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Discovery of Tetrahydrocarbazoles as Dual pERK and pRb Inhibitors

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ABSTRACT:

The extracellular signal-regulated kinase (ERK) is one of the most important molecular targets for cancer that controls diverse cellular processes such as proliferation, survival, differentiation and motility. Similarly, the Rb (retinoblastoma protein) is a tumor suppressor protein and its function is to prevent excessive cell growth by inhibiting cell cycle progression. When the cell is ready to divide, pRb is phosphorylated, becomes inactive and allows cell cycle progression. Herein, we discovered a new series of tetrahydrocarbazoles as dual inhibitors of pERK and pRb phosphorylation. The in-house small molecule library was screened for inhibition of pERK and pRb phosphorylation, which led to the discovery of tetrahydrocarbazole series of compounds as potential leads. *N*-(3-methylcyclopentyl)-6-nitro-2,3,4,4a,9,9a-hexahydro-1*H*-carbazol-2-amine (**1**) is the dual inhibitor lead identified through screening, displaying inhibition of pERK and pRb phosphorylation with IC_{50} values of 5.5 and 4.8 µM, respectively. A short structure-activity relationship (SAR) study has been performed, which identified another dual inhibitor 9-methyl-*N*-(4-methylbenzyl)-2,3,4,4a,9,9a-hexahydro-1*H*-carbazol-2-amine (**16**) with IC_{50} values 4.4 and 3.5 µM for inhibition of pERK and pRb phosphorylation, respectively. This compound has a potential for further lead optimization to discover promising molecularly-targeted anticancer agents.

KEYWORDS

Tetrahydrocarbazole, pERK, pRb, MDR, MAPK, CDKs

1. Introduction

Despite significant advances and progress made in the area of cancer management, multi-drug resistance (MDR) is a growing concern [1, 2]. Currently, molecularly-targeted therapy promises to provide better therapeutic and toxicity profiles [3]. In addition, mechanism-based targeting of the cancer survival and resistance pathways should translate into increased efficacy and reduced MDR. Cancer is a disease that is characterized by uncontrolled cell growth which is manifested due to cellular crosstalk or signaling between and within cells [4]. The aberrations are pleiotropic, and occur due to effects on several different signaling pathways; the most significant among these being mitogen-activated protein kinase (MAPK) pathways, Ras-Raf pathways and cyclin dependent unrestrained cell proliferation [3]. The balance and integration between these signals may widely vary in different tumors, but are important for the outcome and the sensitivity to drug therapy.

The MAPK pathway is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell [5, 6]. The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein resulting in cell division [7, 8]. The pathway includes many proteins, including MAPKs, originally called ERKs (extracellular signal-regulated kinases), which communicate by adding phosphate groups to a neighbouring protein, which acts as an "on" or "off" switch. This pathway is also known as the Ras-Raf-MEK-ERK pathway [8]. Drugs that reverse the "on" or "off" switch are being investigated for cancer treatment [9]. The ERK, one of the key players in this pathway, is a major determinant in the control of diverse cellular processes such as proliferation, survival, differentiation and motility. This pathway is often upregulated in human tumors and as such represents an attractive target for the development of anticancer drugs. Specific blockade of the ERK pathway is expected to result in not only antiproliferative effect but also in antimetastatic and antiangiogenic effects in tumor cells. Recently potent small-molecule inhibitors targeting the components of the ERK pathway have been developed. Among them, BAY 43-900610 (Raf inhibitor) [10], PD184352, PD032590111 [11] and ARRY-14288612 (MEK1/2 inhibitors) [12] have reached the clinical trial stages.

Similarly, the retinoblastoma protein (Rb) is a tumor-suppressor protein that is dysfunctional in several major cancers [13]. The phosphorylated form of this protein (pRb) functions to prevent excessive cell growth by inhibiting cell cycle progression thus preventing cell division. It is also a recruiter of several chromatin-remodeling enzymes such as methylases and acetylases which are implicated in signaling activation of several signaling pathways. Rb is phosphorylated to pRb by certain cyclin-dependent kinases (CDKs). pRb when hyper phosphorylated is unable to complex E2F and therefore, unable to restrict progression from the G1 phase to the S phase of the cell cycle. During the M to G1 transition, pRb is progressively dephosphorylated by PP1, returning to its growth-suppressive hypo phosphorylated state Rb. When it is time for a cell to enter the S phase, complexes of CDKs and cyclins phosphorylate Rb to pRb, inhibiting its activity. The initial phosphorylation is performed by cyclin D/CDK4/CDK6 and followed by additional phosphorylation by cyclin E/CDK2. pRb remains phosphorylated throughout S, G2 and M phases [14, 15]. Due to its key role in the cell cycle progression, it is believed to be a good anticancer target [16, 17]. Modern approaches in cancer therapeutics include combination of ERK pathway inhibitors (cytostatic agents) and conventional anticancer drugs (cytotoxic agents) which may provide

an excellent basis for the development of new chemotherapeutic strategies against cancer [18]. Thus, our study focuses on targeting primary signaling events in the Ras-Raf pathway and cell cycle progression. We believe that mitigating both events simultaneously may lead to improved efficacy in targeting cancer.

In this direction, we have screened our in-house small molecule library of compounds (777 compounds) originally designed for inflammatory targets (TNF-α and IL-6) against pERK and pRb signaling. We tested for pERK, pRb phosphorylation inhibition at 10 µM concentration (n=3) and the compound which demonstrated >70% inhibition were considered as active. 25 compounds showed >70% inhibition in the pERK assay, out of these 6 compound showed consistent activity in dose dependent manner in pERK assay. In pRb assay 14 compound showed >70% inhibition in pRb assay and only 2 compounds demonstrated dual inhibition of pERK and pRb phosphorylation. The dose response effects of these two hits confirmed the activity of compounds and led to the identification of tetrahydrocarbazole based inhibitor **1** (**Figure 1**) as initial hit with pERK (IC_{50}= 5.52 µM) and pRb (IC_{50} = 4.81 µM) inhibitory activity. Tetrahydrocarbazole core is known to possess a wide range of biological activities such as, 5-HT6-receptor antagonists [19], antifungal [20], treatment of human papilloma infection[21-23], treatment of viruses belonging to flaviviruses, pestiviruses, hepaciviruses, prophylaxis dengue fever, yellow fever, west nile virus, HCV[24], treatment of neurological, cardiac and metabolic diseases [25], BTK inhibitor [26], antitumor agents [27], etc. We set out with the primary aim of improving the potency of this core (1) as dual pathway inhibitor. The present study describes the investigations into the SAR, molecular docking and further optimization of **1** as a dual inhibitor of pERK and pRb phosphorylation.

<<<Figure 1 >>>

2. Result and Discusion

2.1 Chemistry. The synthetic route to 1-aminotetrahydrocarbazole analogues of **1** (pERK and pRb phosporylation inhibitor) is shown in **Scheme 1**. The tetrahydrocarbazole core was assembled by cyclization of commercially available 4-(1*H*-indol-3-yl)butanoic acid **2** in presence of polyphosphoric acid (PPA) to the corresponding 2,3,4,9-tetrahydro-1*H*-carbazol-1-one **3** which was converted to 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine **5** by subsequent oxime formation followed by

reduction of the oxime using $LiAlH_4$. Further the amine was converted to the designed analogues of **1** (**6**).

<<<Scheme 1 Here>>>

Syntheses of 2-amino-substituted tetrahydrocarbazole derivatives **13** were achieved by sequential reactions of 1,3-cyclohexadione **7** and phenylhydrazine or 4-methylphenyhydrazine as shown in **Scheme 2**. The intermediate ketone **10** was synthesized according to the reported procedure [28]. 1,3-cyclohexadione (**7**) and phenylhydrazine or 4-methylphenyhydrazine were condensed to give hydrazone 8 which on treatment with *p*TSA and ethylene glycol in toluene gave protected ketone 9. It was further deprotected to the corresponding ketone 10 using 10% aqueous H_2SO_4 or conc. HCl in methanol. Ketone **10** was further converted to oxime **11** by treatment with $NH_2OH.HCl$, which was reduced to the corresponding amine **12** by treatment with $LiAlH_4$. Amine **12** was converted to the designed compounds by reductive amination with respective aldehyde or ketone or by coupling with respective benzoyl chloride or sulfonyl chloride to yield amide or sulfonamide in presence of Et_3N.

<<<Scheme2 Here>>>

In order to synthesize *N*-methyl analogues of **13l**, the reaction sequence shown in **Scheme 3** was followed. *N*-Methylation of the protected ketone **9** was carried out using methyl iodide in presence of NaH led to **14**, which was deprotected to corresponding ketone **15** followed by its conversion to *N*-methyl analogue **16**.

<<<Scheme3 Here>>>

2.2 Biological activity

Our in-house efforts to discover a dual pERK and pRb phosphorylation inhibitor yielded a moderately potent dual inhibitor **1** with IC_{50} values of 5.5 and 4.8 µM, respectively, for pERK and pRb. Initial SAR studies (**Table 1**) showed that only 3-methylcyclopentyl substitution **6c** retained activity compared to the initial hit in both the assays. This led to obvious conclusion that the 6-NO_2 group on the carbazole nucleus contributed little to the biological activity. Substitutions with cyclohexyl and

cyclopentyl led to decrease in the biological activity. Other substituents bearing aromatic sulfonamide groups, were not tolerated (Table 1, compounds **6d-f**). There was no improvement in potency over **1**. Hence, we investigated the effect of changing the position of amine group from benzylic to homobenzylic **13** (Scheme 2). Compound **13a** bearing cyclohexyl substitution exhibited the IC_{50} of 7 µM and 11 µM for inhibition of pERK and pRB phosphorylation, respectively. Compounds **13b**, **13c** having cyclopentyl and 3-methylcyclopentyl substitutions showed moderate activity (IC_{50} = 10–30 µM) in both the assays, and **13d** having tetrahydropyran was inactive in both the assays. This showed that the cycloalkyl groups were not favorable for activity. Sulfonamide **13f** having 4-fluorophenyl substitution showed good activity in both the assays having IC_{50} 7.5 µM and 9.5 µM in pERK and pRb, respectively, indicated the presence of electron-withdrawing group was favorable but at the same time **13g** having electron-withdrawing cyano group retained the activity in pERK (IC_{50} = 7.0 µM) but loss in activity (IC_{50} = >100 µM) in pRb assay was observed. Other benzamides (**13h**, **13i** and **13j**) and sulfonamides (**13e**) were shown to be good to moderately active in pERK assay but were not tolerated in pRb assay.

When cycloalkyls were replaced by benzyl groups (benzyl, p-tolyl), **13k** (pERK IC_{50} = 6.11 µM, pRb IC_{50} = 6.92 µM) and **13l** (pERK IC_{50} = 3.62 µM, pRb IC_{50} = 4.40 µM) showed good activity in both the assays. Compound **13l** having *p*-tolyl substitution showed improved activity compared to the initial hit, **1**. In order to synthesize benzyl-substituted derivatives of **13**, we tried direct reductive amination on ketone **10** with respective benzylamine but we observed unexpected aromatized product as major one. On investigation, we confirmed that it was known in literature [29]. Hence, the same reaction sequence as depicted in **Scheme 2** was followed. Furthermore, we were curious to see effect of substitution on carbazole ring on the biological activity. Thus, we synthesized compound with methyl substitution at position 6. These compounds were synthesized according to **Scheme 2**, the only exception being *p*-tolylhydrazine, which was used instead of phenylhydrazine. With 6-methyl substitution on tetrahydrocarbazole, both the cycloalkyl and benzyl substitutions were active in both the assays (compounds **13m**-**13o**). Compound **13o** having *p*-tolyl substitution showed IC_{50} 4.6 µM and 2.9 µM in pERK and pRb assays, respectively.

<<<Table 1 Here>>>

The SAR showed that methyl substitutions at the 6-position of tetrahydrocarbazole were quite well tolerated. Among the synthesized derivatives of 2-amino substituted tetrahydrocarbazole, **13l** and **13o** were the most potent compounds. Further, we investigated the role of *NH* of tetrahydrocarbazole on the activity by converting *NH* to *N*-Me of the most potent compound **13l**. We observed that the corresponding *N*-methyl derivative **16** was found to be similar in potency for the inhibition of pERK and pRb phosphorylation, compared to the parent **13l** in both the assays. From this, we could conclude that the activity was indifferent to either *NH* free or *N*-methyl substitution.

<<<Table2 Here>>>

<<<Figure 2 Here>>>

2.3 Molecular Docking. We were further interested in understanding the binding mode of these inhibitors in the ligand-binding pocket of ERK2 kinase. A recently deposited high-resolution crystal structure of ERK2 kinase [30] (PDB ID: 5BVD) was used for performing the docking studies of few select molecules (**1**, **13l**, **13o** and **16**). All the molecules along with the crystal structure ligand were docked in the ligand-binding pocket. One of these molecules, compound **16**, exhibited favorable binding with the ERK2 kinase (**Figure 3, Table 3**) as seen from the XP GScore and the MMGBSA binding energy (**Table 3**). The binding modes of other molecules **1**, **13l**, **13o** and **16** along with the crystal structure ligand **17** can be found in the Electronic Supplementary Information (ESI) section. As seen from the IC_{50} values for ERK (**Figure 2 and Tables 2 and 3**), the binding energy values are in agreement with the experimental activity. This only increased our confidence in the molecular docking studies.

The summary of molecular docking analyses (XP GScore) and the corresponding MMGBSA binding energies are listed in **Table 3**. Compound **13o** showed the highest XP GScore of -6.956 while others such as **13l** and **16** were comparable with **1** being the lowest (-5.397). Similarly, the MMGBSA binding energy was found to be the highest for compound **13o** followed by **16**, **1** and **13l**, with the values ranging from -120 to -111 Kcal/mol). The binding modes of these compounds are shown in **Figures 4-7** (can be found in the Electronic Supplementary Information (ESI) section).

Compound **1** exhibited the binding mode similar to crystal structure ligand 17 (**Figure 7**). Two H-bonds were seen with Met106 formed by the exo- and endocyclic Ns. Even though the protonated N was in close proximity to Glu107, no salt-bridge interaction was seen. The 6-NO_2 group did not make any interaction with the ERK2 kinase. In addition, the 3-methyl substituent on the cyclopentyl ring was exposed to the solvent. The tetrahydrocarbazole ring of **1** was found to occupy the same area occupied by the *N*-tetrahydropyranyl 2-aminopyrimidine substructure of 17 (**Figure 7**, alignment not shown).

Unlike the binding mode exhibited by **1**, compounds **13l**, **13o** and **16** showed quite different binding modes, where a consistent salt-bridge interaction of exocyclic protonated N was seen with Asp106 (Figures 4-6). In addition, the protonated exocyclic N in compound **13l** also formed H-bond with Asn164. Another consistent cation-π interaction of Lys52 was found with the benzylic aromatic ring (**Figures 5-7**). The tetrahydrocarbazole ring of **13l**, **13o** and **16** was found to occupy same area of the ERK2 kinase ligand-binding pocket occupied by the 1*H*,2*H*,3*H*,4*H*-pyrrolo[1,2-a]pyrazin-1-one substructure of **17** (**Figure 7**, alignments not shown). We believe that the altered binding mode of **1** and its SAR compounds are responsible for gain in potency over **1**. The docking studies are likely to contribute to our understanding of the inhibition of ERK2 kinase by the lead molecules described in this study.

<<<Table 3 Here>>>

3.0 Conclusions

In the present investigation, we have discovered tetrahydrocarbazoles as a new class of pERK and pRb phosphorylation inhibitors. A series of 1-amino- and 2-amino substituted tetrahydracarbazole derivatives were synthesized, where the initial SAR suggested that the homobenzylamine with benzyl substitution was preferable for the dual inhibition of pERK and pRB phosphorylation. The docking studies of few select molecules with the ERK2 kinase shed some light on the binding modes, and subsequently the binding potency of the SAR molecules compared to the initial hit **1**. The most potent dual inhibitor of pERK and pRb phosphorylation **16** can be a good starting point for an extensive medicinal chemistry program, which may lead to further identification of more potent pERK-pRb dual inhibitors.

4. Experimental Section

4.1 General Chemistry Details.

All the chemicals (intermediates and reagents) and solvents (including dry solvents) were procured from commercial suppliers such as Sigma-Aldrich Chemical Co., Fischer Scientific, VWR International, etc., and were used as received unless otherwise indicated. All reactions were performed under an inert atmosphere (argon or N_2) unless otherwise noted. Analytical silica gel 60 F254-coated TLC plates were purchased from Merck Chemicals, and were visualized with UV light or by treatment with TLC reagents such as ninhydrin, Dragandorff's or phosphomolybdic acid (PMA). Flash-column chromatography was carried out on Combiflash R_f using silica gel (230–400 mesh). ^{1}H-NMR spectra were routinely recorded on Bruker 300 MHz FT NMR, with tetramethylsilane (TMS) as an internal standard. The purity of all compounds was determined by HPLC (Waters 2695 Alliance) system implementing either Method A, Method B or Method C for chromatographic separation. **HPLC solvents:** A: Acetonitrile. B: 0.01MNH_4OAc + 0.5% TEA, pH 5.0 with AcOH. **HPLC Columns:** Column 1: Ascentis TM Express (50 x 4.6 mm I.D.), 2.7 µm operated at 1 mL/min, detection at 288 nm. Column 2: Poroshell 120 EC-C18 (50 X 4.6mm I.D.), 2.7µm operated at 1 mL/min, detection at 276 nm.

HPLC Methods: Method A: Elution with 20-80% linear gradient of A in 6 min followed by 20-80% linear gradient of B in 1 min that is continued using an isocratic elution with 80% B for 3 min using Column 1. **Method B:** Elution with 20-80% linear gradient of B in 6 min followed by 20-80% linear gradient of A in 1 min that is continued using an isocratic elution with 80% A for 3 min using Column 2.

4.1.1. 2,3,4,9-Tetrahydro-1*H*-carbazol-1-one (3): Polyphosphoric acid (20 mL) was added to a stirred solution of 4-(1*H*-indol-3-yl)butanoic acid 2 (20g, 0.0984 mole) in toluene (200 mL) and the resulting reaction mixture was allowed to stir at reflux for 5 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. Toluene was decanted, and oily residue was washed with toluene and discarded toluene layers. Oily residue was diluted with water (200 mL) and stirred at rt for overnight. Reaction mixture was extracted with ethyl acetate (3 x 100 mL), combined organic layers were washed with water (100 mL), brine (100 mL), dried over anhydrous sodium sulphate and filter. Filtrate was concentrated to get crude solid which was purified by

silica gel column chromatography in n- Hexane: Ethyl acetate; 7:3 as elute to get title compound (12.5g, 68.60%) as an Off-White solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.11-2.19 (m, 2H), 2.53-2.57 (m, 2H), 2.93-2.97 (m, 2H), 7.07 (t, *J* = 7.2 Hz, 1H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.67(d, *J* = 7.8 Hz, 1H), 11.58 (s, 1H); MS (ESI) *m/z*: 186.5$[M+H]^+$; HPLC Retention time: 3.764 min., Purity: 89.81%(Method A).

4.1.2. 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine (5): Step-I (Z)-2,3,4,9-tetrahydro-1*H*-carbazol-1-one oxime (4): To a stirred solution of 2,3, 4, 9-tetrahydro-1*H*-carbazol-1-one 3 (1.5 g, 8.10 mmol) in ethanol (20 mL) added subsequently solution of hydroxylamine hydrochloride (1.13 g, 16.2 mmol) in water(10 mL) and a solution of sodium acetate (2.19 g, 26.7 mmol) in water (10 mL). After being stirred at 80°C for 2 h, cooled reaction mixture to 25 – 30°C. Concentrated reaction mixture under reduced pressure and resulting residue was diluted with water (25 mL). Reaction mixture was extracted with ethyl acetate (2 x 50 mL), combined organic layers were washed with brine (50 mL) dried over anhydrous sulphate and filtered. Filtrate was concentrated under reduced pressure to get title compound as a brown solid (1.58g, 97.53%). Step-II: 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine (5): Lithium aluminium hydride (1.0 M in THF, 70mL) was added drop wise to a stirred solution of (Z)-2,3,4,9-tetrahydro-1*H*-carbazol-1-one oxime **4** (1.55g, 7.74 mmol) in THF (80 mL). After being stirred reflux for 7 h. Cooled reaction mixture to 0°C and an excess of $LiAlH_4$ was quenched by addition of sodium sulphate decahydrate until bubbling ceased. Stirred resulting reaction mixture for 0.5 h. precipitated inorganics were removed by filtration through celite bed. Filtrate was concentrated to get crude product which was purified by flash chromatography on silica (dichloromethane : Methanol; 9.5:0.5) to provide 2,3, 4, 9-tetrahydro-1*H*-carbazol-1-amine **5** (1.09g, 85.15%) as a brown Solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.49-1.58 (m, 1H), 1.62-1.74 (m, 1H), 1.89-2.00 (m, 2H), 2.04 - 2.08 (m, 2H), 2.59 (d, *J* = 6.9 Hz, 2H), 3.93(t, *J* = 6.9 Hz, 1H), 6.91 (t, *J* = 7.2 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.33(d, *J* = 7.8 Hz, 1H), 10.67 (s, 1H); MS (ESI) *m/z*: 170.1$[M+H]^+$; HPLC Retention time: 1.737 min., Purity: 98.34%(Method A).

4.1.3. *N*-cyclohexyl-2,3,4,9-tetrahydro-1*H*-carbazol-1-amine hydrochloride (6a)

2,3,4,9-tetrahydro-1*H*-carbazol-1-amine(0.2 g, 1.074 mmol) was treated with cyclohexanone (0.126 g, 1.289 mmol) in tetrahydrofuran (10 mL) at 25-30°C in the presence of trifluoroacetic acid (0.041 mL, 0.537 mmol). After being stirred for 4h.added sodiumcyanoborohydride (0.202 g, 3.22 mmol) and continued stirring at same temperature for 16 h. Reaction mixture was diluted with cold water (50mL) and extracted with ethyl

acetate (2 x 25mL). Combined organic layers were washed with water (25mL), brine (25mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude which was purified by silica gel(60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound (0.12g, 40.76%).Which was converted to the corresponding hydrochloride using IPA. HCl in ethyl acetate using 0.1g of base (*N*-cyclohexyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine).

Yield: 40.76%; White Solid; mp: 119-121°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.08-1.20 (m, 2H), 1.22-1.40 (m, 2H), 1.42-1.59 (m, 2H), 1.60-1.67 (m, 2H), 1.72-1.90 (m, 3H), 1.95-2.22 (m, 5H), 2.69 (s, 2H), 7.02 (t, *J* = 7.2 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 8.82 (s, 1H), 9.38 (s, 1H), 11.29 (s, 1H); MS (ESI) *m/z*: 269.1 $[M+H]^+$; HPLC Retention time: 3.359 min., Purity: 99.41%(Method A).

4.1.4. *N*-cyclopentyl-2,3,4,9-tetrahydro-1*H*-carbazol-1-amine hydrochloride(6b)

2,3,4,9-tetrahydro-1*H*-carbazol-1-amine 5 (0.2 g, 1.074 mmol) was treated with cyclopentanone (0.181 g, 2.148 mmol) in tetrahydrofuran (10 mL) in the presence of TFA (6.12 mg, 0.054 mmol) at 25-30°C. The resulting reaction mixture was stirred for 4 h. at same temperature, then added sodiumcyanoborohydride (0.202 g, 3.22 mmol) continued stirring for 16 h. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25mL), brine (25mL). Dried organic layers over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude. Purification was done by silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound (0.172g, 62.0%) and the base was converted to corresponding hydrochloride salt by using IPA-HCl in ethyl acetate as solvent with 0.1g of free base to yield 0.032 g of hydrochloride **6b**.

Yield: 62.0%; Off-white solid; mp: 114-116°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.57 (s, 2H), 1.80 (s, 5H), 2.06-2.14 (m, 5H), 2.69 (s, 2H), 3.74 (s, 1H), 4.58 (s, 1H), 7.02 (t, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 9.18 (s, 1H), 9.60 (s, 1H), 11.40 (s, 1H); MS (ESI) *m/z*: 255.1 $[M+H]^+$; HPLC Retention time: 3.736 min., Purity: 97.91%(Method A).

4.1.5. *N*-(3-methylcyclopentyl)-2,3,4,9-tetrahydro-1*H*-carbazol-1-aminehydrochloride (6c)

2,3,4,9-tetrahydro-1*H*-carbazol-1-amine **5** (0.2 g, 1.074 mmol) was treated with 3-methylcyclopentanone (0.21 g, 2.148 mmol) in THF (10 mL) in the presence of TFA (9.18 mg, 0.081 mmol) at 25-30°C. After being stirred for 4 h. at 25 – 30 °C added sodiumcyanoborohydride (0.202 g, 3.22 mmol) and continued stirring for 16 h. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 mL). Combined organic layers were washed with water (25mL), brine (25mL), dried over anhydrous and filtered. The filtrate was concentrated under reduced pressure to get crude which was purified by silica gel (60/120) column chromatography in PET. Ether: Ethyl acetate; 7:3 as elute to get title compound (0.168g, 53.16%). Corresponding hydrochloride salt **6c** by using IPA-HCl in ethyl acetate as solvent with 0.1g of free base.

Yield: 53.16%; White solid;137-139°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ0.97-1.01 (m, 2H), 1.05 (d, *J* = 6.3 Hz, 3H), 1.30-1.50 (m, 2H), 1.70-2.26 (m, 5H), 2.68-2.70 (m, 2H), 3.70-3.95 (m, 2H), 4.53-4.54 (m, 2H), 7.02 (t, *J*= 7.5 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 9.01 (s, 1H), 9.35 (s, 1H), 11.25 (s, 1H); MS (ESI) *m/z*: 269.1 $[M+H]^+$; HPLC Retention time: 4.447 min., Purity: 98.79%(Method A).

4.1.6. *N*-(2,3,4,9-tetrahydro-1*H*-carbazol-1-yl)pyridine-3-sulfonamide (6d)

Pyridine-3-sulfonyl chloride (0.157g, 0.885 mmol) was added under inert atmosphere to a stirred solution of 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine **5** (0.15g,0.805 mmol) and triethylamine (0.168mL,1.207mmol) in dry dichloromethane (10 mL) at 0°C. After being stirred at 0°C for 0.5h warmed to rt and stirred for 16 h. After the complete disappearance of starting material on TLC, the reaction mixture was quenched in sat. sodium bicarbonate solution (10 mL). Layers separated and organic layer was washed with water (10 mL), brine (10 mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude which was purified by column chromatography using n- hexane: ethyl acetate; 7:3 as elute to yield title compound (0.203g, 70.34%).

Yield: 70.34; Off-white solid; mp: 159-161°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.51-1.53 (m, 1H), 1.55-1.70 (m, 2H), 1.75-1.90 (m, 1H), 2.49-2.55 (m, 2H, merged with DMSO), 4.65-4.67 (m, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.65-7.69 (m, 1H), 8.29(d, *J* = 7.8 Hz, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 8.54 (d, *J* = 4.2 Hz, 1H), 9.07 (s, 1H), 10.66 (s, 1H); MS (ESI) *m/z*: 328.0$[M+H]^+$; HPLC Retention time: 5.149 min., Purity: 96.82%(Method A).

4.1.7. 4-fluoro-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-1-yl)benzenesulfonamide (6e)

4-Fluorobenzenesulfonyl chloride (0.171g, 0.885 mmol) was added under inert atmosphere to a stirred solution of 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine **5** (0.15g,0.805 mmol) and triethylamine(0.168mL,1.207mmol) in dry dichloromethane (10 mL) at 0°C. After being stirred at 0°C for 0.5h reaction mixture was warmed to rt and stirred for 16h. After the complete disappearance of starting material on TLC, the reaction mixture was quenched in saturated sodium bicarbonate solution (10 mL). Layers separated and organic layers were washed with water (10 mL), brine (10 mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude which was purified by column chromatography using n- Hexane: Ethyl acetate; 7:3 as elute to get title compound (0.167g, 55.18%).

Yield: 55.18%; Off-white solid; mp: 171-173°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.48-1.50 (m, 1H), 1.51-1.58 (m, 2H), 1.61-1.90 (m, 1H), 2.49-2.52 (m, 2H, merged with DMSO), 4.57-4.59 (m, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 7.36-7.38 (m, 2H), 7.47(t, *J* = 8.7 Hz, 2H), 7.96-7.80 (m, 2H), 8.22 (d, *J* = 7.2 Hz, 1H), 10.61 (s, 1H); MS (ESI) *m/z*: 343.1$[M-H]^+$; HPLC Retention time; 6.406 min., Purity: 97.60%(Method A).

4.1.8. 4-Cyano-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-1-yl)benzenesulfonamide (6f)

4-Cyanobenzenesulfonyl chloride (0.178g, 0.885 mmol) was added under inert atmosphere to a stirred solution of 2,3,4,9-tetrahydro-1*H*-carbazol-1-amine **5** (0.15g,0.805 mmol) and triethylamine (0.168mL,1.207mmol) in dry dichloromethane (10 mL) at 0°C. After being stirred at 0°C for 0.5h slowly warmed to 25-30°C and stirred for overnight. After the complete disappearance of starting material on TLC, the reaction mixture was quenched in saturated sodium bicarbonate solution (10 mL). Layers separated and organic layers were washed with water (10 mL), brine (10 mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude, which was purified by column chromatography using n- Hexane: Ethyl acetate; 7:3 as elute to get 4-cyano-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-1-yl)benzenesulfonamide **6f** (0.235g, 75.64%).

Yield: 75.64%; White solid; mp: 200-202°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.17-1.24 (m, 1H), 1.49-1.60 (m, 2H), 1.61-1.79 (m, 1H), 2.50-2.54 (m, 2H, merged with DMSO), 4.60 (s, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 2H), 8.07 (d, J = 8.1 Hz, 2H), 8.12 (d, *J* = 8.4 Hz, 2H), 8.49 (s, 1H), 10.65 (s, 1H); MS (ESI) *m/z*: 352.1$[M+H]^+$; HPLC Retention time: 6.105 min., Purity: 97.24%(Method A).

4.1.9. 3-(2-Phenylhydrazinyl)cyclohex-2-enone (8)

Cyclohexane-1,3-dione (20.0 g, 178 mmol) was dissolved in 125 mL of water at 20°C in a 1.0 liter round bottom flask and apparatus was thereafter evacuated twice and decompressed with nitrogen. A solution of phenylhydrazine (20.06 g, 186 mmol) in 250 mL of water was allowed to run in via dropping funnel at an internal temperature of 20-25°C within the course of 1h. Subsequently, the reaction mixture was further stirred for 3 h. The sandy-like cyclohexane-1,3-dione monophenylhydrazone obtained was filtered off and washed with 200 mL of water. The crude product thus obtained was dried at 60°C under reduced pressure to get hydrazone **8** (33.5g, 93.0%).

4.1.10. 1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] (9)

3-(2-phenylhydrazinyl)cyclohex-2-enone **8** (33 g, 163 mmol) and 4-methylbenzenesulfonic acid hydrate (37.2 g, 196 mmol) was dissolved in a mixture of toluene (500 mL) and ethylene glycol (80mL, 1435 mmol). The reaction mixture was refluxed with a dean stark trap for 24 h. Decanted toluene from reaction, washed out the residue with toluene (2 x 50 mL), combined toluene layers were washed with 5% aq. sodium bicarbonate solution (2 x 100 mL), water (100mL) and with brine (100mL). Dried organic layer over anhydrous sodium sulphate and filtered. The filtrate was concentrated to a get crude product which was purified by trituration in 5% ethyl acetate in n-hexane to get title compound (23.5g, 59.7%) as Off-white solid.

Yield: 59.7%; Off-white solid; ^{1}H-NMR (300MHz, $CDCl_3$): δ 1.59 (s, 1H), 2.06 (t, J = 6.3 Hz, 2H), 2.38-2.47 (m, 1H), 2.90 (t, J = 6.0 Hz, 2H), 3.99 (s, 4H), 7.09 (t, J = 6.9 Hz, 1H), 7.16 (t, J = 6.6 Hz, 1H), 7.38(d, J = 7.5 Hz, 1H), 7.48(d, J = 7.2 Hz, 1H), 7.71 (s, 1H); MS (ESI) *m/z*: 237.0$[M+H]^+$.

4.1.11. 1,3,4,9-Tetrahydro-2*H*-carbazol-2-one (10)

1,3,4,9-Tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] **9** (23 g, 100 mmol) in methanol (330 mL) was treated with 10% aqueous sulphuric acid (100 mL, 100 mmol) for 6 h. at 25 - 30°C. Evaporated methanol on rotavapor and the resulting residue was diluted with cold water (100 mL), the reaction mixture was extracted with ethyl acetate (2 x 100 mL), and combined organic layers were washed with water (100 mL) and brine (100 mL). Dried organic layer over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get a crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 1:1 as elute to get title compound **10** (11.0g, 53.3%) as a white solid.

Yield: 53.3%; White Solid: ^{1}H-NMR (300MHz, $CDCl_3$): δ 2.80 (t, *J* = 6.6 Hz, 2H), 3.13 (t, *J* = 6.6 Hz, 2H), 3.68 (s, 2H), 7.15 (t, *J* = 6.9 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.35(d, *J* = 7.8 Hz, 1H), 7.53(d, *J* = 7.2 Hz, 1H), 7.82 (s, 1H); MS (ESI) *m/z*: 186.1$[M+H]^+$.

4.1.12. (E)-1,3,4,9-tetrahydro-2H-carbazol-2-one oxime(11)

Solution of sodium acetate (5.31 g, 64.8 mmol) in water (10 mL) and hydroxyl amine hydrochloride (2.251 g, 32.4 mmol) in water (5 mL) were added to a stirred solution of 3,4-dihydro-1*H*-carbazol-2(9*H*)-one **10** (3.0 g, 16.20 mmol) in ethanol (50 mL). After being stirred at precipitated solid was collected by filtration and washed with water, ethanol and then with n-hexane. Dried the solid under reduced pressure at 60°C to get intermediate oxime as Off-white solid (9.23g, 70.3%).

Yield: 70.3%; Off-white solid; ^{1}H-NMR (300MHz, $CDCl_3$): δ 2.68 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H), 3.84 (s, 2H), 7.06 (t, *J* = 6.9 Hz, 1H), 7.12 (t, *J* = 6.9 Hz, 1H), 7.30(d, *J* = 7.2 Hz, 1H), 7.45(d, *J* = 7.2 Hz, 1H), 8.40 (s, 1H), 8.94 (s, 1H); MS (ESI) *m/z*: 201.2$[M+H]^+$.

4.1.13. 2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine (12)

Lithium aluminium hydride 1M in THF (32.4 mL, 32.4 mmol) was added to a stirred solution of (Z)-3,4-dihydro-1*H*-carbazol-2(9H)-one oxime (2.30 g, 11.39 mmol) in THF (50.0 mL) at rt and stirred at the same temperature for 0.5 h. The resulting reaction mixture was heated to reflux for 2 h. Progress of the reaction was monitored by TLC. Cooled reaction mixture to 0-5°C an excess $LiAlH_4$ was quenched by addition of the sodium sulphate decahydrate, stirred reaction mixture for 1h., inorganic were removed by filtration through celite bed. The filtrate was concentrated to get a crude which was purified by silica gel (60/120) column chromatography in Chloroform: Methanol; 9.5:0.5 as elute to get title compound (0.75g, 22.66%).

Yield: 22.6%; Brown solid; ^{1}H-NMR (300MHz, $CDCl_3$): δ 2.16-2.56 (m,2H), 2.68-2.88 (m, 2H), 3.04-3.17 (m, 2H), 3.56 (s, 1H), 6.94 (t, *J* = 6.9 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 7.27(d, *J* = 7.8 Hz, 1H), 7.36(d, *J* = 7.5 Hz, 1H), 8.31 (s, 2H), 10.82 (s, 1H); MS (ESI) *m/z*: 187.1$[M+H]^+$.

4.1.14. *N*-cyclohexyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (13a)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with cyclohexanone(0.126 g, 1.289 mmol) in tetrahydrofuran (10 mL) at 25-30°C in the presence

of trifluoroacetic acid (0.041 mL, 0.537 mmol) for 4 h. Then added sodiumcyanoborohydride (0.202 g, 3.22 mmol) and continued stirring at same temperature for overnight. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25mL), brine (25mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude which was purified by silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get *N*-cyclohexyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **13a** (0.03g, 10.19%).

Yield: 10.19%; White solid; mp: 172-174°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 0.80-1.08 (m, 2H), 1.08-1.27 (m, 3H), 1.55-1.62 (m, 2H), 1.66-1.70 (m, 2H), 1.83-1.87 (m, 2H), 1.96-2.09 (m, 1H), 2.40-2.49 (m, 1H), 2.50-2.73 (m, 3H), 2.92 2.97 (m, 1H), 3.14 (s, 1H), 6.88-6.99 (m, 2H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 7.2 Hz, 1H), 10.57 (s, 1H); MS (ESI) *m/z*: 269.1[M+H]$^+$; HPLC Retention time; 3.422 min., Purity: 97.88%(Method A).

4.1.15. *N*-cyclopentyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (13b)

2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with cyclopentanone (0.181 g, 2.148 mmol) in tetrahydrofuran (10 mL) in the presence of TFA (6.12 mg, 0.054 mmol) at 25-30°C. After being stirred for 4 h. at the same temperature added sodiumcyanoborohydride (0.202 g, 3.22 mmol) and continued stirring for 16 h. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 ml), combined organic layers were washed with water (25ml), brine (25ml) dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude. Purification was done by silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound **13b** (0.134g, 48.5%).

Yield: 48.5%; White solid; mp: 180-182°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.59-1.65 (m, 3H), 1.66-1.74 (m, 3H), 1.84-1.90 (m, 1H), 2.00-2.12 (m, 2H), 2.22-2.28 (m, 1H), 2.62-2.90 (m, 3H), 3.20-3.25 (m, 1H), 3.60 – 3.80 (m, 2H), 3.75 (s, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 7.04 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.38 (d, *J* = 7.5 Hz, 1H), 10.79 (s, 1H); MS (ESI) *m/z*: 255. [M+H]$^+$; HPLC retention time: 3.085 min., Purity: 99.28% (Method A).

4.1.16. 2,3,4,9-Tetrahydro-*N*-(3-methylcyclopentyl)-1*H*-carbazol-2-amine (13c)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine (0.3 g, 1.611 mmol) was treated with 3-methylcyclopentanone (0.316 g, 3.22 mmol) in THF (10 mL) in presence of TFA (9.18 mg, 0.081 mmol) at 25-30°C. The reaction mixture stirred for 4 h. at 25 - 30°C. Charged

sodiumcyanoborohydride (0.202 g, 3.22 mmol) and stirred at the same temperature for overnight. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 mL). Combined organic layers were washed with water (25mL) and brine (25mL). Dried organic layers over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude product. Purification was done silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound (0.168g, 38.9%). Hydrochloride salt was prepared in ethyl acetate with 140 mg of base by using methanolic HCl to get hydrochloride **13c** as white solid (0.11g, 57.89%).

Yield: 57.89%; White solid; mp: 213-215°C; ^{1}H-NMR (300MHz, DMSO-d_3): δ 0.97 (d, *J* = 4.5 Hz, 3H), 1.17-1.34 (m, 1H), 1.32-1.50 (m, 1H), 1.54-1.66 (m, 2H), 1.74-1.88 (m, 2H), 2.01-2.05 (m, 2H), 2.41-2.42 (m, 1H), 2.67 -2.72 (m, 2H), 2.92-2.97 (m, 2H), 3.18-3.24 (m, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 6.9 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 10.58 (s, 1H); MS (ESI) *m/z*: 269.1$[M+H]^+$; HPLC Retention time: 3.481 min., Purity: 99.09%(Method A).

4.1.17. 2,3,4,9-tetrahydro-*N*-(tetrahydro-2H-pyran-4-yl)-1*H*-carbazol-2-amine (13d)

2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12** (0.3 g, 1.611 mmol) was treated with dihydro-2Hpyran-4(3*H*)-one (0.242 g, 2.416 mmol) in THF (10 mL) in the presence of TFA (9.18 mg, 0.081mmol) at 25-30°C. The reaction mixture stirred for 4 hrs at 25 - 30°C. Charged sodiumcyanoborohydride (0.202 g, 3.22 mmol) and stirred at the same temperature for overnight. The reaction mixture was diluted with cold water (50mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25mL) and brine (25mL). Dried organic layers over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get which was purified by silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound **13d** (0.132g, 27.5%). Hydrochloride salt was prepared in ethyl acetate by using methanolic HCl with 100 mg of base (0.038g, 29.92%).

Yield: 29.92%; Brown solid; mp: 155-157°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.17-1.23 (m, 1H), 1.30-1.69 (m, 2H), 1.70-1.82 (m, 1H), 1.90-1.99 (m, 4H), 2.18-2.27 (m, 2H), 2.55-2.73 (m, 4H), 3.87-3.90 (m, 2H), 6.95 (t, *J* = 7.2 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 10.72 (s, 1H); MS (ESI) *m/z*: 271.2$[M+H]^+$; HPLC Retention time: 2.464 min., Purity: 90.82%(Method A).

4.1.18. *N*-(2,3,4,9-tetrahydro-1*H*-carbazol-2-yl)pyridine-3-sulfonamide (13e)

2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with pyridine-3-sulfonyl chloride (0.229 g, 1.289 mmol) in the presence of triethylamine (0.326 g, 3.22 mmol) at 25-30°C for 4 h. The reaction mixture was diluted with chloroform (25 mL), washed with water (25mL), brine (25mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get a crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get title compound **13e** (0.08g, 21.98%).

Yield: 21.98%; White solid; mp: 168-170°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.61-1.79 (m, 1H), 1.81-1.86 (m, 1H), 2.55-2.63 (m, 2H), 2.64-2.80 (m, 2H), 3.56-3.58 (m, 1H), 6.90 (t, *J* = 7.2 Hz, 1H), 6.97 (t, *J* = 6.9 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.66 (t, *J* = 7.5 Hz, 1H), 8.17 (d, *J* = 6.3 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.83 (d, *J* = 4.5 Hz, 1H), 9.12 (s, 1H), 10.59 (s, 1H); MS (ESI) *m/z*: 328.0$[M+H]^+$; HPLC Retention time: 4.774 min., Purity: 96.58%(Method A).

4.1.20. 4-Fluoro-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-2-yl)benzenesulfonamide (13f)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with 4-fluoroobenzene-1-sulfonyl chloride (0.230 g, 1.181 mmol) in dichloromethane (5 mL) in the presence of triethylamine (0.449 mL, 3.22 mmol) at 25-30°C for 2 h. The progress of the reaction was monitored by TLC. The reaction mixture was diluted with dichloromethane (25 mL), washed with water (25mL) and brine (25mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to a get crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get title compound **13f** (0.118g, 31.6%).

Yield: 31.6%; Off-white solid; mp:180-182°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.20-1.70 (m, 1H), 1.80-1.86 (m, 1H), 2.54-2.62 (m, 2H), 2.68-2.76 (m, 2H), 3.41-3.49 (m, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.46 (t, *J* = 8.7 Hz, 2H), 7.94 (t, *J* = 8.1 Hz, 3H), 10.59 (s, 1H); MS (ESI) *m/z*: 367.5$[M+H]^+$; HPLC Retention time: 5.924 min., Purity: 98.96%(Method A).

4.1.21. 4-Cyano-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-2-yl)benzenesulfonamide (13g)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine (0.2 g, 1.074 mmol) was treated with 4-cyanobenzene-1-sulfonyl chloride (0.238 g, 1.181 mmol) in dichloromethane (5 mL) in

presence of the triethylamine (0.326 g, 3.22 mmol) at 25-30°C for 2 h. The reaction mixture was diluted with dichloromethane (25 mL), washed with water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get 4-cyano-*N*-(2,3,4,9-tetrahydro-1*H*-carbazol-2-yl)benzenesulfonamide **13g** (0.072g, 18.79%).

Yield: 18.79%; Off-white solid; mp: 93-95°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.61-1.69 (m, 1H), 1.80-1.90 (m, 1H), 2.56-2.61 (m, 2H), 2.68-2.71 (m, 2H), 3.51-3.61 (m, 1H), 6.90 (t, *J* = 7.2 Hz, 1H), 6.98 (t, *J* = 6.9 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 8.11 (d, *J* = 8.1 Hz, 2H), 8.23 (s, 1H), 10.58 (s, 1H); MS (ESI) *m/z*: 352.0$[M+H]^+$; HPLC Retention time: 5.692 min., Purity: 98.47%(Method A).

4.1.22. 3-Fluoro-*N*-(6,7,8,9-tetrahydro-5*H*-carbazol-7-yl)benzamide (13h)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with 3-fluorobenzoyl chloride (0.204 g, 1.289 mmol) in dichloromethane (5 mL) in the presence of triethyl amine (0.326 g,3.22 mmol) at 25-30°C for 1 h. The progress of reaction was monitored by TLC. The reaction mixture was diluted with dichloromethane (25 mL), washed with water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get 3-fluoro-*N*-(6,7,8,9-tetrahydro-5*H*-carbazol-7-yl)benzamide **13h** (0.15g, 39.16%).

Yield: 39.16%; White solid; mp: 172-174 °C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.83-1.89 (m, 1H), 2.05-2.19 (m, 1H), 2.60-2.73 (m, 1H), 2.78-2.86 (m, 2H), 3.01-3.09 (m, 1H), 4.31-4.40 (m, 1H), 6.93 (t, *J* = 7.2 Hz, 1H), 7.01 (t, *J* = 6.9 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.34-7.42 (m, 2H), 7.54 (q, *J* = 7.5 Hz, 1H), 7.68 (s, 1H), 7.74 (t, *J* = 7.8 Hz, 1H), 8.56 (d, J = 7.2 Hz, 1H), 10.69 (s, 1H); MS (ESI) *m/z*: 331.1$[M+H]^+$; HPLC Retention time: 5.652 min., Purity: 99.09% (Method A).

4.1.23. *N*-(6,7,8,9-tetrahydro-5*H*-carbazol-7-yl)-3-methoxybenzamide(13i)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.976 mmol) was treated with 3-methoxybenzoyl chloride (0.371 g, 2.174 mmol) in Chloroform (5 mL) in the presence of triethyl amine (1.104 g, 5.93 mmol) at 25-30°C for 1 h. The reaction mixture was diluted with chloroform (25 mL), washed with water (25mL) and brine (25mL). The organic layer

was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get a crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get title compound **13i** (0.135g, 36.98%).

Yield: 36.98%; White solid; mp: 190-192°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.85-1.88 (m, 1H), 2.05-2.19 (m, 1H), 2.60-2.69 (m, 1H), 2.78-2.86 (m, 3H), 3.00-3.07 (m, 1H), 3.82 (s, 3H), 4.30-4.40 (m, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 6.9 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.34-7.48 (m, 4H), 8.47 (d, *J* = 7.2 Hz, 1H), 10.68 (s, 1H); MS (ESI) *m/z*: 343.1[M+H]$^+$; HPLC Retention time: 5.341 min., Purity: 97.33% (Method A).

4.1.24. 2-Fluoro-*N*-(6,7,8,9-tetrahydro-5*H*-carbazol-7-yl)benzamide (13j)

2,3,4,9-Tetrahydro-1*H*-carbazol-2-amine **12** (0.2 g, 1.074 mmol) was treated with 2-fluorobenzoyl chloride (0.187 g, 1.181 mmol) in Chloroform (10 mL) in the presence of triethylamine (0.150 mL, 1.074 mmol) at 25-30°C for 1 h. The reaction mixture was diluted with chloroform (25 mL), washed with water (25mL) and brine (25mL). The organic layer was dried over anhydrous sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 8:2, as elute to get title compound **13j** (0.14g, 39.54%).

Yield: 39.54%; Off-white solid; mp: 168-170°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.10-1.90 (m, 1H), 1.99-2.07 (m, 1H), 2.61-2.72 (m, 1H), 2.73-2.86 (m, 2H), 3.03-3.10 (m, 1H), 4.30-4.40 (m, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 7.27 (t, *J* = 8.7 Hz, 3H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.51 (t, *J* = 6.3 Hz, 1H), 7.60 (t, *J* = 7.2 Hz, 1H), 8.44 (d, *J* = 7.2 Hz, 1H), 10.67 (s, 1H); MS (ESI) *m/z*: 331.1[M+H]$^+$; HPLC Retention time: 5.596 min., Purity: 99.47%(Method A).

4.1.25. *N*-benzyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (13k)

Concentrated H_2SO_4 (7.94 mg, 0.081 mmol) was added to a stirred solution of 3,4-dihydro-1*H*-carbazol-2(9*H*)-one **10** (0.3 g, 1.620 mmol) and phenylmethanamine (0.191 g, 1.782 mmol) in THF (2 mL) at 25-30°C. Being stirred at the same temperature for 3 h added sodium cyanoborohydride (0.153 g, 2.430 mmol) and stirred for another 1.5 h. Diluted reaction mixture with water (25 mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated to get crude

which was purified by silica gel (60/120) column chromatography in PET. Ether: ethyl acetate; 1:1 as elute to get *N*-benzyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **13k** (0.032g, 7.08%).

Yield: 7.08%; Brown solid; ^{1}H-NMR (300MHz, $CDCl_3$): δ 1.77-1.85 (m, 1H), 2.12-2.25 (m, 1H), 2.61-2.80 (m, 2H), 2.81-2.88 (m, 1H), 3.00-3.10 (m, 1H), 3.15-3.25 (m, 1H), 3.95 (s, 2H), 7.09-7.14 (m, 2H), 7.31-7.37 (m, 5H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.72-7.74 (m, 1H); MS (ESI) *m/z*: 277.2$[M+H]^+$; HPLC Retention time: 3.183 min., Purity: 98.80%(Method B).

4.1.26. *N*-(4-methylbenzyl)-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (13l)

Concentrated H_2SO_4 (7.94 mg, 0.081 mmol) was added to a stirred solution of 3,4-dihydro-1*H*-carbazol-2(9*H*)-one **10** (0.3 g, 1.620 mmol) and *p*-tolylmethanamine (0.216 g, 1.782 mmol) in THF (2 mL) at 25-30°C. after being stirred at same temperature for 3 h., added sodium cyanoborohydride (0.153 g, 2.430 mmol) and stirred for another 1.5 h. Diluted reaction mixture with water (25 mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude which was purified by silica gel (60/120) column chromatography in PET. Ether: ethyl acetate; 1:1 as elute to get title compound **13l** (0.132g, 28.20%).

Yield: 28.2%; Off-white solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.50-1.68 (m, 1H), 2.01-2.11 (m, 2H), 2.27 (s, 3H), 2.66-2.80 (m, 2H), 2.90-3.00 (m, 2H), 3.78 (s, 2H), 6.89 (t, *J* = 7.5 Hz, 1H), 6.94 (t, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 2H), 7.30 (d, *J* = 9.0 Hz, 1H), 10.62 (s, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 19.55, 21.16, 29.93, 30.58, 50.52, 53.40, 108.17, 111.01, 117.61, 118.46, 121.41, 127.39, 128.36(2C), 129.18(2C), 133.93, 135.82, 136.49, 138.64; MS (ESI) *m/z*: 291.2$[M+H]^+$; HPLC Retention time: 3.253 min., Purity: 98.03%(Method A).

4.1.27. 3-(2-*p*-Tolylhydrazinyl)cyclohex-2-enone (8a)

p-Tolylhydrazine (11.33 g, 93 mmol) was added to a stirred solution of cyclohexane-1,3-dione(10 g, 89 mmol) in water (300 mL) at 15-20°C and resulting reaction mixture was stirred at the same temperature for 1h. Precipitated solid was collected by filtration. Washed solid with water and Pet. Ether, dried under reduced pressure in rotating flask at 50°C to get title compound (16.0g, 83.0%).

4.1.28. 6-Methyl-1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] (9a)

To a stirred suspension of 3-(2-(*p*-tolyl)hydrazinyl)cyclohex-2-enone **8a** (15 g, 69.4 mmol) in toluene (300 mL) charged 4-methylbenzenesulfonic acid hydrate (15.83 g, 83 mmol) and ethane-1,2-diol (37.9 g, 610 mmol). The resulting mixture was refluxed with a dean stark trap for 24 h. Decanted toluene from reaction, washed out the residue with toluene (2 x 50mL), combined toluene layers were washed with 5% aq. sodium bicarbonate solution (2 x 100mL), water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get a crude product which was purified by trituration in 5% ethyl acetate in n-hexane to get title compound **9a** (9.2g, 54.1%) as Off-white solid.

Yield: 54.1%; Off-white solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.89 (t, J = 6.0 Hz, 2H), 2.34(s, 3H), 2.67 (t, J = 5.7 Hz, 2H), 2.87 (s, 2H), 3.95 (s, 4H), 6.80 (d, J = 8.1 Hz, 1H), 7.11-7.13 (m, 2H), 10.49(s, 1H); MS (ESI) *m/z*: 244.1$[M+H]^+$; HPLC Retention time: 4.815 min., Purity: 99.23%(Method A).

4.1.29. 6-Methyl-1,3,4,9-tetrahydro-2*H*-carbazol-2-one (10a)

6-Methyl-1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] **9a** (3.0 g, 12.33 mmol) in methanol (330 mL) was treated with concentrated hydrochloric acid (5 mL) for overnight at 25 - 30°C. Evaporated methanol on rotavapor, residue was diluted with cold water (100 mL) and extracted with ethyl acetate (2 x 100mL). Combined organic layers were washed with water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 1:1 as elute to get title compound **10a** (1.3g, 53.06).

Yield: 53.06%; White solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 2.36(s, 3H), 2.66 (t, J = 6.3 Hz, 2H), 2.92 (t, J = 6.0 Hz, 2H), 3.61 (s, 2H), 6.86 (d, J = 7.8 Hz, 1H), 7.16-7.19 (m, 2H), 10.67(s, 1H); MS (ESI) *m/z*: 200.1$[M+H]^+$; HPLC Retention time: 4.196 min., Purity: 99.58%(Method A).

4.1.30. (*Z*)-6-methyl-1,3,4,9-tetrahydro-2H-carbazol-2-one oxime(11a)

To a stirred solution of 6-methyl-3,4-dihydro-1*H*-carbazol-2(9*H*)-one **10a** (0.52 g, 2.61 mmol) in Ethanol (20 mL) charged solution of sodium acetate (0.856 g, 10.44 mmol) in water (1 mL) and hydroxylamine hydrochloride (0.363 g, 5.22 mmol) in water (1 mL). The

resulting reaction mixture was stirred at 25 -30°C for 0.5h. Precipitated solid was collected by filtration, washed with water and ethanol and then with n-hexane. Dried solid under vacuum at 60°C to get oxime **11a** (0.55g, 98.38%).

4.1.31. 6-methyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (12a)

Lithium aluminium hydride (2.61 mL, 5.22 mmol) 1M in THF was added to a stirred solution of (Z)-6-methyl-3,4-dihydro-1*H*-carbazol-2(9*H*)-one oxime **11a** (0.55g, 2.56 mmol) in THF(50 mL) at 25-30°C and stirred for 0.5h. Then heated resulting reaction mixture to reflux for 2 h. Progress of the reaction was monitored by TLC. Cooled reaction mixture to 0-5°C and quenched an excess of $LiAlH_4$ with sodium sulphate decahydrate. Stirred reaction mixture for 1h.at 25-30°C and inorganic were removed by filtration through celite bed. Evaporated filtrate to get crude product which was purified by silica gel (60/120) column chromatography using chloroform: Methanol; 9.5:0.5as elute to get 6-methyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12a** (0.45g, 86.0%).

Yield: 86.0%; Brown solid; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.35(s, 1H), 1.48-1.57 (m, 1H), 1.65-1.75 (m, 1H), 1.90-1.94 (m, 1H), 2.33 (s, 3H), 2.38-2.41(m, 1H), 2.55-2.70 (m, 2H), 2.82-2.89 (m, 1H), 3.05-3.12 (m, 1H), 6.78 (d, J = 8.1 Hz, 1H), 7.08-7.10 (m, 2H), 10.43(s, 1H); MS (ESI) *m/z*: 201.1$[M+H]^+$; HPLC Retention time: 2.159 min., Purity: 94.55%(Method A).

4.1.32. 6-methyl-*N*-(3-methylcyclopentyl)-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine (13m)

2,2,2-Trifluoroacetic acid (5.69 mg, 0.050 mmol) was added to a stirred solution 6-methyl-2,3,4,9-tetrahydro-1*H*carbazol-2-amine **12a** (0.2g, 0.999mmol) and 3-methylcyclopentanone (0.118 g, 1.198 mmol) in THF (5 mL) and stirred for 1 h. Charged sodium cyanotrihydroborate (0.126 g, 1.997 mmol) and the resulting reaction mixture was stirred overnight. Diluted reaction mixture with water (25 mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by silica gel (60/120) column chromatography in dichloromethane: methanol; 9:1 as elute to get title compound **13m** (0.15g, 53.1%).

Yield: 53.1%; White solid; mp: 214-216°C; ^{1}H-NMR (300MHz, $CDCl_3$): δ 0.87-0.99 (m, 1H), 1.03 (d, J = 6.3 Hz, 3H), 1.26-1.36 (m, 2H), 1.43-1.47 (m, 2H), 1.72-1.81 (m, 2H), 1.89-

2.00 (m, 2H), 2.09-2.18 (m, 2H), 2.44 (s, 3H), 2.54-2.62 (m, 1H), 2.71-2.74 (m, 1H), 2.78-2.84 (m, 1H), 2.98-3.03 (m, 1H), 3.16 (s, 1H), 3.31 -3.45 (m, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.27 (s, 1H), 7.60 (s, 1H); MS (ESI) *m/z*: 283.1$[M+H]^+$; HPLC Retention time: 3.221 min., Purity: 99.87%(Method A).

4.1.33. *N*-benzyl-2,3,4,9-tetrahydro-6-methyl-1*H*-carbazol-2-amine hydrochloride (13n)

2,2,2-Trifluoroacetic acid (4.27 mg, 0.037 mmol) was added to a stirred solution of 6-methyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12a** (0.15 g, 0.749 mmol) and benzaldehyde (0.095 g, 0.899 mmol) After being stirred at rt for 1 h added sodium cyanotrihydroborate (0.094 g, 1.498 mmol) and continued stirring overnight at same temperature. Diluted reaction mixture with water (25 mL), extracted with ethyl acetate (2x25 mL), combined organic layers were washed with water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by making hydrochloride salt **13n** (0.09g, 36.8%) by using IPA-HCl in ethyl acetate.

Yield: 36.8%; Off-white solid; mp: 208-210°C; ^{1}H-NMR (300MHz, DMSO-d_6): δ 1.91-1.98 (m, 1H), 2.34 (s, 3H), 2.40-2.44 (m, 1H), 2.55-2.65 (m, 1H), 2.78-2.83 (m, 1H), 3.01-3.04 (m, 1H), 3.22-3.28 (m, 1H), 3.56 (s, 1H), 4.29 (s, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 2H), 7.44-7.63 (m, 5H), 9.40 (s, 1H), 9.59 (s, 1H), 10.68 (s, 1H); 13C-NMR(100 MHz, $CDCl_3$): δ 19.18, 21.68, 25.98, 26.48, 48.03, 54.41, 107.47, 110.99, 117.69, 122.60, 127.15, 127.18, 129.11(2C), 129.32, 130.62(2C), 131.09, 132.75, 135.04; MS (ESI) *m/z*: 291.1$[M+H]^+$; HPLC Retention time: 3.193 min., Purity: 98.79%(Method A).

4.1.34. 6-methyl-*N*-(4-methylbenzyl)-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine hydrochloride (13o)

2,2,2-trifluoroacetic acid (4.27 mg, 0.037 mmol) was added to a stirred solution of 6-methyl-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine **12a** (0.15 g, 0.749 mmol) and 4-methylbenzaldehyde (0.108g, 0.899 mmol) in tetrahydrofuran (5 mL) After being stirred for stirred for 1h.at rt. Added sodium cyanotrihydroborate (0.094 g, 1.498 mmol) and continued stirring for overnight. Diluted reaction mixture with water (25 mL), extracted with ethyl acetate (2 x 25 mL), combined organic layers were washed with water (25 mL) and brine (25 mL). Organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by silica gel column chromatography in Pet. Ether:Ethyl acetate; 8:2 as elute to get 6-methyl-*N*-(4-

methylbenzyl)-2,3,4,9-tetrahydro-1H-carbazol-2-amine which was converted to corresponding hydrochloride **13o** (0108g, 36.8%) by using IPA-HCl and ethyl acetate as solvent.

Yield: 36.8%; Off-white solid; mp: 210-212 °C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.91-1.92 (m, 1H), 2.32-2.33(m, 2H), 2.34 (s, 3H), 2.50-2.60 (m, 1H), 2.77-2.83 (m, 1H), 2.95-2.99 (m, 1H), 3.20-3.33 (m, 1H), 3.53 (s, 1H), 4.23 (s, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 7.2 Hz, 2H), 7.49 (d, *J* = 7.2 Hz, 2H), 7.26-7.38 (bs, 2H), 10.62 (s, 1H); ^{13}C-NMR(100 MHz, DMSO-d_6): δ 19.18, 21.28, 21.70, 25.98, 26.46, 47.72, 54.16, 107.46, 111.00, 117.69, 122.60, 127.14, 129.64(3C), 130.59(3C), 131.1, 135.02, 138.75; MS (ESI) *m/z*: 305.1$[M+H]^+$; HPLC Retention time: 3.551 min., Purity: 93.05%(Method A).

4.1.35. 9-Methyl-1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] (14)

Sodium hydride (0.698 g, 17.45 mmol) was added to a stirred solution of 1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] **9** (2.0 g, 8.72 mmol) in THF (50 mL) at 0-5°C. Stirred reaction mixture for 10 min. and added slowly iodomethane (1.636 mL, 26.2 mmol). After being stirred at same temperature for 1 h stirred reaction mixture overnight at 25 – 30 °C. Quenched an excess of sodium hydride with methanol, evaporated solvent on rotavapor to thick residue which was diluted with water (50 mL), extracted with ethyl acetate (2 x 50mL). Combined organic layers were washed brine (50 mL), dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by silica gel (60/120) column chromatography in Pet. Ether: Ethyl acetate; 1:1 as elute to get title compound **14** (1.7g, 80.0%).

Yield: 80.0%; Viscous Oil; ^{1}H-NMR (300MHz, $CDCl_3$): δ2.05 (t, *J* = 6.3 Hz, 2H), 2.91 (t, *J* = 6.3 Hz, 2H), 3.00 (s, 2H), 3.62 (s, 3H), 4.09 (s, 4H), 7.08 (t, *J* = 6.9 Hz, 1H), 7.17 (t, *J* = 6.6 Hz, 1H), 7.26(d, *J* = 9.3 Hz, 1H), 7.49(d, *J* = 7.8 Hz, 1H); MS (ESI) *m/z*: 252.1$[M+H]^+$.

4.1.36. 9-Methyl-1,3,4,9-tetrahydro-2*H*-carbazol-2-one (15)

To a stirred suspension of 9-methyl-1,3,4,9-tetrahydrospiro[carbazole-2,2'-[1,3]dioxolane] **14** (1.6g, 6.58 mmol) in methanol (30 mL) charged slowly 10% aqueous sulphuric acid (20 mL) at 25 - 30°C and stirred resulting reaction mixture at same temperature for 30 h. Diluted reaction mixture with water(150 mL) and extracted with ethyl acetate(2 x 100mL). Combined organic layers were washed with water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified silica gel (60/120) column

chromatography in Pet. Ether: Ethyl acetate; 1:1 as elute to get 9-methyl-1,3,4,9-tetrahydro-2*H*-carbazol-2-one **15** (0.4g, 30.5%).

Yield: 30.5%;^{1}H-NMR (300MHz, $CDCl_3$): δ 2.80 (t, *J* = 6.3 Hz, 2H), 3.13 (t, *J* = 6.6 Hz, 2H), 3.63 (s, 2H), 3.64 (s, 3H), 7.15 (t, *J* = 6.9 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.32(d, *J* = 8.1 Hz, 1H), 7.53(d, *J* = 7.8 Hz, 1H); MS (ESI) *m/z*: 200.1$[M+H]^+$.

4.1.37. 9-Methyl-*N*-(4-methylbenzyl)-2,3,4,9-tetrahydro-1*H*-carbazol-2-amine hydrochloride 16)

Mixture of p-Tolylmethanamine (0.182 g, 1.506 mmol) and 9-methyl-3,4-dihydro-1*H*-carbazol-2(9H)-one **15**(0.3 g, 1.506 mmol) was heated to 100 °C in preheated oil bath for 30 min. Then resulting reaction mixture was cooled to 25-30°C and diluted with THF (10 mL) followed by added sodium cyanoborohydride (0.189 g, 3.01 mmol) and continued stirring for overnight at rt. Diluted reaction mixture with water(25 mL), extracted with ethyl acetate (2x25 mL), combined organic layers were washed with water(25mL) and brine(25mL). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to get crude which was purified by silica gel column chromatography in Chloroform: Methanol; 9:1 as elute to get base which was converted to corresponding hydrochloride **16** (006g, 13.6%) by using IPA-HCl and ethyl acetate as solvent.

Yield: 13.6%; White solid; mp: 222-224 °C; ^{1}H-NMR (300MHz, DMSO-d_6): δ1.86-1.99 (m, 1H), 2.33 (s, 3H), 2.40-2.50(m, 1H), 2.60 -2.73 (m, 1H), 2.83-2.99 (m, 3H), 3.55-3.56 (m, 1H), 3.65 (s, 3H), 4.21-4.39 (m, 2H), 6.99 (t, *J* = 7.2 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 2H), 9.28 (s, H), 9.44 (s, 1H); ^{13}C-NMR (100MHz, DMSO-D_6): δ 19.15, 21.28, 25.12, 26.14, 29.53, 47.80, 54.13, 107.87, 109.65, 118.11, 119.06, 121.22, 126.36, 129.62, 129.69(2C), 130.59(2C), 132.51, 137.49, 138.84; MS (ESI) *m/z*: 305.8$[M+H]^+$; HPLC Retention time: 6.156 min., Purity: 99.68%(Method A).

4.2. Biological Assay

4.2.1. pERK assay: Briefly, 25,000 MDAMB231 cells were grown in 96 well transparent plate and kept in CO2 incubator for 24 h.. After 24 h. cells were serum starved for 6 h. and later treated with 10µM concentration of drug for 1 h., after treatment cells were fixed with 3.7% formaldehyde and permeabilized with 0.1% triton X, then the cells were stained with P-

p44/p42 MAPK (T202/Y204) antibody and secondary ECLTM Anti-Rabbit IgG antibody. Color was developed with TMB substrate and read on SpectraMax.

4.2.2. pRB assay: Briefly, 20,000 MDAMB231 cells were grown in black 96-well transparent bottom plates and kept in CO2 incubator for 24 h. After incubation the cells were treated with 10µM concentration drug for 16 h.. and treated cells were fixed with 3.7% formaldehyde and permeabilized with 0.1% triton X, then the cells were stained with anti-pRb antibody and secondary Dylight 549 antibodies. Cells were fluorescently stained and analyzed using the Cellomics ArrayScan HCS Reader (Cellomics/Thermo Fisher). Cell nuclei were stained with Hoechst 33342 to identify individual cells and to optimize focusing.

4.3. Molecular Docking

4.3.1. Software. All the molecular modeling studies described herein were performed using Small-Molecule Drug Discovery Suite 2015-4[31] and the modules included therein for performing various molecular modeling operations are described below.

4.3.2. Protein Structure. The crystal structure of ERK2 kinase, retrieved from the Protein Data Bank (PDB ID 5BVD [30]), was subjected to *Protein Preparation wizard* using default settings as implemented in Schrödinger suite 2015-4 [32]. The termini were capped, missing side chains were added using Prime version 4.2 and all the water molecules were deleted. H-bonds were optimized and in the end, restrained minimization was performed wherein the heavy atoms were converged to root mean square deviation (RMSD) 0.3 Å. The prepared protein was then subjected to further minimization using Prime 4.2 (default settings) [33, 34]. Glide version 6.9[35], as implemented in Schrödinger suite 2015-4, was used for performing docking studies of few select molecules **1** (Figure 1), **13l** and **13o** (Table 2) and **16** (Figure 2).

4.3.3. Ligand Structures. The ligand structures were built in Maestro 10.4. All the structures were then optimized using the LigPrep (version 2.6) module, as implemented in Schrödinger suite 2015-4 using the default settings. This set of molecules was then subjected to the docking studies. The aim of the study was to investigate the binding mode of few synthesized molecules in the binding site of the ERK2 kinase.

4.3.4. Receptor Grid Generation. The refined protein structure was used to define the active site using *Receptor Grid Generation* module. The native ligand present in the target structure (compound **17**, Figure 3) was used for placing the enclosing box. The grid was then used for docking the ligands in the ligand-binding site.

For docking studies, Extra Precision (XP) (Ref. 35a) mode was used. The docked poses were minimized and RMSD to input ligand geometries were calculated. Table 3 lists the docking scores for all the molecules used in the docking studies. Figures 4-6 show the docking poses of representative molecules into the binding cavity of the ERK2 kinase. After docking studies, the pose viewer file was then used as input for Prime-MMGBSA calculations. The MMGBSA binding energies of the top-ranked pose for each molecule are given in Table 3.

5. Acknowledgements

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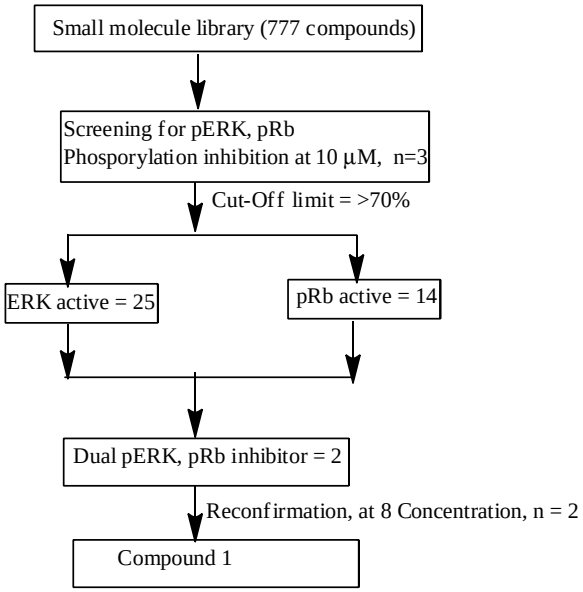
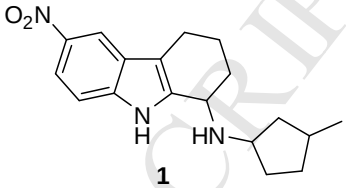
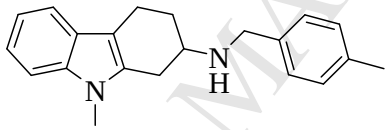




1

Figure 1. Screening strategy and molecular structure of the initial hit obtained from in-house library screening



16

pERK IC_{50} = 4.4 µM
pRb IC_{50} = 3.5 µM

Figure 2. Molecular structure of **16** along with the pERK and pRb inhibition data

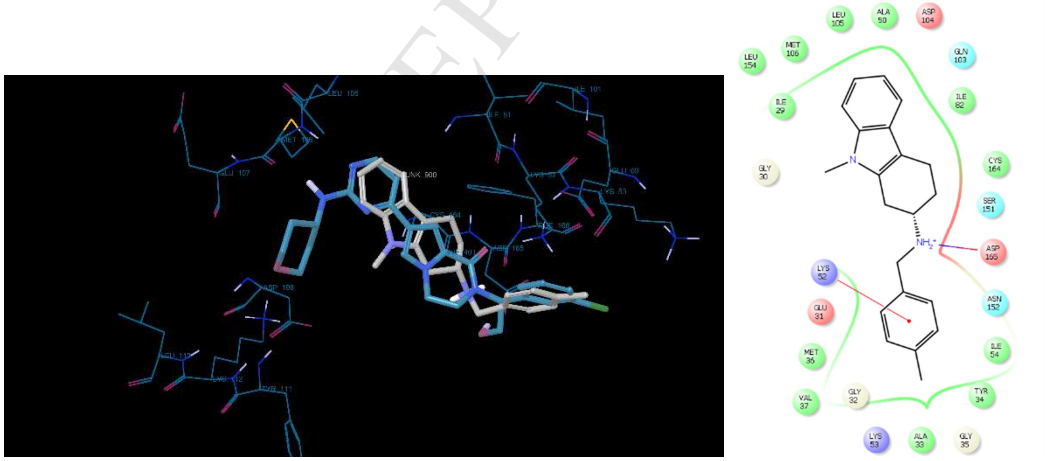
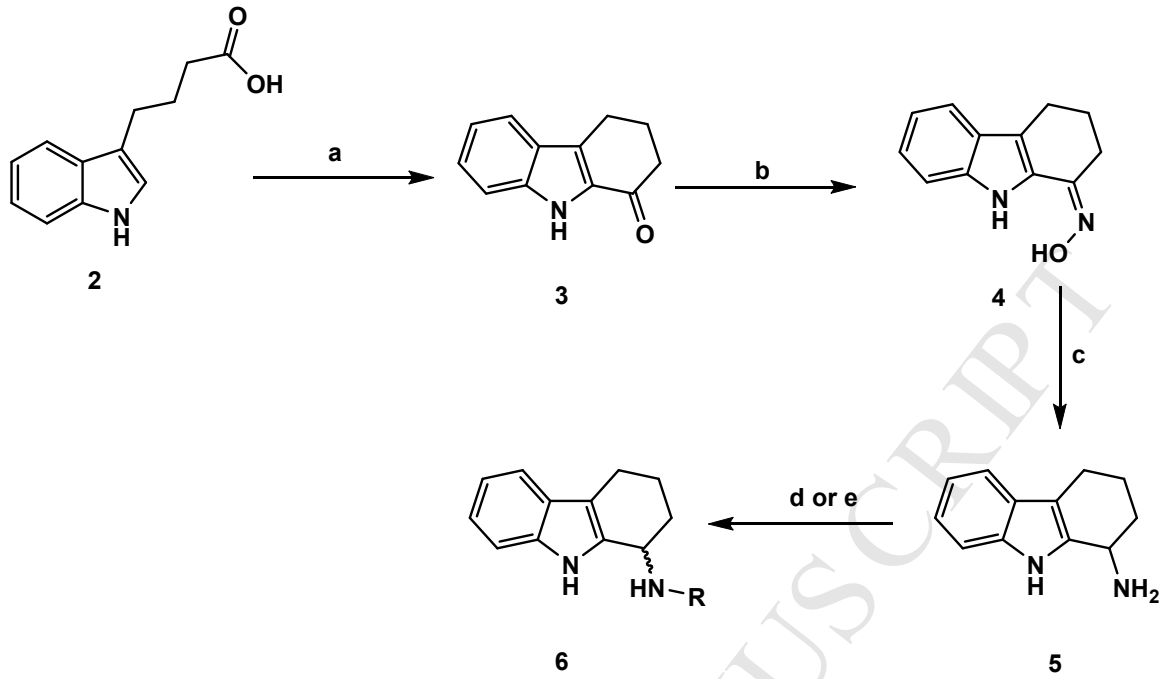


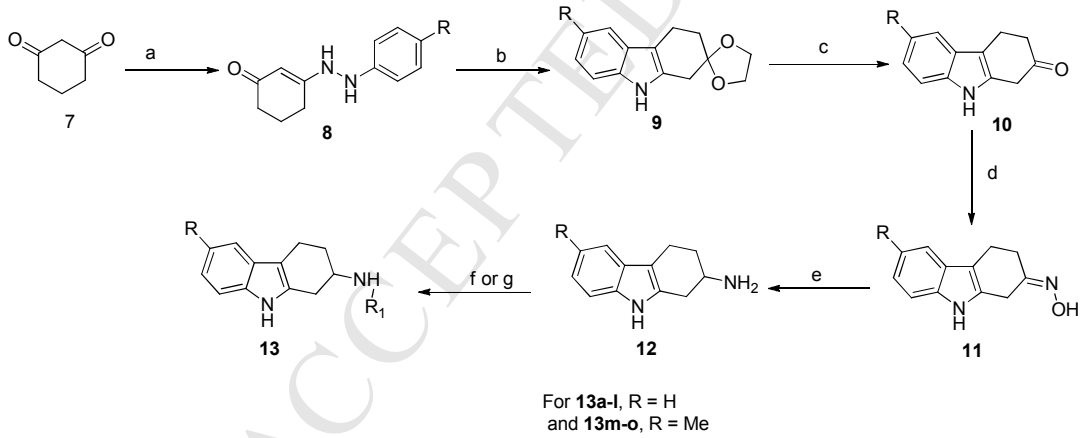
Figure 3. Binding mode of **16** in the ligand-binding pocket of ERK2 kinase

Scheme 1: Synthesis of inhibitors 6



Scheme 1. Reagents and conditions: a) PPA, Toluene, Reflux, 40 h, 68.6%; b) Hydroxylamine hydrochloride, ethanol, rt, 3 h, 97.5%; c) $LiAlH_4$, THF, Reflux, 2 h, 85.1%; d) i) aldehyde/ketone, TFA, THF, 0 °C – rt, 2 h; ii) $NaBH_3CN$, rt, 16h, 40.0 – 63.0%; e) Sulfonyl chloride, triethylamine, DCM, 0 °C - rt, 1-2 h, 55.0 – 75.0%.

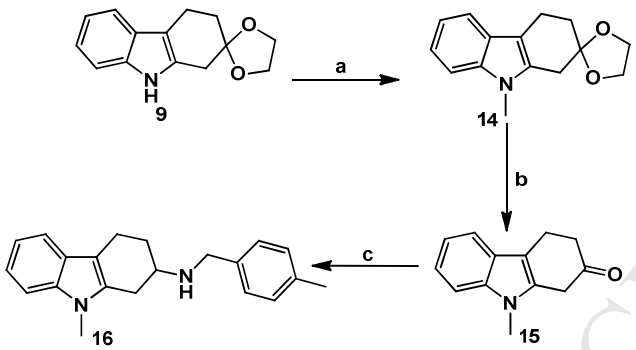
Scheme 2: Synthesis of inhibitors **13**



Scheme 2. Reagents and conditions: a) Phenyl hydrazine or *p*-tolylhydrazine, water, rt, 4 h, 93.0%; b) *p*TSA, ethyleneglycol, toluene, Reflux, 24 h, 59.7%; c) Concentrated HCl, or 10% aq. H_2SO_4, methanol, rt, 16 h, 53.3%; d) Hydroxylamine hydrochloride, rt, 3 h, 70.3 – 98.3%; e) $LiAlH_4$, THF, Reflux, 2 h, 22.0 – 86.0%; f) i) aldehyde/ketone, TFA or Conc.H_2SO_4, THF, 0 °C –

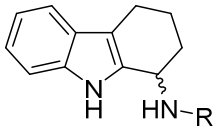
rt, 2 h; ii) $NaBH_3CN$, rt, 16 h, 7.0 - 53.0%; g) Sulfonyl chloride / Benzoyl chloride, triethylamine, rt, 2-3 h, 18.8 - 39.2%.

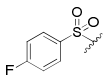
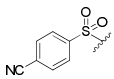
Scheme 2: Synthesis of inhibitor **16**



Scheme 3. Reagents and conditions: a) CH_3I, NaH, THF, 0 °C- rt, 2 h, 54.1%; b) 6N HCl, methanol, rt, overnight, 53.6%; c) i) 4-Methylbenzalamine, 100 °C, 0.5 h; ii) $NaBH_3CN$, THF, rt, 16 h, 13.6%.

Table 1. Inhibition of pERK and pRb phosphorylation by 1-aminosubstituted tetrahydrocarbazole derivatives 6

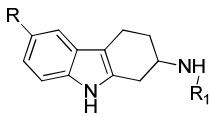


Sr. No.	Compound No.	R	pERK IC_{50} (μM)	pRb IC_{50} (μM)
1	**6a**[#]		9[a]	15[a]
2	**6b**		21	10
			30[a]	16[a]
3	**6c**[#]		3	9
			3[a]	15[a]
4	**6d**		>100	>100
5	**6e**		>100	9.2
6	**6f**		>100	>100
pERK standard	Sorafenib	--	0.063	--

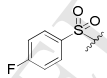
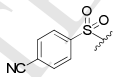
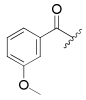
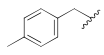
pRB standard	Staurosporine	--	--	0.066

[a] Values for corresponding hydrochloride salts [#] compound **6a**, **6c** and **1** was studied for treatment of CNS disorder and HPCV virus[22, 24, 25

Table 2. Inhibition of pERK and pRb phosphorylation by 2-aminosubstituted tetrahydrocarbazole derivatives 13



For **13a-l** R = H
13m-o R = Me

Sr. No.	Compound No.	R_1	pERK IC_{50} (µM)	pRb IC_{50} µM)
1	**13a**		7.0	11.0
2	**13b**		21.0	10.0
3	**13c**		>30.0[a]	17.3[a]
4	**13d**		>100[a]	>100[a]
5	**13e**		69.86	>100
6	**13f**		7.5	9.5
7	**13g**		7.0	>100
8	**13h**		8.1	100
9	**13i**		9.0	>100
10	**13j**		29.68	>100
11	**13k**		6.11	6.92
12	**13l**		3.62	4.10
13	**13m**		6.0 5.5[a]	2.0 3.6[a]
14	**13n**		5.5[a]	2.2[a]

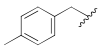
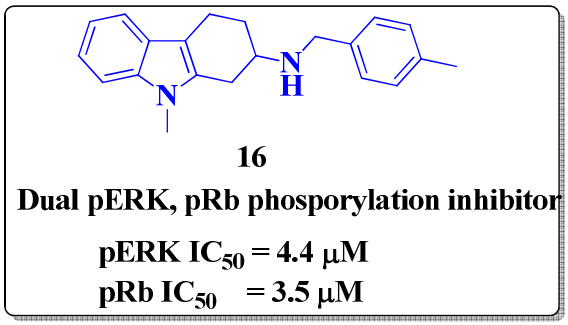
15	**13o**		4.6[a]	2.9[a]
pERK standard	Sorafenib	--	0.063	--
pRB standard	Staurosporine	--	--	0.066

Table 3. Summary of molecular docking results and binding energy calculations

Sr. No.	Compound No.	XP GScore	MMGBSA ΔG_{bind} (Kcal/mol)
1	1	-5.397	-117.080
2	13l	-6.674	-111.283
3	13o	-6.956	-120.916
4	16	-6.574	-120.016

Graphical Abstract:



Research Highlight:

- Concept for development of dual pERK and pRb phosphorylation inhibitor.
- Discovery of *N*-(3-methylcyclopentyl)-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazol-1-amine(**1**) tetrahydrocarbazole class of compound as dual pERK, pRb phosphorylation inhibitor.
- A Short SAR led to the identification of another hit as dual pERK, pRb phosphorylation inhibitor (**16**)