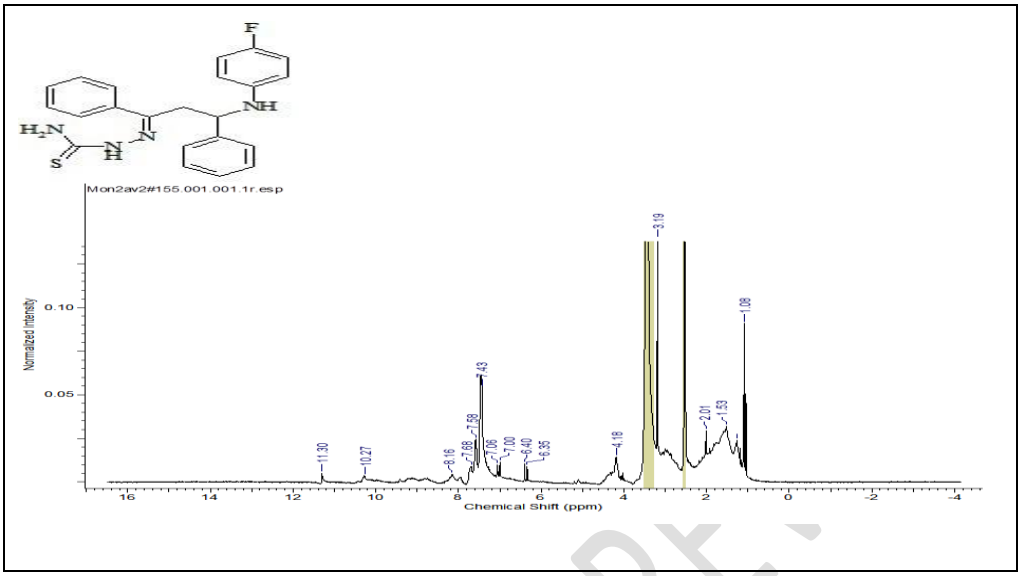


Figure 6: NMR of compound B4

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.

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**

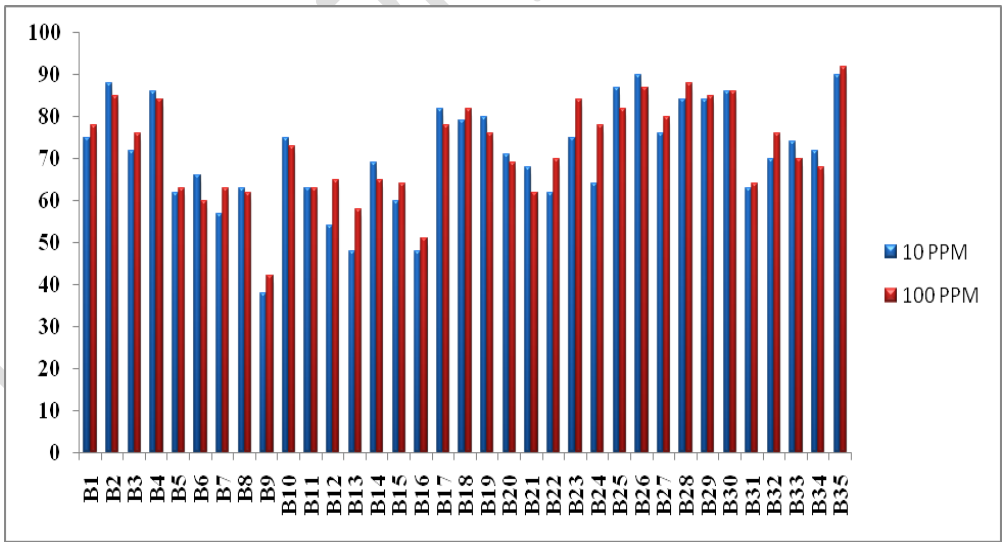


Figure 7: Graphical representation of Potato Disk Assay

DISCUSSION:

Compounds like B2 and B4 in which all components used for synthesis of mannich 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.

CONCLUSION:

From bioassay results it can be concluded that thiosemicarbazide derivatives of mannich 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 mannich bases of thiosemicarbazide as anticancer drugs as well as new chemical entity needs to be done.

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