Design and Synthesis of Positive Allosteric Modulators of CB1 Cannabinoid Receptor

Master’s Thesis Dissertation

By

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I dedicate this thesis to my grandparents Mr. Shripad Geed and Mrs. Kamal Geed for instilling the thirst for knowledge in me and for having faith in me all these years. They played a pivotal role in shaping my childhood and youth and made me what I am today.
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1. Abbreviations:

1. GPCR – G- Protein Coupled Receptor
2. Δ⁹ THC - Δ⁹ Tetrahydrocannabinol
3. MAPK – Mitogen-Activated Protein Kinases
4. 2-AG – 2- Arachidonoyl Glycerol
5. FAAH- Fatty Acid Amide Hydrolase
6. MGL – Monoacyl glycerol lipase
7. CB1 – Cannabinoid Receptor Type 1
8. CB2 – Cannabinoid Receptor Type 2
9. CNS – Central Nervous System
10. ECS – Endocannabinoid System
11. AEA – Anandamide (N-Arachidonoyl ethanolamine)
12. ACPA – Arachidonoylcyclopropylamide
13. GABA – Gamma Aminobutyric Acid
14. HIV – Human Immunodeficiency Virus
15. TMH - Transmembrane Helices
16. ICL - Intracellular Loop
17. cAMP – cyclic Adenosine monophosphate
18. TMS – Trimethylsilane
19. NMR- Nuclear Magnetic Resonance
20. FT-IR – Fourier Transform Infrared Spectrometry
21. PAM – Positive Allosteric Modulator
22. NAM – Negative Allosteric Modulator
23. DMF – Dimethylformamide
24. NaOH – Sodium Hydroxide
25. rt- Room Temperature
26. NMP – N-methyl-2-pyrrolidone
27. HCl – Hydrochloric Acid
28. MeOH – Methanol
29. EtOH - Ethanol
30. EtOAc – Ethyl Acetate
31. MS – Mass Spectrometry
32. h – Hour
33. MW – Microwave
34. EFC – Enzyme Fragment Complementation
35. RLU – Relative luminescence Unit
36. NaH – Sodium Hydride
37. DAT – Dopamine Transporter
38. cAMP – Cyclic Adenosine Monophosphate
39. HTS – High Throughput Screening
40. CINV – Chemotherapy Induced Nausea and Vomiting
41. EDG – Endothelial Differentiation Gene
42. HBSS – Hank’s Balanced Salt Solution
43. DMSO – Dimethyl Sulfoxide
44. CDCl₃ – Deuterated Chloroform
45. CH₃OD – Deuterated Methanol
46. HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
47. ICRS - International Cannabinoid Research Society
48. SAR – Structure- Activity Relationship
49. PTSD – Post-Traumatic Stress Disorder
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**Scheme 1** - Synthesis of 3-(2-(9H-carbazol-9-yl) ethyl)-4-substituted-1H-1,2,4-triazole5(4H)-oxo/thione analogs

**Scheme 2** - Synthesis of 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione

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**Scheme 4** - Synthesis of 9-(2-(4-(2-methylallyl)-5-(methylthio)-4H-1,2,4-triazol-3-yl)ethyl)-9H-carbazole

**Table 1**- Proposed list of analogs

**Table 2**- List of compounds synthesized by scheme 1

**Table 3**- List of compounds synthesized by scheme 3

**Table 4**- Consolidated list of compounds with biological evaluation
3. **ABSTRACT:**

The endocannabinoid system which consists of CB1 and CB2 receptors that belong to type A GPCRs, endogenous cannabinoids (endocannabinoids) and enzyme machinery involved in their biosynthesis, transportation and metabolism is associated with pathophysiology of plethora of disorders ranging from obesity, multiple sclerosis, anorexia nervosa and Huntington’s disease. A large number of orthosteric ligands have been developed for CB1 receptors for the treatment of various disorders, but their therapeutic applications are limited due to the problems such as receptor desensitization and CNS side effects. CB1 allosteric modulators have the potential of offering same therapeutic advantages as agonists and are likely to be devoid of agonist-related problems. These ligands act at a site (allosteric), distinct from the traditional orthosteric site. The allosteric site upon activation induces conformational changes in the orthosteric site which ‘fine-tunes’ the response of the response of the orthosteric ligands. In most of the cases, allosteric ligands cannot precipitate its action in absence of orthosteric ligands. The fine tuning of receptor helps reduce the dose of the orthosteric ligand which in turn would reduce dose related side effects and also address the receptor de-sensitization issue. In 2007 a HTS lead (AZ-5, GAT236, Figure 4) was reported to act as CB1 positive allosteric modulator (PAM) in the preliminary assays. Since then no further analogs, biochemical or pharmacological studies on this template has been reported. The objective of the present study was to develop newer analogs of this lead by exploration of this scaffold and evaluation of these analogs for their PAM activity. Four sites of variation were identified in the lead carbazole template. For the scope of current study, carbazole ring was kept unaltered and rest of the structure was explored by structure-activity relationship (SAR) studies. A novel synthetic route was developed to ensure rapid synthesis of the compounds with acceptable yields. Fifteen analogs thus synthesized, were tested for the potentiation of EC$_{20}$ agonist dose in β-arrestin and cAMP assays. All the compounds were found to be active selectively in cAMP assay with insignificant potentiation of agonist response β-arrestin assay. None of the compounds displayed more PAM activity compared to the original lead molecule. This study is involved in development of novel route for rapid synthesis of GAT236 analogs. The preliminary data obtained from the functional testing of the synthesized molecules has provided useful information for further lead optimization.
4. Introduction:

Active research in cannabinoid pharmacology started with the discovery of Δ⁹-THC (Figure 1), the psychoactive constituent of marijuana. It exerts its pharmacological effects through cannabinoid receptors in the brain and periphery. Two cannabinoid receptor subtypes are known to exist, CB1 and CB2. These receptors belong to the superfamily of G-protein-coupled receptor (GPCR). CB2 receptors are expressed primarily in immune cells, whereas CB1 receptors exist predominantly in the brain and also in peripheral tissues such as testis and adipose tissue. To date a number of endogenous cannabinoids and synthetic CB subtype selective ligands have been identified and many of these have been (pre)clinically shown to be useful in a variety of disorders including obesity, nicotine and alcohol dependence, multiple sclerosis, cancer, diarrhea and cardiovascular diseases.

4.1 The Endocannabinoid System:

The Endocannabinoid system (ECS) consists of cannabinoid receptors, CB1 and CB2, their endogenous ligands (endocannabinoids) N-arachidonoylethanol amine (AEA) and 2-arachidonoyl glycerol (2-AG, Figure 1) and enzymes and proteins involved in synthesis, transportation and metabolic inactivation (FAAH and MGL). Abundantly expressed in hippocampus, cerebellum and striatum and scarcely in GIT lining, pancreas, liver, eyes and adipose tissues, CB1 receptors play pivotal role in cognition, memory and sensory function in brain and in energy balance, metabolism and nociception in peripheral nervous system. Further, the adverse effects of Δ⁹ THC too are mediated by the CB1 receptors in the brain. The CB2 receptors are mainly expressed in cells of immune system and are up regulated when demand arises as in case of inflammation. Both CB1 and CB2 receptors are Gᵢₒ coupled in most of the cases but can be Gₛ coupled in some cases. Activation of CB1 receptors causes a cascade of downstream signaling which starts with inhibition adenylate cyclase, stimulation of potassium channels and activation of MAPK.
Endocannabinoids are naturally occurring cannabinoid ligands that are known for their retrograde signaling, meaning they are synthesized by post-synaptic neurons and activate the pre-synaptic neurons when the need arises (physiological and pathological stimuli), which causes inhibition of both excitatory and inhibitory neurotransmitters and are enzymatically degraded after the demand satiates. Monoacyl glycerol lipase (MGL) and Fatty Acid Amide Hydrolase (FAAH) are the two key enzymes shown to be responsible for deactivation of endocannabinoids. MGL selectively deactivates 2-AG whereas, FAAH deactivates AEA as well as 2-AG. MGL and FAAH inhibitors such as URB597 (Figure 2) have also been explored for therapeutics of disease states in which it is beneficial to have increased activity of ECS for the disease state such as pain\textsuperscript{6,7} reducing blood pressure and blocking cell proliferation.

**Figure 1: $\Delta^8$-THC and endogenous cannabinoids**

Overall the ECS functions as a neuromodulator and influences activities such as cognitive learning, pain and appetite control. The endocannabinoid binding sites are expressed primarily in the brain and immune system\textsuperscript{8}.

The CB1 and CB2, which belong to rhodopsin type or class A of the GPCR superfamily and are $G_{i/o}$ coupled. They share 44% overall homology and 68% homology in their transmembrane
domain. CB1 and CB2 share common signaling pathways such as adenylyl cyclase inhibition and stimulation of mitogen-activated protein kinase (MAPK). Activation of CB1, but not CB2 mediates inhibition of N- and P/Q-type calcium channel and stimulation of potassium channels.

4.2 Allosteric Modulators:

Studies over the past decade have demonstrated several GPCRs having ligand binding sites distinct from the orthosteric site, which are referred as allosteric site. The allosteric site influences the orthosteric site by inducing conformational changes in the latter. Majority of drug discovery programs have focused on the development of orthosteric ligands of CB1 receptors. However, they suffer from disadvantages such as tolerance, addiction and abuse potential. Orthosteric sites of GPCR subtypes activated by the same ligand are often highly conserved; therefore, achieving subtype selectivity may be more challenging when targeting this site for drug design. Allosteric ligands act through less conserved sites and thus have a greater potential for receptor subtype selectivity. Allosteric ligands mediate their effects by modifying receptor conformation leading to a change in the binding and/or functional properties of orthosteric ligands.

Generally, allosteric modulator may only elicit its effects when the endogenous agonist is present, thereby resulting in a selective ‘tuning’ of biological response when and where they are required. This is in contrast to orthosteric ligands, which may also continuously affect receptor, function for as long as they are present. The binding of an allosteric modulator delivers a conformational change which affects the affinity and/or efficacy of the orthosteric ligand, thereby fine-tuning the pre-existing actions of the endogenous ligands. There is ample evidence that the levels of endocannabinoids are increased under (patho)physiological conditions, in which an autoreceptor action of the endocannabinoids has been also implicated. By triggering activation of the CB1 without causing the unwanted psychotropic effects, allosteric enhancers of the CB1 receptor would signify a nexus in cannabinoid research. Proof-of-principle for the concept of fine tuning of the endocannabinoid signaling is a beneficial therapeutic strategy can be found in the effects of inhibitors of the enzymes responsible for the rapid intracellular hydrolysis of anandamide and 2-AG. FAAH inhibitors are anxiolytic and antidepressant, and both FAAH and MAG lipase inhibitors are antinociceptive. The nature of the effect of allosteric
modulators is known to be ligand dependent.\textsuperscript{18} There is substantial evidence for CB1 agonist-selective G protein signaling, such that different ligands differentially direct cellular signal transduction pathways. Despite the fact that both anandamide and 2-AG have nanomolar affinity for the CB1 receptor, they might well have different physiological and pathophysiological roles, being released in different tissues and initiating diverse signaling events. Although agonist-specific trafficking of cellular responses associated with the endocannabinoids has not been fully elucidated, it is conceivable that allosteric modulators could be designed to affect anandamide and 2-AG differentially, thereby affording ligand-dependent modulation that maintains the dynamic tissue-specific signaling associated with these endocannabinoids.\textsuperscript{19} As more potent compounds become available, this will undoubtedly be the subject of future research.\textsuperscript{13,20}

Allosteric modulators display a number of theoretical advantages over orthosteric ligands as potential therapeutic agents, such as:

1. Allosteric modulators, except the ones belonging to ago-PAM class, do not display any response in the absence of orthosteric ligand. Thus, allosteric modulators have the potential to maintain activity dependence and both temporal and spatial aspects of endogenous physiological signaling.\textsuperscript{21}

2. Allosteric ligands may have greater receptor selectivity. This is possible because the sequence for orthosteric site is highly conserved as compared to allosteric site\textsuperscript{18} Alternatively, selectivity might be engendered by combining both orthosteric and allosteric pharmacophores within the same molecule to yield a novel class of 'bitopic' GPCR ligand.\textsuperscript{18}

3. Allosteric modulators with limited positive or negative cooperativity would impose a 'ceiling' on the magnitude of their allosteric effect.\textsuperscript{22} This means that large doses of allosteric modulators can be administered with a lower propensity towards target-based toxicity than orthosteric agonists or antagonists. Further, limited cooperativity modulators introduce a new level of pharmacological responsiveness, whereby they can allow for a subtle re-setting of endogenous agonist activity.\textsuperscript{12,20}

Recently, two GPCR allosteric modulators have entered the market, generating further excitement in this field. The first such drug, Cinacalcet (Amgen), a PAM of the calcium sensing receptor, is used to treat hyperparathyroidism. The other, Maraviroc (Pfizer), a negative allosteric modulator (NAM) of chemokine receptor CCR5, was launched for the treatment of HIV
Several other GPCR allosteric modulators are currently undergoing preclinical studies and clinical trials, thereby suggesting that the approach of targeting allosteric mechanism to be sound and tractable.

### 4.3 Therapeutic Potential of CB1 Allosteric Modulators:

Benzodiazepines are a classic example of positive allosteric modulators (PAMs) of GABA receptors. Also it was recently shown that co-administration of a α4β2 neuronal nicotinic acetylcholine receptor agonist and its PAM in experimental models of pain in rats showed potentiation of analgesic efficacy but not side effects. Allosteric modulators of the CB1 receptor might also have effects on receptor trafficking, thereby affecting desensitization, surface expression, tolerance and dependence.

CB1 PAMs would have a unique profile and an advantage over direct agonists due to following key characteristics:

1. Allosteric CB1-receptor modulation has the potential to improve endogenous cannabinoid tone to therapeutic levels with required subtype specificity and less side-effect risk/enhanced safety.

2. Although agonist-specific trafficking of cellular responses associated with endocannabinoids have not been fully elucidated, it is conceivable that allosteric modulators could be designed to affect AEA and 2-AG differentially, thereby affording ligand-dependent modulation that maintains the dynamic, tissue-specific signaling associated with these endocannabinoids.

3. Allosteric CB1 modulators are expected to be devoid of desensitization, tolerance, dependence (addiction) liability, and adverse influence on receptor cell-surface expression/recycling.
Medicinal Chemistry research led to optimization of the structure of Δ⁹-THC which paved way for the development of three cannabinoid medications. The first was Nabilone (Figure 3), a synthetic analogue of Δ⁹-THC and a CB1/CB2 agonist, for the treatment of chemotherapy-induced nausea and vomiting (CINV) in cancer patients.²⁴ Recently, Nabilone has also been evaluated successfully for the management of treatment-resistant nightmares in posttraumatic stress disorder.¹⁸,²⁵

Synthetic (-)-Δ⁹-THC (Marinol®, Dronabinol, Solvay, Marietta, GA, USA), has been marketed as an anti-emetic for the treatment of CINV, appetite stimulant for treatment of cachexia (weight loss and wasting) in AIDS patients and is undergoing Phase II clinical trials for the treatment of marijuana addiction (sponsored by NIDA).

Sativex® (GW pharmaceuticals, Salisbury, Wiltshire, UK), an oromucosal spray formulation of cannabidiol and Δ⁹-THC for symptomatic relief of neuropathic pain in Multiple Sclerosis was launched in Canada in 2006. In 2007, Sativex was also approved as an adjunctive analgesic in patients with advanced cancer who experience moderate to severe pain during the highest tolerated dose of opioid therapy for persistent background pain.¹²,²⁴

4.4 Background of CB1 PAMs reported in literature:

Research in CB1 PAM area began with the presentation of AZ-1 (PAM-1, Figure 4) and AZ-5 (GAT236, Figure 4) by AstraZeneca in ICRS 2007.²⁶ In 2009 dopamine transporter (DAT) inhibitors (RTI-371 and JHW007, Figure 4) that do not produce the expected locomotors behavioral stimulation in mice were shown to be also acting as CB1 PAMs.²⁷ However these compounds have a 3-phenyltropane scaffold (shown in blue, Figure 4) which is well-known to have off-target activities.²⁸
Lipoxins are short lived endogenously produced nonclassic eicosanoids whose appearance in inflammation signals the resolution of inflammation. Recently Lipoxin A4 was shown to have potent CB1 PAM activity. However this scaffold has role in inflammation and other related biochemical pathways. In our laboratory detail biochemical and pharmacological characterization around PAM-1 has been carried out together with the structure-activity relationship studies (Kulkarni & Thakur, unpublished). The preliminary studies confirmed therapeutic utility of CB1 PAMs in treating glaucoma, PTSD, pain and other CB1 mediated disorders without having CB1 related side effects such as hypothermia, catalepsy and addiction potential (microdialysis). Since the presentation by AstraZeneca in 2007, no further pharmacological, biochemical or SAR studies were published on this lead. Encouraged by these findings, GAT236 was synthesized in Dr. Thakur’s laboratory and was found to potentiate the of CP-55,940 (Figure 5) in preliminary biochemical evaluation.

![Chemical structures](image)

**Figure 4: Literature reported CB1 PAMs**
Figure 5: Orthosteric agonist, CP-55,940
5. **Objectives:**

The lead molecule GAT236 was synthesized in Thakur laboratory by Dr. Pushkar Kulkarni, was tested and shown to act as CB1 PAM in preliminary assays. The objective of my master’s research project is:

1. To develop analogs of GAT236 template (Figure 6) with:
   a. Variation in carbon chain length (Site II)
   b. Replacing the sulfur atom at Site IV, with oxygen or carrying out thiomethylation.
   c. Replacement the Site III, with various branched chain, substituted, unsubstituted, aromatic and bulky groups.

![Figure 6: Site Variation](image)

2. Evaluation of the synthesized analogs in cAMP and β-arrestin assays to confirm their PAM activity at CB1, by measuring the shift in dose versus activity graph of the PAM compared to the EC\textsubscript{20} dose of orthosteric agonist (CP-55,940), and while doing so, exploring and overcoming the encountered synthetic chemistry challenges.

3. To develop preliminary SAR on the basis of results of biological evaluation
6. Rationale:

Like many other GPCRs, CB1 receptor has also been recently demonstrated to possess allosteric site(s).\textsuperscript{13,21,27} CB1 receptor being a transmembrane protein has been difficult to crystallize and as of now its crystal structure has not been developed. Limited information exist about the amino acid residues involved at the allosteric site.\textsuperscript{29}

GAT236 bears a carbazole scaffold which is linked with a carbon spacer to a triazole moiety. To date GAT236 is the only molecule known as CB1 PAM in this scaffold with micro-molar activity profile. Therefore, there was a need for exploration around this scaffold to understand structural requirements for CB1 PAM activity and to get more potent and efficacious compounds.

Hence the objective of this work is to develop analogs of GAT236 with improved activity and physicochemical properties. This can be achieved by making structural modifications at key sites of the molecule.

The molecule was divided into following four key sites as described in the objective section above. In the present work site-I (carbazole, Figure 3) will be maintained and systematic variations have been made at sites II, III and IV.

6.1. Site variations:

Site II:

The site II variation is the site of carbon chain length variation. Analogs varying in chain length from 1-3 were synthesized and were biochemically evaluated for CB1 PAM activity.

Site III: The 4-position nitrogen atom of the triazole ring is the place for site III of variation. The variations were proposed at this site to explore the stereoelectronic requirements at the receptor site. The methylallyl group was replaced by functional groups with varying steric, and electronic properties. The proposed analogs yielded the information about change in activity of the molecule after substitution by substituted, unsubstituted, aromatic and bulkier functional groups.

Site IV: The thione functionality embedded in the triazole ring was the place for site IV variation. It was proposed to substitute sulfur atom with oxygen atom as well as to make thioalkyl derivatives to study the effect of such substitution on activity.
6.2. List of proposed molecules (Table-1):

**General Structure**

![General Structure Diagram]

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*Table 1: List of proposed molecules*
7. Retrosynthetic strategy for the synthesis of proposed analogs:

Scheme A : Retrosynthetic plan

We proposed the synthesis of common hydrazide intermediate (c) which can be transformed to desired site-III analogs (a) by treating with suitable isothiocyanates or isocyanates followed by base catalyzed cyclization, to give the corresponding compounds (a). Synthon (d) can in turn be synthesized by alkylating carbazole nitrogen using strong base and suitable bromoalkyl ester to give intermediate (d) which can be converted to hydrazide using hydrazine hydrate.
8. Chemicals and Materials:

All the reagents and solvents were purchased from Aldrich and Alfa Aesar, unless otherwise specified, and used without further purification. All anhydrous reactions were performed under an argon or nitrogen atmosphere in flame-dried glassware using scrupulously dry solvent. Flash column chromatography employed silica gel 60 (230-400 mesh) and was performed on Interchim Puriflash 450. All compounds were demonstrated to be homogenous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F245 on glass, layer thickness 250μm), and chromatogram were visualized by phosphomolybdic acid or anisaldehyde reagent staining. Melting points were determined on a micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum RX - 1 FT-IR spectrometer. NMR spectra were recorded in CDCl₃, CD₃OD, DMSO-d₆ or Acetone-d₆, on Varian 500 MHz and Varian 400 Mass spectrometer, and the chemical shifts are reported in units of δ ppm relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and coupling constant (J) are reported in hertz (Hz). Low resolution mass spectra were performed in the Department of Chemistry and Chemical Biology and Barnett Institute of Chemical Analysis at Northeastern University.
9. Chemistry:

9.1 Scheme 1: Synthesis of 3-(2-(9\textit{H}-carbazol-9-yl)ethyl)-4-substituted-1\textit{H}-1,2,4-triazole-5(4\textit{H})-oxo/thione analogs

![Scheme 1](image)

Reagents and conditions: i) methyl 3-bromopropanoate, NaOH, anh. DMF, 150°C, 40min, MW; ii) HCl in anh. MeOH, 4h, RT; iii) hydrazine hydrate (99%) in MeOH reflux, 6h; iv) anh. dioxane, reflux 6-12h; v) 5% aq. NaOH solution, reflux; vi) dil. HCl (pH 1.0).

The synthesis of analogs with ethyl spacer between carbazole and triazole ring (3-(2-(9\textit{H}-carbazol-9-yl)ethyl)-4-substituted-1\textit{H}-1,2,4-triazole-5(4\textit{H})-oxo/thione analogs) is summarized in scheme-1.

Commercially available carbazole 1 was \textit{N}-alkylated\textsuperscript{30} with methyl-3-bromopropionate and sodium hydroxide in anhydrous DMF under microwave heating to give the carboxylic acid 2 (70% yield). Esterification of acid 2 using HCl in anhydrous MeOH yielded ester 3 (95% yield) which upon reflux with hydrazine monohydrate gave hydrazide compound 4 (63% yield). Reaction of 4 with the required isothiocyanates or isocyanates gave corresponding semicarbazide/thiosemicarbazides 5 (in good yields). Base catalyzed cyclization using sodium hydroxide followed by acidic workup gave target compound 6 (61 to 86 % yields).
List of compounds synthesized using scheme 1 are given in the table 2 below:

**General Structure**

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<th>Yield %</th>
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*Table 2: Compounds synthesized using scheme 1*
9.2 Scheme 2: Synthesis of 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione

Reagents and conditions: a) γ-butyrolactone, NaOEt, anh. NMP, microwave, 40min, 200 °C; b) HCl in anh. MeOH; c) hydrazine hydrate (99%) in MeOH, reflux, 6h; d) anh. Dioxane, reflux 6-12h; e) 5% aq. NaOH solution, reflux; f) dil. HCl (pH 1.0).

Compounds 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione, i.e. compound with three carbon spacer between carbazole and triazole ring was synthesized as depicted in scheme 2.

Carbazole was microwaved with γ-butyrolactone and sodium ethoxide to give the carboxylic acid 13 (85% yield). The acid 13 was esterified using HCl in MeOH to give the ester 14 (88% yield) which was treated with hydrazine monohydrate to give compound 15 (69% yield). Reaction of methyl allyl isothiocyanate with hydrazide 15 gave the thiosemicarbazide 16 which on cyclization with sodium hydroxide gave 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione 17 (65% yield).
9.3 Scheme 3: Synthesis of 3-(2-(9H-carbazol-9-yl)methyl)-4-substituted-1H-1,2,4-triazole-5(4H)-oxo/thione analogs

Where: i) KOH, ethyl 2-bromoacetate, anhydrous DMF, MW 120°C, 45min; ii) 0.5M HCl in anh. MeOH, 25°C , 12h; iii) hydrazine hydrate, MeOH; iv) anh. EtOH, reflux, 6h; v) 5% aq. NaOH, reflux, 12h; vi) dil. HCl (pH 1.0).

The scheme 3 describes method to synthesize compounds with one carbon spacer. Carbazole 1 was microwaved with ethyl-2-bromoacetate to give the carboxylic acid 18 (59% yield). The acid 18 was esterified using HCl in MeOH to give the ester 19 (89% yield) which was treated with hydrazine monohydrate to give compound 20 (89% yield). The compound 20 was reacted required isothiocyanates to give the corresponding thiosemicarbazide 21 in good yields, which on cyclization with sodium hydroxide gave final corresponding compound 22 in good yields.
List of compounds synthesized using scheme 3 are given in the table 3 below:

### General Structure

![General Structure Diagram]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Code</th>
<th>R₁</th>
<th>R₂</th>
<th>Yield %</th>
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*Table 3: Compounds synthesized using scheme 3*
9.4 Scheme 4: Synthesis of 9-(2-(4-(2-methylallyl)-5-(methylthio)-4\textsubscript{H}-1,2,4-triazol-3-yl)ethyl)-9\textsubscript{H}-carbazole

Synthesis of 9-(2-(4-(2-methylallyl)-5-(methylthio)-4\textsubscript{H}-1,2,4-triazol-3-yl)ethyl)-9\textsubscript{H}-carbazole has been depicted in scheme 4. 6a was reacted with iodomethane in presence of potassium carbonate and methanol at room temperature to yield the thiomethylated product. (77\% yield)
10. Biological Evaluation:

PAMs of CB1 receptors can be evaluated for their potentiation of agonist response using different in-vitro assays such as β-Arrestin, cAMP, $[^{35}\text{S}]\text{GTP}\gamma\text{S}$, etc. It is well-known that CB1 agonists have influence on β-Arrestin pathway and its signaling. It has also been proved that CB1 agonists increase cAMP levels by manipulating the cAMP pathway. Therefore these assays have been widely used for quantification and pharmacological characterization of CB1 agonists. In this study, the same assays were performed with PAMs being administered with EC$_{20}$ value of the orthosteric ligand dose, to eliminate the possibility of receptor sensitization by hyper-stimulation or receptor internalization. The potentiation i.e. potency (left shift of dose-response curve or EC$_{50}$) and efficacy (E$_{max}$) of EC$_{20}$ agonist dose was used for quantification and pharmacological characterization of CB1 PAMs.

10.1 β-Arrestin assay:

β-arrestin belongs to a family of proteins that is responsible for signal transduction especially in GPCRs and in turn in CB1 receptors too. β-arrestin plays an important part in receptor recycling on an account of receptor sensitization. β-arrestin binds to the cytoplasmic end of the GPCRs and masks it to prevent its binding to the G-Protein. Further, arrestin links the receptor to the Clathrin like internalization machinery which is responsible for receptor recycling and de-sensitization. The property of β-arrestin to bind to receptor on activation is exploited for quantification of agonist response in CB1. Upon stimulation, β-arrestin are employed for receptor desensitization. The stimulus in this case is by orthosteric ligand CP-55,940, which is considered to be the base signal. The compounds that are to be tested are PAMs then they would not give any signal because they will not stimulate GPCRs in absence of orthosteric ligand. On co-administration with orthosteric ligands (CP-55,940) these compounds if PAM, will increase the signal intensity of the orthosteric ligand. This increase in efficacy is proportional to the potency of the allosteric modulator. Recently, has been proved that β-arrestin pathway is not only involved in receptor internalization as earlier thought, but are also involved in downstream signaling. It has also been showed that several ligands and receptors are biased towards either G-protein or β-arrestin pathway for signaling.

The PathHunter β-Arrestin assay monitors the activation of a GPCR in a homogenous, non-imaging assay format using a technology developed by DiscoveRx called complementation. As
shown below, this utilizes an enzyme fragment complementation (EFC) assay with β-galactosidase (β-Gal) as the functional reporter. The enzyme is split into two complementary portions expressed as fusion proteins in the cell. The Enzyme Acceptor (EA) is fused to β-Arrestin and the ProLink donor peptide is fused to the GPCR of interest.\(^{36}\)

Upon GPCR stimulation, β-Arrestin is recruited to the receptor for desensitization\(^{34}\), bringing the two fragments of β-Gal together and allowing complementation to occur. This will generate an active enzyme that can convert a chemiluminescent substrate and generate an output signal detectable on a standard microplate reader (Figure 7).\(^{36}\)

![Figure 7:β-Arrestin Assay](image)

**10.2 cAMP assay:**

DiscoveRx have developed a panel of cell lines stably expressing non-tagged GPCRs that signal through cAMP. The Hit Hunter cAMP Hunter assay monitors the activation of a GPCR via Gi and Gs secondary messenger signaling in a homogenous, non-imaging assay format using a technology developed by DiscoveRx called complementation. This utilizes an enzyme fragment complementation (EFC) assay with β-galactosidase (β-Gal) as the functional reporter. The enzyme is split into two complementary portions. Pro-Label donor peptide is fused to cAMP and in the assay competes with cAMP generated by cells for binding to a cAMP-specific antibody. Active β-Gal is formed by complementation with EA to any unbound ED-cAMP. The active enzyme can convert a chemiluminescent substrate to generate an output signal detectable on a standard microplate reader (Figure 8).
Figure 8: cAMP Assay

10.1.1 Assay Design: GPCR Arrestin

10.1.1.1 Cell Handling

1. PathHunter cell lines were expanded from freezer stocks in T25 flasks according to standard procedures and maintained in selective growth media prior to assay.

2. Once it was established that the cells were healthy and growing normally, cells were passaged from flasks using cell dissociation reagent and seeded into white walled clear bottom 384-well microplates for compound profiling.

3. For profiling, cells were seeded at a density of 5000 cells per well in a total volume of 20 µL and were allowed to adhere and recover overnight prior to compound addition.

10.1.1.2 Agonist Format:

1. Intermediate dilution of compound stocks were generated such that 5 µL of 5X compound could be added to each well with a final DMSO concentration of 1 % of total volume.

2. For profiling compound in agonist mode, the cells were incubated in the presence of compound at 37°C for 90 minutes (180 minutes for EDG2 and EDG8).
10.1.1.3 Positive Allosteric Modulation Format

1. Agonist (CP-55,940) dose curves were performed the morning of profiling to determine the EC$_{20}$ value for the following allosteric testing with compounds. 5 µL of 5X agonist was added to each well with an equal concentration of vehicle present.

2. EC$_{20}$ agonist concentration was determined directly from agonist dose curve (Fig.7)

3. For allosteric determination, cells were co-incubated with compound in the presence of EC$_{20}$ agonist.

4. 5 µL of 5X compound in 5X EC$_{20}$ agonist was added to cells and incubated at 37°C for 90 minutes (180 minutes for EDG2 and EDG8).

10.1.1.4 Signal Detection

1. Assay signal was generated through a single addition of 12.5 or 15 µL (50 % v/v) of PathHunter Detection reagent cocktail for agonist and antagonist assays respectively followed by one hour incubation at room temperature.

2. Microplates were read following signal generation with a PerkinElmer Envision™ instrument for chemiluminescent signal detection.

10.1.1.5 Data Analysis

1. Dose curves in the presence and absence of compound were plotted using GraphPad Prism or Activity Base.

2. For agonist / PAM mode assays, percentage activity was calculated using the following formula:

\[
\text{% Activity} = 100\% \times \frac{\text{Mean RLU of test sample} - \text{mean RLU of vehicle control [EC}_{20}\text{ for PAM]}}{\text{mean MAX RLU control ligand} - \text{mean RLU of vehicle control [EC}_{20}\text{ for PAM]}}.
\]
10.2.1 Assay Design: cAMP

10.2.1.1 Cell Handling

1. cAMP Hunter cell lines were expanded from freezer stocks in T25 flasks according to standard procedures and maintained in selective growth media prior to assay.

2. Once it was established that the cells were healthy and growing normally, cells were passaged from flasks using cell dissociation reagent buffer and seeded into white walled clear bottom 384-well microplates for compound profiling.

3. For profiling, cells were seeded at a density of 10000 cells per well in a total volume of 20 µL and were allowed to adhere and recover overnight prior to compound addition.

4. Cells were treated the following day using the protocols shown below. cAMP modulation was determined using the DiscoveRx HitHunter cAMP XS+ assay.

10.2.1.2 Gi Agonist Format

1. Media was aspirated from cells and replaced with 15 µL 2:1 HBSS/HEPES : cAMP XS+ Ab reagent.

2. Forskolin dose curves were performed to determine the EC$_{80}$ concentration for subsequent testing with agonist. 5 µL of 4X forskolin was added to each well with an equal concentration of vehicle present.

3. EC$_{80}$ forskolin concentration was determined directly from agonist dose curve.

4. Intermediate dilution of compound stocks were generated such that 5 µL of 4X compound with 4x EC$_{80}$ forskolin could be added to each well with a final vehicle concentration of 1 % of total volume.

5. For profiling compound in agonist mode, the cells were incubated in the presence of compound at 37°C for 30 minutes.
10.2.1.3 Positive Allosteric Modulation Format

1. Media was aspirated from cells and replaced with 10 µL 1:1 HBSS/HEPES: cAMP XS+ Ab reagent.

2. Agonist (CP-55,940) dose-response curves were performed to determine the EC$_{20}$ value for the following testing with compounds. 5 µL of 4X agonist was added to each well with an equal concentration of vehicle present.

3. EC$_{20}$ agonist concentration was determined directly from agonist dose curve. (Fig.8)

4. For allosteric determination, cells were pre-incubated with compound followed by agonist challenge at the EC$_{20}$ concentration.

5. 5 µL of 4X compound was added to cells and incubated at 37°C for 30 minutes.

6. 5 µL of 4X EC$_{20}$ agonist was added to cells and incubated at 37°C for 30 minutes. For G$_i$ coupled GPCRs, EC$_{80}$ forskolin was included.

10.2.1.4 Signal Detection

1. After appropriate compound incubation, assay signal was generated through incubation with 20 µL cAMP XS+ ED/CL lysis cocktail for one hour followed by incubation with 20 µL cAMP XS+ EA reagent for three hours at room temperature.

2. Microplates were read following signal generation with a PerkinElmer Envision™ instrument for chemiluminescent signal detection.
11. Result and Discussion:

GAT236 was a HTS lead compound which was synthesized in our lab and this report mainly focused on optimization of this lead by making variations in defined sites (sites II, site III and site IV) maintaining site I carbazole as constant.

The synthesis of planned compounds had major common steps like a) N-alkylation of carbazole giving required hydrazide in few steps; b) formation of semicarbazide/thiosemicarbazides; c) cyclization to give required triazole analogs.

It was planned to vary chain length between carbazole and triazole rings by 1, 2 and 3 carbon spacers. This was successfully achieved and is shown in schemes 1, 2 and 3.

Alkylation of carbazole is reported by various methods like abstraction of proton from carbazole nitrogen using NaH, followed by treatment with bromoalkylesters. This method was low yielding and required stringent inert conditions and tedious purification. In our hands base catalyzed alkylation using KOH under microwave heating gave better yields in Scheme-1 for 3-(2-(9H-carbazol-9-yl)ethyl)-4-substituted-1H-1,2,4-triazole-5(4H)-oxo/thione analogs. These compounds were mainly characterized using NMR and MS. A typical spectra of final compounds in DMSO(d_6) showed a triazole ‘NH’ as singlet was observed around δ13.7. Typical carbazole ring proton pattern was observed as one doublet corresponding to two protons around δ8.15 which was coupled to another doublet around δ7.55. This was followed by two triplets corresponding for two protons each at 7.45 and 7.22 respectively. In short, 1H NMR signal pattern of two doublets and two triplets was common characteristic of all intermediates of this scheme. With changes in substitution one doublet and triplet overlapped to give a mixed signal which appeared as a multiplet.

Base assisted N-alkylation under MW conditions gave carboxylic acid instead of desired ester, however this reaction was faster, product was easily isolable by simple acidification and filtration, hence was preferred over other methods.

However, this method of N-alkylation did not work in the synthesis of 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione (Scheme-2), hence an alternative route using gamma butyrolactone using sodium methoxide worked well giving good yields of required butanoic acid.
This carboxylic acid in HCl gas in dry methanol gave quantitative yields of desired methyl ester which was converted to hydrazide. Hydrazide was scaled up on gram scale as it was common starting material for the synthesis of variations at sites III and IV. Hydrazide was reacted with isocyanates/isothiocyanates under inert conditions in alcohol to give corresponding substituted semicarbazide/thiosemicarbazides which crystallized out from the alcoholic medium in ice bath. Suspension of these semicarbazide/thiosemicarbazides were taken in aqueous base like NaOH and refluxed for required time. The suspended semicarbazide/thiosemicarbazides slowly dissolved to give clear solution. Upon completion of reaction the pH was adjusted to 1 using cold dil.HCl. The product precipitated out. Recrystallization from methanol can give pure compound. Analytical samples were obtained using flash chromatography.
Table 4: Consolidated list of Compounds with activity data

General Structure

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Table 4: Consolidated list of Compounds with activity data

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11.1 Structure-Activity Relationship:

1. When the chain length (i.e. spacer between carbazole and triazole ring at site II is increased or decreased by one carbon a decrease in potency is seen. However, the E\text{max} was observed to be constant in cAMP assay as seen in GAT236, GAT242 and GAT249.

2. Bulkier groups like phenyl ethyl, adamantyl, benzyl, morpholine at site III were not tolerated and reduced the cAMP response as observed in GAT239, GAT241, GAT247, GAT254 and GAT256.

3. Saturation of parent methylallyl side chain at site III reduced the cAMP response as observed in GAT238, GAT244.

4. Unsaturated groups like allyl and methylallyl at site III were tolerated and increased the cAMP response as exemplified in GAT236, GAT240 and GAT249.

5. Substituting oxygen in place of sulfur at site IV reduced the cAMP response.

6. S-methylation at site IV lead to complete loss of activity which underlines the importance of thione group in the molecule as seen in GAT255.

7. All the compounds were observed to be almost inactive in β-arrestin assay, which indicates functional selectivity of the compounds towards cAMP pathway.
12. Conclusion:

A series of novel positive allosteric modulators of CB1 receptor with carbazole scaffold were successfully synthesized. The molecules synthesized gave an initial idea about the steric, electronic and hydrophobic-hydrophilic requirement of binding of allosteric compounds at the CB1 allosteric site. This study also serves as an initial guideline for the synthesis of newer analogs.

It appears that sites II, III and IV have valuable contribution towards activity and any changes in parent structure at these sites is not tolerated. These compounds when given with EC_{20} dose of orthosteric ligand CP-55,940, potentiated the action of CP-55,940 in multifolds. This potentiation of CP-55,940 response was a measure of quantification of PAM activity.

The EC_{50} and E_{max} were used to develop a short SAR study which provides preliminary information for lead optimization.

From this study it is evident that the changes made in sites II, III and IV did not yield compounds with improved activity profiles in comparison with parent lead GAT236. Hence, it can be hypothesized that changes in site I, i.e. the carbazole ring could be made to improve the PAM activity.
13. Experimental:

3-(9H-carbazol-9-yl)propanoic acid: (2)

In a 5 mL microwave vial 9H-carbazole (1.25 g, 7.48 mmol), potassium hydroxide (3 g, 53.5 mmol) were taken in 3ml of anhydrous DMF. The vial was heated at 120°C for 10 min under microwave conditions then cooled to room temperature. To it was added methyl 3-bromopropanoate (1.5 g, 8.98 mmol), reaction vial was sealed and heated under microwave conditions for 30 min. It was cooled to room temperature, diluted with water, solid precipitated out. It was separated by filtration and filtrate was acidified to pH 2 using ice cold dilute HCl, product precipitated out as a white solid. It was filtered, washed with ice cold water, air dried to give pure 3-(9H-carbazol-9-yl)propanoic acid (1.25 g, 69.9 % yield). M.P: 172-173°C. 1H NMR (500 MHz, CDCl₃) δ 12.38(s, 1H), 8.14(d, 2H, J = 7.5Hz), 7.63(d, 2H, J = 8Hz), 7.46(t, 2H, J = 7Hz), 7.21(t, 2H, J = 7Hz), 4.64(t, 2H, J = 7.5Hz), 2.75(t, 2H, J = 7.5Hz).

3-(9H-carbazol-9-yl)propanoate: (3)

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoic acid (1 g, 4.18 mmol) was taken in 60ml of 0.5M HCl in anhydrous methanol. Reaction was stirred under inert conditions at room temperature for 4h. Solvents were removed under vacuum. Residue was washed with 10% NaHCO₃ solution. Residue was diluted with water, partitioned in DCM: Water, washed with brine. Organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to give methyl 3-(9H-carbazol-9-yl)propanoate (1.01g, 95 % yield). 1H NMR (500 MHz, CDCl₃) δ 8.10(d, 2H, J = 10Hz), 7.51-7.45(m, 4H), 7.28-7.23(m,2H), 4.66(t, 2H, J = 8.5Hz), 3.64(s, 3H), 2.86(t, 2H, J = 9Hz).

3-(9H-carbazol-9-yl)propanehydrazide: (4)

In a 100 ml round bottom flask methyl 3-(9H-carbazol-9-yl)propanoate (980 mg, 3.87 mmol), hydrazine (9919 mg, 310 mmol) were taken in 99% hydrazine hydrate and reaction was heated at 105°C under inert conditions for 6h. It was cooled to room temperature. Solvents were removed under high vacuum. Residue was washed with ice cold water and recrystallized for methanol to
give 3-(9H-carbazol-9-yl)propanoylhydrazide (620mg, 63.3% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.07(d, 2H, J = 8Hz), 7.48-7.42(m, 4H), 7.26-7.22(m, 2H), 6.47(bs, 1H), 4.68(t, 2H, J = 7Hz), 3.62(bs, 2H), 3.48(s, 1H), 2.62(t, 2H, J = 7Hz).

**2-(3-(9H-carbazol-9-yl)propanoyl)-N-(2-methylallyl)hydrazinecarbothioamide: (5a)**

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoylhydrazide (500mg, 1.974 mmol), 3-isothiocyanato-2-methylprop-1-ene (223 mg, 1.974 mmol) were taken in 20ml of anhydrous ethanol and reaction was heated at 105°C under inert conditions for 12h. It was cooled to room temperature. Solvents were removed under high vacuum. The residue was diluted with ice cold water, filtered to give white solid, which was crude 2-(3-(9H-carbazol-9-yl)propanoyl)-N-(2-methylallyl)hydrazinecarbothioamide which was taken for next step.

**2-(3-(9H-carbazol-9-yl)propanoyl)-N-isobutylhydrazinecarbothioamide: (5b)**

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoylhydrazide (500 mg, 1.974 mmol), 1-isothiocyanato-2-methylpropane (341 mg, 2.96 mmol) were taken in 30ml of anhydrous EtOH and reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature, solvents were removed under vacuum, residue was washed with ice cold water, air dried to give 2-(3-(9H-carbazol-9-yl)propanoyl)-N-isobutylhydrazinecarbothioamide (595mg, 82% yield). Product was taken for next step without further purification.

**2-(3-(9H-carbazol-9-yl)propanoyl)-N-phenethylhydrazinecarbothioamide: (5c)**

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoylhydrazide (250mg, 0.987 mmol), (2-isothiocyanatoethyl)benzene (242 mg, 1.480 mmol) were taken in 30ml of anhydrous EtOH and reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature, solvents were removed under vacuum, residue was washed with ice cold water, air dried to give 2-(3-(9H-carbazol-9-yl)propanoyl)-N-phenethylhydrazinecarbothioamide (365mg, 89% yield). Product was taken for next step without further purification.
2-(3-(9H-carbazol-9-yl)propanoyl)-N-allylhydrazinecarbothioamide: (5d)

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoylhydrazide (250 mg, 0.987 mmol), 3-isothiocyanatoprop-1-ene (147 mg, 1.480 mmol) were taken in 30ml of anhydrous EtOH and reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature, solvents were removed under vacuum, residue was washed with ice cold water, air dried to give 2-(3-(9H-carbazol-9-yl)propanoyl)-N-allylhydrazinecarbothioamide (245mg, 70.4 % yield). Product was taken for next step without further purification.

2-(3-(9H-carbazol-9-yl)propanoyl)-N-((3s,5s,7s)-adamantan-1-yl)hydrazinecarbothioamide: (5e)

In a 100 ml round bottom flask 3- (9H-carbazol-9-yl)propanoylhydrazide (100mg, 0.395 mmol), (3s,5s,7s)-1-isothiocyanatoadamantane (99 mg, 0.513 mmol) were taken in 30ml of anhydrous EtOH and reaction was heated at 110 °C under inert conditions for 12h. Reaction flask was cooled to room temperature, solvents were removed under vacuum, residue was washed with ice cold water, air dried to give 2-(3-(9H-carbazol-9-yl)propanoyl)-N-((3s,5s,7s)-adamantan-1-yl)hydrazinecarbothioamide (145mg, 82 % yield). Product was taken for next step without further purification.

2-(3-(9H-carbazol-9-yl)propanoyl)-N-benzylhydrazinecarbothioamide: (5f)

In a 100 ml round bottom flask 3-(9H-carbazol-9-yl)propanoylhydrazide (200mg, 0.790 mmol), (isothiocyanatomethyl)benzene (165 mg, 1.105 mmol) were taken in 20ml, of anhydrous EtOH, reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum, residue was triturated with ice cold water to give white solid, filtered, air dried to give crude 2-(3-(9H-carbazol-9-yl)propanoyl)-N-benzylhydrazinecarbothioamide (260mg, 82 % yield). Product was taken for next step without further purification.
2-(3-(9\textit{H}-carbazol-9-yl)propanoyl)-\textit{N}-(2-morpholinoethyl)hydrazinecarbothioamide: (5g)

In a 100 mL round bottom flask 3-(9\textit{H}-carbazol-9-yl)propanoylhydrazide (250 mg, 0.987 mmol), 4-(2-isothiocyanatoethyl)morpholine (204 mg, 1.184 mmol) in EtOH (Volume: 50 ml), reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature, organic solvents were removed under vacuum, residue was diluted with cold water, filtered, air dried to give crude 2-(3-(9\textit{H}-carbazol-9-yl)propanoyl)-\textit{N}-(2-morpholinoethyl)hydrazinecarbothioamide (335 mg, 80 % yield) which was taken for next step.

2-(3-(9\textit{H}-carbazol-9-yl)propanoyl)-\textit{N}-benzylhydrazinecarboxamide: (5j)

In a 100 ml round bottom flask 3-(9\textit{H}-carbazol-9-yl)propanoylhydrazide (200 mg, 0.790 mmol), (isocyanatomethyl)benzene (147 mg, 1.105 mmol) were taken in 20 ml anhydrous EtOH, reaction was heated at 110°C under inert conditions for 12h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum, residue was triturated with ice cold water to give white solid, filtered, air dried to give crude 2-(3-(9\textit{H}-carbazol-9-yl)propanoyl)-\textit{N}-benzylhydrazinecarboxamide (260 mg, 85 % yield). Product was taken for next step without further purification.

3-(2-(9\textit{H}-carbazol-9-yl)ethyl)-4-(2-methylallyl)-1\textit{H}-1,2,4-triazole-5(4\textit{H})-thione: (6a)

In a 100 ml round bottom flask 2-(3-(9\textit{H}-carbazol-9-yl)propanoyl)-\textit{N}-(2-methylallyl) hydrazinecarbothioamide (500 mg, 1.364 mmol) was suspended in 60 ml of 5% NaOH solution and reaction was refluxed under inert conditions for 10h. It was cooled to room temperature. Volatiles were removed under high vacuum. Residue was diluted with ice cold water, acidized to pH 2 using 10% HCl maintaining temperature at 0°C to give white solid. It was filtered, washed with ice cold water, air dried and recrystallized from methanol to give pure 3-(2-(9\textit{H}-carbazol-9-yl)ethyl)-4-(2-methylallyl)-1\textit{H}-1,2,4-triazole-5(4\textit{H})-thione (365 mg, 77 % yield). $^1$H NMR (500 MHz, DMSO d$_6$) $\delta$ 13.68 (s, 1H), 8.15 (d, 2H, $J = 7.5$Hz), 7.55 (d, 2H, $J = 8$Hz), 7.45 (t, 2H, $J = 8.5$Hz), 7.22(t, 2H, $J = 7.5$Hz), 4.78 (t, 2H, $J = 7.5$Hz), 4.69 (s, 1H), 4.34 (s, 1H), 4.26 (s, 1H), 3.07 (t, 2H, $J = 7.5$Hz), 1.51 (s, 3H). Mass Spectrum m/z [M+H]$^+$ - 349.46
3-(2-(9H-carbazol-9-yl)ethyl)-4-isobutyl-1H-1,2,4-triazole-5(4H)-thione: (6b)

In a 100 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-isobutylhydrazinecarbothioamide (900mg, 2.442 mmol) was taken in 60ml 5% NaOH solution and reaction was refluxed for 8h, then cooled to room temperature. Volatiles were removed under high vacuum. Residue was diluted with ice cold water, acidified to pH 2 using ice cold HCl. The product precipitated out. It was filtered, washed with ice cold water, air dried, recrystallized from methanol to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-isobutyl-1H-1,2,4-triazole-5(4H)-thione (690 mg, 81 % yield). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3} ) δ 12.18 (s, 1H), 8.11 (d, 2H, \( J = 7.5\)Hz), 7.47 (t, 2H, \( J = 8.5\)Hz), 7.36 (d, 2H, \( J = 8.5\)Hz), 7.26(t, 2H, \( J = 7.5\)Hz), 4.79 (t, 2H, \( J = 7.5\)Hz), 3.4 (d, 2H, \( J = 7.5\)Hz), 3.10 (t, 2H, \( J = 7.5\)Hz), 2.11 (sep, 1H), 0.75 (d, 6H, \( J = 6.5\)Hz). Mass Spectrum m/z [M+H]\textsuperscript{+} - 351.48

3-(2-(9H-carbazol-9-yl)ethyl)-4-phenethyl-1H-1,2,4-triazole-5(4H)-thione: (6c)

In a 100 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-phenethylhydrazinecarbothioamide (800mg, 1.921 mmol) was taken in 60ml of 5%NaOH solution and reaction was refluxed under inert conditions for 8h, cooled to room temperature, volatiles were removed under high vacuum, residue was diluted with ice cold water, acidified to pH 2 using ice cold HCl, product precipitated out, filtered, washed with ice cold water, air dried, recrystallized from methanol to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-phenethyl-1H-1,2,4-triazole-5(4H)-thione (610mg, 80 % yield). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3} ) δ 10.63 (s, 1H), 8.09 (d, 2H, \( J = 8\)Hz), 7.46 (t, 2H, \( J = 7\)Hz), 7.27 (t, 2H, \( J = 7.5\)Hz), 6.86(dd, 2H, \( J = 4.5\)Hz, \( J = 1.5\)Hz ), 4.53 (t, 2H, \( J = 7.5\)Hz), 4.09 (t, 2H, \( J = 7\)Hz), 3.72 (t, 2H, \( J = 7\)Hz), 2.90 (t, 2H, \( J = 7\)Hz), 2.43 (t, 2H, \( J = 7.5\)Hz). Mass Spectrum m/z [M+H]\textsuperscript{+} - 399.52

3-(2-(9H-carbazol-9-yl)ethyl)-4-allyl-1H-1,2,4-triazole-5(4H)-thione: (6d)

In a 100 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-allylhydrazinecarbothioamide (850mg, 2.412 mmol) was taken in 60ml of 5% NaOH solution and reaction was refluxed under inert conditions for 8h, cooled to room temperature. Volatiles
were removed under high vacuum. Residue was diluted with ice cold water, acidified to pH 2 using ice cold HCl, product precipitated out. It was filtered, washed with ice cold water, air dried, recrystallized from methanol to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-allyl-1H-1,2,4-triazole-5(4H)-thione (605mg, 75 % yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 11.16 (s, 1H), 8.09 (d, 2H, \(J = 8\text{Hz}\)), 7.47 (t, 2H, \(J = 7.5\text{Hz}\)), 7.34 (d, 2H, \(J = 8.5\text{Hz}\)), 7.27 (t, 2H, \(J = 7.5\text{Hz}\)), 5.74-5.66 (m, 1H), 5.13 (d, 1H, \(J = 10.5\text{Hz}\)), 4.87 (d, 1H, \(J = 17.5\text{Hz}\)), 4.76 (t, 2H, \(J = 7\text{Hz}\)), 4.29 (d, 2H, \(J = 5.5\text{Hz}\)), 3.11 (t, 2H, \(J = 7.5\text{Hz}\)). Mass Spectrum m/z [M+H]\(^+\) - 335.44

3-(2-(9H-carbazol-9-yl)ethyl)-4-((3s,5s,7s)-adamantan-1-yl)-1H-1,2,4-triazole-5(4H)-thione: (6e)

In a 100 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-((3s,5s,7s)-adamantan-1-yl) hydrazinecarbothioamide (300mg, 0.672 mmol) was taken in 60ml of 5%NaOH solution and reaction was refluxed under inert conditions for 8h, cooled to room temperature. Volatiles were removed under high vacuum. The residue was diluted with ice cold water, acidified to pH 2 using ice cold HCl, product precipitated out. It was filtered, washed with ice cold water, air dried, recrystallized from methanol to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-((3s,5s,7s)-adamantan-1-yl)-1H-1,2,4-triazole-5(4H)-thione (175mg, 60.8 % yield). \(^1\)H NMR (500 MHz, DMSO d\(_6\)) \(\delta\) 9.82 (s, 1H), 8.99 (s, 1H), 7.47 (t, 2H, \(J = 7.5\text{Hz}\)), 7.34 (d, 2H, \(J = 8.5\text{Hz}\)), 7.27 (t, 2H, \(J = 7.5\text{Hz}\)), 4.6 (t, 2H, \(J = 6.5\text{Hz}\)), 2.72 (t, 2H, \(J = 6\text{Hz}\)), 2.05-1.78 (m, 9H), 1.55 (bs, 6H). Mass Spectrum m/z [M+H]\(^+\) - 429.59

3-(2-(9H-carbazol-9-yl)ethyl)-4-benzyl-1H-1,2,4-triazole-5(4H)-thione: (6f)

In a 250 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-benzylhydrazinecarbothioamide (250mg, 0.621 mmol) was taken in 30ml of 5%NaOH solution to it was added 80ml ethanol to give a clear solution, reaction was heated at 135°C for 12h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum. The residue was diluted with cold water, cooled to 0°C, acidified to pH 1 using ice cold dilute HCl. The product precipitated out. It was filtered, washed with ice cold water, air dried to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-benzyl-1H-1,2,4-triazole-5(4H)-thione (205 mg, 86 % yield). \(^1\)H
NMR (500 MHz, DMSO d₆) δ 13.76(s, 1H), 8.13 (d, 2H, J = 8Hz), 7.42-7.33 (m, 4H), 7.32-7.25 (m, 3H), 7.19 (t, 2H, J = 8Hz), 7.15 (d, 2H, J = 7.5Hz), 5.13 (s, 2H), 4.61 (t, 2H, J = 7.5Hz), 3.01 (t, 2H, J = 7Hz). Mass Spectrum m/z [M+H]+ - 385.50

3-(2-(9H-carbazol-9-yl)ethyl)-4-(2-morpholinoethyl)-1H-1,2,4-triazole-5(4H)-thione: (6g)

In a 100 mL round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-(2-morpholinoethyl) hydrazinecarbothioamide (130mg, 0.305 mmol), sodium hydroxide (12.22 mg, 0.305 mmol) were taken in 60ml of 20% NaHCO₃ and reaction was heated at 120°C for 10h. Reaction was cooled to room temperature, concentrated on vacuum to remove volatile impurities, diluted with water, acidified to pH 2 using dil. HCl, product precipitated out, filtered, washed with ice cold water, air dried to give crude product, purified by flash chromatography using LUKNOVA column (2:1 Hex/EtOAc; 40g column) to give pure 3-(2-(9H-carbazol-9-yl)ethyl)-4-(2-morpholinoethyl)-1H-1,2,4-triazole-5(4H)-thione (90mg, 72.3 % yield). ¹H NMR (500 MHz, CDCl₃) δ 11.95(s, 1H), 8.1 (d, 2H, J = 8Hz), 7.45(t, 2H, J = 8.5Hz), 7.32(d, 2H, J = 8.5Hz), 7.26 (t, 2H, J = 6.5Hz), 4.77 (t, 2H, J = 7Hz), 3.53-3.47(m, 6H), 3.19 (t, 2H, J = 7Hz), 2.39 (t, 2H, J = 6Hz), 2.22 (t, 4H, J = 5Hz). Mass Spectrum m/z [M+H]+ - 408.53

9-(2-(4-(2-methylallyl)-5-(methylthio)-4H-1,2,4-triazol-3-yl)ethyl)-9H-carbazole: (6h)

In a 100 ml round bottom flask 3-(2-(9H-carbazol-9-yl)ethyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione (100mg, 0.287 mmol), iodomethane (50.9 mg, 0.359 mmol), potassium carbonate (159 mg, 1.148 mmol) were taken in MeOH (60 ml), and reaction was stirred at room temperature under inert conditions for 4hr. Complete conversion of starting material was monitored by TLC. Solvents were removed under vacuum, residue was diluted with water, partitioned in DCM: Water, washed with brine, the organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to give crude product, purified by flash chromatography using LUKNOVA column (4:1 Hex/EtOAc; 40g column) to give pure 9-(2-(4-(2-methylallyl)-5-(methylthio)-4H-1,2,4-triazol-3-yl)ethyl)-9H-carbazole (80mg, 77 % yield). ¹H NMR (500 MHz, CDCl₃) δ 8.07 (d, 2H, J=8Hz), 7.04 (t, 2H, J=8Hz), 7.26-7.20 (m, 4H), 4.76 (t, 2H, J=7Hz), 4.67 (s, 1H), 4.07 (s, 1H), 3.41 (s, 2H), 3.13 (t, J=7Hz, 2H), 2.50 (s, 1H), 1.35 (s, 1H).
3-(2-(9H-carbazol-9-yl)ethyl)-4-phenethyl-1H-1,2,4-triazol-5(4H)-one: (6i)

In a 250 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-phenethylhydrazinecarboxamide (250mg, 0.624 mmol) was taken in 50ml of 5% NaOH solution, reaction was refluxed for 8h. Reaction flask was cooled in ice bath, acidified to pH 1 using ice cold dilute HCl to give white solid. It was filtered, washed with ice cold water, air dried to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-phenethyl-1H-1,2,4-triazol-5(4H)-one (185 mg, 80 % yield). 1H NMR (500 MHz, DMSO d6) δ 11.52(s, 1H), 8.17 (d, 2H, J = 8Hz), 7.47-7.42(m, 4H), 7.23-7.14(m, 4H), 6.93(dd, 2H, J = 8Hz, J = 2Hz), 4.57(t, 2H, J = 7Hz), 3.45(t, 2H, J = 7Hz), 2.63(m, 4H). Mass Spectrum m/z [M+H]+ - 369.43

3-(2-(9H-carbazol-9-yl)ethyl)-4-benzyl-1H-1,2,4-triazol-5(4H)-one: (6j)

In a 250 ml round bottom flask 2-(3-(9H-carbazol-9-yl)propanoyl)-N-benzylhydrazinecarboxamide (250mg, 0.647 mmol) was taken in 30ml of 5% NaOH solution to it was added 80ml ethanol to give a clear solution, reaction was heated at 135°C for 12h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum. The residue was diluted with cold water, cooled to 0°C, acidified to pH 1 using ice cold dilute HCl. The product precipitated out. It was filtered, washed with ice cold water, air dried to give 3-(2-(9H-carbazol-9-yl)ethyl)-4-benzyl-1H-1,2,4-triazol-5(4H)-one (195mg, 82 % yield). 1H NMR (500 MHz, DMSO d6) δ 13.75(s, 1H), 8.13 (d, 2H, J = 8Hz), 7.41-7.32 (m, 4H), 7.31-7.25 (m, 3H), 7.19 (t, 2H, J = 8Hz), 7.15 (d, 2H, J = 7Hz), 5.12 (s, 2H), 4.61 (t, 2H, J = 7Hz), 3.01 (t, 2H, J = 7Hz). Mass Spectrum m/z [M+H]+ - 369.43

4-(9H-carbazol-9-yl)butanoic acid: (7)

In a 20 mL microwave vial 9H-carbazole (2 g, 11.96 mmol), dihydrofuran-2(3H)-one (2.059 g, 23.92 mmol), sodium ethoxide (3.26 g, 47.8 mmol) were taken in anhydrous NMP, the vial was sealed and reaction was heated at 200°C under microwave conditions for 40min, cooled to room temp, diluted with water, partitioned in DCM: Water, water layer was cooled to 0°C was acidified to pH 1 using cold dilute HCl, product precipitated out as white solid, washed with cold
water, air dried to give 4-(9H-carbazol-9-yl)butanoic acid (2.58g, 85 % yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.11 (d, 2H, $J = 8$Hz), 7.49-7.41(m, 4H), 7.23(dt, 2H, $J = 7$ Hz, $J = 1.0$ Hz), 4.41(t, 2H, $J = 7.5$ Hz), 2.43(t, 2H, $J = 7.5$ Hz), 2.23(q, 2H, $J = 7$Hz).

**methyl 4-(9H-carbazol-9-yl)butanoate: (8)**

In a 100 ml round bottom flask 4-(9H-carbazol-9-yl)butanoic acid (1.45 g, 5.72 mmol) was dissolved in 60ml (0.5M) HCl in anhydrous MeOH and the reaction was stirred at room temperature under inert conditions for 6h. Organic solvents were removed under vacuum, residue was neutralized with saturated NaHCO$_3$ solution and diluted with water, partitioned in DCM: Water, combined DCM layers were washed with brine, the organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to give crude product which was purified by flash chromatography column (4:1 Hex/EtOAc; 80g column) to give pure methyl 4-(9H-carbazol-9-yl)butanoate (1.35g, 88 % yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.10 (d, 2H, $J = 8$Hz), 7.49-7.40(m, 4H), 7.23(dt, 2H, $J = 7$ Hz, $J = 1.0$ Hz), 4.40(t, 2H, $J = 7.5$ Hz), 3.67(s, 3H), 2.36(t, 2H, $J = 7.5$ Hz), 2.22(q, 2H, $J = 7$Hz).

**4-(9H-carbazol-9-yl)butanehydrazide: (9)**

In a 250 ml round bottom flask methyl 4-(9H-carbazol-9-yl)butanoate (2 g, 7.48 mmol) was taken in 80ml MeOH, to it was added 15ml of 98% hydrazine hydrate, reaction was heated at 105°C for 8h. Reaction flask was cooled to room temperature, solvents were removed under vacuum and residue was triturated with ice cold water to give white solid, which was sufficiently pure to be taken for next step.

**2-(4-(9H-carbazol-9-yl)butanoyl)-N-(2-methylallyl)hydrazinecarbothioamide: (10)**

In a 100 ml round bottom flask 4-(9H-carbazol-9-yl)butanoylhydrazide (250mg, 0.935 mmol), 3-isothiocyanato-2-methylprop-1-ene (127 mg, 1.122 mmol) were taken in anhydrous EtOH, reaction was heated at 105 °C under inert conditions for 12h. Reaction flask was cooled to room temperature, organic solvents were removed under vacuum. Residue was triturated in ice cold
water to give white solid, filtered, washed with ice cold water, air dried to give white solid product 2-(4-(9H-carbazol-9-yl)butanoyl)-N-(2-methylallyl)hydrazinecarbothioamide which was not isolated and taken for next step.

3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione: (11)

In a 250 ml round bottom flask 2-(4-(9H-carbazol-9-yl)butanoyl)-N-(2-methylallyl)hydrazinecarbothioamide (175mg, 0.460 mmol) was taken in 5% NaOH and the reaction was heated at 120°C for 12h. Reaction flask was cooled in ice cold water, acidified to pH 1 using ice cold dil. HCl, white solid precipitated out. It was filtered, washed with cold water, air dried to give crude product which was purified by flash chromatography column (3:1 Hex/EtOAc; 40g column) to give pure 3-(3-(9H-carbazol-9-yl)propyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione (108mg,, 64.8 % yield). 1H NMR (500 MHz, DMSO d 6) δ 13.62(s, 1H), 8.14 (d, 2H, J = 7.5Hz), 7.59 (d, 2H, J = 7.5Hz), 7.43 (t, 2H, J = 7.5Hz), 7.19 (t, 2H, J = 7.5Hz), 4.66(s, 1H), 4.49 (t, 2H, J = 7.5Hz), 4.40 (s, 1H), 4.25 (s, 1H), 2.59 (t, 2H, J = 6.5Hz), 2.49 (s, 2H), 2.14 (t, 2H, J = 7.5Hz), 5.54 (s, 3H). Mass Spectrum m/z [M+H]+ - 363.49

2-(9H-carbazol-9-yl)acetic acid: (12)

In a 20 mL microwave vial 9H-carbazole (1.5 g, 8.97 mmol), potassium hydroxide (2.013 g, 35.9 mmol) were taken in 10ml of anhydrous DMF and the reaction was heated at 120°C for 15 min under microwave conditions, cooled to room temperature, followed by addition of ethyl 2-bromoacetate (1.498 g, 8.97 mmol), the vial was sealed and reaction was heated at 120°C under microwave conditions for 30min. Cooled to room temperature, diluted with water, solid separated was filtered. Filtrate was acidified to pH 2 using cold dilute HCl to give white precipitate. It was washed with ice cold water to give pure 2-(9H-carbazol-9-yl)acetic acid (1.2 g, 59.4 % yield). M.P: 212-214°C. 1H NMR (500 MHz, CDCl3) δ 8.15(d, 2H, J = 7Hz), 7.54(d, 2H, J = 8Hz), 7.43(dd, 2H, J = 8Hz), 5.22(s, 2H).
methyl 2-(9\textit{H}-carbazol-9-yl)acetate: (13)

In a 100 ml round bottom flask 2-(9\textit{H}-carbazol-9-yl)acetic acid (1.0 g, 4.44 mmol) was dissolved in 0.5M HCl in 80ml of anhydrous MeOH, reaction was stirred at room temperature under inert conditions for 12h. Reaction was quenched by neutralizing with saturated NaHCO$_3$ solution. Solvents were removed under vacuum. Residue was diluted with water. Product partitioned in DCM: Water, washed with brine, the organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to give crude product methyl 2-(9\textit{H}-carbazol-9-yl)acetate (945 mg, 89 % yield). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.10(d, 2H, $J = 8$Hz), 7.47(dd, 2H, $J = 8$Hz, $J = 1.5$Hz), 7.33(d, 2H, $J = 8.5$Hz), 7.26(d, 2H, $J = 7.5$Hz), 5.01(s, 2H), 3.71(s, 2H).

2-(9\textit{H}-carbazol-9-yl)acetohydrazide: (14)

In a 100 mL round bottom flask methyl 2-(9\textit{H}-carbazol-9-yl)acetate (1.8 g, 7.52 mmol) was dissolved in 50ml of MeOH, 10ml of 98% hydrazine was added and reaction was heated at 110°C for 5h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum. Residue was diluted with minimal of ice cold water, white product precipitated out. It was filtered, air dried to give 2-(9\textit{H}-carbazol-9-yl)acetohydrazide (1.6 g, 89 % yield), this was taken to next step without further purification.

2-(2-(9\textit{H}-carbazol-9-yl)acetyl)-N-(2-methylallyl)hydrazinecarbothioamide: (15a)

In a 100 ml round bottom flask 2-(9\textit{H}-carbazol-9-yl)acetohydrazide (400 mg, 1.672 mmol), 3-isothiocyanato-2-methylprop-1-ene (229 mg, 2.023 mmol) were taken in 30ml of anhydrous ethanol. The reaction was refluxed under inert conditions for 6h. Organic solvents were removed under vacuum. Reaction flask was cooled in ice bath, triturated with ice cold water to give white solid. It was filtered, air dried to give 2-(2-(9\textit{H}-carbazol-9-yl)acetyl)-N-(2-methylallyl)hydrazinecarbothioamide (510mg, 87 % yield) this was taken for next step without further purification.
2-(2-(9H-carbazol-9-yl)acetyl)-N-phenethylhydrazinecarbothioamide: (15b)

In a 100 ml round bottom flask 2-(9H-carbazol-9-yl)acetohydrazide (350mg, 1.463 mmol), (2-isothiocyanatoethyl)benzene (287 mg, 1.755 mmol) were taken in 25ml of anhydrous EtOH. The reaction was heated at 105°C under inert conditions for 6h. Organic solvents were removed under vacuum, reaction flask was cooled in ice bath, triturated with ice cold water to give white solid, filtered, air dried to give 2-(2-(9H-carbazol-9-yl)acetyl)-N-phenethylhydrazinecarbothioamide (390mg, 66.2 % yield). Product was taken for next step without further purification.

2-(2-(9H-carbazol-9-yl)acetyl)-N-isobutylhydrazinecarbothioamide: (15c)

In a 100 ml round bottom flask 2-(9H-carbazol-9-yl)acetohydrazide (350mg, 1.463 mmol), 1-isothiocyanato-2-methylpropane (202 mg, 1.755 mmol) were taken in 30ml of anhydrous EtOH, reaction was heated at 105°C under inert conditions for 6h. Organic solvents were removed under vacuum. The reaction flask was cooled in ice bath and triturated with ice cold water to give white solid, filtered, air dried to give 2-(2-(9H-carbazol-9-yl)acetyl)-N-isobutylhydrazinecarbothioamide (360mg, 69.4 % yield). Product was taken for next step without further purification.

2-(2-(9H-carbazol-9-yl)acetyl)-N-allylhydrazinecarbothioamide: (15d)

In a 100 ml round bottom flask 2-(9H-carbazol-9-yl)acetohydrazide (300mg, 1.254 mmol), 3-isothiocyanatoprop-1-ene (149 mg, 1.505 mmol) were taken in 25ml of anhydrous EtOH, reaction was heated at 105°C under inert conditions for 6h. Reaction flask was cooled to room temperature. Organic solvents were removed under vacuum, residue was triturated with ice cold water to give white solid, filtered, air dried to give crude 2-(2-(9H-carbazol-9-yl)acetyl)-N-allylhydrazinecarbothioamide (385mg, 91 % yield). Product was taken for next step without further purification.
3-((9H-carbazol-9-yl)methyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione: (16a)

In a 250 ml round bottom flask 2-(2-(9H-carbazol-9-yl)acetyl)-N-(2-methylallyl)hydrazinecarbothioamide (200mg, 0.567 mmol) was taken in 80ml of 5% NaOH solution, reaction was refluxed for 12h. Reaction flask was cooled in ice bath, acidified to pH 1 using ice cold dilute HCl to give white solid. It was filtered, washed with ice cold water, air dried to give 3-((9H-carbazol-9-yl)methyl)-4-(2-methylallyl)-1H-1,2,4-triazole-5(4H)-thione (110mg, 58.0 % yield). $^1$H NMR (500 MHz, DMSO d$_6$) $\delta$ 13.5 (d, 1H, $J = 2.5$Hz), 8.16 (d, 2H, $J = 8$Hz), 7.53 (d, 2H, $J = 8.5$Hz), 7.45 (t, 2H, $J = 8$Hz), 7.23 (t, 2H, $J = 8$Hz), 5.72 (s, 2H), 4.53 (s, 2H), 4.44 (s, 1H), 3.94 (s, 1H), 1.37 (s, 1H). Mass Spectrum m/z [M+H]$^+$ - 335.44

3-((9H-carbazol-9-yl)methyl)-4-phenethyl-1H-1,2,4-triazole-5(4H)-thione: (16b)

In a 250 ml round bottom flask 2-(2-(9H-carbazol-9-yl)acetyl)-N-phenethylhydrazinecarbothioamide (200mg, 0.497 mmol) was taken in 80ml of 5% NaOH solution. The reaction was refluxed for 12h. Reaction flask was cooled in ice bath and acidified to pH 1 using ice cold dilute HCl to give white solid. It was filtered, washed with ice cold water, air dried to give 3-((9H-carbazol-9-yl)methyl)-4-phenethyl-1H-1,2,4-triazole-5(4H)-thione (100mg, 52.3 % yield). $^1$H NMR (500 MHz, DMSO d$_6$) $\delta$ 13.76 (s, 1H), 8.2 (d, 2H, $J = 8$Hz), 7.50-7.43 (m, 4H), 7.28-7.23 (m, 5H), 6.76 (m, 2H), 5.60 (s, 2H), 4.06 (t, 2H, $J = 8$Hz), 2.42 (t, 2H, $J = 8$Hz). Mass Spectrum m/z [M+H]$^+$ - 385.50

3-((9H-carbazol-9-yl)methyl)-4-isobutyl-1H-1,2,4-triazole-5(4H)-thione: (16c)

In a 250 ml round bottom flask 2-(2-(9H-carbazol-9-yl)acetyl)-N-isobutylhydrazinecarbothioamide (200mg, 0.564 mmol) was taken in 80ml of 5% NaOH solution. The reaction was refluxed under inert conditions for 12h. Reaction flask was cooled in ice bath, acidified to pH 1 using ice cold dilute HCl to give white solid. It was filtered, washed with ice cold water, air dried to give 3-((9H-carbazol-9-yl)methyl)-4-isobutyl-1H-1,2,4-triazole-5(4H)-thione (140mg, 73.7 % yield). $^1$H NMR (500 MHz, DMSO d$_6$) $\delta$ 13.63 (s, 1H), 8.12 (d, 2H, $J = 8$Hz), 7.6 (d, 2H, $J = 8$Hz), 7.46 (t, 2H, $J = 7.5$Hz), 7.24 (t, 2H, $J = 7.5$Hz), 5.82 (s, 2H),
3.77 (d, 2H, \( J = 8 \text{Hz} \)), 2.185(sep, 1H), 0.73 (d, 6H, \( J = 7 \text{Hz} \)). Mass Spectrum m/z \([\text{M+H}]^+\) - 337.45

3-((9H-carbazol-9-y1)methyl)-4-allyl-1H-1,2,4-triazole-5(4H)-thione:(16d)

In a 250 ml round bottom flask 2-(2-(9H-carbazol-9-yl)acetyl)-N-allylhydrazinecarbothioamide (200 mg, 0.591 mmol) was taken in 80ml of 5% NaOH solution, reaction was refluxed for 8h. Reaction flask was cooled in ice bath and acidified to pH 1 using ice cold dilute HCl, to give white solid. It was filtered, washed with ice cold water, air dried to give pure 3-((9H-carbazol-9-yl)methyl)-4-allyl-1H-1,2,4-triazole-5(4H)-thione (135mg, 71.3 % yield). \(^1\)H NMR (500 MHz, DMSO d\(_6\)) \( \delta \) 13.69(s, 1H), 8.17 (d, 2H, \( J = 8 \text{Hz} \)), 7.56 (d, 2H, \( J = 8.5 \text{Hz} \)), 7.45 (t, 2H, \( J = 8.5 \text{Hz} \)), 7.23 (d, 2H, \( J = 8 \text{Hz} \)), 5.75 (s, 2H), 5.62-5.5(m, 1H), 4.95 (d, 1H, \( J = 10.5 \text{Hz} \)), 4.74 (d, 1H, \( J = 17 \text{Hz} \)), 4.66 (d, 2H, \( J = 5 \text{Hz} \)). Mass Spectrum m/z \([\text{M+H}]^+\) - 321.41
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