INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

# SYNTHESIS AND ANTI-INFLAMATORY ACTIVITY OF 2-[(1H-BENZIMIDAZOLE-2YL METHYL) SULFONYL] –N- (PHENYL METHYLIDINE) ACETOHYDRAZIDE DERIVATIVES

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**ABSTRACT**

Benzimidazole derivates play vital role in biological field such as antimicrobial, antiviral, antidiabetic, and anticancer activity. Therapeutic significance of these clinically useful drugs in treatment of microbial infections encouraged the development of some potent and significant compounds. A series of 2-substituted benzimidazoles derivatives (**Va-g**) were synthesized and evaluated for their possible anti-inflammatory activity. The structures of the synthesized compounds were confirmed on the basis of their spectral data and elemental analysis. Majority of the compounds were active in Carrageenan induced hind paw edema method test and compounds **Vd** and **Vf** had shown high potency in terms of % inhibition and are moderately potent to that of standard drug diclofenac (20 mg/ kg body weight).

**Keywords:** Benzimidazoles, Anti-inflammatory, Carrageenan-induced hind paw edema.

## INTRODUCTION

Benzimidazole derivatives are of broad interest because of their various biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio.[1-3] Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-$B_{12}$.[4] It is evident from the literature that the benzimidazole nucleus is present in numerous antioxidant[5], antiparasitic[6], antihelmintics[7], antiproliferative[8], anti-HIV[9], anticonvulsant[10], anti-inflammatory[11], antihypertensive[12], antineoplastic[13], and antitrichinellosis[14] activities. Varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities.

2-substituted analogs of benzimidazoles are known to be more potent biologically active compounds. Jincheng Huang et-al reported the synthesis of 4 - (2 – pyridyl) piperazine – 1-benzimidazoles are used as potent TRPV1 antagonists for the treatment of anti-inflammatory and neuropathic pain.[15]

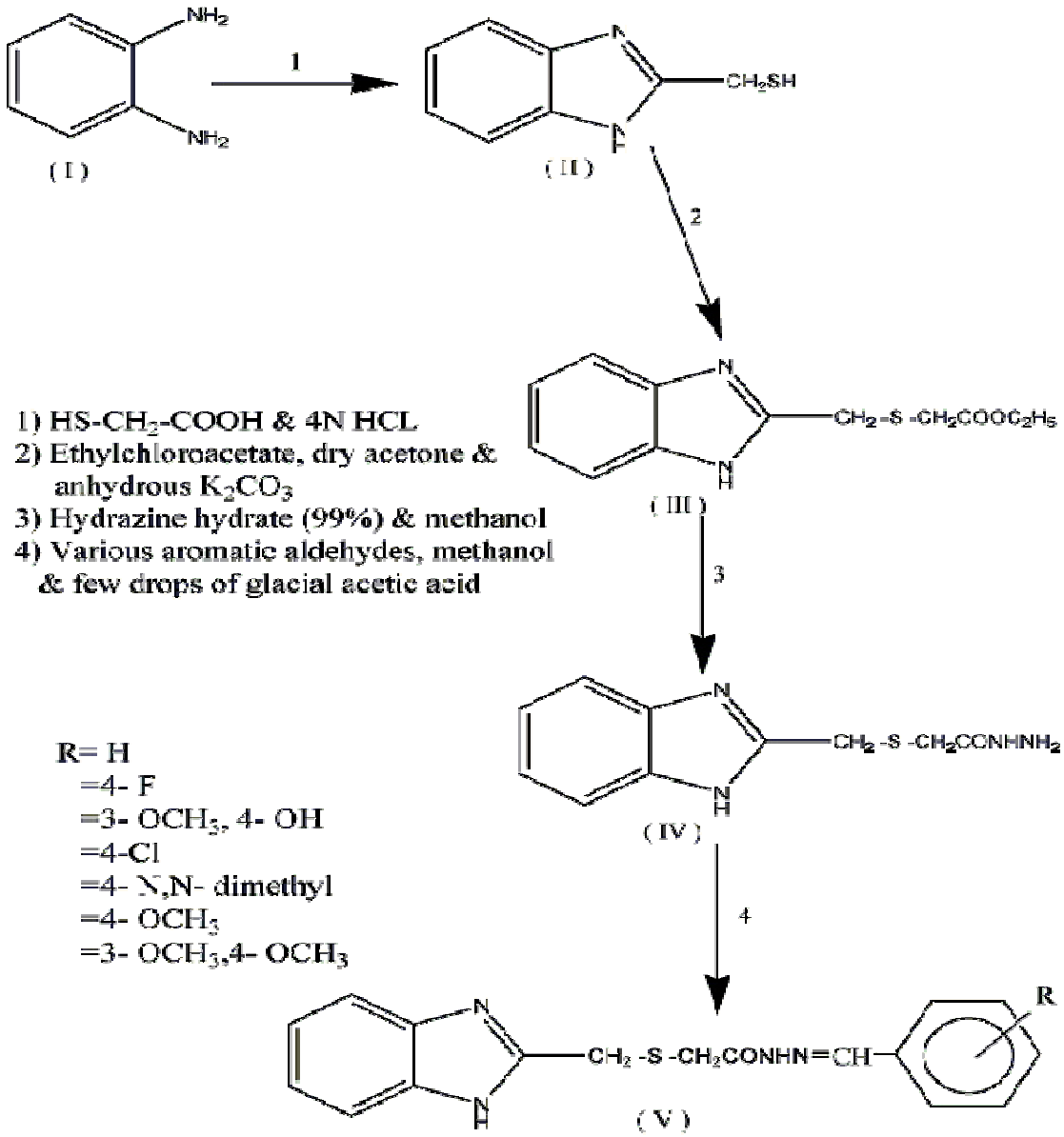
The present work was aimed to plan the synthesis of new benzimidazoles derivatives containing different schiff's bases as derivatives led to formation of biologically more active compounds. Such reactions are not reported so far. To evaluate new products for anti-inflammatory activity.

### Drugs and Chemicals

All the chemicals and solvents obtained from local firms from India:

1. Carrageenan - Sd.Fine Chem.Ltd
2. OPDA (ortho phenylene diamine) - Moly Chem.
3. Thioglycollic acid – Himedia
4. Acetone – Merck
5. Chloroform – Merck
6. NaOH – Merck
7. Ethylchloroacetate - Sd.Fine Chem. Ltd.
8. Methanol – Merck
9. Glacial acetic acid – Merck
10. Anhydrous $K_2CO_3$ - Universal Laboratories
11. Hydrazene hydrate (99%) - Sd Fine Chem Ltd.

**SCHEME**



## EXPERIMENTAL PROCEDURE

**1: Synthesis of 1H-Benzimidazol-2-ylmethane thiol (II)**

3.5gms of o-phenylenediamine was taken in 250ml round bottom flask. It is dissolved in 30-40 ml of 4N Hcl .Then Add 1.5ml of thioglycolicacid.This reaction mixture was kept for reflux for about 3-4hours. The solution was made alkaline by adding saturated sodium hydroxide solution (2-3ml). The base precipitated was filtered and dried.

The compound was recrystalised from Acetone or chloroform [Yield: 85%] as a light brown solid mp 154-160$^0$C Mobile phase – chloroform:methanol = 2:0.5ml

**2**: **Synthesis of Ethyl [(1H-benzimidazol-2-ylmethyl) sulfanyl] acetate (III)**

1H-Benzimidazol -2-ylmethane thiol(II) was dissolved in 40-50ml of dry acetone in 250ml round bottom flask then add 0.65ml of Ethylchloroacetate drop wise ,add potassium carbonate(0.01m) and shaken thoroughly. It was refluxed for 12-16hours by taking proper care that no moisture should enter in to the flask. Calcium guard tube was fixed to the condenser and temperature was maintained at 56$^0$C.

The compound was recrystalised from methanol [Yield: 70%] as a dark brown solid. Mobile phase-chloroform: methanol=2:0.5

**3**: **Synthesis of 2-[(1H-benzimidazol-2-yl methyl) sulfanyl] acetohydrazide (IV)**

Ethyl 1H-benzimidazol-2-yl methyl) sulfanyl] acetate (III, 0.01m) was taken in 250ml round bottom flask and dissolved in 30ml of methanol. To this hydrazine hydrate (0.01m) was added drop wise and shaken well. The reaction mixture was refluxed for 1-2hours by maintaining temperature 64$^0$C.Methanol was evaporated and the compound was collected and dried.

Recrysalised from ethanol (Yield: 74%) as a light brown solid. mobile phase-chloroform: methanol=2:0.5ml.

**4**: **Synthesis of 2-[(1H-benzimidazol-2yl methyl) sulfanyl)] –N$^1$-(phenylmethylidene) acetohydrazide (V)**

A mixture of compound (**IV,** 0.01 mol.) and an appropriate aromatic aldehyde (0.01 ml) in methanol ( 50ml) containing 3-4 drops of glacial acetic acid was reflexed on a water bath for about 2 hours and cooled . After cooling the resulting solid was filtered, washed thoroughly with small quantities of methanol, dried and recrystalized from suitable solvent (s).

Adapting this procedure 7 different compound was prepared by following the above detailed procedure and their physical data is presented in table I.

**Acute Oral Toxicity Test**

Acute oral toxicity of the synthesized compound was determined using female albino mice. The animals were fasted for 3 h prior to the experiment according to the recommended procedure (OECD guideline no. 425). As per the guidelines, the animals were observed for 48 h for any mortality following oral administration of the different doses of preparation. Based on this observation, a dose of 100 mg/kg was selected for the anti-inflammatory studies.

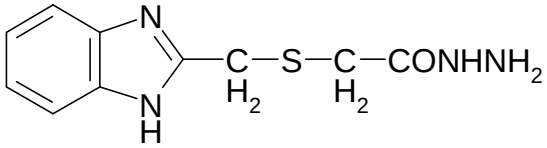
**Experimental Section**: The protocol followed was approved by the Institutional Animal Ethical Committee, University College of Pharmaceutical Sciences, Warangal.

**ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED RAT HIND PAW EDEMA METHOD**

Wister strain albino rats weighing between 180-250gm, fasted 24 hours before the test, were divided into eight groups of five animals each. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading. Compounds were tested in the dose of 100mg/kg body weight. Diclofenac 20mg/kg was used as standard. The compounds were administered as suspensions in sodium CMC (0.1%w/v) intraperitonially 1 hr before the injection of carrageenan. Control group of animals received a suspension of sodium CMC only. 0.1ml of 1.0%w/v carrageenan suspension in normal saline was injected into the plantar region (aponeurosis) of the right hind paw. The swelling produced after injection of the phlogistic agent was measured at hourly intervals for 4 hrs. Percentage inhibition of edema was calculated using the formula given below and the results were presented in table II.

**% inhibition of edema = mean edema of control group–mean edema of treated group x 100**
**Mean edema of control group**

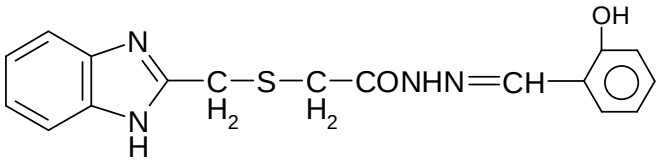
**SPECTRAL DATA**



**(IV)**

The IR Spectrum of (IV) exhibits characteristic peaks at ($cm^{-1}$) (FIG. 1)
H-N-H Stretching (3322.88); N-H Stretching (3282.48); =C-H (2977.23); C-H (2931.10); C=O (1665.65); C=C (1627.00); C=N (1525.81).

The MASS Spectrum of the compound (IV) exhibited its molecular ion peak at m/z 236 (FIG. 2).



**(Va)**

The NMR spectrum of compound (Va) exhibits its charectaristic proton signals at 5.5 (s, OH); 4.5(s,2H, -$CH_2$ -SH); 5.1(s,2H,S –$CH_2$– CO); 6.4(s,1H,HC – Ar); 6.8 – 7.4 (m, 8H, Ar-H); 10.0(s,1H, -CO – NH) (FIG.3).

The MASS Spectrum of compound (Va) exhibited its molecular ion peak (m+1) at 340 (FIG. 4).

## RESULTS AND DISCUSSION

The anti-inflammatory activity of 7 test compounds has been evaluated and the data are presented in Table II using Diclofenac (20 mg/kg) as the standard.

The compounds **Vd** and **Vf** showed the percentage inhibition of 55.80 and 48.75 respectively. These two compounds are the most relatively potent of all the compounds tested for anti-inflammatory activity. The compounds **Ve** & **Vg** showed moderate anti-inflammatory activity with percentage inhibition of 48.75 & 42.50 respectively. The compounds **Vb,Vc**, and **Va** showed minimum anti-inflammatory activity with percentage inhibition of 24.36,24.14,16.60 respectively.

## CONCLUSION

The following conclusions have been drawn from the results of these investigations:

- All the new Benzimidazole derivatives were evaluated for anti-inflammatory activity and the results were found to be encouraging.
- All the new synthesized compounds exhibited mild to moderate anti-inflammatory activity.
- Compound with methyl group at 3rd position, hydroxyl group at 4th position of phenyl ring exhibited maximum activity with percentage inhibition of 55.80. Compounds **Vd**, **Vf, Ve, Vg,Vb,Vc** and **Va**, were found to be next in the order of activity.
- The promising results gave us scope for further work in this area. It has been felt necessary from the results of

the present anti-inflammatory activity that there is a need for further advanced studies, at least on the few of the test compounds which are found to be superior.

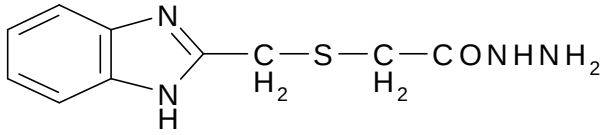
**TABLE I: PHYSICAL DATA OF {2 (1H – BENZIMIDAZOL -2 YL METHYL) SULFANYL} – N - ( PHENYLMETHYLIDENE) ACETOHYDRAZIDE**

| S. No. | COMPOUND | R | M.F. | Mol. wt | M.P (°C ) | % Yield |
|---|---|---|---|---|---|---|
| 1 | Va | OH | $C_{17}H_{16}N_4SO$ | 306 | 197-200 | 70 |
| 2 | Vb | Cl | $C_{17}H_{15}ClN_4SO$ | 337.5 | 190-192 | 45 |
| 3 | Vc | F | $C_{17}H_{16}FN_4SO$ | 302 | 170-173 | 60 |
| 4 | Vd | 3-$OCH_3$, 4-OH | $C_{18}H_{18}N_4SO_3$ | 353 | 232-235 | 54 |
| 5 | Ve | 4-N,N-dimethyl | $C_{19}H_{21}N_5SO$ | 349 | 226-230 | 65 |
| 6 | Vf | 4-$OCH_3$ | $C_{18}H_{18}N_4SO_2$ | 336 | 195-198 | 67 |
| 7 | Vg | 3-$OCH_3$, 4-$OCH_3$ | $C_{19}H_{22}N_4SO_3$ | 367 | 254-257 | 56 |

**TABLE II: ANTI- INFLAMMATORY ACTIVITY OF {2 (1H – BENZIMIDAZOL – 2 – YL METHYL) SULFANYL} – N - (PHENYLMETHYLIDENE) ACETOHYDRAZINE (V) DERIVATIVES**

| S. No. | COMPOUND | R | CONTROL | TEST | DIFFERENCE | % INHIBITION |
|---|---|---|---|---|---|---|
| 1 | Va | OH | 4.1 | 3.421 | 0.681 | 16.60 |
| 2 | Vb | 4-Cl | 4.1 | 3.201 | 0.999 | 24.36 |
| 3 | Vc | 4-F | 4.1 | 3.113 | 0.990 | 24.14 |
| 4 | Vd | 3-OCH3, 4-OH | 4.1 | 1.812 | 2.228 | 55.80 |
| 5 | Ve | 4-N,N-dimethyl | 4.1 | 2.103 | 1.999 | 48.75 |
| 6 | Vf | 4-OCH3 | 4.1 | 1.98 | 2.102 | 50.02 |
| 7 | Vg | 3-OCH3, 4-OCH3 | 4.1 | 2.32 | 1.742 | 42.50 |
| 8 | Diclofenac sodium 20mg/kg | | 4.1 | .812 | 3.288 | 80.19 |

Dose of test compound = 100mg / kg



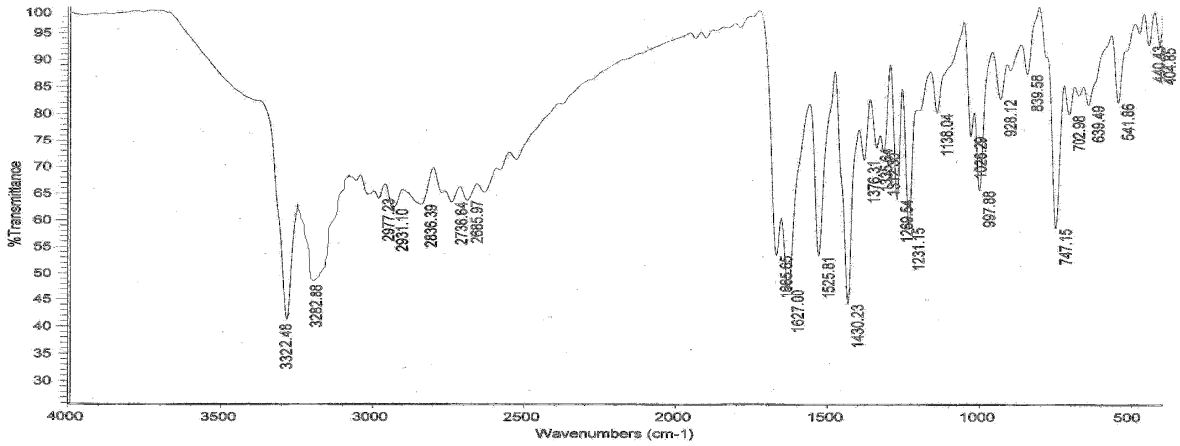




**FIG. 1: IR spectrum of 2 - [(1H - benzimidazol – 2 - yl methyl) sulfanyl] acetohydrazide (IV)**

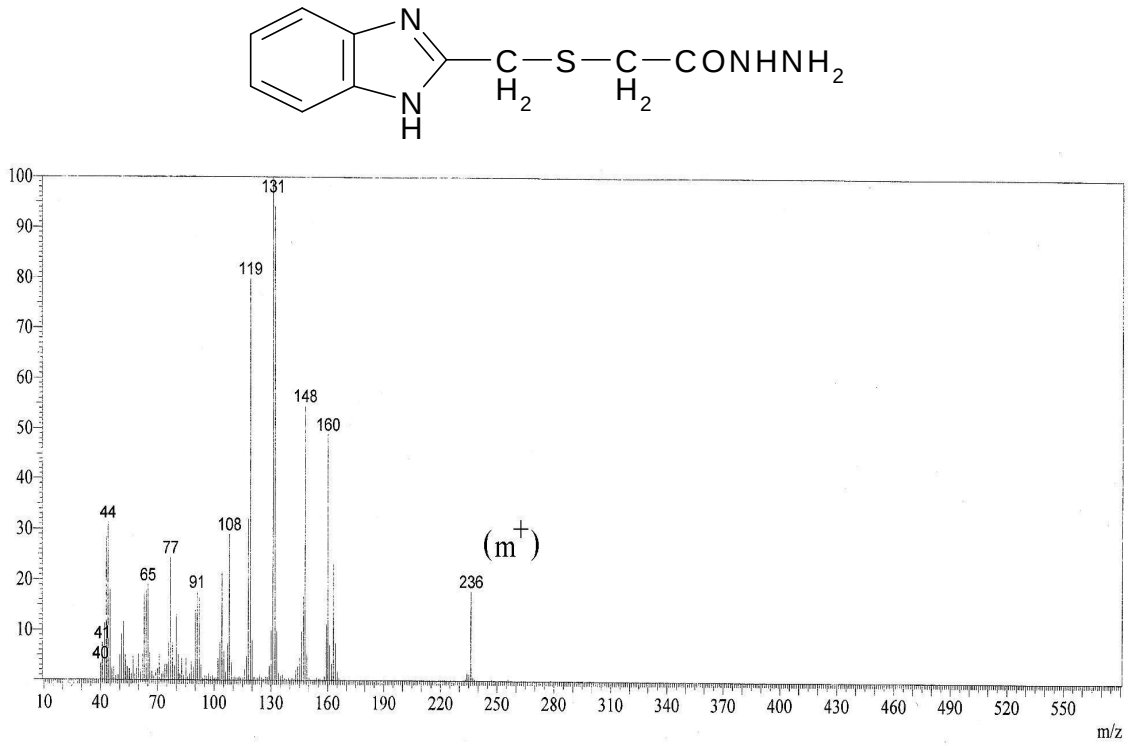



**FIG. 2: Mass spectrum of 2-[(1H- benzimidazol – 2 - yl methyl) sulfanyl] acetohydrazide (IV)**

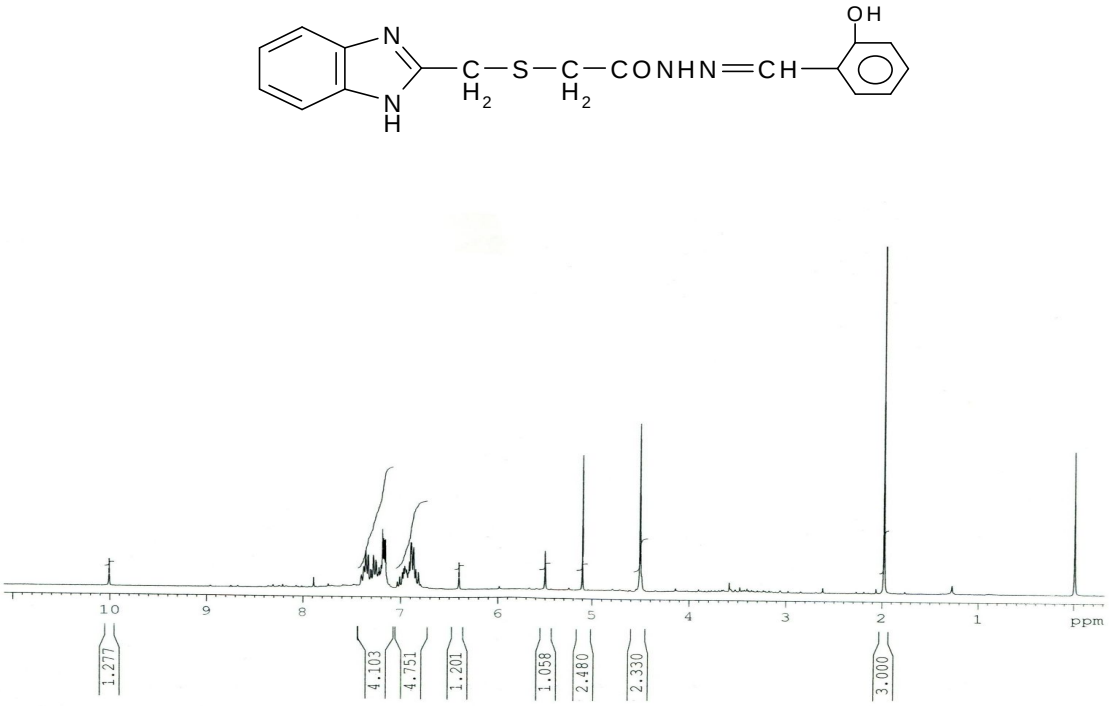
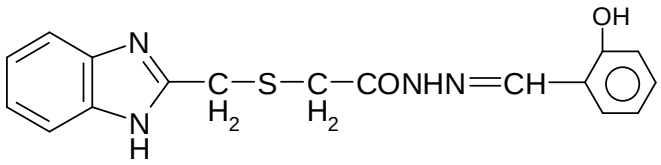



**FIG. 3: $^1$H NMR spectrum of 2-[(1H- benzimidazol - 2yl methyl) sulfanyl)]- N-(phenylmethylidene) acetohydrazide (Va)**



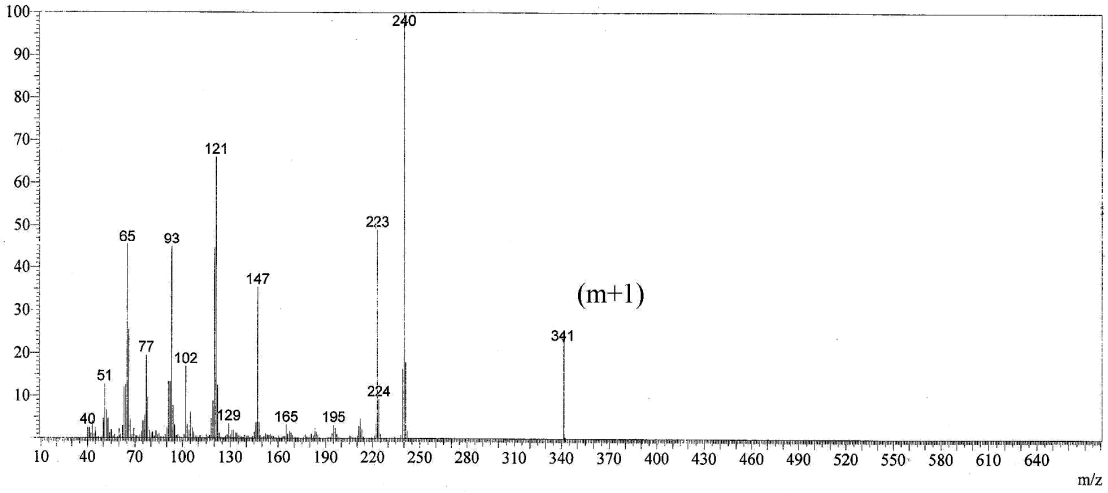




**FIG. 4: Mass spectrum of 2-[(1H-benzimidazol - 2yl methyl) sulfanyl)]- N-phenyl methylidene) acetohydrazide (Va)**

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