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Synthesis, Characterization and Evaluation of N-Substituted Tetrahydrocarbazoles for anti-oxidant activity

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ABSTRACT

A New Class of N-Substituted Tetrahydrocabazole derivatives are prepared by in presence of reagents like glacial acetic acid leads the formation of Intermediate compounds substituted tetrahydrocabazole (3a, 3b), by using cyclohexanone and phenyl hydrazine's using as starting material. The Intermediate compounds upon treating with 10% sodium hydroxide and substituted 4amino-benzoyl chlorides (4a, 4b) gives (4aminobenzoyl) 1, 2, 3, 4 tetrahydro carbazole derivatives. (5a, 5b). The structures of new derivatives are characterized by ^{1}H NMR, IR, and Mass spectral data. All the newly synthesized compounds were evaluated for their in-vitro anti-oxidant activity. Among these compounds the 5b shows good anti-oxidant activity due to the presence of the methyl functional group at 8th position.

Keywords: Tetra hydro carbazole, Cyclohexanone, Phenyl hydrazine's, Anti-Oxidant.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions [1-10]. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects. Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, 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 [11-15]. Various classes of carbazoles are given in Figure. 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 [16-18], pyrido-carbazolequinone, tetrahydrocarbazoles, benzocarbazoles, furo-carbazoles, pyridocarbazoles, pyrrolo-carbazoles, indolocarbazoles, oxazolinyl 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-oxidant activity for the *N*-substituted carbazoles [19-23].

Tetrahydrocabazole condensed with indole, furan, pyrimidine, pyrazoline, and thiophene, moieties have been known to processes wide spectrum biological activities. A simple and efficient method for the synthesis of these pharmaceutically important classes of compounds is highly desirable precluding the usage of organic solvents. There have been many methods of synthesis [24-28]. In general the carbazoles synthesis is carried out by multistep Fisher reaction which requires the usage of organic solvents with very good product yields. Initially substituted phenyl hydrazines 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 CH3COOH, ZnCl2 and Hcl lesser amount of the desired product was obtained [29-35]. 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 be90°C. 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. The tetrahydrocabazole ring system has been the structural subunit of many naturally occurring alkaloids, biologically active molecules and medicinal important synthetic analogues [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, 1H NMR (CDCl3, 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 carbazole derivatives. (5a, 5b).

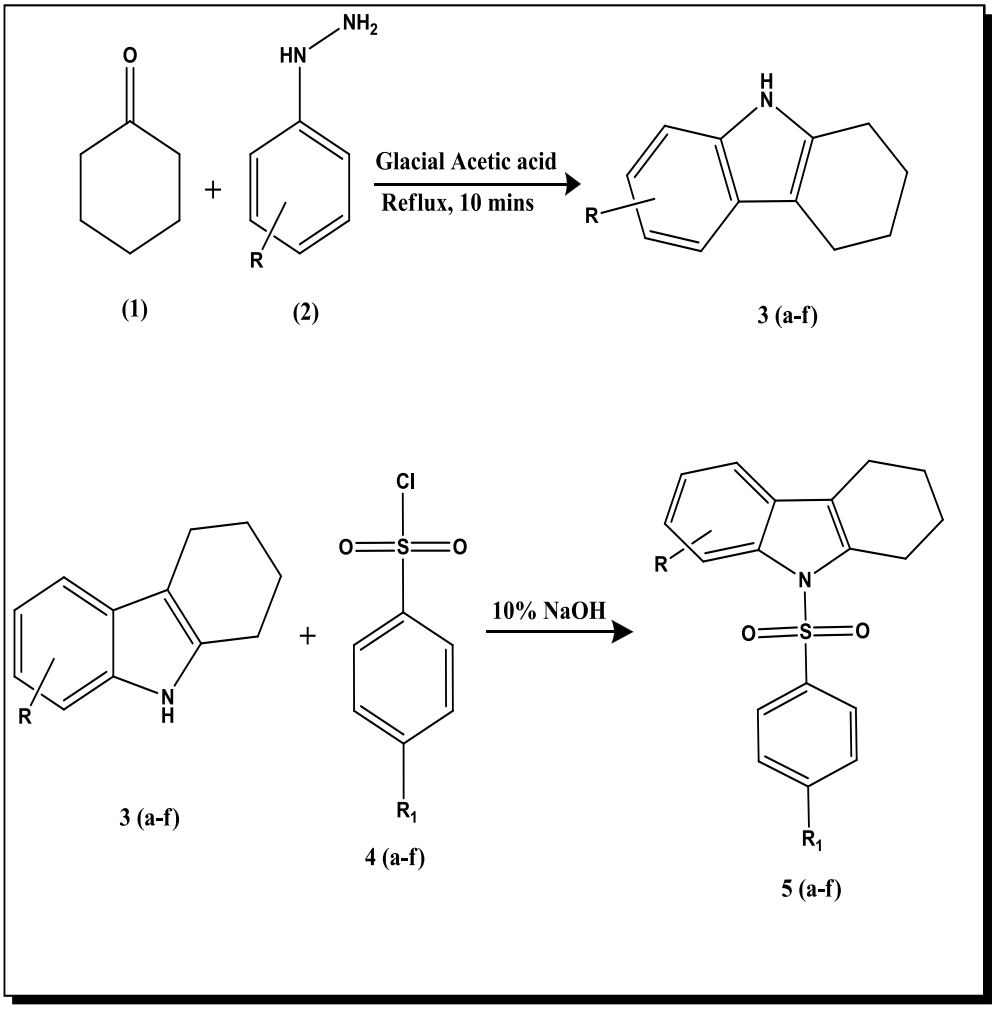
Step I

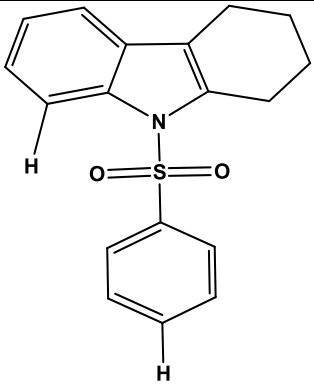
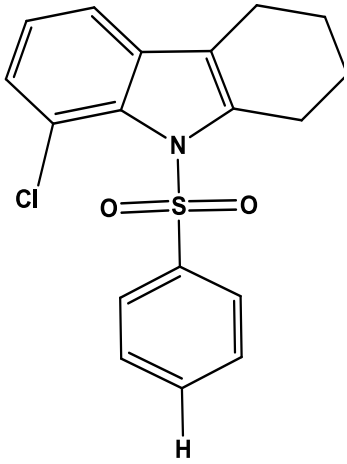
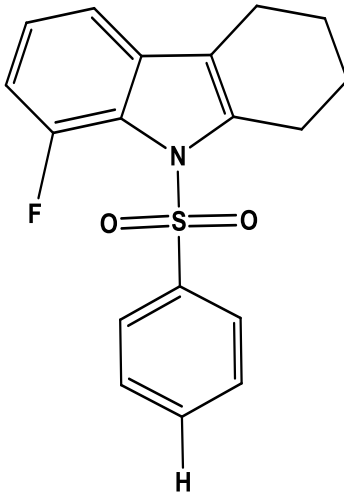
Synthesis of 1,2,3,4 tetrahydrocabazole (3a, 3b) (The Fischer's indolisation reaction): Dissolve cyclohexanone (1) (9.8 gm, 0.1mol) in (34.65 gm,0.6 mole) of glacial acetic acid, substituted phenyl hydrazine's(2a, 2b)(10.8 gm,0.1mol) and the solution was refluxed for 10 minutes. Reaction mixture was 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.

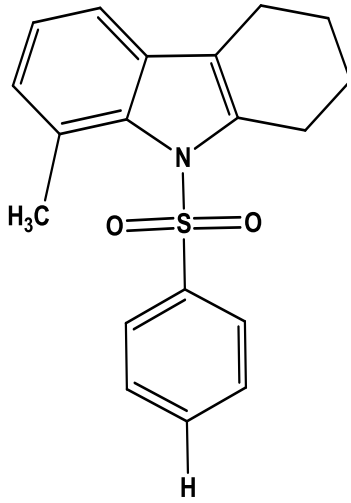
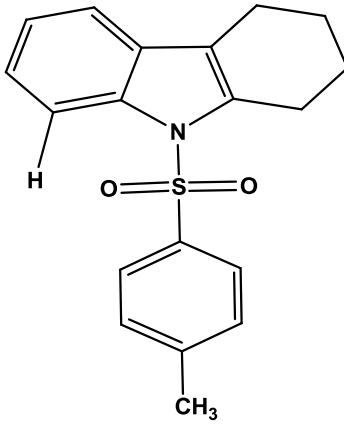
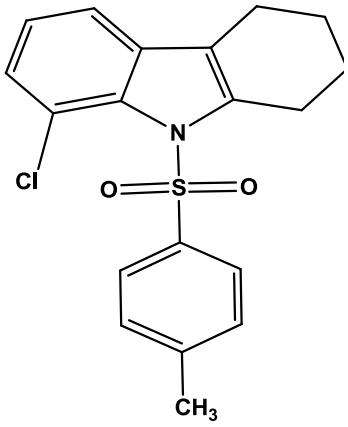
Step II

Synthesis of N-(4 –amino benzoyl) 1,2,3,4-tetrhydroacabazole 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_1=H		92
2	R=Cl	R_1=H		84
3	R=F	R_1=H		86

4	**R=CH_3**	**R_1=H**		**80**
5	**R=H**	**R_1=CH_3**		**76**
6	**R=Cl**	**R_1=CH_3**		**73**

BIOLOGICAL EVALUATION

Antioxidant activity by DPPH free radical scavenging method

DPPH solution (0.004% w/v) was prepared in 95% methanol. All the test compounds **(5a-j)** were mixed with 95% methanol to prepare the stock solutions (10 mg/100 mL or 100 µg/mL). 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & the final volume was made up to 10 mL whose concentration was then 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL & 100 µg/mL respectively. Freshly prepared DPPH solution was added in each of these test tubes and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference standard.

% scavenging of the DPPH free radical was measured using the following equation

$$\%\ \text{DPPH radical} - \text{scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \text{X100}$$

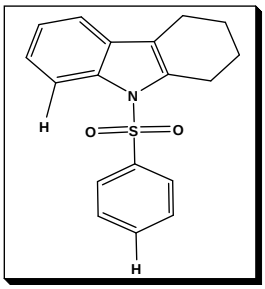
Results of % DPPH scavenging activity of the titled compounds were given in **Table 1**

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^{-1} indicating the presence of Aromatic C-H Stretching vibration, peak at 1718.39 cm^{-1} indicating the presence of C-C (in ring) of aromatic, and 3 peaks at 1597.94 cm^{-1}, 1517.50 cm^{-1}, 1441.66 cm^{-1} indicates the presence of C=C of aromatic, peak range at 1265.66-1398.69 cm^{-1} indicates the presence of S=O group, peak range at 659.85-919.23 cm^{-1} indicates the presence of C-H out of plane bending and vibration of mono, di substituted aromatic ring.

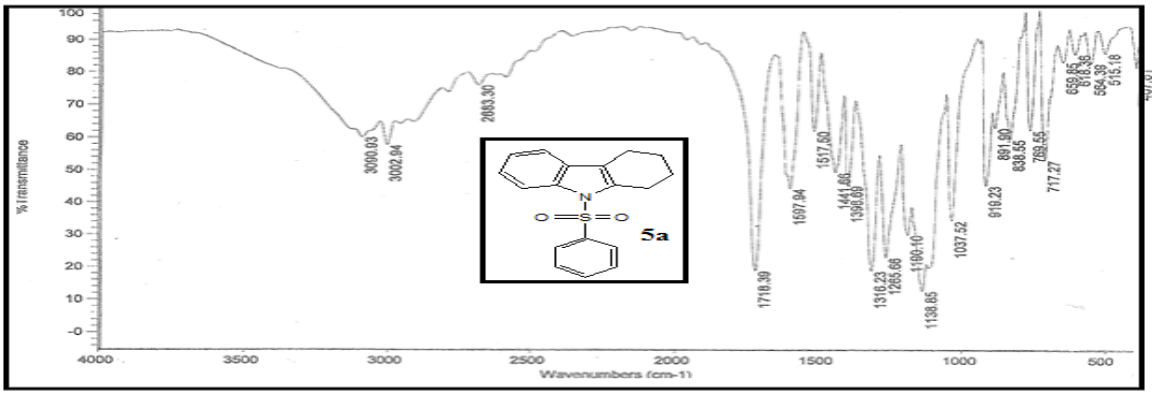
COMPOUND 5a



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

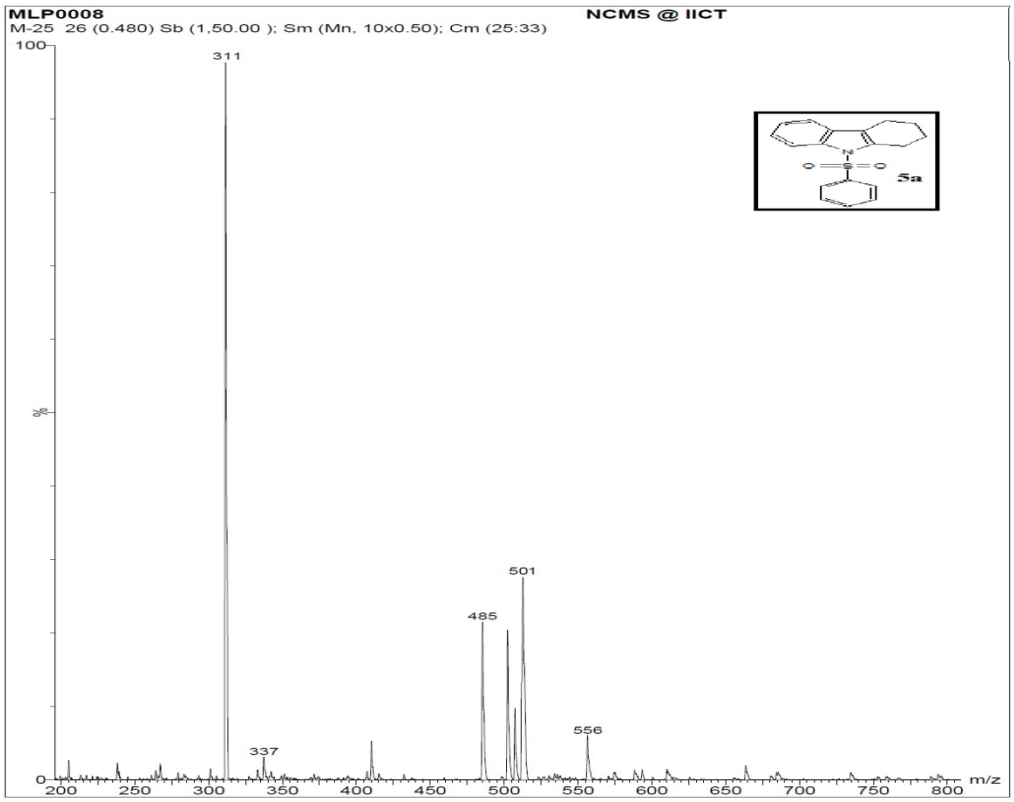
Formula : $C_{18}H_{17}NO_2S$
Colour : Yellow
Nature : solid
Yield : 92 %.
M.P : 130 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

IR (KBr): 3090.93 cm^{-1} Aromatic C-H Str, 1718.39 cm^{-1} Aromatic C-C Str (in ring), 3 peaks at 1597.94 cm^{-1}, 1517.50 cm^{-1}, 1441.66 cm^{-1} Aromatic C=C, 1265.66-1398.69 cm^{-1} S=O group, 659.85-919.23 cm^{-1} C-H out of plane bending and mono/di substituted aromatic ring.



IR spectrum of compound 5a

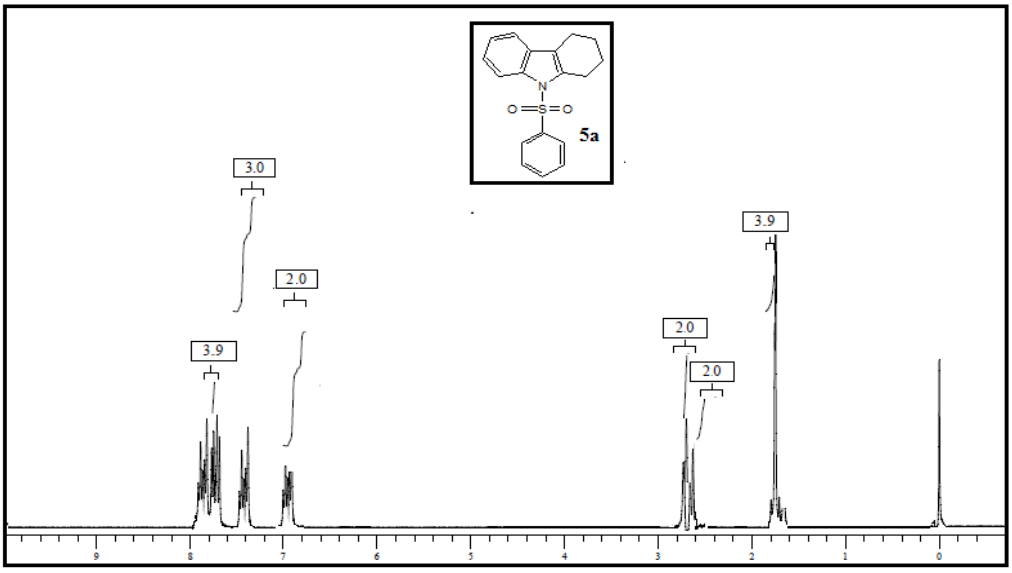
MASS: 311, m /z molecular ion peak [M^+] 311



Mass spectrum of compound 5a

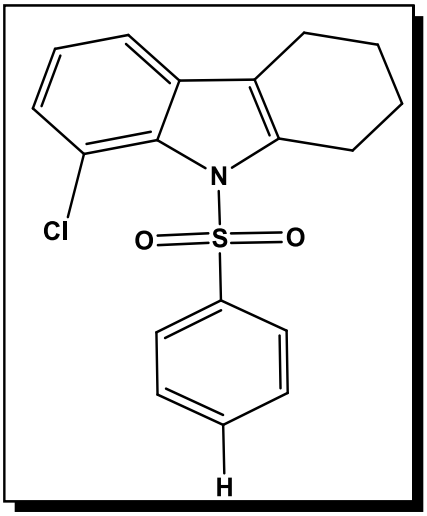
H^1-NMR ($CDCl_3$, 300 MHz) spectrum

δ 1.78 (s, 4H, Alicyclic-C<u>H</u>$_2$), δ 2.61 (s, 2H, Alicyclic-C<u>H</u>$_2$), δ 2.76 (s, 2H, Alicyclic-C<u>H</u>$_2$), δ 6.95 (m, 2H, Ar-<u>H</u>), δ 7.4 (m, 3H, Ar-<u>H</u>), δ 7.8 (m, 4H, Ar-<u>H</u>)



^{1}H-NMR spectrum of compound 5a

COMPOUND 5b



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

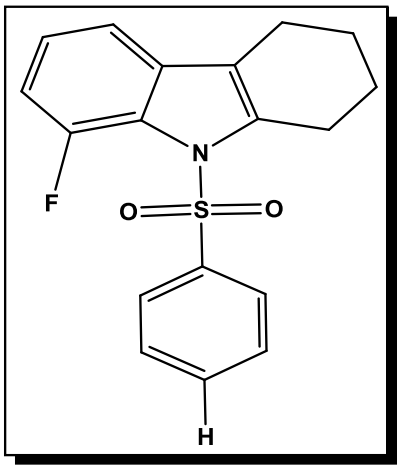
Formula : $C_{18}H_{16}ClNO_2S$
Colour : White
Nature : Crystal
Yield : 84 %.
M.P : 139 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

IR (KBr): 3100 cm^{-1} Aromatic C-H Str, 1585 cm^{-1} Aromatic C-C Str (in ring), 3 peaks at 1560 cm^{-1}, 1513 cm^{-1}, 1496 cm^{-1} Aromatic C=C, 1380 cm^{-1} S=O group, 900-650 cm^{-1} C-H out of plane bending and mono/di substituted aromatic ring, 750-600 cm^{-1} C-Cl str.
MASS: 345, m /z molecular ion peak $[M+H]^+$ 346.

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

δ 1.93 (m, 4H, Alicyclic-C<u>H</u>$_2$), δ 2.81 (t, 4H, Alicyclic-C<u>H</u>$_2$), δ 6.05 (d, 2H, Ar-<u>H</u>), δ 6.30 (t, 2H, Ar-<u>H</u>), δ 6.56 (t, 1H, Ar-<u>H</u>), δ 7.00 (d, 1H, Ar-<u>H</u>), δ 7.30 (t, 1H, Ar-<u>H</u>), δ 7.61 (d, 1H, Ar-<u>H</u>).

COMPOUND 5c



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

Formula : $C_{18}H_{16}FNO_2S$
Colour : White
Nature : Crystal
Yield : 86 %.
M.P : 166 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

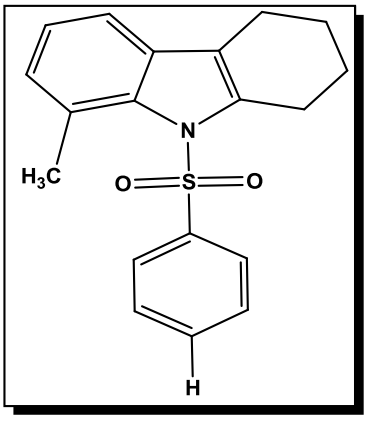
<u>IR (KBr) :</u> 3000 cm^{-1} Aromatic C-H Str, 1660 cm^{-1} Aromatic C-C Str (in ring), 3 peaks at 1550 cm^{-1}, 1481 cm^{-1}, 1450 cm^{-1} Aromatic C=C, 1400 cm^{-1} C-F str, 1360 cm^{-1} S=O group, 930-600 cm^{-1} C-H out of plane bending and mono/di substituted aromatic ring.

<u>MASS</u>: 329, m /z molecular ion peak [M^+] 329.

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

δ 1.80-1.91 (m, 2H, Alicyclic-C<u>H</u>$_2$), δ 2.0-2.3 (m, 2H, Alicyclic-C<u>H</u>$_2$), δ 2.6-2.8 (m, 4H, Alicyclic-C<u>H</u>$_2$), δ 6.45 (d, 2H, Ar-<u>H</u>), δ 6.61 (t, 2H, Ar-<u>H</u>), δ 6.94 (t, 1H, Ar-<u>H</u>), δ 7.6 (d, 1H, Ar-<u>H</u>), δ 7.96 (d, 1H, Ar-<u>H</u>), δ 8.11 (t,1H, Ar-H).

COMPOUND 5d



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

Formula : $C_{19}H_{19}NO_2S$
Colour : white
Nature : solid
Yield : 80 %
M.P : 136 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

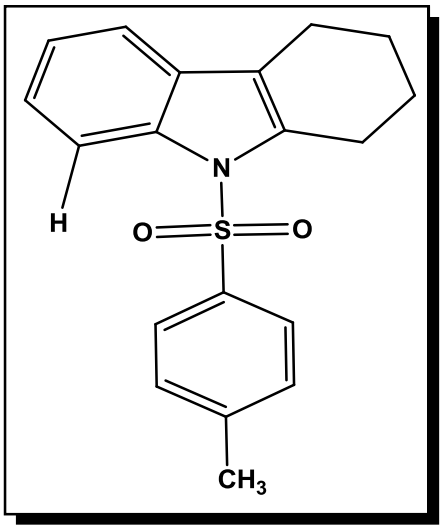
IR (KBr): 3000 cm^{-1} Aromatic C-H Str, 1550 cm^{-1} Aromatic C-C Str(in ring), 3 peaks at 1600 cm^{-1}, 1556 cm^{-1}, 1450 cm^{-1} Aromatic C=C, 1300 cm^{-1} S=O group, 900-650 cm^{-1} C-H out of plane bending and mono/di substituted aromatic ring.

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

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

δ 1.96 (s, 4H, Alicyclic-CH_2), δ 2.3 (m, 4H, Alicyclic-CH_2), δ 2.4 (s, 3H, Ali-CH_3), δ 6.11 (d, 1H, Ar-H), δ 6.25 (d, 1H, Ar-H), δ 6.5 (t, 2H, Ar-H), δ 6.85 (t, 1H, Ar-H), δ 7.1 (d, 1H, Ar-H), δ 7.46 (t, 1H, Ar-H), δ 7.92 (d, 1H, Ar-H)

COMPOUND 5e



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

Formula : $C_{19}H_{19}NO_2S$
Colour : Light brown
Nature : Solid
Yield : 76%
M.P : 144 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

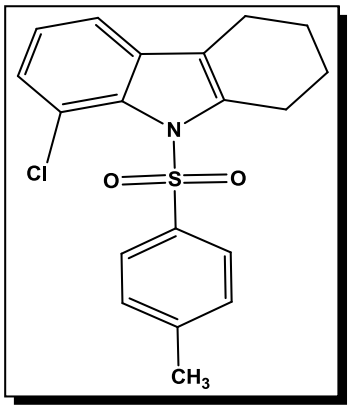
IR (KBr) : 3080 cm^{-1} Aromatic C-H Str, 1590 cm^{-1} Aromatic C-C Str(in ring), 3 peaks at 1645 cm^{-1}, 1610 cm^{-1}, 1580 cm^{-1} Aromatic C=C, 1200 cm^{-1} S=O group, 938-694 cm^{-1} C-H out of plane bending and mono/di substituted aromatic ring.

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

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

δ 1.72 (s, 2H, Alicyclic-CH_2), δ 2.0 (s, 2H, Alicyclic-CH_2), δ 2.4 (s, 3H, -CH_3), δ 2.61 (t, 2H, Alicyclic-CH_2), δ 2.8 (t, 2H, Alicyclic-CH_2), δ 6.09 (d, 2H, Ar-H), δ 6.38 (d, 2H, Ar-H), δ 6.85 (d, 2H, Ar-H), δ 7.26 (t, 1H, Ar-H), δ 7.59 (s, 1H, Ar-H).

COMPOUND 5f



Name: 8-chloro-9-tosyl-2,3,4,9-tetrahydro-1*H*-carbazole

Formula : $C_{19}H_{18}ClNO_2S$
Colour : brown
Nature : solid
Yield : 73 %
M.P : 120 ^oC
The structure of the compound was confirmed by IR, Mass and NMR

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

MASS: 359, m /z molecular ion peak $[M+Na]^+$ 382.

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

δ 1.81 (d, 4H, Alicyclic-CH_2), δ 2.2 (s, 3H, Alicyclic-CH_2), δ 2.52 (s, 2H, Ali-CH_3), δ 2.77 (t, 2H, Alicyclic-CH_2), 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 ^{1}H-NMR studies.

BIOLOGICAL EVALUATION

Antioxidant activity

DPPH free radical scavenging activity

DPPH free radical scavenging activity results displayed that title compounds **(5a-f)** are able to show marked antioxidant activity. Among which **5d** showed highest antioxidant activity, whereas compounds **5a, 5b** showed good activity. Remaining all the compounds have shown **5c, 5e, 5f** moderate activity. The IC_{50} values of all compounds **(5a-f)** were found between 25.46-90.66 µg/mL with antioxidant activity. These compounds have showed less antioxidant potential with the standard ascorbic acid.

Table-1. Antioxidant activity of the synthesized compounds (5a-f)

Compound	DPPH free radical scavenging activity (%)					
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	IC_{50} (µg/mL)
5a	45.23±0.7	51.11±0.1	67.39±0.1	69.46±0.5	71.48±0.2	29.13
5b	37.59±0.6	46.10±0.6	51.92±0.1	67.31±0.6	70.50±0.5	49.23
5c	34.11±0.3	39.43±0.4	41.78±0.3	46.81±0.1	53.10±0.3	90.66
5d	47.19±0.6	54.84±0.2	68.43±0.4	74.68±0.5	81.87±0.2	25.46
5e	36.23±0.1	41.65±0.4	44.11±0.2	48.02±0.2	54.90±0.4	82.96
5f	31.23±0.2	37.54±0.4	48.05±0.2	52.12±0.2	63.70±0.2	69.15
Ascorbicacid	55.12±0.2	65.08±0.2	75.26±0.2	85.82±0.4	93.74±0.2	8.96

Ascorbic acid (reference antioxidant compounds) was used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates (mean±SD, n=3).

CONCLUSION

"A novel synthesis of *N*-substituted tetrahydrocabazole analogs and their biological evaluation anti-oxidant activity" by adopting new methodology. The structures were confirmed by IR, ^{1}H-NMR and Mass spectral studies. The IC_{50} values of all compounds **(5a-f)** were found between 25.46-90.66 µg/mL with antioxidant activity. These compounds have showed less antioxidant potential with the standard ascorbic acid. Among which **5d** showed highest antioxidant activity.

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