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## Evaluation of anti-cancer activity on N-Substituted Tetrahydrocarbazoles

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### ABSTRACT

A New series of N-Substituted Tetrahydrocarbazole derivatives are prepared by cyclohexanone (1) treated with substituted phenyl hydrazine's (2a, 2b) refluxed at 60°C for 10 mins undergoes cyclisation with the loss of ammonia, in presence of reagents like glacial acetic acid leads the formation of substituted tetrahydrocarbazole (3a, 3b), this upon treating with 10% sodium hydroxide and substituted 4-amino-benzoyl chlorides (4a, 4b) gives (4-aminobenzoyl) 1, 2, 3, 4 tetrahydro carbazole derivatives. (5a, 5b). The structures of new derivatives should be purified by the different chromatographic techniques and assigned on the basis of <sup>1</sup>H NMR, IR, and Mass spectral data. All the newly synthesized compounds were evaluated for their in-vitro anti-cancer activity.

**Keywords:** Tetra hydro carbazole, Cyclohexanone, Anti-cancer, Phenyl hydrazine.

### INTRODUCTION

Cancer has emerged as one of the most alarming disease in the last few decades throughout the world. It is a multifactorial disease contributing towards uncontrolled growth and invasion of abnormal cells leading to the formation of tumors [1-8]. Pim-kinases control various proteins involved in significant biological processes such as cell cycle progression and apoptosis. Over expression of pim-kinases have been observed in human leukaemia and lymphoma, prostate, pancreatic and colon cancer contributed to tumorigenesis. For these reasons, pim-kinases are considered as important targets for the development of new anticancer drugs. Carbazole is an aromatic

heterocyclic organic compound. It has a tricyclic structure [9-10], consisting of two six membered benzene ring fused on either side with a five membered nitrogen-containing ring. Carbazole and its derivatives are an important type of nitrogen containing heterocyclic compounds that are widespread in nature. Various classes of carbazoles are given in Figure [11-15]. The Carbazole ring is present in a variety of naturally occurring medicinally active substances e.g., carbazomycins and murrayafoline A. Series of carbazole derivatives including oxazinocarbazoles, isoxazolocarbazolequinone, pyrido-carbazolequinone, tetrahydrocarbazoles, benzocarbazoles, furo-carbazoles,

pyridocarbazoles, pyrrolo-carbazoles, indolocarbazoles, oxazoliny carbazoles, thienocarbazoles, imidazocarbazoles, thiazolocarbazoles, benzopyrano-carbazoles, benzofurano-carbazoles and *N*-substituted carbazoles have been synthesized and are well known for their pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, antitumor, anticonvulsant, antipsychotic, antidiabetic, larvicidal properties, *etc.* Keeping in view the so vast therapeutical potential of carbazoles, this research will summarize the anti-cancer activity for the *N*-substituted carbazoles [20-23].

The tetrahydrocabazole ring system has been the structural subunit of many naturally occurring alkaloids, biologically active molecules and medicinal important synthetic analogues. Tetrahydrocabazole condensed with indole, furan, pyrimidine, pyrazoline, and thiophene, moieties have been known to processes wide spectrum biological activities. There have been many methods of synthesis. In general the carbazoles synthesis is carried out by multistep Fisher reaction which requires the usage of organic solvents with very good product yields [24-26]. Hence a simple and efficient method for the synthesis of these pharmaceutically important classes of compounds is highly desirable precluding the usage of organic solvents [27-30]. Initially substituted phenyl hydrazine's were used to optimize their action conditions such as different acids, solvents, and reaction temperature. Finally we found that glacial acetic acid given excellent yields. In presence of CH<sub>3</sub>COOH, ZnCl<sub>2</sub> and HCl lesser amount of the desired product was obtained [31-33]. The effect of solvents was also investigated and the highest yield was observed in glacial acetic acid, when the reaction was conducted at lower temperatures lower yields were obtained. Ideal temperature for the reaction was found to be 90°C [34-35]. In the presence of electron releasing groups present in the Para position of phenyl hydrazine's observed more yield comparatively presence of electron withdrawing groups. To the best of our knowledge this is a first report for the efficient and economic synthesis of carbazoles using readily available laboratory reagents with short reaction times [36-38].

## MATERIALS AND METHODS

All the chemicals were of AR grade and were obtained from Sigma–Aldrich and SD Fine Chemicals. Melting points (m. p) were determined in open capillaries on OptiMelt automated melting point system and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) with silica gel F254 (Merck) with visualization by UV-light. The compounds are purified by using column chromatography on silica gel (60-120 mesh). The instruments used for obtaining the spectroscopic data were: FT-IR spectrophotometer SHIMADZU-435, <sup>1</sup>H NMR (CDCl<sub>3</sub>, Avance 300 MHz). Mass spectral analysis experiments are performed using a quadruple time-of-flight mass spectrometer (QSTAR XL, Applied Bio systems/MDS Sciex, Foster City, CA, USA), equipped with an ESI source.

### Experimental Methods

#### Chemistry

In the below representative scheme, cyclohexanone (1) treated with substituted phenyl hydrazine's (2a, 2b) refluxed at 60°C for 10 mins undergoes cyclisation with the loss of ammonia, in presence of reagents like glacial acetic acid leads the formation of substituted tetrahydrocabazole (3a, 3b), this upon treating with 10% sodium hydroxide and substituted 4amino-benzoyl chlorides (4a,4b) gives (4amino benzoyl) 1,2,3,4 tetrahydro cabazole derivatives. (5a, 5b).

#### Step I

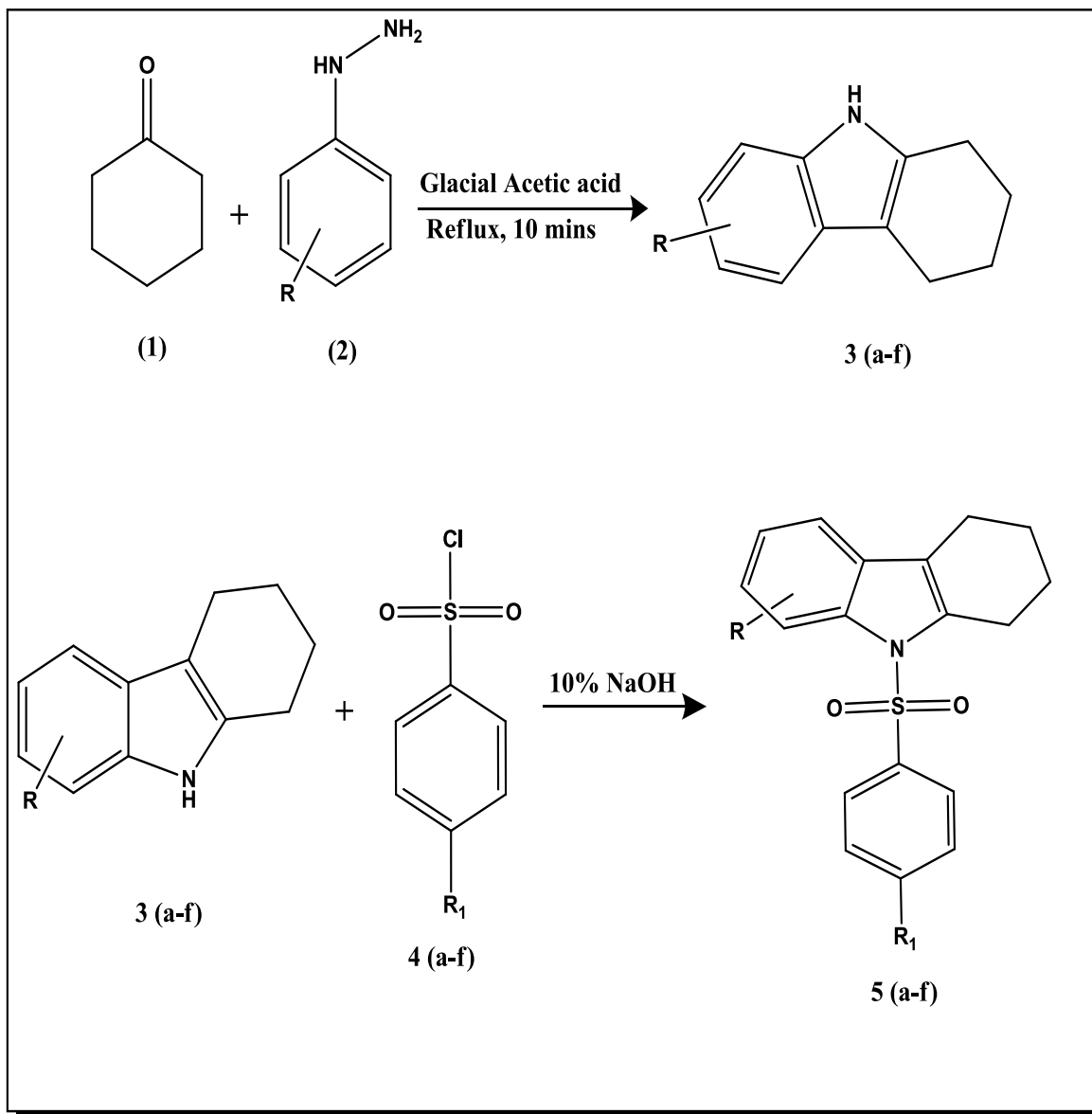
Synthesis of 1,2,3,4 tetrahydrocabazole (3a, 3b) (The Fischer's indolisation reaction): Dissolve cyclohexanone (1) (9.8 gm, 0.1mol) in (34.65 gm, 0.6 mole) of glacial acetic acid, substituted phenyl hydrazine's (2a, 2b) (10.8 gm, 0.1mol) and the solution was refluxed for 10 minutes. Reaction mixture was cooled, where the tetrahydrocabazole (3a, 3b) was crystallized out, filtered at the pump, drained well and recrystallized from aqueous ethanol. The recrystallization was performed rapidly, since tetrahydrocarbazoles under goes atmospheric oxidation in hot solution which has melting point of 146°C.

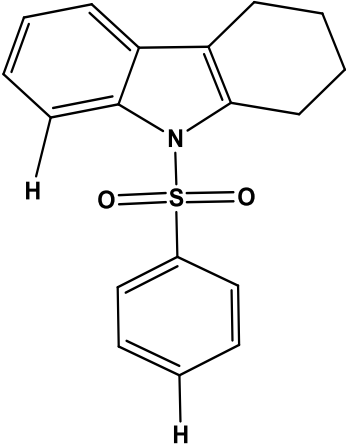
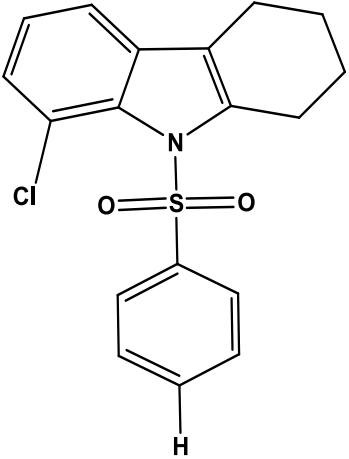
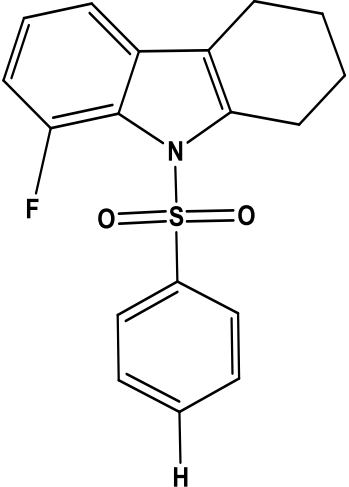
## Step II

Synthesis of N-(4 -amino benzoyl) 1,2,3,4-tetrahydroacabazole 1,2,3,4-Tetrahydrocarbazoles (3a, 3b) (1 gm,5.78 mole) was added to 10% NaOH solution in a well cooled conical flask and then 2ml of 4-amino benzoyl chloride(4)was added with

constant shaking, cooled in water and shaken vigorously for 10 minutes until the odour of the benzoyl chloride was disappeared. Solid was filtered off and N-substituted derivative (5a, 5b), washed with a little cold water and recrystallized from ethanol.

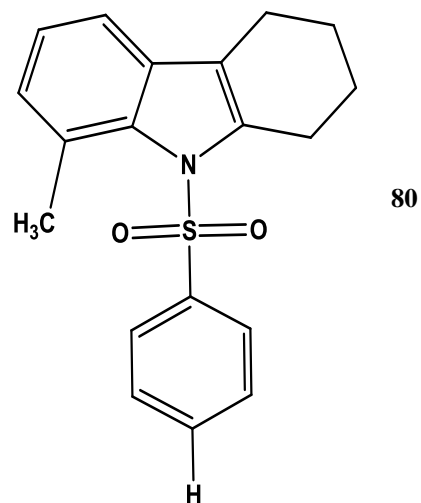
## Scheme



S.No	Substituted -1,2,3,4-tetrahydrocarbazole 3 (a-f)	Substituted benzene sulfonyl chloride 4 (a-f)	Final compounds 5 (a-f)	Yield (%)
1	R= H	R <sub>1</sub> =H		92
2	R=Cl	R <sub>1</sub> =H		84
3	R=F	R <sub>1</sub> =H		86

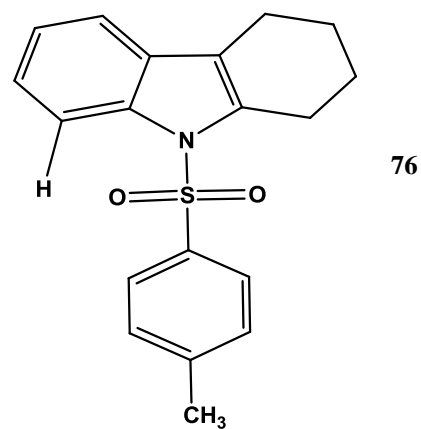
4 R=CH<sub>3</sub>

R<sub>1</sub>=H



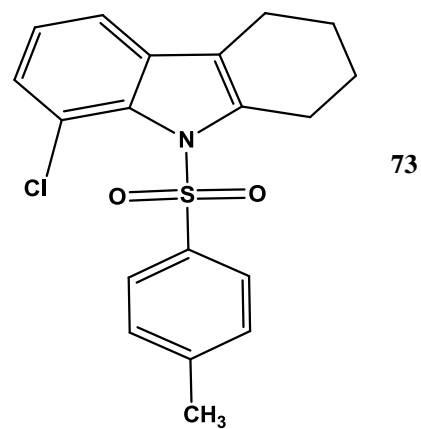
5 R=H

R<sub>1</sub>=CH<sub>3</sub>



6 R=Cl

R<sub>1</sub>=CH<sub>3</sub>



## BIOLOGICAL EVALUATION

### Anticancer activity by MTT assay

#### Cell culture and maintenance

Cytotoxicity of all newly synthesized hybrid compounds was evaluated *in vitro* against a panel of human tumour cell lines according to the procedures described in the literature. The panel consisted of lung carcinoma (A-549), cervical cancer (He La), prostate cancer (DU-145), breast carcinoma (MCF-7).

#### Cytotoxic assay experiment by MTT method

The stock solutions of test compounds were prepared in DMSO. After 24 h incubation, different concentrations up to 50  $\mu$ l of compounds were added in 48 h incubation. The final concentration of DMSO was 0.01% in each well. A separate well containing 0.01% DMSO only was run as DMSO control, which was found inactive under applied conditions. The cell growth was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma) reduction assay, which is based on ability of viable cells to reduce a soluble yellow tetrazolium salt to blue formazan crystal. Briefly, after 48 h of treatments, the 10  $\mu$ l of MTT dye, prepared in phosphate buffered saline (PBS) were added to all wells. The plates were then incubated for 4h at 37°C. Supernatant from each well was carefully removed, formed crystals were dissolved in 100  $\mu$ l of DMSO

and absorbance at 540 nm wavelength was recorded and each concentration was tested in threefold.

$$\text{Cell toxicity (\% of control)} = \frac{[(O.Dt - O.Db) / (O.Dc - O.Db)] \times 100}{}$$

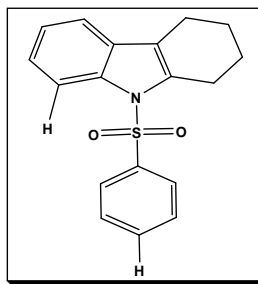
Where, O.Dt –mean optical density of treated wells, O.Db- mean optical density of blank wells, O.Dc - mean optical density of control wells. The results are summarized in **Table 1** accordingly. The IC<sub>50</sub> values were determined as concentration of compounds that inhibited cell growth by 50%.

## RESULTS AND DISCUSSION

With a view to obtain biologically active (*N*-Substituted -amino benzene sulphonyl) **1, 2, 3, 4-tetrahydrocarbazole derivatives (5a-f)**, series of compounds have been synthesized. The structures of all the synthesized compounds were established by spectral methods as discussed below.

IR spectrum shows characteristic absorption at 3090.93 cm<sup>-1</sup> indicating the presence of Aromatic C-H Stretching vibration, peak at 1718.39 cm<sup>-1</sup> indicating the presence of C-C (in ring) of aromatic, and 3 peaks at 1597.94 cm<sup>-1</sup>, 1517.50 cm<sup>-1</sup>, 1441.66 cm<sup>-1</sup> indicates the presence of C=C of aromatic, peak range at 1265.66-1398.69 cm<sup>-1</sup> indicates the presence of S=O group, peak range at 659.85-919.23 cm<sup>-1</sup> indicates the presence of C-H out of plane bending and vibration of mono, di substituted aromatic ring.

### Compound 5a



**Name:** 9-(phenylsulfonyl)-2, 3, 4, 9-tetrahydro-1H-carbazole

Formula: C<sub>18</sub>H<sub>17</sub>NO<sub>2</sub>S

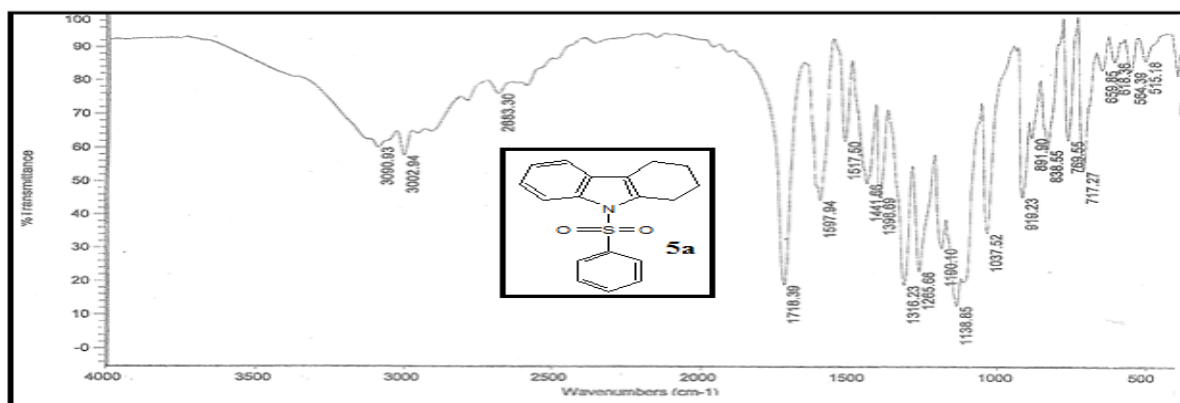
Colour: Yellow

Nature: solid

Yield: 92 %.

M.P: 130 °C

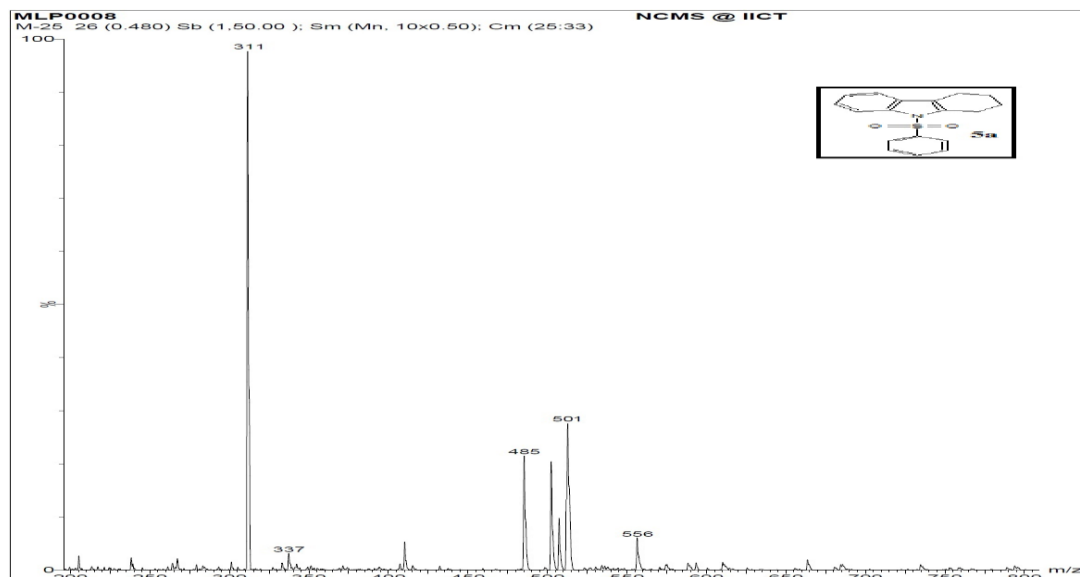
The structure of the compound was confirmed by IR, Mass and NMR IR (KBr): 3090.93 cm<sup>-1</sup> Aromatic C-H Str, 1718.39 cm<sup>-1</sup> Aromatic C-C Str (in ring), 3 peaks at 1597.94 cm<sup>-1</sup>, 1517.50 cm<sup>-1</sup>, 1441.66 cm<sup>-1</sup> Aromatic C=C, 1265.66-1398.69 cm<sup>-1</sup> S=O group, 659.85-919.23 cm<sup>-1</sup> C-H out of plane bending and mono/di substituted aromatic ring.



**IR spectrum of compound 5a**

### Mass

311, m/z molecular ion peak [ $M^+$ ] 311

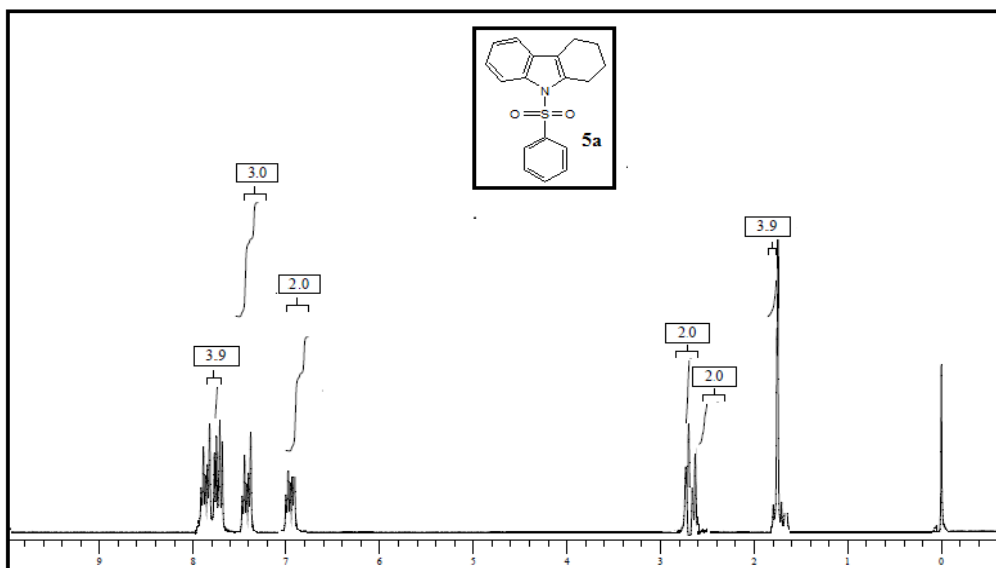


**Mass spectrum of compound 5a**

### $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum

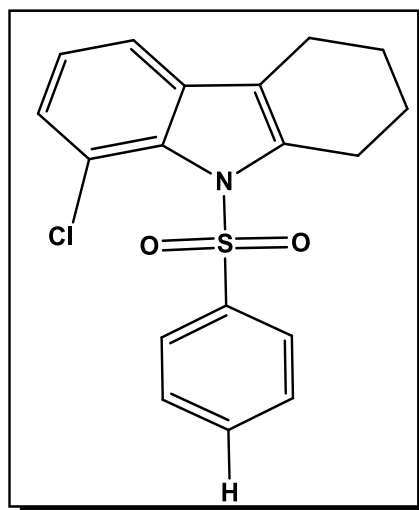
$\delta$  1.78 (s, 4H, Alicyclic- $\text{CH}_2$ ),  $\delta$  2.61 (s, 2H, Alicyclic- $\text{CH}_2$ ),  $\delta$  2.76 (s, 2H, Alicyclic- $\text{CH}_2$ ),  $\delta$

6.95 (m, 2H, Ar- $\text{H}$ ),  $\delta$  7.4 (m, 3H, Ar- $\text{H}$ ),  $\delta$  7.8 (m, 4H, Ar- $\text{H}$ )



<sup>1</sup>H-NMR spectrum of compound 5a

## COMPOUND 5b



### Name: 8-chloro-9-(phenylsulfonyl)-2, 3, 4, 9-tetrahydro-1H-carbazole

Formula: C<sub>18</sub>H<sub>16</sub>ClNO<sub>2</sub>S

Colour: White

Nature: Crystal

Yield: 84 %.

M.P: 139 °C

The structure of the compound was confirmed by IR, Mass and NMR

### IR (KBr)

3100 cm<sup>-1</sup> Aromatic C-H Str, 1585 cm<sup>-1</sup> Aromatic C-C Str (in ring), 3 peaks at 1560 cm<sup>-1</sup>, 1513 cm<sup>-1</sup>, 1496

cm<sup>-1</sup> Aromatic C=C, 1380 cm<sup>-1</sup> S=O group, 900-650 cm<sup>-1</sup> C-H out of plane bending and mono/di substituted aromatic ring, 750-600 cm<sup>-1</sup> C-Cl str.

### MASS

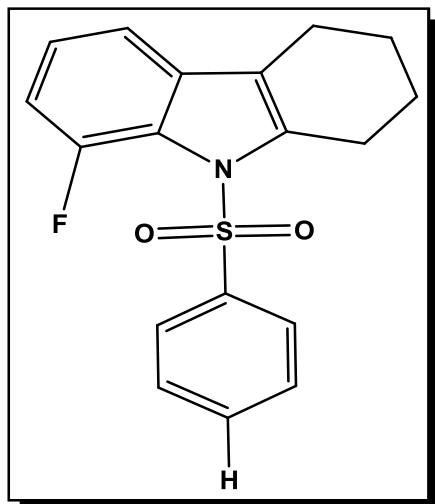
345, m/z molecular ion peak [M+H]<sup>+</sup> 346.

### <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) spectrum

δ 1.93 (m, 4H, Alicyclic-CH<sub>2</sub>), δ 2.81 (t, 4H, Alicyclic-CH<sub>2</sub>), δ 6.05 (d, 2H, Ar-H), δ 6.30 (t, 2H, Ar-H), δ 6.56 (t, 1H, Ar-H), δ 7.00 (d, 1H, Ar-H), δ 7.30 (t, 1H, Ar-H), δ 7.61 (d, 1H, Ar-H).



## COMPOUND 5c



### Name: 8-fluoro-9-(phenylsulfonyl)-2, 3, 4, 9-tetrahydro-1H-carbazole

Formula:  $C_{18}H_{16}FNO_2S$

Colour: White

Nature: Crystal

Yield: 86 %.

M.P: 166 °C

The structure of the compound was confirmed by IR, Mass and NMR

### IR (KBr)

3000  $cm^{-1}$  Aromatic C-H Str, 1660  $cm^{-1}$  Aromatic C-C Str (in ring), 3 peaks at 1550  $cm^{-1}$ , 1481  $cm^{-1}$ , 1450  $cm^{-1}$  Aromatic C=C, 1400  $cm^{-1}$  C-F

str, 1360  $cm^{-1}$  S=O group, 930-600  $cm^{-1}$  C-H out of plane bending and mono/di substituted aromatic ring.

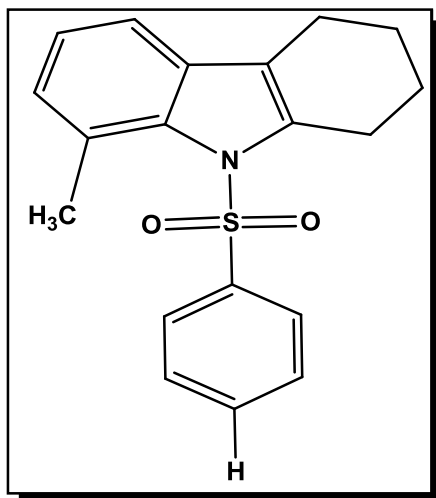
### MASS

329, m/z molecular ion peak [ $M^+$ ] 329.

### $H^1$ -NMR ( $CDCl_3$ , 300 MHz) spectrum

$\delta$  1.80-1.91 (m, 2H, Alicyclic- $\underline{CH_2}$ ),  $\delta$  2.0-2.3 (m, 2H, Alicyclic- $\underline{CH_2}$ ),  $\delta$  2.6-2.8 (m, 4H, Alicyclic- $\underline{CH_2}$ ),  $\delta$  6.45 (d, 2H, Ar- $\underline{H}$ ),  $\delta$  6.61 (t, 2H, Ar- $\underline{H}$ ),  $\delta$  6.94 (t, 1H, Ar- $\underline{H}$ ),  $\delta$  7.6 (d, 1H, Ar- $\underline{H}$ ),  $\delta$  7.96 (d, 1H, Ar- $\underline{H}$ ),  $\delta$  8.11 (t, 1H, Ar-H).

## COMPOUND 5d



**Name: 8-methyl-9-(phenylsulfonyl)-2,3,4,9-tetrahydro-1H-carbazole**

Formula:  $C_{19}H_{19}NO_2S$

Colour: white

Nature: solid

Yield: 80 %

M.P: 136 °C

The structure of the compound was confirmed by IR, Mass and NMR

**IR (KBr)**

3000  $cm^{-1}$  Aromatic C-H Str, 1550  $cm^{-1}$  Aromatic C-C Str(in ring), 3 peaks at 1600  $cm^{-1}$ , 1556  $cm^{-1}$ , 1450  $cm^{-1}$  Aromatic C=C, 1300  $cm^{-1}$

S=O group, 900-650  $cm^{-1}$  C-H out of plane bending and mono/di substituted aromatic ring.

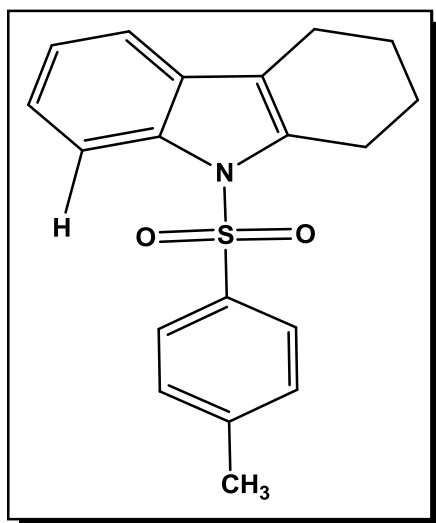
**MASS**

325, m/z molecular ion peak  $[M^+]$  325,  $[M+Na]^+$  348.

**$^1H$ -NMR ( $CDCl_3$ , 300 MHz) spectrum**

$\delta$  1.96 (s, 4H, Alicyclic-CH<sub>2</sub>),  $\delta$  2.3 (m, 4H, Alicyclic-CH<sub>2</sub>),  $\delta$  2.4 (s, 3H, Ali-CH<sub>3</sub>),  $\delta$  6.11 (d, 1H, Ar-H),  $\delta$  6.25 (d, 1H, Ar-H),  $\delta$  6.5 (t, 2H, Ar-H),  $\delta$  6.85 (t, 1H, Ar-H),  $\delta$  7.1 (d, 1H, Ar-H),  $\delta$  7.46 (t, 1H, Ar-H),  $\delta$  7.92 (d, 1H, Ar-H)

**COMPOUND 5e**



**Name: 9-tosyl-2, 3, 4, 9-tetrahydro-1H-carbazole**

Formula:  $C_{19}H_{19}NO_2S$

Colour: Light brown

Nature: Solid

Yield: 76%

M.P: 144 °C

The structure of the compound was confirmed by IR, Mass and NMR

**IR (KBr)**

3080  $cm^{-1}$  Aromatic C-H Str, 1590  $cm^{-1}$  Aromatic C-C Str(in ring), 3 peaks at 1645  $cm^{-1}$ , 1610  $cm^{-1}$ , 1580  $cm^{-1}$  Aromatic C=C, 1200  $cm^{-1}$

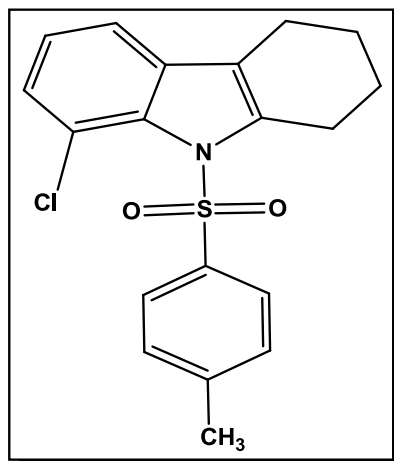
S=O group, 938-694  $cm^{-1}$  C-H out of plane bending and mono/di substituted aromatic ring.

**MASS**

327, m/z molecular ion peak  $[M+H]^+$  328.

**$^1H$ -NMR ( $CDCl_3$ , 300 MHz) spectrum**

$\delta$  1.72 (s, 2H, Alicyclic-CH<sub>2</sub>),  $\delta$  2.0 (s, 2H, Alicyclic-CH<sub>2</sub>),  $\delta$  2.4 (s, 3H, -CH<sub>3</sub>),  $\delta$  2.61 (t, 2H, Alicyclic-CH<sub>2</sub>),  $\delta$  2.8 (t, 2H, Alicyclic-CH<sub>2</sub>),  $\delta$  6.09 (d, 2H, Ar-H),  $\delta$  6.38 (d, 2H, Ar-H),  $\delta$  6.85 (d, 2H, Ar-H),  $\delta$  7.26 (t, 1H, Ar-H),  $\delta$  7.59 (s, 1H, Ar-H).

**COMPOUND 5f****Name: 8-chloro-9-tosyl-2, 3, 4, 9-tetrahydro-1H-carbazole**Formula: C<sub>19</sub>H<sub>18</sub>ClNO<sub>2</sub>S

Colour: brown

Nature: solid

Yield: 73 %

M.P: 120 °C

The structure of the compound was confirmed by IR, Mass and NMR

**IR (KBr)**

3075 cm<sup>-1</sup> Aromatic C-H Str, 1538 cm<sup>-1</sup> Aromatic C-C Str (in ring), 3 peaks at 1595cm<sup>-1</sup>, 1536 cm<sup>-1</sup>, 1499 cm<sup>-1</sup> Aromatic C=C, 1250-1350 cm<sup>-1</sup> S=O group, 900-650 cm<sup>-1</sup> C-H out of plane bending and mono/di substituted aromatic ring, 730-639 cm<sup>-1</sup> C-Cl str.

**MASS**359, m/z molecular ion peak [M+Na]<sup>+</sup> 382.**<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) spectrum**

δ 1.81 (d, 4H, Alicyclic-CH<sub>2</sub>), δ 2.2 (s, 3H, Alicyclic-CH<sub>2</sub>), δ 2.52 (s, 2H, Ali-CH<sub>3</sub>), δ 2.77 (t,

2H, Alicyclic-CH<sub>2</sub>), δ 6.32 (d, 1H, Ar-H), δ 6.55 (d, 2H, Ar-H), δ 6.92 (s, 1H, Ar-H), δ 7.38 (d, 1H, Ar-H), δ 7.41 (d, 1H, Ar-H), δ 7.71 (m, 1H, Ar-H).

All the synthesized final compounds (**5a**, **5b**, **5c**, **5d**, **5e**, **5f**) structures were confirmed by IR, MASS and <sup>1</sup>H-NMR studies.

**BIOLOGICAL EVALUATION****Anticancer activity****Cytotoxic assay experiment by MTT method**

Cytotoxicity studies have shown that compound **5c** (-F) showed highest activity i.e lowest IC<sub>50</sub>, against A-549. Whereas **5b** (-Cl), **5a** (-H) substitutions also showed next highest activities. Electron donating -CH<sub>3</sub> group (**5d**) substitution and modifications on sulfonyl aryl moiety have decreased the activity of the compound.

Overall all the six compounds were found to have markable anticancer activity against the screened cell line.

**Table-1. Cytotoxicity studies of few of the titled compounds.**

Compound	Lung Cancer
	A – 549 (IC <sub>50</sub> in μM)
<b>5a</b>	<b>22.17</b>
<b>5b</b>	<b>17.59</b>
<b>5c</b>	<b>10.35</b>
<b>5d</b>	<b>32.35</b>
<b>5e</b>	<b>&gt;40</b>
<b>5f</b>	<b>&gt;40</b>
<b>Doxorubicin</b>	<b>0.05</b>

## CONCLUSION

“A novel synthesis of *N*-substituted tetrahydrocabazole analogs and their biological evaluation for anti-cancer activity” by adopting new methodology. The structures were confirmed by IR, <sup>1</sup>H-NMR and Mass spectral studies. The process involves the eco-friendly method of

synthesizing analogues with high yields and relatively pure compounds which increases a wide scope in the field of medicinal chemistry. Synthesized compounds were screened for anticancer activity and found to have markable biological activity.

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