WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 13, 1567-1592. Research Article ISSN 2277– 7105

SYNTHESIS AND EVALUATION OF CYTOTOXIC ACTIVITY OF SOME ISATIN DERIVATIVES

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Article Received on
05 Sept. 2021,

Revised on 26 Sept. 2021,
Accepted on 17 October 2021

DOI: 10.20959/wjpr202113-22089

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ABSTRACT

Isatin nucleus was found to possess many pharmacological activities like anti-inflammatory, antihistaminic, antiviral, anticancer, anticonvulsant, antioxidant etc. Hence attempts were made to synthesize isatin analogues with cytotoxic activity. Purity of the compounds were determined by TLC and melting point. Formation of all the derivatives were confirmed by FT-IR, ^{1}HNMR, Mass spectrum. *In vitro* cytotoxic effect of synthesized compounds **IVa-d** was evaluated on the growth of allium cepa root meristems and the effect was compared with standard cytotoxic drug cyclophosphamide. Compound **IVa-d** possesses poor to good cytotoxic activity at the concentration of 10 mg/mL. All the compounds are almost ineffective at 1 mg/mL concentration. Cytotoxic activity exhibited by cyclophosphamide is very significant as compared to all the derivatives at both the concentrations.

KEYWORDS: Isatin, FTIR, cytotoxic activity, cyclophosphamide.

1. INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today scientists in this field are equally concerned with the creation of new synthetic drug compounds, possibly based on newly discovered mechanisms. The focus on development of new synthetic drug compounds has resulted in the incorporation of many other disciplines, such

as biochemistry and molecular biology into medicinal chemistry. Medicinal chemistry is almost geared toward drug discovery and development.[1]

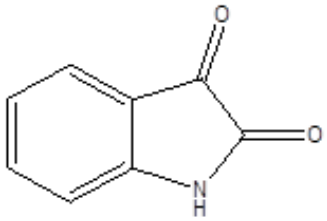
Medicinal chemistry concerned the drug discovery, the development, the identification and the interpretation of mode of action of biologically active compounds at the molecular level. Drug discovery is nothing but the structural investigation. The drugs used in medicines are developed from so called lead compounds which serve as the starting point for synthesis of analogues. The approach to the drug design depends on the objectives of the design team, which range from changing the pharmacokinetic of an existing drug to a completely new compound.

Drug discovery process can be defined as a process that starts with the investigation of the disease and therapeutic target of interest which includes methodology and assay development, lead identification and characterization *in vitro*, formulation, animal pharmacology studies, pharmacokinetics and safety studies. When synthetic chemicals overtook the number of natural products, synthetic compounds offered opportunity to medicinal screening of compounds. Medicinal chemistry is the discipline concerned with determining the influence of chemical structure on biological activity.[2]

Heterocyclic compounds have been used for long time in history for curing different disease. The prevalence of heterocyclic ring among drugs and biological agents of mammalian origin can lead to assumption that the presence of such rings in drugs constituents part of pharmacophore, replacement of the particular ring system in such case leads to loss of desirable biological activity. Recognition of pharmacophoric functions is still largely an empirical art.[3] It can be interfered that ring system itself is primarily a molecular scaffold upon which the characteristics pharmacophore for the various receptor is involved. It is interesting to note that range of biological activities involved is different substantially from those seen with the benzofused five membered heterocycles.[4]

1.2 ISATIN

Isatin was first obtained by Erdman and Laurent in 1841 as a product from oxidation of indigo by nitric acid.



Isatin

The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. The interest in this compound has stemmed from the wide spread biological and pharmacological properties of its derivatives.[10]

1.3 Applications of Isatin in organic synthesis

Many synthetic methodologies have been described for the conversion of isatins to other heterocyclic systems.

a) Partial or total reduction of the heterocyclic ring, leading to indoles and derivatives.
b) Oxidation of the heterocyclic ring
c) Nucleophilic addition at position C-3, which may be further followed by a cyclization process, with or without N1-C2 bond cleavage.
d) Nucleophilic substitution at position C-2, leading to the opening of the heterocyclic ring. This process may be followed by an intramolecular or by an intermolecular *exo-trig* cyclization.

4. EXPERIMENTAL AND RESULTS

4.1 Identification and Purification of chemicals[58]

1. 4-fluoroaniline

a) Appearance

Brown color liquid.

b) Solubility tests

Soluble in alcohol and ether.

c) Boiling point

The boiling point of 4-fluoroaniline was determined by capillary method.

Result: The boiling point of 4-fluoroaniline was found to be 186 ^{0}C.

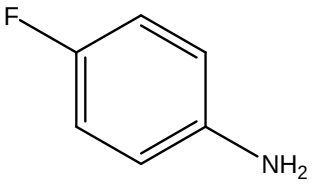
d) Thin layer chromatography

Purity of compound was checked by TLC method. Single spot was obtained when the sample run under the solvent system of chloroform : methanol in the ratio 6:4

Result: The R_f value was found to be 0.58

e) Infrared absorption spectrum

The potassium bromide pellet containing 4-fluoroaniline was prepared to record the spectrum in the range of 4000-500 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No.7** and spectral analysis is shown in **Table No. 1**



4-Fluoroaniline

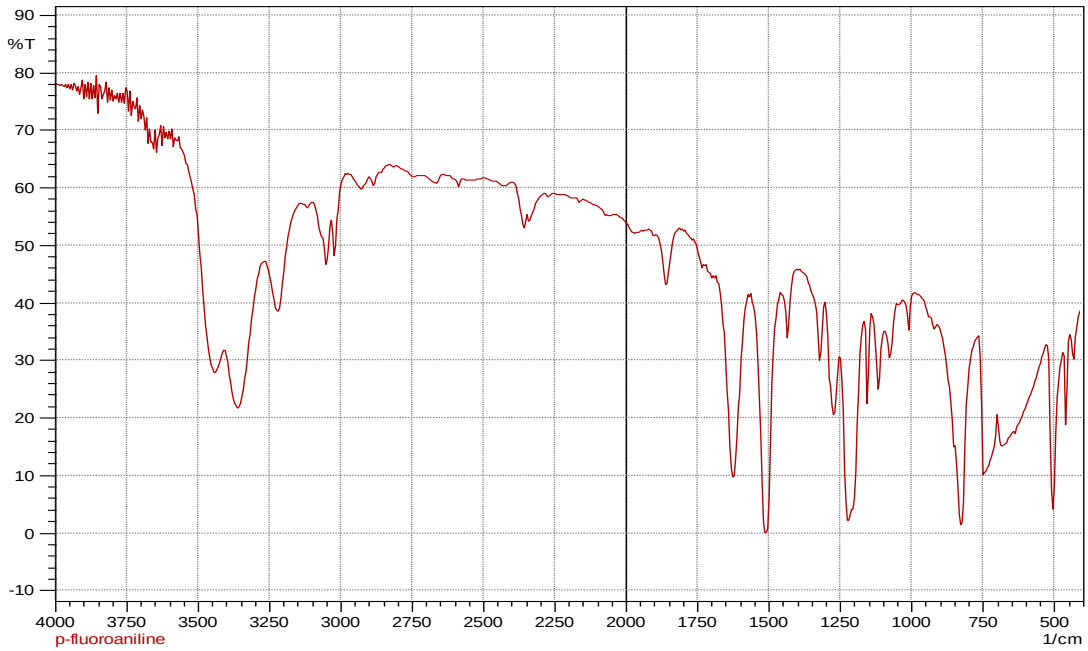


Fig No.7: FT-IR spectrum of 4-fluoroaniline.

Table No.1: FT-IR spectrum analysis of 4-fluoroaniline.

Frequency (cm^{-1})	Assignment
3440.77, 3359.77	N-H stretching (Asym., sym.)
3051.18	Aromatic C-H stretching
1623.95	Aromatic C=C stretching
1512.09	N-H deformation

2. Chloral hydrate

a) Melting point

The melting point of chloral hydrate was determined by capillary method.

Result: The melting point of chloral hydrate was found to be in the range of 52-54^{0}C.

3. Cinnamaldehyde

a) Boiling point

The boiling point of cinnamaldehyde was determined by capillary method.

Result: The boiling point of cinnamaldehyde was found to be 248^{0}C.

4. 4-toluldehyde

a) Boiling point

The boiling point of 4-toluldehyde was determined by capillary method.

Result: Boiling point of 4-toluldehyde was found to be 204^{0}C

5. 4-hydroxy benzaldehyde

a) Melting point

The melting point of 4-hydroxy benzaldehyde was determined by capillary method.

Result: Melting point of 4-hydroxy benzaldehyde was found to be in the range of 110-112^{0}C.

6. 4-dimethylamino benzaldehyde

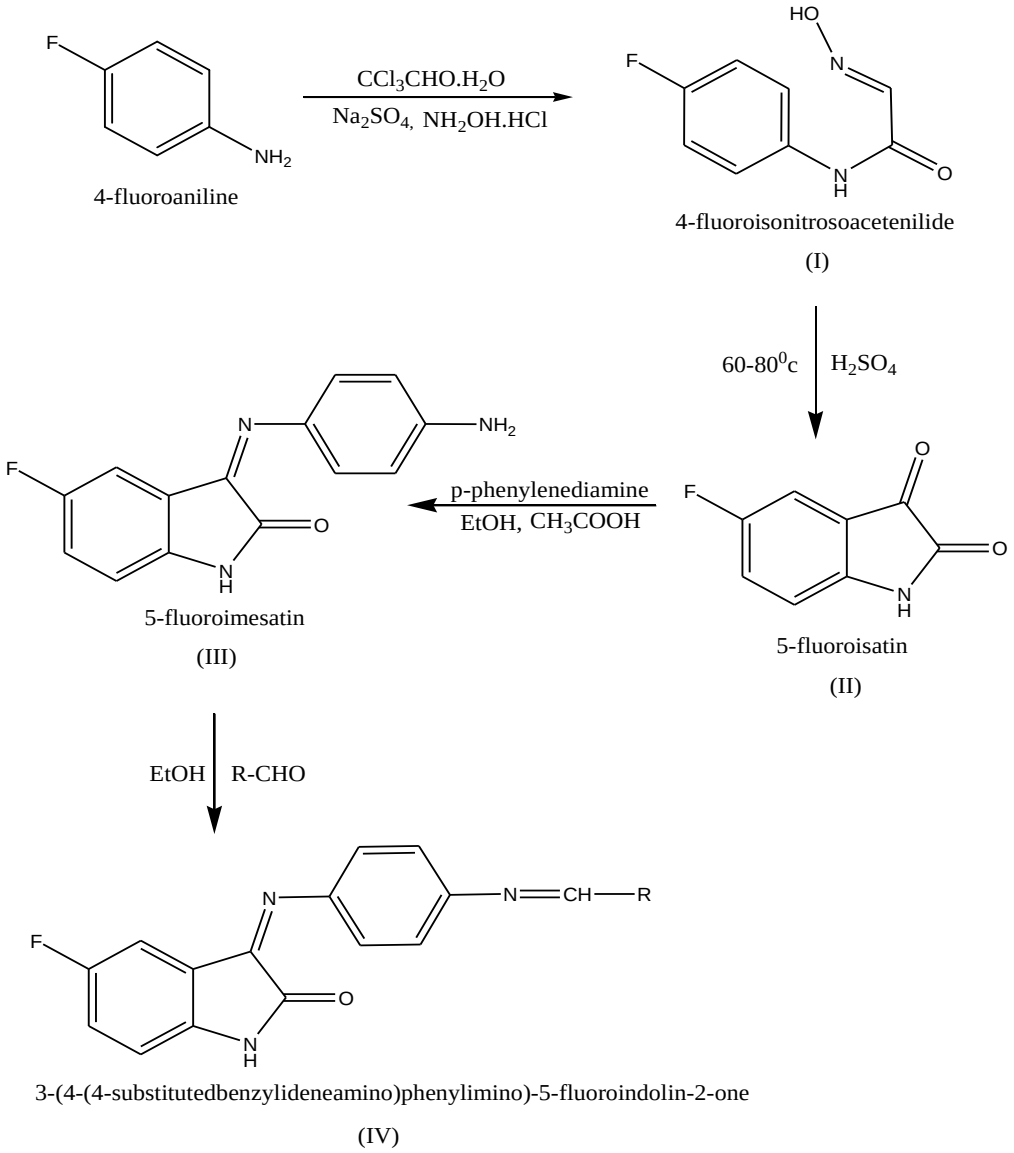
a) Melting point

The melting point of 4-dimethylamino benzaldehyde was determined capillary method.

Result: Melting point of 4-dimethylamino benzaldehyde was found to be in the range of 70-72^{0}C.

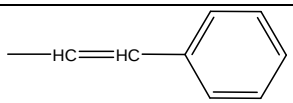
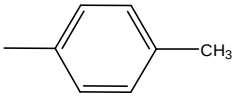
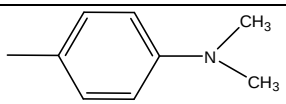
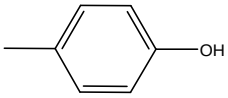
All the melting / boiling points and Rf value are uncorrected.

4.1.1: Synthesis of Isatin derivatives[59]



Synthesis of Schiff bases of isatin derivatives

Table No.2: Various Substitution Used.

Compound	R
IVa	
IVb	
IVc	
IVd	

4.2: Synthesis of 3-(4-(4-substitutedbenzylideneamino)phenylimino)-5-fluoroindolin-2-one IV (a)[60,61]

4.2.1: STEP 1: Synthesis of compound 4-fluoroisonitrosoacetanilide (I).

In a one litre round bottom flask 15g (0.54 mol) of chloral hydrate was dissolved in 240 ml of water and then 260g of crystalline sodium sulphate was added gradually. To this 11g (0.5 mol) of 4-fluoroaniline in 60 ml of water and 10.2g (8.01ml) of concentrated hydrochloric acid were added to dissolve amine completely. Finally a solution of 22g (1.58 mol) of hydroxylamine hydrochloride in 100 ml of water was added. The flask containing reaction mixture heated on a wire gauge using burner till boiling occurs (about 40 minutes). After one to two minutes of boiling reaction was completed. During the heating period some crystals of 4-fluoroisonitrosoacetanilide were separated. On cooling under running tap water remaining compound crystallised. Then it was filtered with suction and dried in air.

4.2.2: STEP 2: Synthesis of 5-fluoroisatin (II)

64.41gm (35ml) of concentrated sulphuric acid was warmed to 50^0C in a 250 ml round bottomed flask and to this 9g (0.46 mol) of dry 4-fluoroisonitrosoacetalinide(I) was added at a such rate as to keep the temperature between 60-70^0C but not higher. External cooling applied at this stage so that the reaction could be carried out more rapidly. After complete addition of the isonitroso compound the solution was heated to 80^0C and kept at this temperature for about 10 minutes to complete the reaction. Then the reaction mixture was cooled at room temperature and poured upon ten to twelve times its volume on crushed ice. After standing for about one and half hour, the 5-fluoroisatin was filtered with suction, washed several times with cold water to remove sulphuric acid, and then dried in the air.

4.2.3: STEP 3: Synthesis of 5-fluoroimesatin (III)

A mixture of equimolar (0.01 mol) quantities of 5-fluoroisatin (II) and p-phenylenediamine was dissolved in 30 ml of ethanol in presence of acetic acid and refluxed for 1 hour then kept aside for 24 hours, the product which was separated out was filtered, dried and recrystallized with ethanol.

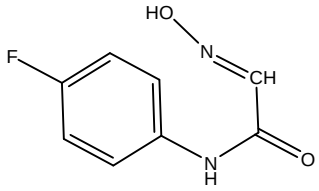
4.2.4: General procedure for synthesis of Schiff base of isatin (IV)

A mixture of equimolar (0.01 mol) quantities of 5-fluoroimesatin (III) and different aromatic aldehydes was dissolved in 30 ml of ethanol and refluxed for 8 hours. After standing for 24-48 hours at room temperature the product of different substituted isatin derivatives were separated out was filtered and recrystallized with absolute ethanol.

4.3: Characterization

a) Infrared absorption spectrum (I, II III)

The potassium bromide pellets containing compound I, II, III were prepared separately to record the spectrum in the range of 4000-400 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No. 8, 9 and 10** and spectral analysis is shown in **Table No. 3, 4 and 5** respectively.



4-fluoroisonitrosoacetanilide

(I)

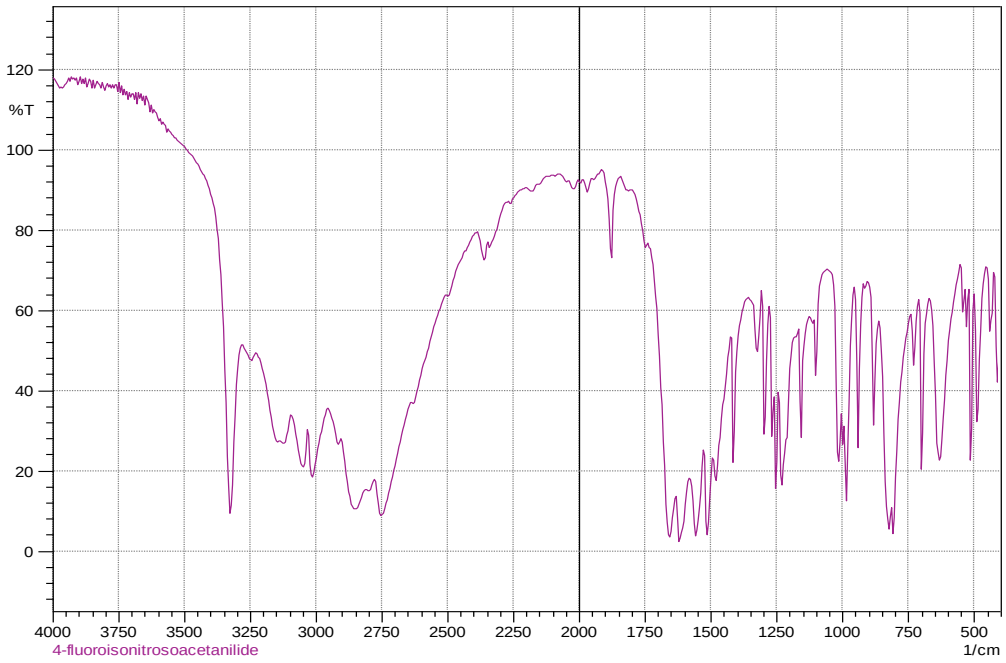
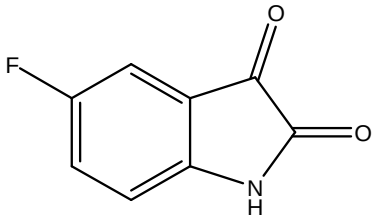


Fig No.8: FT-IR Spectrum of compound I.

Table No.3: FT-IR spectrum analysis of compound I.

Frequency (cm^{-1})	Assignment
3400-2850	OH band (broad)
3325.05	N-H Stretching (sec.)
3049.25	Aromatic C-H stretching
2852.52, 2914.24	Aliphatic C-H stretching
1695.04	C=O stretching
1654.81	C=N stretching
1556.45	C=C stretching



5-fluoroisatin

(II)

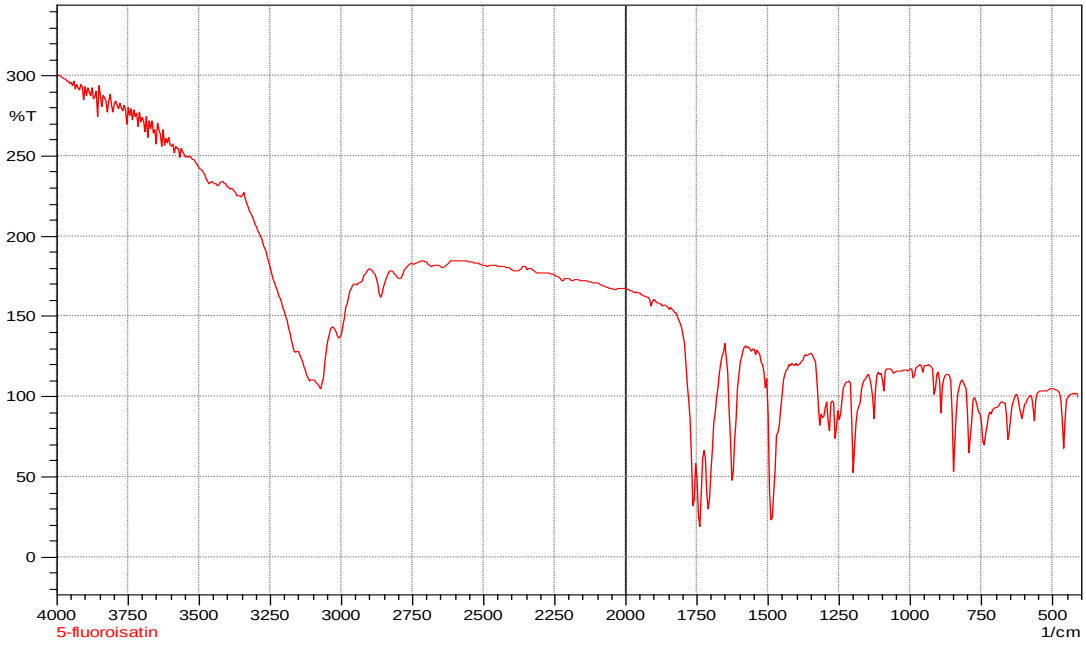
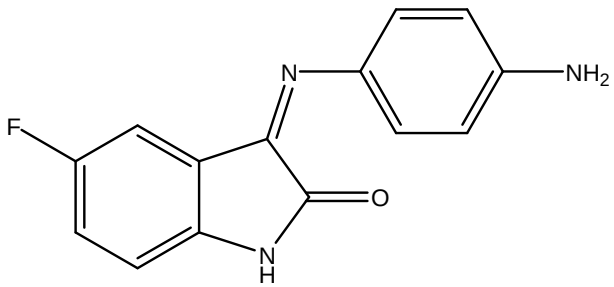


Fig No.9: FT-IR Spectrum of II.

Table No.4: FT-IR spectrum analysis of II.

Frequency (cm^{-1})	Assignment
3159.18	N-H stretching
3072.39	Aromatic C-H stretching
1708.81	C=O stretching
1599.65	C=C stretching



5-fluoroimesatin

(III)

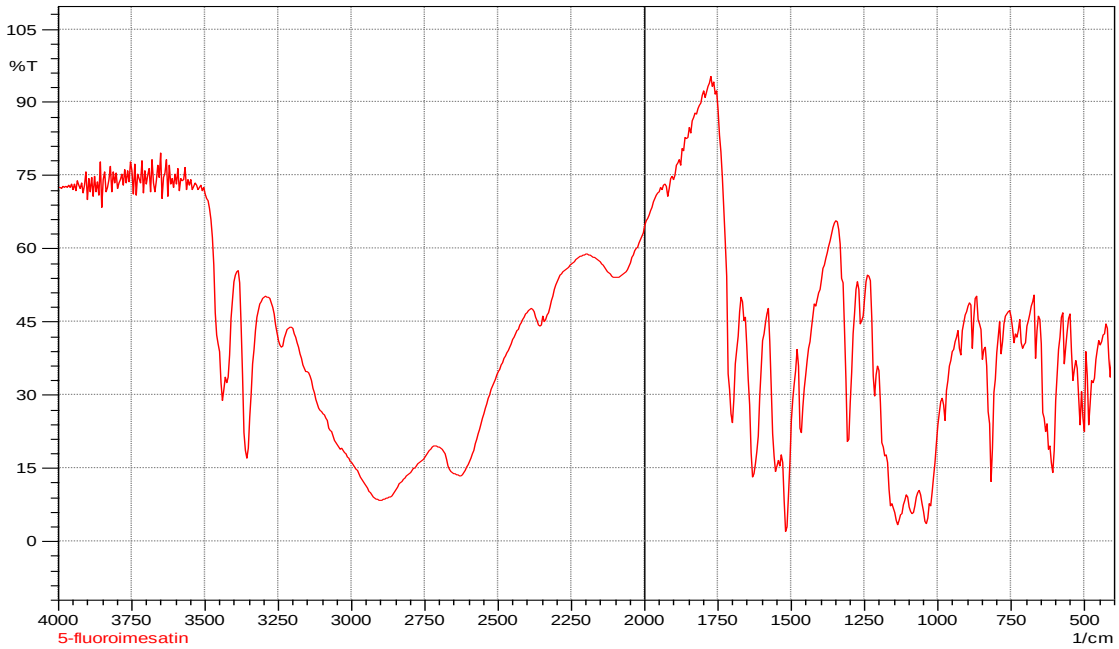


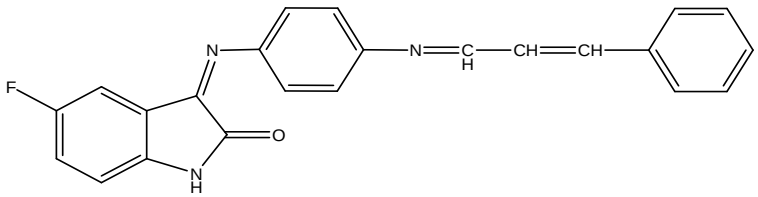
Fig. No.10: FT-IR Spectrum of III.

Table No.5: FT-IR spectrum analysis of III.

Frequency (cm^{-1})	Assignment
3438.84, 3423.41, 3357.84	N-H stretching
3037.68	Aromatic C-H stretching
1703.03	C=O stretching
1683.74	C=N stretching
1552.29	C=C stretching

4.4: SPECTRAL DATA OF DERIVATIVES

5.4.1: Synthesis of 3-(4-(3-phenylallylideneamino)phenylimino)-5-fluoroindolin-2-one (IVa).



3-(4-(3-phenylallylideneamino)phenylimino)-5-fluoroindolin-2-one (IVa)

a) Infrared absorption spectrum (IVa)

The potassium bromide pellet containing compound IVa was prepared to record the spectrum in the range of 4000-400 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No.10** and spectral analysis is shown in **Table No.6**

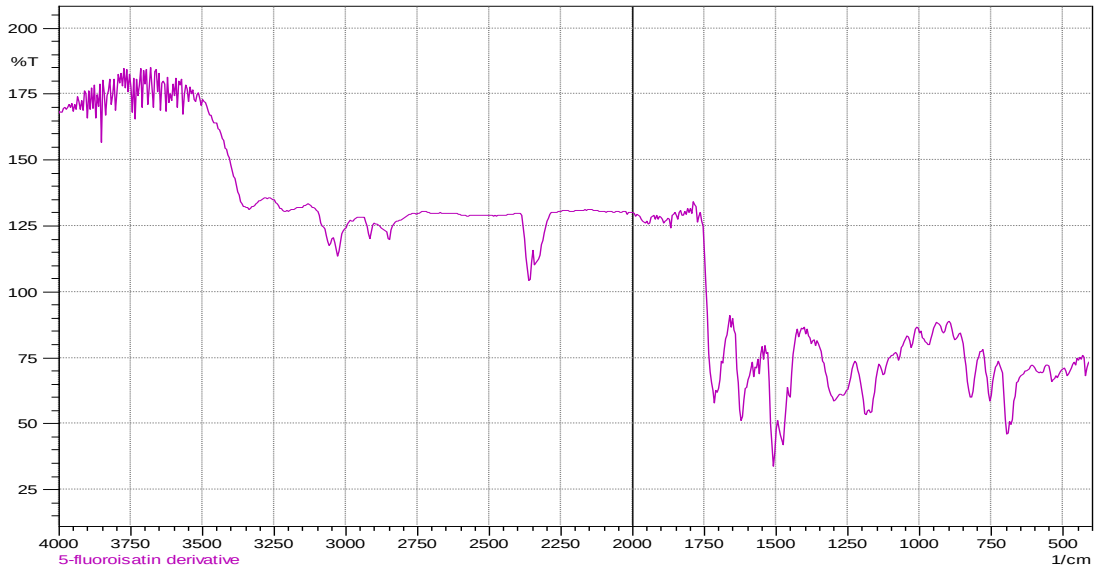


Fig No.11: FT-IR Spectrum of Iva.

Table No.6: FT-IR spectrum analysis of Iva.

Frequency (cm^{-1})	Assignment
3344.34	N-H stretching
3056.96	Aromatic C-H stretching
2848.67, 2916.17	Aliphatic C-H stretching
1697.24	C=O stretching
1683.74	C=N stretching
1595.02	C=C stretching

b) Mass spectrum (IVa)

Mass spectrum of compound (IVa) was taken by GC-MS shown in fig.No.11

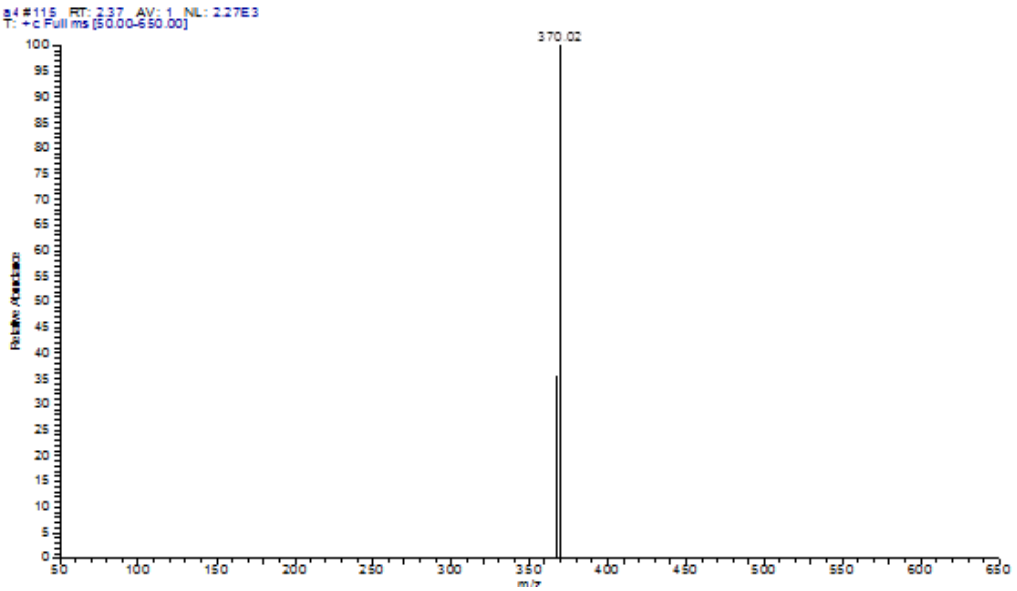


Fig. No.12: Mass spectrum of Iva.

(m/z):370.02

c) ^{1}H NMR Spectrum (IVa)

The ^{1}H NMR Spectrum of compound (IVa) was taken using $CDCl_3$ as a solvent is shown in fig.No.8 and spectral analysis is shown in table No. 7

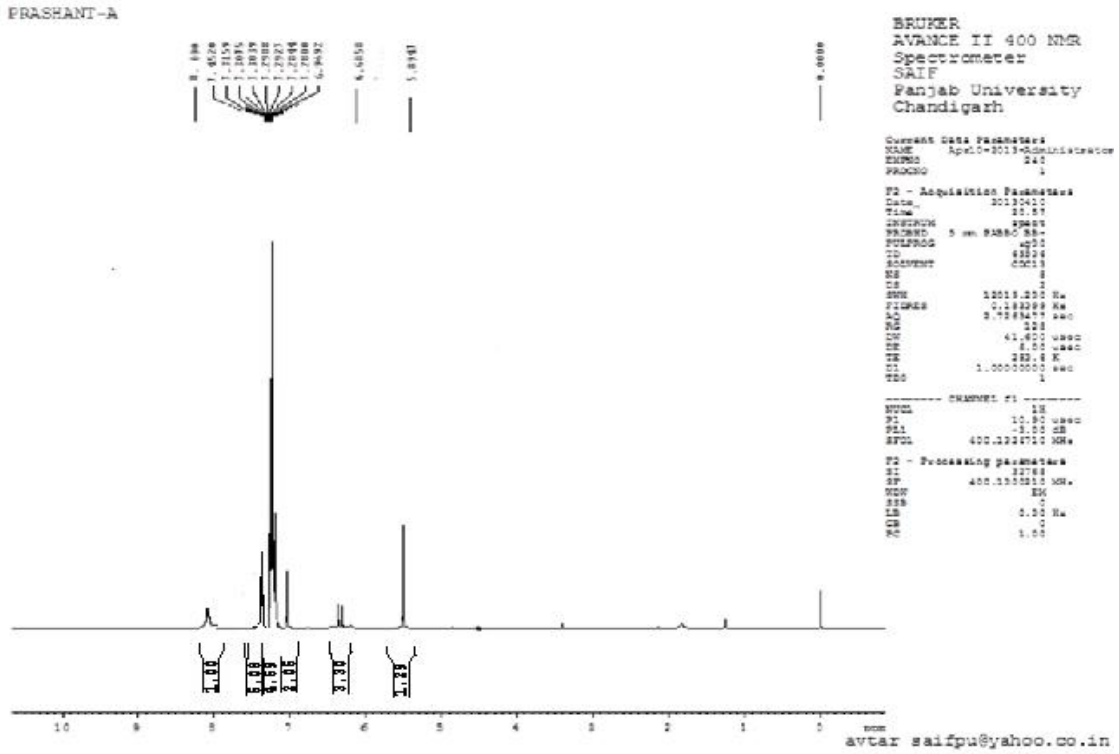
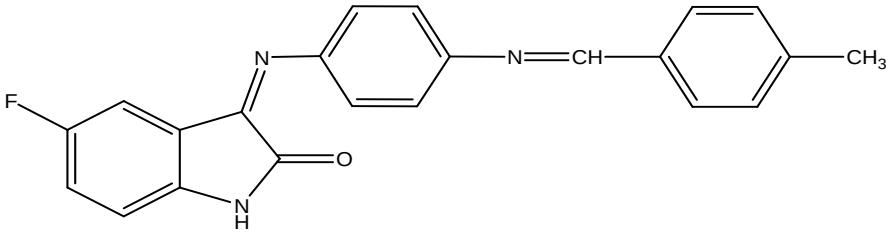


Fig. No.13: ^{1}H NMR Spectrum (IVa).

Table No.7: ^{1}H NMR Spectrum analysis of Iva.

δ (ppm)	Assignment
-H of C_6H_5-CH-C<u>H</u>=	5.8947
-H of C_6H_5-C<u>H</u>-CH=	6.6858
-H of N=CH	7.4520
m-Ar-CH	6.9692-7.3159
-H of –NH-	8.000

4.4.2: Synthesis of 3-(4-(4-(methyl)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVb)



3-(4-(4-(methyl)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVb**)**

a) Infrared absorption spectrum (IVb)

The potassium bromide pellet containing compound IVb was prepared to record the spectrum in the range of 4000-400 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No. 13** and spectral analysis is shown in **Table No.8**

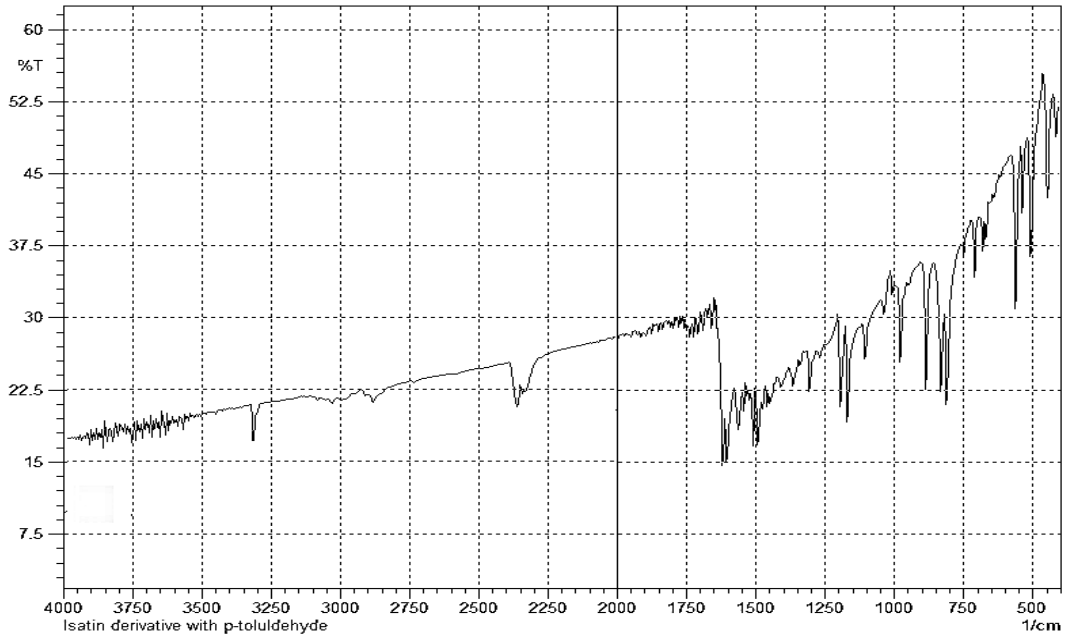


Fig. No.14: FT-IR spectrum of IVb.

Table No.8: FT-IR spectrum analysis of IVb.

Frequency (cm^{-1})	Assignment
3220.90	N-H stretching
3028.03	Aromatic C-H stretching
2881.45	Aliphatic C-H stretching
1695.27	C=O stretching
1688.17	C=N stretching
1596.87	C=C stretching

b) Mass spectrum (IVb)

Mass spectrum of compound (IVb) was taken by GC-Ms shown in fig.No.14

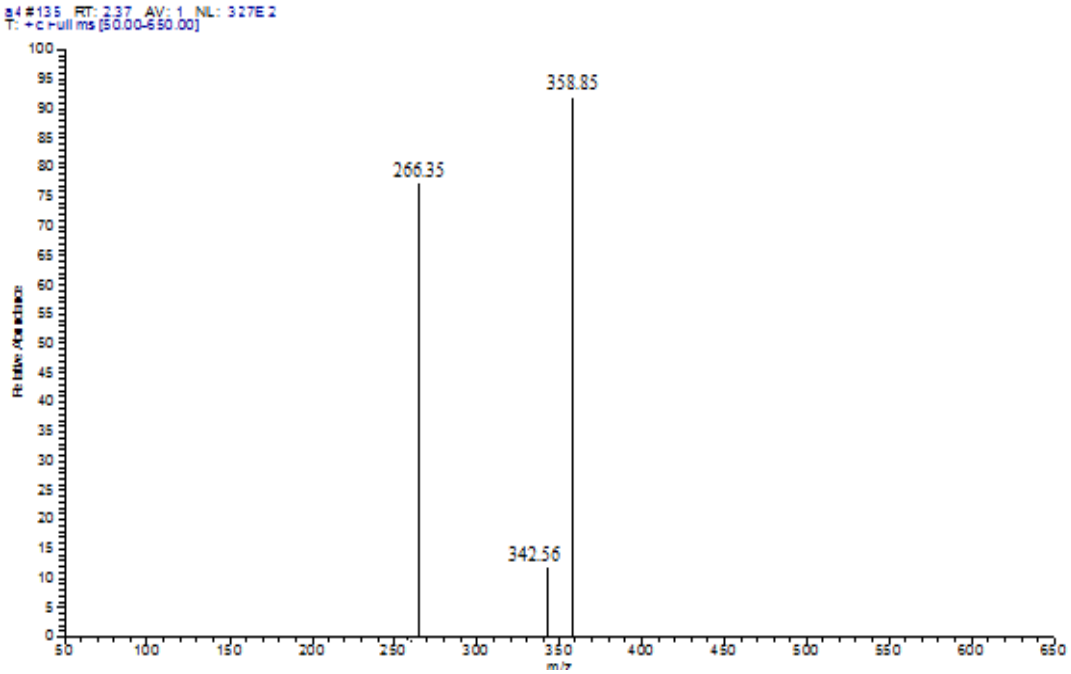


Fig No.15: Mass spectrum of IVb

(m/z): 358.85

c) ^{1}H NMR Spectrum (IVb)

The ^{1}H NMR Spectrum of compound (IVb) was taken using $CDCl_3$ as a solvent is shown in fig.No.15 and spectral analysis is shown in table No. 9

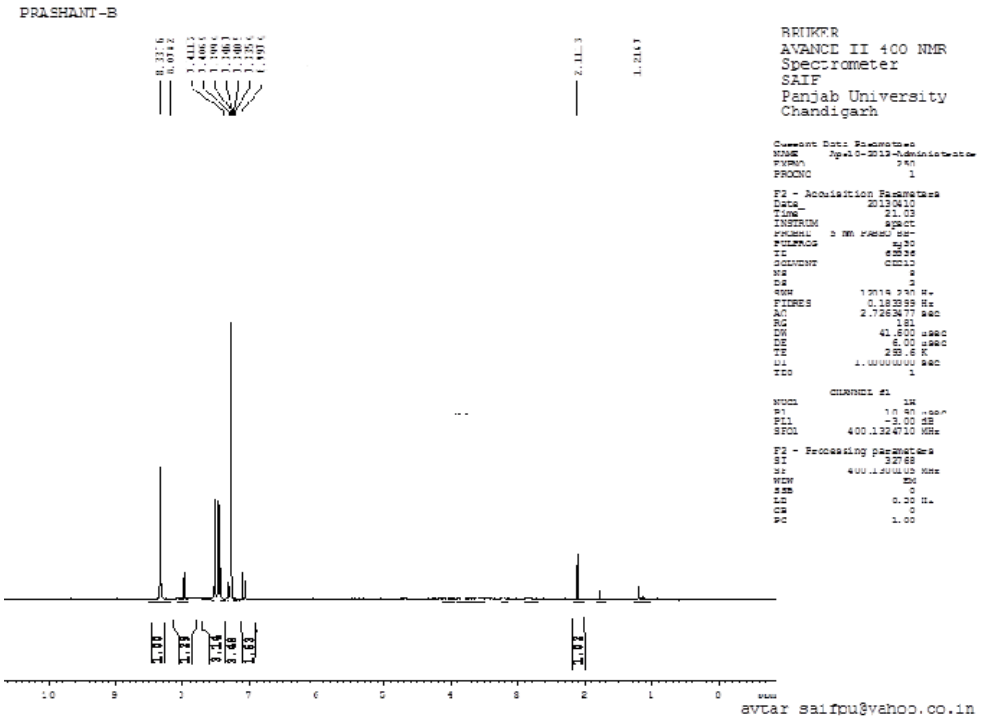
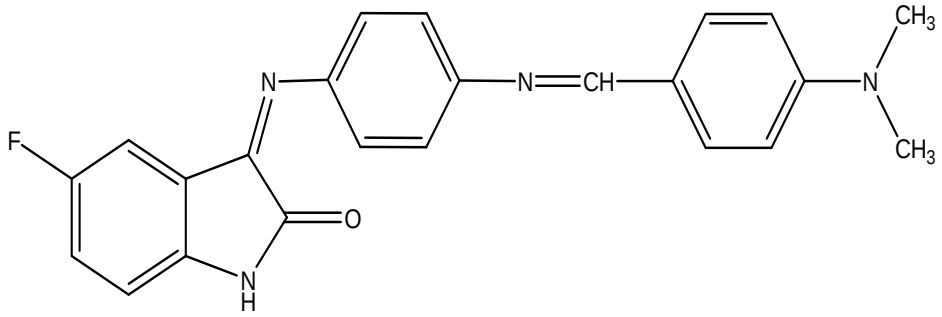


Fig. No.16: ^{1}H NMR Spectrum (IVb).

Table. No.9: ^{1}HNMR Spectrum analysis of IVb.

δ(ppm)	Assignment
-H of $–CH_3$	2.1113
m-Ar-CH	6.9974-7.4152
-H of –NH-	8.0782
-H of –N=CH-	8.3376

4.4.3: Synthesis of 3-(4-(4-(dimethylamino)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVc)



3-(4-(4-(dimethylamino)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVc)

a) Infrared absorption spectrum (IVc)

The potassium bromide pellet containing compound IVc was prepared to record the spectrum in the range of 4000-400 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No. 16** and spectral analysis is shown in **Table No.10**

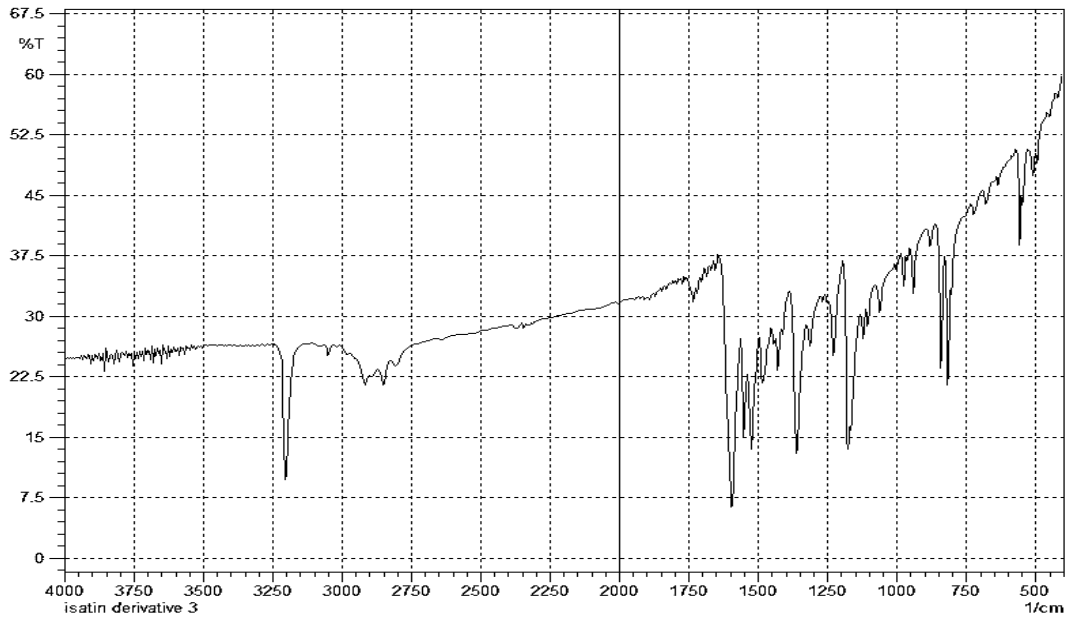


Fig No.17: FT-IR spectrum of IVc.

Table No.10: FT-IR spectrum analysis of IVc.

Frequency (cm^{-1})	Assignment
3207.78	N-H stretching
3070.10	Aromatic C-H stretching
2891.10, 2916.17	Aliphatic C-H stretching
1697.03	C=O stretching
1680.15	C=N stretching
1562.23	C=C stretching

b) Mass spectrum (IVc)

Mass spectrum of compound (IVc) was taken by GC-Ms shown in fig.No.17

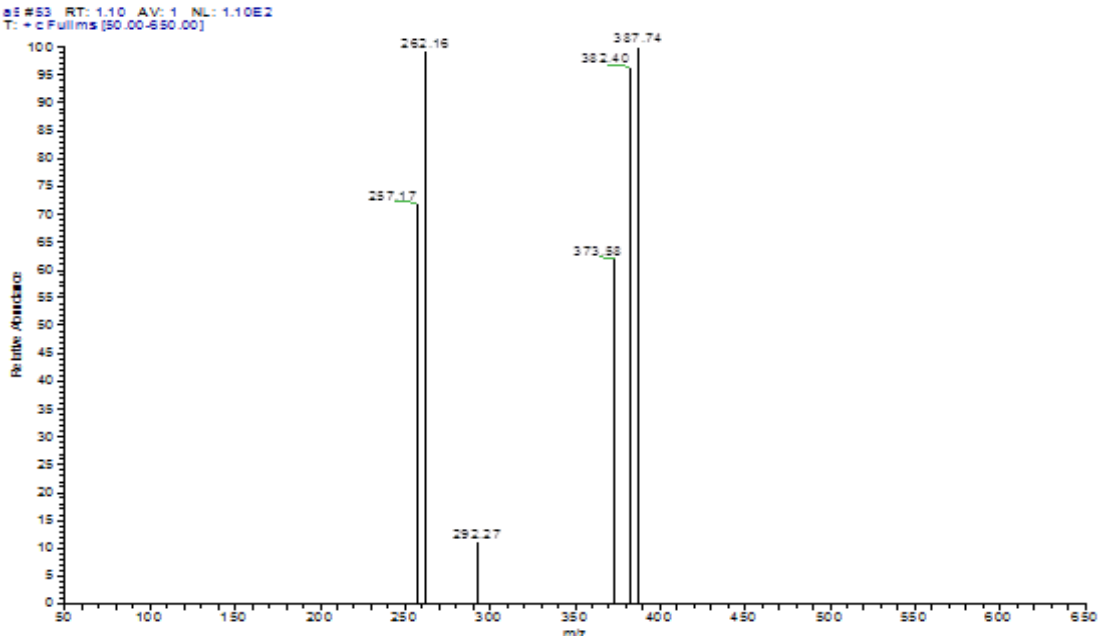


Fig No.18: Mass spectrum of IVc.

(m/z): 387.74

c) ^{1}H NMR Spectrum (IVc)

The ^{1}H NMR Spectrum of compound (IVc) was taken using $CDCl_3$ as a solvent is shown in fig.No.18 and spectral analysis is shown in table No. 11.

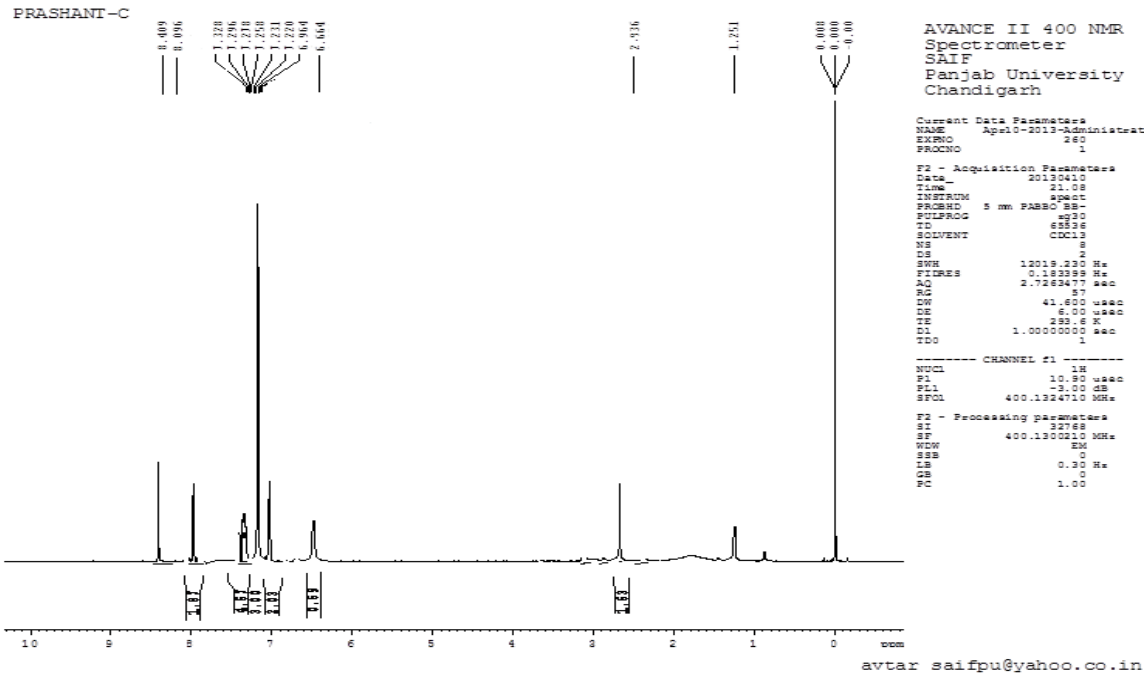
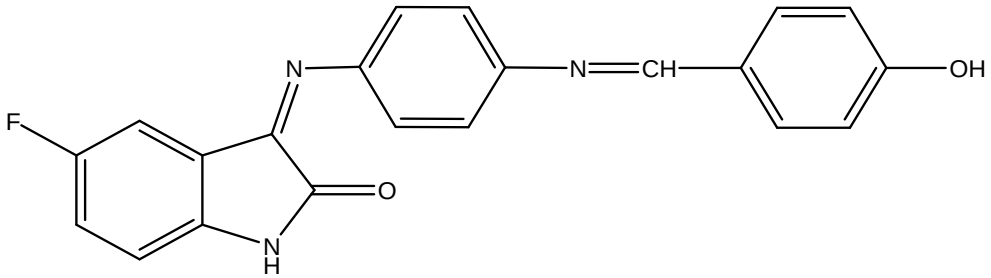


Fig.No. 19: ^{1}H NMR Spectrum (IVc).

Table No.11: ^{1}HNMR Spectrum analysis of IVc.

δ(ppm)	Assignment
-H of $-N(CH_3)_2$	2.9363
m, Ar-CH	6.6645-7.3283
-H of –NH-	8.0964
-H of –N=CH-	8.4097

4.4.4: Synthesis of 3-(4-(4-(hydroxy)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVd)



3-(4-(4-(hydroxy)benzylideneamino)phenylimino)-5-fluoroindolin-2-one (IVd)

a) Infrared absorption spectrum (IVd)

The potassium bromide pellets containing compound **IVd** was prepared separately to record the spectrum in the range of 4000-400 cm^{-1} by using FT-IR spectrophotometer as shown in **Fig No. 19** and spectral analysis is shown in **Table No.12**

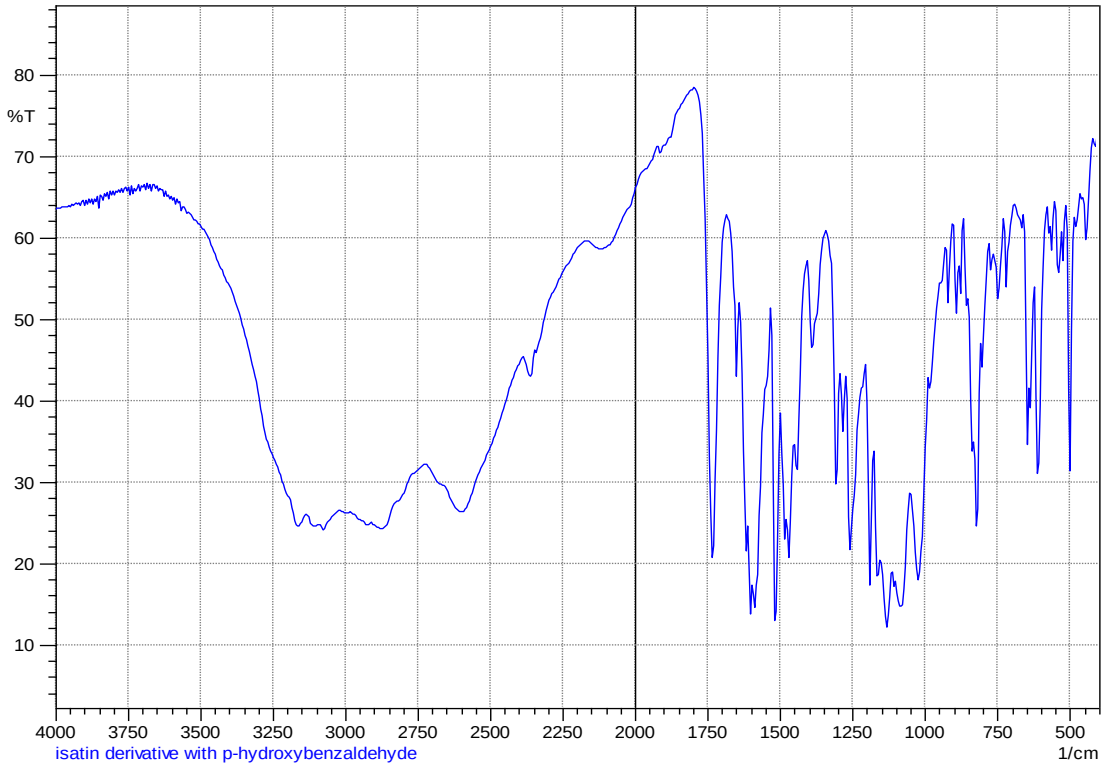


Fig. No.20: FT-IR spectrum of IVd.

Table No.12: FT-IR spectrum analysis of IVd.

Frequency (cm^{-1})	Assignment
3400-3000	OH band (Broad)
3164.97	N-H stretching
3074.32	Aromatic C-H stretching
2877.60, 2923.88	Aliphatic C-H stretching
1691.96	C=O stretching
1645.24	C=N stretching
1589.23	C=C stretching

c) Mass spectrum (IVd)

Mass spectrum of compound (IVd) was taken by GC-Ms shown in fig.No.20

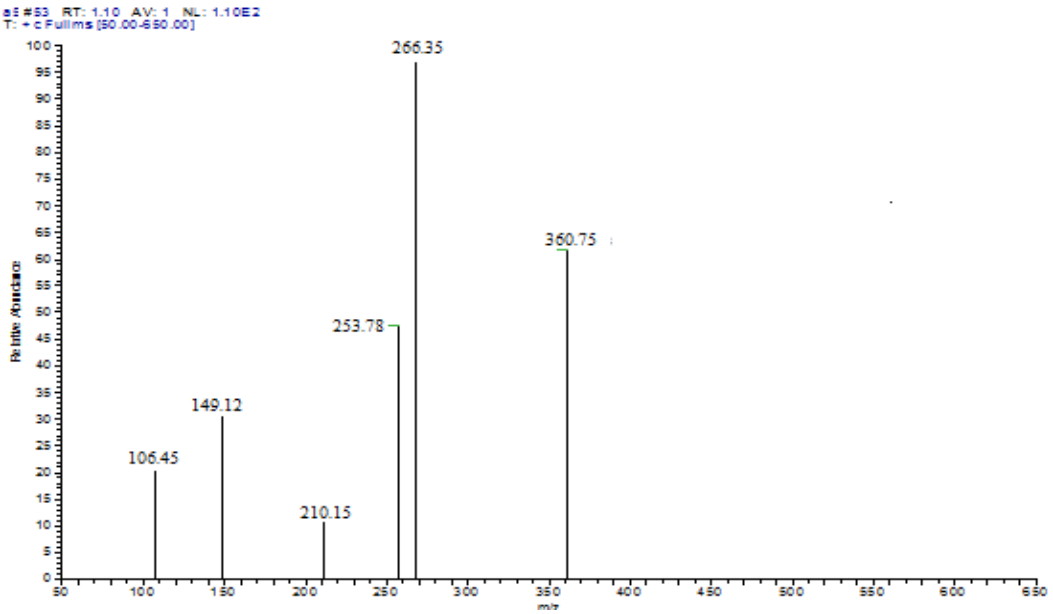


Fig No.21: Mass spectrum of IVd

(m/z): 360.75

d) ^{1}H NMR Spectrum (IVd)

The ^{1}H NMR Spectrum of compound (IVd) was taken using $CDCl_3$ as a solvent is shown in fig.No.21 and spectral analysis is shown in table No. 13

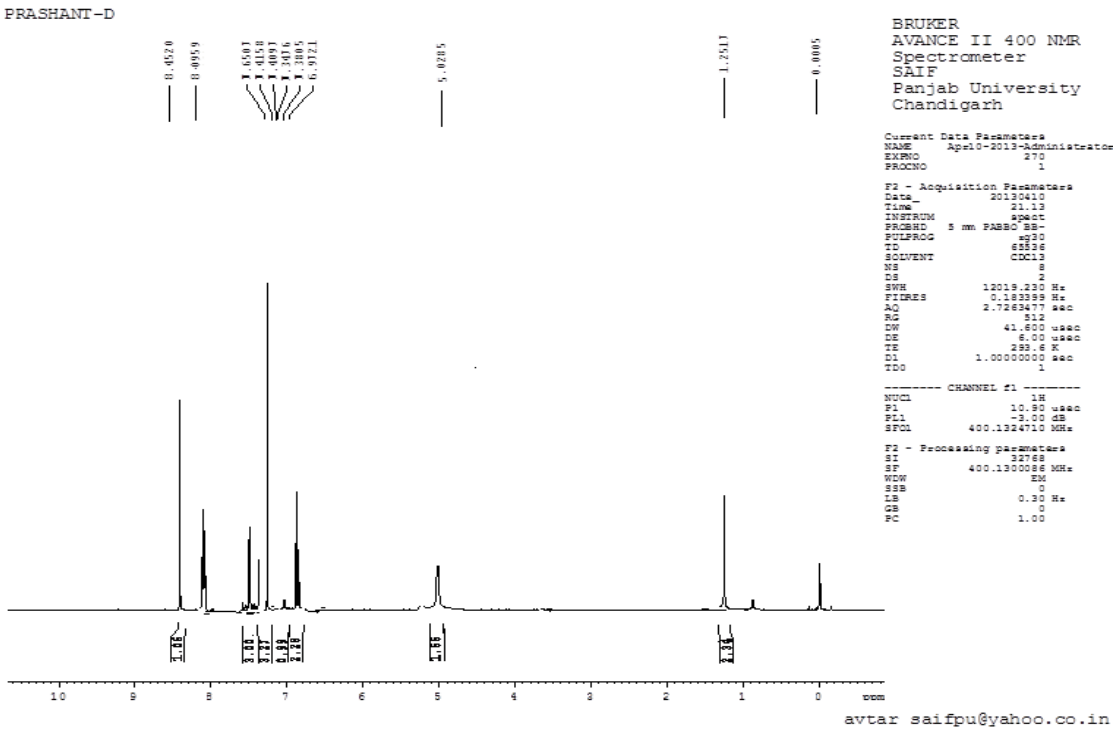


fig.No.22: ^{1}H NMR Spectrum (IVd).

Table No. 13: ^{1}HNMR Spectrum analysis of IVd.

δ (ppm)	Assignment
Ar-OH	5.0285
m-Ar-CH	6.9747-7.6507
H of NH	8.0959
-Hof N=CH	8.4520

Table No. 14: Color, percentage yield, melting point and R_F value of synthesized derivatives (IVa-IVd).

Product	Colour	Melting point* (^{0}C)	Time required for reflux	Yield (%)	R_f value*
IVa	Red	138-140	8 hrs	71.23	0.80
IVb	Yellow	114-116	8 hrs	72.35	0.75
IVc	Orange	198-200	8 hrs	70.13	0.70
IVd	Yellow	220-224	8.5 hrs	71.19	0.78

*** Uncorrected, * mobile phase chloroform: methanol (9:1)**

Table No.15: Elemental analysis of synthesized compound (IVa-IVd).

Compound	Elemental Analysis Calculated (%)		
	C	H	N
IVa	74.78	4.37	11.38
IVb	73.94	4.51	11.76
IVc	71.49	4.96	14.50
IVd	70.19	3.93	11.69

4.5: CYTOTOXIC SCREENING[62]

Onion root model was used for studying cytotoxic activity. Alliun cepa root tip meristems have been widely used for the evaluation of cytotoxic and antimitotic activity.

The inhibitory effect of synthesized compounds was evaluated on the growth of allium cepa root meristems and the effect was compared with standard anticancer drug cyclophosphamide.

4.5.1 Growing Allium cepa meristems

Locally available *allium cepa* bulbs were grown in dark over 100 mL tap water at ambient temperature until the roots have grown to approximately 3-4 cm. the water was changed daily.

4.5.2 Conditions for drug incubation

Working dilutions of all the drugs were made in tap water. Standard drug cyclophosphamide and compound IVa-d were used at 1mg/ml and 10 mg/ml concentration. The bulbs with root

tips grown up to 3-4 cm were placed over drug solution and incubation was carried out at ambient temperature.

Control (Water)

Standard (Cyclophosphamide)

Compound IVa

Compound IVb

Compound IVc

Compound IVd

Fig. 22: Photographs showing growth of onion in the presence of water, cyclophosphamide and compound IVa-d.

Table 16: Root length attained after incubation with water, cyclophosphamide & compounds IVa-d.

Groups	Root length after hour				
	0 h	**24 h**	**48 h**	**72 h**	**96 h**
Control	3.417 ± 0.20	3.567 ± 0.21	3.724 ±0.17	3.81 ± 0.22	4.017 ±0.26
Comp IVa 1 mg/ml	3.283 ±0.25	3.40 ±0.30	3.51 ±0.32	3.733 ±0.19	3.817 ±0.17
Comp IVa 10 mg/ml	3.417 ±0.24	3.45 ±0.27	3.667 ±0.18	3.48 ±0.27	3.38** ±0.20
Comp IVb 1 mg/ml	3.340 ±0.29	3.467 ±0.18	3.63 ±0.19	3.683 ±0.20	3.817 ±0.17
Comp IVb 10 mg/ml	3.617 ±0.30	3.66 ±0.16	3.633 ±0.24	3.45 ±0.27	3.75 ±0.18
Comp IVc 1 mg/ml	3.417 ±0.28	3.50 ±0.28	3.71 ±0.19	3.90 ±0.14	4.017 ±0.14
Comp IVc 10 mg/ml	3.117 ±0.27	3.31 ±0.11	3.46 ±0.18	3.45 ±0.34	3.25** ±0.25
Comp IVd 1 mg/ml	3.267 ±0.338	3.28 ±0.26	3.48 ±0.24	3.6 ±0.14	3.84 ±0.14
Comp IVd 10 mg/ml	3.217 ±0.24	3.46 ±0.16	3.617 ±0.19	3.833 ±0.38	3.6** ±0.06
Standard 1 mg/ml	3.267 ±0.33	3.25 ±0.21	3.367* ±0.15	3.25** ±0.24	3.10** ±0.20
Standard 10 mg/ml	3.43 ±0.28	3.33** ±0.17	3.283** ±0.21	3.1** ±0.26	2.85** ±0.12

#Values are represented as Mean±SD; (n = 6).

*p<0.05 is considered significant and **p<0.01 is considered very significant when compared with control group.

5. CONCLUSION

The isatin derivatives prepared successfully with good yield. This was prepared by the procedure reported in the literature. Different derivatives of isatin **(IVa to IVd)** were synthesized in four steps. All the derivatives were characterized in terms of FT-IR, ^{1}H NMR, MASS, TLC and Melting point to ensure the formation and conformation of the synthesised compounds. *In-vitro* cytotoxic study was also carried out.

The evaluation of synthesized derivatives for cytotoxic activity indicated that compound **IVa-d** possess poor to good cytotoxic activity at the concentration of 10 mg/mL. All the compounds are ineffective at 1 mg/mL concentration. Cytotoxic activity exhibited by cyclophosphamide is very significant as compared to all the derivatives at both the concentrations.

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