RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Design and Synthesis of Novel Carbazolo-Thiazoles by Molecular Hybridization Approach as Potential Anti-mycobacterial Agents

Mahamadhanif S. Shaikh, Mahesh B. Palkar, Harun M. Patel, Rajesh A. Rane, Wesam S. Alwan, Mahidansha M. Shaikh, Iqbal M. Shaikh, Girish A. Hampannavar, Rajshekhar Karpoormath*

Department of Pharmaceutical Chemistry, Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban – 4000, South Africa.

***Corresponding author**

E-mail: karpoormath@ukzn.ac.za, rvk2006@gmail.com

Tel no.: +27(0)312607179, +27721107207; Fax No.:+27(0)312607792

Abstract

Various substituted carbazolo-thiazoles **6** (**a-o**) have been synthesized in good yields by molecular hybridization approach. These synthesized compounds were evaluated for their *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain at National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA. Among the tested series, compound **6c** (MIC = 21 μM) exhibited the most promising anti-mycobacterial activity. A brief structure activity relationship (SAR) studies revealed that electron donating groups (OCH_3 and OH) especially on the phenyl ring of thiazole motif exhibited positive correlation with anti-mycobacterial activity. In addition, they displayed low cytotoxicity against a mammalian Vero cell line using MTT assay, thereby providing a high therapeutic index. This study reveals the significance of molecular hybridization and the scope for the development of carbazole clubbed thiazole compounds as potential anti-mycobacterial agents.

Keywords: Carbazole; Thiazole; Anti-mycobacterialactivity; Cytotoxicity; NIAID.

1 Introduction

Tuberculosis is the leading cause of mortality from a single infectious agent and is responsible for more than three million deaths worldwide every year.[1] Despite half a century of anti-tubercular chemotherapy, there are still 8-10 million new cases of active tuberculosis each year and nearly two billion individuals are believed to harbor latent tuberculosis.In coincidence with the spread of HIV infection,[2] tuberculosis is today amongst the universal health threats. India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDR-TB.[3] It is estimated that nearly 5, 00,000 people die from the disease more than 1000 per day.[4] The combination of long treatment duration (6–9 months), increased incidence of (multi or extensive) drug resistance, co-morbidity with HIV-AIDS and lack of investment in anti-infective drug discovery has led to a situation, where the discovery, development and introduction of new treatments for tuberculosis has become critical.[5] Therefore the present-day situation necessitates the re-engineering and repositioning of old drug families for developing new anti-mycobacterial to achieve effective TB control even against the resistant forms of TB.[6-8]

Natural products or their direct derivatives, play crucial role in modern day chemotherapy of tuberculosis.[9] The promising natural anti-mycobacterials include carbazole compounds (**I** to **III**) and carbazole alkaloids, such as Clausine (**IV**) and Micromeline (**V**), isolated independently from several sources[10] (**Fig. 1**). Synthetic analogues of natural carbazole alkaloid exhibited significantly improved *in vitro* as well as *in vivo* anti-mycobacterial activity against *M. tuberculosis*H_{37}Rv.[11-15] On the other hand, thiazole conjugated with a wide range of heterocyclic moieties (**VII** to **IX**) were reported to exhibit potent anti-mycobacterial activity[16, 17](**Fig. 1**). In view of these facts and as a part of our enduring studies in the area of anti-mycobacterial agents[18-21] it was envisaged to synthesize biologically active hybrid pharmacophores (carbazole

and thiazole) by integrating them in one molecular platform for biological evaluation. This approach was an attempt to investigate the possible synergistic influence of such structural hybridizations on the anticipated activity, hoping to discover a new lead molecule. In the present study, we report the synthesis and *in vitro* anti-mycobacterial activity of some novel carbazolo-thiazoles **6(a-o)** hybrid molecules against *M. tuberculosis* $H_{37}Rv$ and their *in vitro* cytotoxicity profile.

2 Chemistry

The synthesis of a novel series of thiazolyl substituted carbazole hydrazine analogues **6** (**a-o**) was achieved through an efficient and versatile synthetic route as illustrated in **Scheme 1**. It is quite clear that unique final steps were involved in the synthesis of target compounds, which have structural variations on the hydrazine bond attached to 3rd position of carbazole ring. The various starting material i.e. 9-methyl-carbazole (**1**) was prepared from commercially available carbazole by *N*-methylation process using iodo-methane and sodium hydride. Further, compound (**1**) was subjected to formylation reaction (Vilsmayer Hack) in the presence of $POCl_3$ and DMF to afford 9-methyl-carbazole-3-carbaldehyde (**2**),which was further treated with thiosemicarbazide in the presence of methanol and catalytic glacial acetic acid to yield 1-((9-methyl-carbazol-6-yl methylene)thiosemicarbazide (**3**). In addition, the desired title compounds **6** (**a-o**) were synthesized by Hantzsch cyclo-condensation reaction of compound (**3**) with various appropriately substituted α-Bromoaromatic/heteroaromatic ketones **5** (**a-o**) in the presence of methanol.[22] Structures of key intermediate compound (**3)** and its corresponding thiazolyl substituted carbazole hydrazine derivatives **6** (**a-o**) were established on the basis of their physicochemical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and HRMS) data.

3 Results and Discussion

3.1 Spectral Studies

All the newly synthesized compounds exhibited acceptable analysis of their anticipated structures, which are summarized in experimental section. In general, the IR spectrum of compound (**3**) revealed typical absorption bands around 3373.23 cm^{-1} for N-H (of NH_2 group), 1619.92 cm^{-1} for C=N and1145.80cm^{-1} for C=S groups. This was further substantiated from ^1H NMR spectrum of compound (**3**), which exhibited the presence of singlet signals at around δ11.38, 8.58, 8.21 and 3.89 ppm for the N-H proton of CH= N protons, 4th proton of carbazole ring and *N*-CH_3 protons indicating its formation by a simple carbon- nitrogen bond formation process in the presence of thiosemicarbazide and acetic acid as catalyst.[22] The IR spectrum of the compounds **6** (**a**-**o**) showed disappearance of the typical peaks due to NH_2 group (N-H Str) and appearance of moderately strong bands around 3319-3074 cm^{-1} and 1629.47-1572.25 cm^{-1}, which are characteristic of the N-H and C=N groups respectively. In the case of compounds **6i** and **6j**, the peaks appeared around 3454.28-3342.53 cm^{-1}due to OH group, while the prominent bands around 1721.35-1711.27 cm^{-1} was attributed to C=O in compounds **6n** and **6o**.^{1}H-NMR spectrum (400 MHz) of these title compounds **6** (**a-o**) recorded in DMSO-d_6 displayed some distinctive singlet signals at around δ 12.59-8.38 ppm for N-H proton, δ 8.45-8.19 ppm for CH=N proton at 3rd position of the carbazole and δ 8.36-8.20 ppm for a aromatic proton at 4th position of the carbazole nucleus. Further, the most informative singlet signal resonated between δ 7.92-7.20 ppm, which was attributed to the aromatic proton (H-5) of the thiazole ring indicating its formation through cyclo-condensation process. This observation was consistent with previously reported similar compounds.[22] The unique singlet signal resonating around δ 3.91-3.87 ppm indicated the presence of methyl protons (*N*-CH_3) on the 9th position of the

carbazole ring. Various aromatic/hetero-aromatic proton signals were observed between δ 8.31-6.92 ppm. These findings were further substantiated from their respective ^{13}C-NMR spectra of the title compounds. The characteristic ^{13}C-NMR signals observed at around δ 148.72-141.12 and 110.21-101.48 ppm were assigned to carbons of Schiff base (CH=N) and C-5 of thiazole ring. A prominent carbon signal observed around δ 29.65-28.62 ppm, indicated the presence of N-CH_3 carbon in the title compounds. In case of compounds **6n** and **6o**, a characteristic signal appeared around δ 167.86-158.75 ppm due to the presence of carbonyl carbon (C=O) of coumarin ring, while various aromatic/hetero-aromatic carbons resonated around δ 134.76-111.08 ppm.

3.2 Antimycobacterial Activity

In-vitro evaluation of the anti-mycobacterial activity of newly synthesized title compounds [(**3**, and **6** (**a-o**) was carried out at Infectious Disease Research Institute (IDRI) within the **National Institute of Allergy and Infectious Diseases** (NIAID) screening program, Bethesda, MD, USA. Minimum Inhibitory Concentration (MIC) was determined against *M. tuberculosis* strain H_{37}Rv grown under aerobic conditions by using a dual read-out (OD_{590} and fluorescence) assay procedure.[23-25] The assay was based on measurement of growth in liquid medium of a fluorescent reporter strain of H37Rv, where the readout was either optical density (OD) or fluorescence. The use of two readouts minimizes problems caused by compound precipitation or auto fluorescence. The purpose of the screening program was to provide a resource whereby new experimental compounds could be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*. The result of anti-mycobacterial activity is presented in **Table 1**. All the synthesized compounds exhibited an interesting activity profile with MIC ranging from 21 to 200 μM against the tested mycobacterial strain. We also studied the effects of various aromatic/hetero-aromatic substituents

at 4th position of the thiazole ring, which was in turn attached to 3rd position of 9-methyl carbazole through a hydrazine linkage. Amongst them compound **6c** (MIC=21 μM) with 3,4-dimethoxyl (OCH_3) groups on aromatic nucleus exhibited most promising anti-mycobacterial activity with IC_{50} value of 28 μM. In the case of compound **6b** (MIC=32 μM), with an unsubstituted aromatic ring exhibited good inhibitory activity of IC_{50} value of 35μM. The activity was considerably affected by substituents at position-4 on the thiazole nucleus. In general, the electron donating groups on aromatic ring have greatly contributed for anti-mycobacterial activity while the electron withdrawing substituents have caused decrease in activity. It was observed that compounds **6i** (MIC = 31 μM) and **6j** (MIC = 37 μM) with hydroxyl (OH) group at 2nd and 4th position of aromatic nucleus displayed notable activity with IC_{50} values 39 and 41 μM respectively. Compounds **6a**, **6d**, **6e**, **6f**, **6h**, **6k** and **6l** having either nitro or halogen groups on aromatic ring found to be least active with MIC value >200 μM. Replacing substituted phenyl group at 4th position of thiazole ring with heterocyclic groups such as thiophene (**6m**) and coumarin (**6n** and **6o**) resulted in no significant change in anti-mycobacterial activity with MIC value of >200 μM. In three cases we have replaced the aromatic ring with thiophene (**6m**) and coumarin (**6n** and **6o**) ring systems, which resulted in activity with a MIC value of >200 μM.

3.3 Cytotoxic Activity

The synthesized title compounds [(**3**, and **6** (**a-o**)] were further screened for cytotoxicity (IC_{50}) in a mammalian Vero cell line. After 72 h of exposure, viability of cell was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay and results are summarized in **Table 1**. The sixteen tested derivatives showed IC_{50} values ranging from 202.4 to 440.8 μM. All the synthesized compounds

displayed no significant activity against mammalian Vero cell line at concentrations <100 μM. Among the series tested, compounds **6a**, **6b**, **6e**, **6h**, **6i**, **6n** and **6o** demonstrated lower toxicity with IC_{50} values of >300 μM. These results are important, since compounds with increased cytoliability are much attractive in the development of new chemical entities for the treatment of TB. This is primarily due to the fact that the treatment of TB requires a lengthy course of drug regimen leading to several side effects. Thus, the need for an agent with a high margin of safety becomes a primary concern.

4 Conclusion

In conclusion, herein we report the synthesis, spectral studies and preliminary *in-vitro* anti-mycobacterial activity of a novel series of thiazolyl substituted carbazolehydrazine analogues **6(a-o)** by molecular hybridization approach. Structure activity relationship study reveal that the electron donating groups (OCH_3 and OH) on the phenyl ring of thiazole moiety resulted in excellent anti-mycobacterial activity, while the electron withdrawing substituents decreased the activity. Among the synthesized compounds, compound (**6c**, MIC = 21μM) emerged as a promising compound endowed with good anti-mycobacterial activity. Further, the title compounds were also assessed for their cytotoxic activity (IC_{50}) against mammalian Vero cell line using MTT assay. The results indicated that these compounds exhibit anti-tubercular activity at non-cytotoxic concentrations. These results indicate the importance of molecular hybridization and the development of carbazole clubbed thiazole based lead candidates as potential anti-mycobacterial agents. The anti-mycobacterial activity can further be enhanced by slight modifications in the ring substituents and/or extensive additional functionalization, which warrants further investigation. We believe that the observed outcome should contribute to the

global efforts in the discovery of new lead compounds with improved anti-mycobacterial activity.

5 Experimental

5.1 Chemistry Protocol

All research chemicals were purchased from Sigma–Aldrich and Merck Millipore, South Africa. Commercially available chemicals 4-bromo phenacyl bromide (**5a**) and phenacyl bromide (**5b**) were procured from Sigma–Aldrich (South Africa). Solvents except laboratory reagent grade were dried and purified according to the literature when necessary. The progress of reactions and purity of compounds were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates procured from E. Merck and Co. (Darmstadt, Germany) using ethyl acetate (10%) in dichloromethane as mobile phase and iodine vapours as visualizing agent.

Melting points of synthesized compounds were determined in Thermo Fisher Scientific (IA9000, Essex, Great Britain) digital melting point apparatus and are uncorrected. IR spectra were recorded on Bruker Alpha FT-IR Spectrometer (Billerica, MA, USA) by using ATR technique. The ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCE 400 (Bruker, Rheinstetten/Karlsruhe, Germany) using $CDCl_3$ and/or DMSO-d_6. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. HRMS were recorded on Autospec Mass Spectrometer under the electron impact at 70 eV.

5.1.1 Synthesis of 9-methylcarbazole (1) [26]

To a constantly stirred solution of carbazole (5 g, 0.025 mol, 1 equiv.) in *N*,*N*-dimethylformamide (20 mL) maintained at 0°C, NaH (0.028 mol, 1.1 eq) was slowly and carefully added in portions and stirring was continued for 30 min. To this reaction mass, methyl iodide (4.34 g, 0.0306 mol, 1.2 equiv.) was added and stirred at room temperature for 5 h. the

progress of reaction was monitored by TLC. Further, the reaction mixture was poured into ice cold water, the solid precipitated was filtered and washed with cold water to yield white solid of compound (**1**). Yield: 93.33%, M.P.; 86-88 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3048.18 (Ar-H Str.), 2953.18 (C-H Str. of CH_3); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.13 (d, J = 7.80 Hz, 2H, Ar-H),7.50 (m, 2H, Ar-H), 7.43 (d, J = 8.20 Hz, 2H, Ar-H), 7.25 (m,2H, Ar-H), 3.87 (s, 3H,*N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 140.97, 125.63, 122.74, 120.27, 118.79, 108.38,29.02 (N-CH_3).

5.1.2 Synthesis of 9-methyl-carbazole-3-carbaldehyde (2)[26]

Vilsmeier-Haack reagent was freshly prepared by the careful addition of $POCl_3$ (2ml, 0.021 mol) in DMF (8 ml, 0.103 mol) at 0 °C with constant stirring. To this, 9-Methyl carbazole (**1**, 5.0 g, 0.0238 mol, 1 equiv.) was added to the reagent with continuous stirring maintaining the temperature at 0 °C for initial 30 min and later stirred at RT for 2 h, and finally at 60 °C for another 2 h. The reaction mixture was then poured in sodium carbonate solution and stirring was continued at 90 °C for 2 h. After cooling to RT, the reaction mixture was suspended into water, extracted with dichloromethane (20 ml X 3 times) and the collective extracts were washed with water and dried over anhydrous sodium sulphate. The residue obtained after the *in-vacuo* removal of dichloromethane was further recrystallized from ethanol to afford the desired compound (**2**) as off white crystalline solid. Yield: 75 %, M.P.; 74-76 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3022 (Ar-H Str.) 2993 (C-H Str. of CH_3), 1684.01 (C = O Str.); ^{1}H-NMR (400 MHz,DMSO-d_6, δ ppm): 10.09 (s, 1H, CHO),8.15 (d, 1H, J = 7.76 Hz, Ar-H), 8.02 (dd, 1H, J = 8.52 Hz, ArH), 7.53 (t, 1H, Ar-H), 7.45 (dd, 2H, J = 8.28 Hz, Ar-H), 7.34 (t, 1H, Ar-H), 3.88 (s, 3H,*N*-

CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 191.80 (C = O), 144.51, 141.70, 128.54, 127.24, 126.76, 123.83, 122.99, 120.65, 109.11, 29.38 (*N*-CH_3).

5.1.3 Synthesis of 1-((9-methyl-carbazol-6-yl methylene) thiosemicarbazide (3)[22]

To a constantly stirred solution of compound **2** (4.0 g, 0.0191 mol, 1 equiv.) and thiosemicarbazide (1.91 g, 0.021 mol, 1.1 equiv.) in anhydrous methanol (40 mL), a catalytic amount of glacial acetic acid (0.15 equiv.) was added. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the solid separated was filtered and washed with cold methanol to afford off white crystalline solid of compound (**3**). Yield: 81%, M.P.; 225-226 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3373.23, 3261.31 (N-H Str. of NH_2), 3174.17(N-H Str. of NH), 3036.85 (Ar-H), 1619.92 (C = N Str.), 1145.80(C = S); ^{1}H-NMR (400 MHz,DMSO-d_6, δ ppm): 11.38 (s, 1H, NH), 8.58 (s, 1H, CH = N), 8.23 (d, 1H, *J* = 7.76, Ar-H), 8.21 (s, 1H, H-4 of carbazole), 8.15-7.98 (s, 2H, NH_2), 7.96 (dd, 1H, *J* = 8.60 Hz, Ar-H), 7.62 (d, 2H, *J* = 8.88 Hz, Ar-H), 7.49 (t, 1H, *J* = 8.10 Hz, Ar-H), 7.26 (t, 1H, *J* = 7.48 Hz, Ar-H), 3.89 (s, 3H, *N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 176.92, 143.04 (CH=N),141.06, 140.56, 125.60, 124.60, 121.67, 119.94, 118.76, 108.86, 28.62 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{15}H_{14}N_4S$ $[M+H]^+$: 282.0939 found 283.0931

5.1.4 General procedure for the synthesis of 2-bromo-1-substituted phenylethanone 5(c-h)

27

To a cooled solution of appropriately substituted acetophenones **4(c-h)** (1 equiv.) in chloroform/AcOH was added bromine (1.2 equiv.). The solution was stirred at room temperature for 3-5 h until TLC showed full consumption of starting materials. The reaction mass was poured

in ice cold water and extracted with 10 mL of DCM (3 times). Anhydrous Na_2SO_4 was added to the combined organic layer, filtered and the excess solvent was removed *in-vacuo* under reduced pressure. The resultant solid/liquid obtained was washed with hexane to yield compounds **5**(**c**-**h**).

5.1.4.1 2-bromo-1-(3,4-dimethoxyphenyl)ethanone (5c)

Off white solid; Yield: 93%; ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.61-7.58 (dd, 1H, J = 8.40 Hz, Ar-H), 7.52 (s, 1H, Ar-H), 6.90 (d, 1H, J = 8.36 Hz, Ar-H), 4.39 (s, 2H, CH_2Br), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3).

5.1.4.2 2-bromo-1-(4-chlorophenyl)ethanone (5d)

Off white solid; Yield: 80%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.91 (d, 2H, J = 8.52 Hz, Ar-H), 7.48 (d, 2H, J = 8.52 Hz, Ar-H), 4.65 (s, 2H, CH_2Br).

5.1.4.3 2-bromo-1-(4-nitrophenyl)ethanone (5e)

Yellow solid; Yield: 90 %, ^{1}H NMR (400 MHz, $CDCl_3$,δ ppm): 8.35 (d, 2H, J = 8.84 Hz, Ar-H), 8.16 (d, 2H, J = 8.88 Hz, Ar-H), 4.46 (s, 2H, CH_2Br)

5.1.4.4 2-bromo-1-(4-fluorophenyl)ethanone (5f)

White solid; Yield: 75 %, ^{1} H NMR (400 MHz, $CDCl_3$,δ ppm): 8.04 (m, 2H, Ar-H), 7.14 -7.20 (m, 2H, Ar-H), 4.40 (s, 2H, CH_2Br)

5.1.4.5 2-bromo-1-(*p*-tolyl)ethanone (5g)

White solid; Yield: 78 %, ^{1}H NMR (400 MHz, $CDCl_3$,δ ppm): 7.88 (d, 2H, J = 8.28Hz, Ar-H), 7.29 (d, 2H, J = 8.12 Hz, Ar-H), 4.42 (s, 2H, CH_2Br), 2.42 (s, 3H, CH_3)

5.1.4.6 2-bromo-1-(3-nitrophenyl)ethanone (5h)

Yellow solid; Yield: 65%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.82 (t, 1H, J = 7.86 Hz, Ar-H), 8.48 (dd, 1H, J = 8.08 Hz, Ar-H), 8.31-8.34 (m, 1H, Ar-H), 7.75-7.71 (t, 1H, J = 7.98 Hz, Ar-H), 4.47 (s, 2H, CH_2Br).

5.1.5 General Procedure for the α-Bromination of aromatic/heteroaromatic ketones 5(i-o) [28]

To a hot solution of copper(II)bromide (1.07 g, 0.007 mol, 2 equiv.) in ethyl acetate (10 mL) was added a solution of appropriately substituted aromatic/heteroaromatic ketones **4** (**i-o**) (0.003 mol, 1 equiv.) in chloroform (10 mL) drop wise over 30 min. The reaction mixture was then refluxed for 12 h and cooled to room temperature, filtered through Celite bed. The filtrate was washed with saturated $NaHCO_3$/brine solution and dried over anhydrous Na_2SO_4. The resultant solution was concentrated under reduced pressure to afford the desired compounds **5** (**i-o**).

5.1.5.1 2-bromo-1-(2-hydroxyphenyl)ethanone (**5i**)

Yelow oil; Yield: 65%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 12.25 (s, 1H, OH), 7.75 (m, 2H, Ar-H) 7.54 (m, 2H, Ar-H), 4.44 (s, 2H, CH_2Br).

5.1.5.2 2-bromo-1-(4-hydroxyphenyl)ethanone (**5j**)

Yellow oil; Yield:75 % , ^{1}HNMR (400 MHz , $CDCl_3$, δ ppm): 11.72 (s, 1H, OH), 7.94 – 7.90 (dd, 2H, *J* = 8 .72- 8.53 Hz, Ar-H), 6.95 – 6.91 (t, 2H, *J* = 8.78 Hz, Ar-H), 4.41 (s, 2H, CH_2Br).

5.1.5.3 2-bromo-1-(3-chlorophenyl)ethanone (**5k**)

Off white solid; Yield: 67 % , ^{1}H NMR (400 MHz , $CDCl_3$, δ ppm): 7.95 (s, 1H, Ar-H), 7.86 – 7.82 (d, 1H, *J* = 7.76 Hz, Ar-H), 7.58-7.55 (dd, 1H, *J* = 8.00 Hz, Ar-H) , 7.45 – 7.38 (m, 1H, Ar-H), 4.41 (s, 2H, CH_2Br).

5.1.5.4 2-bromo-1-(3-fluorophenyl)ethanone (**5l**)

White solid; Yield: 70%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.86-7.84 (d, 1H, *J* = 5.88 Hz, Ar-H), 7.76-7.75 (d, 2H, *J* = 6.28 Hz, Ar-H), 7.49-7.47 (d, 1H, *J* = 5.40 Hz, Ar-H), 4.42 (S, 2H, CH_2Br).

5.1.5.5 2-bromo-1-(thiophen-2-yl)ethanone (5m)

Yellow oil; Yield: 80%, ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.81- 7.80 (dd, 1H, *J* = 4.00 Hz, Ar-H), 7.72- 7.71 (dd, 1H, *J* = 5.00 Hz, Ar-H), 7.18 -7.16 (dd, 1H, *J* = 7.76 Hz, Ar-H), 4.36 (s, 2H, CH_2Br)

5.1.5.6 3-(2-bromoacetyl)-2*H*-chromen-2-one (5n)

Yellow solid; Yield: 90%, 1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.63 (s, 1H, Ar-H), 7.72-7.70 (d, 1H, *J* = 7.48 Hz, Ar-H), 7.41 (m, 3H, ArH), 4.75 (s, 2H, CH_2Br)

5.1.5.7 6-bromo-3-(2-bromoacetyl)-2*H*-chromen-2-one (5o)

Yellow solid; Yield, 90% , ^{1}H NMR (400 MHz , $CDCl_3$, δ ppm): 8.52 (s, 1H, Ar-H), 7.82-7.80 (d, 1H, *J* = 7.24 Hz, Ar-H), 7.78-7.77 (d, 1H, *J* = 7.36 Hz, Ar-H), 7.30-7.28 (d, 1H, *J* = 8.84 Hz, Ar-H), 4.71 (s, 2H, CH_2Br).

5.1.6 General procedure for the synthesis of thiazolyl substituted carbazole hydrazine derivatives 6 (a-o) [22]

A solution of carbazole thiosemicarbazide compound (**3**, 150 mg, 0.531 mol, 1 equiv.) and α-bromoaromatic/heteroaromatic ketones **5** (**a-o**), (0.638 mol, 1.2 equiv.) in anhydrous methanol (10 mL) was refluxed for 3-5 h until TLC showed full consumption of starting materials. The residue was collected by filtration and was stirred in saturated $NaHCO_3$ solution for 30 min. The resultant solid was filtered, dried and purified by recrystallization with ethanol to afford the title compounds **6** (**a-o**).

5.1.6.1 4-(4-bromophenyl)-2-(2-((9-methyl-9*H*-carbazol-3yl)methylene)hydrazinyl)thiazole (6a)

Brown solid, Yield: 62%, M.P.; 262-264ºC; FTIR (ATR, ν_{max}, cm^{-1}): 3319.96 (N-H Str.), 3043.75 (Ar-H Str.), 2965.52 (C-H Str. of CH_3), 1618.20 (C = N Str.); ^{1}H-NMR (400

MHz,DMSO-d_6, δppm): 12.39 (s, 1H, NH), 8.38 (s, 1H, CH = N), 8.22 (s, 1H, H-4 of carbazole), 8.20 (s, 1H, Ar-H), 7.88-7.87 (dd, 1H, *J* = 8.56 - 8.60 Hz, Ar-H), 7.85- 7.81 (d, 2H, *J* = 8.48 Hz, Ar-H), 7.67-7.59 (m, 4H, Ar-H), 7.52-7.50 (t, 1H, *J* = 7.60 Hz, Ar-H), 7.40 (s, 1H, H-5 of thiazole), 7.25-7.23 (t, 1H, *J* = 7.54 Hz, Ar-H), 3.90 (s, 3H, *N*-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.05, 148.72(CH = N), 142.60, 140.79, 140.58, 133.44, 131.05, 127.07, 125.67, 124.89, 123.08, 121.64, 119.99, 119.06, 118.83, 109.27, 103.69 (C-5 of thiazole), 28.69 (*N*-CH_3). HR ESI *m/z*: calculated for $C_{23}H_{17}BrN_4S$ $[M+H]^+$: 460.0357 found 461.0359.

5.1.6.2 2-(2-((9-methyl-9H-carbazol-3-yl) methylene)hydrazinyl)-4-phenylthiazole (6b)

brown solid, Yield: 58%, M.P.;246-248°C; FTIR (ATR, ν_{max}, cm^{-1}): 3295.05 (N-H Str.), 3049.53 (Ar-H Str.), 2915.12 (C-H Str. of CH_3), 1620.14 (C = N Str.); ^{1}H-NMR (400 MHz,DMSO-d_6, δ ppm): 12.00 (s,1H, NH), 8.37 (s, 1H,CH = N), 8.24 (s, 1H, H-4 of carbazole), 8.21 (s, 1H, Ar-H), 7.88-7.86 (d, 2H, *J* = 8.44 Hz, Ar-H), 7.67-7.60 (m, 2H, Ar-H), 7.52-7.48 (t, 2H, *J* = 7.72 Hz, Ar-H), 7.41-7.39 (t, 2H, *J* = 7.62 Hz, Ar-H), 7.32 (s,1H, H-5 of thiazole), 7.25-7.23 (m, 2H, Ar-H), 3.90 (S,3H,*N*-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.37, 142.74 (CH = N), 141.09, 141.02, 134.76, 128.56, 127.44, 126.10, 125.47, 123.49, 122.09, 121.88, 120.43, 119.44, 109.71, 109.45, 103.18 (C-5 of thiazole), 55.99, 29.19 (*N*-CH_3), 18.51. HR ESI *m/z*: calculated for $C_{23}H_{18}N_4S$ $[M+H]^+$: 382.1252 found 383.1253.

5.1.6.3 4-(3,4-dimethoxyphenyl)-2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazole (6c)

brown solid, Yield: 50%, M.P.; 242-244°C; FTIR (ATR, ν_{max}, cm^{-1}): 3074.36 (N-H), 3008.28 (Ar-H), 2930.41 (C-H Str. of CH_3), 1616.36 (C = N); ^{1}H-NMR (400 MHz,DMSO-d_6, δ ppm): 8.38 (s, 1H, NH), 8.31 (s, 1H,CH = N), 8.23 (s, 1H, H-4 of carbazole), 8.21-8.20 (d, 1H, *J* = 7.88 Hz, Ar-H), 7.89-7.87 (dd, 1H, *J* = 8.68 -8.60 Hz, Ar-H), 7.67-7.63 (m, 2H, Ar-H), 7.52-7.48 (m, 1H,

Ar-H), 7.43-7.41 (m, 2H, Ar-H), 7.27-7.25 (t, 1H, *J* = 7.52 Hz, Ar-H), 7.20 (s, 1H, H-5 thiazole), 7.00-6.98 (d, 1H, *J* = 8.36 Hz, Ar-H), 3.91 (s, 3H, *N*-CH_3), 3.82 (s, 3H, OCH_3), 3.78 (s, 3H,OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.16, 148.68(CH=N), 141.26, 126.12, 125.31, 123.56, 122.10, 121.89, 120.44, 119.57, 118.03, 111.79, 109.72, 101.48 (C-5 of thiazole), 55.51, 29.14 (*N*-CH_3). HR ESI *m/z*: calculated for $C_{25}H_{22}N_4O_2S$ $[M+H]^+$: 442.1463 found 443.1464.

5.1.6.4 4-(4-chlorophenyl)-2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazole (6d)

brown solid, Yield: 48%, M.P.; 236-238°C; FTIR (ATR, ν_{max}, cm^{-1}): 3181.95 (N-H), 3054.74 (Ar-H), 2926.22 (C-H Str. of CH_3), 1592.71 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6, δ ppm): 12.08 (s, 1H,NH), 8.36 (s, 1H,CH = N), 8.22 (s, 1H , H-4 of carbazole), 8.20 (s, 1H, Ar-H), 7.89-7.85 (m, 3H, Ar-H), 7.67-7.64 (d, 1H, *J* = 8.60 Hz, Ar-H), 7.62-7.60 (d, 1H, *J* = 8.16 Hz, Ar-H), 7.51-7.49 (m, 3H, Ar-H), 7.37 (s, 1H, H-5 of thiazole), 7.26-7.22 (t, 1H, *J* = 7.36 Hz), 3.90(s, 3H, *N*-CH_3), ^{13}C-NMR(100MHz, DMSO-d_6, δppm): 168.51, 149.14, 143.04 (CH=N), 141.23, 141.02, 133.54, 131.84, 128.58, 127.19, 126.12, 125.34, 123.53, 122.09, 121.87, 120.42, 119.49, 119.28, 109.71, 109.46, 104.03 (C-5 of thiazole), 56.00, 29.11 (*N*-CH_3), 18.49; HR ESI *m/z*: calculated for $C_{23}H_{17}ClN_4S$ $[M+H]^+$: 416.0862 found 417.0865

5.1.6.5 2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)-4-(4-nitrophenyl)thiazole (6e)

brown solid, Yield: 55%, M.P.; 236 – 238°C; FTIR (ATR, ν_{max}, cm^{-1}): 3312.86 (N-H), 3046.57 (Ar-H), 2928.85 (C-H Str. of CH_3), 1621.16 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6, δ ppm): 12.16 (s, 1H, N-H) , 8.38 (s, 1H, CH = N), 8.29 - 8.26 (d, 2H, *J* = 8.92 Hz, AR-H), 8.24 (s, 1H, H-4 of carbazole) , 8.22-8.21 (d, 1H, *J* = 7.76 Hz, Ar-H), 8.13-8.11 (d, 2H, *J* = 8.9 Hz, AR-H), 7.88-7.86 (dd, 1H, *J* = 8.62 Hz, Ar-H), 7.70 (s, 1H, H-5thiazole), 7.67-7.65 (d, 1H, *J* = 8.64, Ar-H), 7.63-7.61(d, 1H, *J* = 8.16 Hz, Ar-H), 7.50-7.48 (t, 1H, *J* = 8.16 Hz, Ar-H), 7.27-7.25 (t,

1H, *J* = 7.50 Hz, Ar-H), 3.90 (s, 3 H, *N*-CH_3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 168.78, 148.52, 146.14, 143.30 (CH = N), 141.27, 141.03, 140.75, 126.29, 126.13, 125.26, 124.08, 123.55, 122.10, 121.88, 119.58, 119.29, 109.72, 109.47, 108.17 (C-5 of thiazole), 29.13 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{17}N_5O_2S$ $[M+H]^+$: 427.1103 found 428.1107

5.1.6.6 4-(4-fluorophenyl)-2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazole (6f)

dark brown solid Yield: 60 %, M.P ; 228-229 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3180 (NH),3047.51 (Ar-H), 2926.67 (C-H Str. of CH_3), 1624.30 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6, δ ppm): 11.69 (s, 1H ,N-H), 8.37 (s, 1H, CH = N), 8.23 (s, 1H, H-4 of carbazole), 8.22-8.20 (d, 1H, *J* = 7.76 Hz, Ar-H), 7.91-7.85 (m, 3H, Ar-H), 7.67-7.64 (d, 1H, *J* = 8.59 Hz, Ar-H), 7.62-7.60 (d, 1H, *J* = 8.28 Hz, Ar-H), 7.51-7.47 (t, 1H *J* = 7.72 Hz, Ar-H), 7.28 (s, 1H,H-5 of thiazole), 7.26-7.24 (m, 3 H, Ar-H), 3.90 (s,3H, *N*-CH_3), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.47, 160.35, 149.20, 143.06 (CH=N), 141.23, 141.01, 131.24, 127.52, 127.44, 126.13, 125.33, 123.53, 122.08, 121.87, 120.41, 119.48, 119.28, 115.51, 115.29, 109.70, 109.45, 102.98 (C-5 of thiazole), 29.12 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{17}FN_4S$ $[M+H]^+$: 400.1158 found 401.1156

5.1.6.7 2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)-4-(p-tolyl)thiazole (6g)

off white solid, Yield: 60%, M.P; 252–254°C; FTIR (ATR, ν_{max}, cm^{-1}): 3109.40 (N-H), 3048.24 (Ar-H), 2917.32 (C-H Str. of CH_3), 1613.95 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6, δppm): 12.30 (s, 1H, N-H), 8.38 (s, 1H, CH = N), 8.24 (s, 1H, H-4 of carbazole) , 8.22-8.21 (d, 1H, *J* = 7.68 Hz, Ar-H), 7.89-7.88 (dd, 1H, *J* = 8.56 Hz, Ar-H), 7.86-7.85 (d, 2H, *J* = 8.08 Hz, Ar-H), 7.76-7.74 (d, 1H, *J* = 8.60 Hz, Ar-H), 7.67-7.65 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.63-7.61 (t, 1H, *J* = 7.52 Hz, Ar-H), 7.26-7.24 (m, 3H, Ar-H), 7.21 (s, 1H, H-5 of thiazole) 3.90 (s, 3H, *N*-CH3), 2.32 (s,CH_3), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.28, 141.26 (CH=N), 141.02, 136.89,

131.67, 129.15, 128.96, 127.37, 126.12, 125.50, 125.31, 123.56, 122.11, 121.88, 120.43, 119.56, 119.28, 109.72, 109.46, 102.38 (C-5 of thiazole), 29.13 (N-CH_3), 20.78 (Ar-CH_3); HR ESI *m/z*: calculated for $C_{24}H_{20}N_4S$ $[M+H]^+$: 396.1409 found 397.1409

5.1.6.8 2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)-4-(3-nitrophenyl)thiazole (6h)

Yellow solid, Yield: 68 %, M.P.; 220– 222°C; FTIR (ATR, ν_{max}, cm^{-1}): 3107.31 (N-H), 3085.87 (Ar-H) , 2925.61 (C-H Str. of CH_3), 1572.25 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6 ,δ ppm): 8.68 (s, 1H, NH), 8.36 (s, 1H, CH = N), 8.31-8.29 (d, 1H, *J* = 7.84 Hz, Ar-H), 8.22-8.21 (s, 1H, H-4 of carbazole), 8.19 (d, 1H, *J* = 7.72 Hz, Ar-H), 8.14-8.12 (dd, 1H, *J* = 8.04 Hz, Ar-H), 7.88- 7.85 (dd, 1H, *J* = 8.56 – 8.60 Hz, Ar-H), 7.71-7.60 (m, 4H, Ar-H), 7.57 (s, 1H, H-5 of thiazole) 7.51 -7.47 (t, 1H, *J* = 7.56 Hz, Ar-H), 7.26-7.22 (t, 1H, *J* = 7.44 Hz, Ar-H), 3.89 (s, 3 H, *N*-CH3), ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 169.14, 148.24, 148.07, 142.99 (CH = N), 141.19, 141.01, 136.33, 131.50, 130.15, 126.10, 125.44, 123.53, 122.09, 121.88, 120.40, 119.86, 119.42, 119.26, 109.68, 109.43, 105.70 (C-5 of thiazole), 30.64, 29.11 (N-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{17}N_5O_2S$ $[M+H]^+$: 427.1103 found 428.1106

5.1.6.9 2-(2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazol-4-yl)phenol (6i)

Brown solid, Yield: 65%, M.P.; 245 – 247°C; FTIR (ATR, ν_{max}, cm^{-1}): 3342.53 (OH), 3116.66 (NH), 3057.16 (Ar-H), 2894.84 (C-H Str. of CH_3), 1621.58 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) : 12.59 (s, 1H, N-H), 8.41 (s, 1H,CH = N), 8.29 (s, 1H, H-4 of carbazole), 8.23-8.21 (d, 1H, *J* = 7.68 Hz, Ar-H), 7.91-7.89 (dd, 1H, *J* = 8.64 – 8.72 Hz, Ar-H), 7.80 - 7.78 (dd, 1H, *J* = 7.84 - 7.80 Hz, Ar-H), 7.69-7.66 (d, 1H, *J* = 8.64 Hz, Ar-H), 7.63-7.61 (d, 1H, *J* = 8.20 Hz, Ar-H), 7.52-7.47 (t, 1H, *J* = 7.30 Hz, Ar-H), 7.40 (s, 1H, H-5 of thiazole), 7.27-7.23 (t, 1H, *J* =7.48 Hz, Ar-H), 7.20-7.18 (t, 1H , *J* = 8.42 Hz, Ar-H), 6.92-6.88 (m, 2H,Ar-H), 5.10 (s, 1H, OH), 3.91 (s, 3H, *N*-CH_3) , ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 167.67, 156.65,

155.13, 141.12 (CH=N), 141.04, 129.20, 127.19, 126.16, 124.98, 123.71, 122.09, 121.87, 120.43, 119.91, 119.35, 119.11, 116.70, 109.75, 109.51, 103.70 (C-5 of thiazole), 30.66, 29.15 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{18}N_4OS$ $[M+H]^+$: 398.1201 found 399.1203

5.1.6.10 4-(2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazol-4-yl)phenol (6j)

Brown solid, Yield: 55%, M.P.; 284 – 285°C; FTIR (ATR, ν_{max}, cm^{-1}): 3454.28 (OH), 3124.46 (N-H), 3059.35 (Ar-H), 2940.97 (C-H Str. of CH_3), 1629.47 (C = N); ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm) : 12.40 (s, 1H, N-H), 8.37 (s, 1H, CH = N), 8.23 (s, 1H, H-4 of carbazole), 8.21 (s, 1H, Ar-H), 7.88-7.86 (d, 1H, *J* = 8.56 Hz, Ar-H), 7.65-7.59 (m, 4H, Ar-H), 7.52-7.50 (t, 1H, *J* = 7.76 Hz, Ar-H), 7.26-7.24 (t, 1H, *J* = 7.42 Hz, Ar-H), 7.05 (s, 1H, H-5 of thiazole), 6.80-6.74 (d, 2H, *J* = 8.56 Hz, Ar-H), 4.66 (s, OH), 3.91 (s, 3H, *N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 157.68, 141.80 (CH = N), 141.57, 129.41, 127.51, 126.62, 125.86, 124.12, 122.64, 122.43, 120.93, 119.79, 115.81, 110.21, 109.97, 100.80 (C-5 of thiazole), 29.65 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{18}N_4OS$ $[M+H]^+$: 398.1201 found 399.1204

5.1.6.11 4-(3-chlorophenyl)-2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazole (6k)

Brown solid, Yield: 48%, M.P.; 238 – 240 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3215.93 (N-H), 3052.00 (Ar-H), 2936.70 (C-H Str. of CH_3), 1593.86 (C = N); ^{1}HNMR (400 MHz,DMSO-d_6, δ ppm): 12.29 (s, 1H, N-H), 8.37 (s, 1H, CH = N), 8.31 (s, 1H, H-4 of carbazole), 8.22-8.21 (d, 1H, *J* = 7.56 Hz, Ar-H), 7.92 (s, 1H, H-5 of thiazole), 7.88 -7.85 (dd, 1H, *J* = 8.72 Hz, Ar-H), 7.84-7.82 (d, 1H, *J* = 7.76 Hz, Ar-H), 7.67-7.65 (d, 1H , *J* = 8.64 Hz, Ar-H), 7.63-7.61 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.52-7.44 (m, 3H, Ar-H), 7.36-7.34 (m, 1H, Ar-H), 7.26-7.23 (t, 1H, Ar-H), 3.91 (s, 3H, *N*-CH_3), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 141.76 (CH = N), 141.57, 133.94, 130.97, 126.61, 125.70, 124.51, 124.06, 122.64, 120.93, 119.78, 110.21, 109.96 (C-5 of thiazole), 29.65 (N-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{17}ClN_4S$ $[M+H]^+$: 416.0862 found 417.0864

5.1.6.12 4-(3-fluorophenyl)-2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazole (6l)

Brown solid, Yield: 45%, M.P.; 235 – 237^{0}C; FTIR (ATR, ν_{max}, cm^{-1}): 3187.06 (N-H), 3046.62 (Ar-H), 2929.54 (C-H Str. of CH_3), 1590.14 (C = N); ^{1}H NMR (400 MHz,DMSO-d_6, δ ppm) : 12.24 (s, 1H,NH), 8.37 (s, 1H,CH = N), 8.22 (s, 1H, H-4 of carbazole), 8.21 (s, 1H, Ar-H), 7.88-7.86 (dd, 1H, *J* = 8.58 Hz ,Ar-H), 7.73-7.71 (d, 1H, *J* = 7.84 Hz, Ar-H), 7.67-7.61 (m, 3H, Ar-H), 7.50-7.46 (m, 2H, Ar-H), 7.44 (s, 1H, H-5 of thiazole), 7.26 -7.23 (t, 1H, *J* = 7.18 Hz, Ar-H), 7.15 -7.10 (t, 1H, *J* = 8.45 Hz, Ar-H), 3.91 (s, 3H, *N*-CH_3) ; ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.47, 163.70, 142.93 (CH = N), 141.22, 141.02, 130.59, 130.51, 126.10, 125.38, 123.50, 122.10, 121.89, 121.48, 120.43, 119.49, 119.26, 112.09, 111.86, 109.71, 109.45, 104.69 (C-5 of thiazole), 29.13 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{23}H_{17}FN_4S$ $[M+H]^+$: 400.1158 found 401.1159

5.1.6.13 2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)-4-(thiophen-2-yl)thiazole (6m)

Brown solid, Yield; 45%, M.P.; 241– 243 $^{\circ}$C; FTIR (ATR, ν_{max}, cm^{-1}): 3152 (N-H) 3055.87 (Ar-H), 2928.05 (C-H Str. of CH_3), 1621.29 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6 , δ ppm): 12.19 (s, 1H, NH), 8.37 (s, 1H, CH = N), 8.22-8.20 (d, 1H, *J* = 7.84 Hz, Ar-H), 8.19 (s, 1H, H-4 of carbazole), 7.87-7.84 (dd, 1H, *J* = 8.44-8.48 Hz, Ar-H), 7.66-7.64 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.62-7.60 (d, 1H, *J* = 8.60 Hz, Ar-H), 7.51-7.45 (m, 3H, Ar-H), 7.26-7.22 (m, 1H, Ar-H), 7.15 (s, 1H, H-5 of thiazole), 7.09-7.07 (m, 1H, Ar-H), 3.90 (s, 3H, *N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):168.2, 145.1, 143.0 (CH=N), 141.2, 141.0, 138.9, 127.8, 126.1, 125.3, 123.1, 122.0, 121.8, 120.4, 119.5, 119.2, 109.7, 101.5 (C-5 of thiazole), 29.13 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{21}H_{16}N_4S_2$ $[M+H]^+$: 388.0816 found 389.0814

5.1.6.14 3-(2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (6n)

Yellow solid; Yield: 67%, M.P.; 267– 269 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3164.52 (N-H), 3015.73 (Ar-H), 2871.89 (C-H Str. of CH_3), 1721.35 (C=O), 1604.68 (C = N); 1H NMR (400 MHz, DMSO-d_6, δ ppm) : 8.52 (s, 1H, N-H), 8.35 (s, 1H, CH = N), 8.23 (s, 1H, H-4 of carbazole), 8.20 (s, 1H, H-4 of coumarin), 7.86-7.84 (dd, 1H, *J* = 8.60 Hz, Ar-H) , 7.83-7.81 (dd, 1H, *J* = 7.80 Hz, Ar-H), 7.75 (s, 1H, H-5 of thiazole), 7.64-7.58 (m, 3H, Ar-H), 7.50-7.48 (m, 4H, Ar-H), 7.26-7.22 (t, 1H, *J* = 7.48 Hz, Ar-H), 3.91 (s, 3H, *N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 158.75 (C=O), 152.22, 150.22, 143.81 (CH = N), 143.32, 142.92, 141.24, 138.09, 132.61, 131.64, 129.13, 128.73, 126.13, 125.25, 124.80, 123.55, 122.07, 120.38, 119.29, 117.77, 116.04, 112.91, 110.21 (C-5 of thiazole), 29.09 (*N*-CH_3), 18.47; HR ESI *m/z*: calculated for $C_{26}H_{18}N_4O_2S$ $[M + Na]^+$: 450.1150 found 473.1153

5.1.6.15 6-bromo-3-(2-(2-((9-methyl-9H-carbazol-3-yl)methylene)hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (6o)

Yellow solid; Yield: 67%, M.P.; 267– 269 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3201.01, 3119.73 (N-H), 3042.34 (Ar-H), 2929.74, 1711.27 (C = O), 1627.14 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) : 12.11 (s, 1H, N-H), 8.45 (s, 1H, CH = N), 8.36 (s, 1H, H-4 of carbazole), 8.24 (s, 1H, H-4 of coumarin), 8.21-8.19 (d, 1H, *J* =7.64 Hz, Ar-H), 8.10-8.09 (d, 1H, *J* = 7.31 Hz, Ar-H), 7.87-7.84 (dd, 1H, *J* = 8.64 Hz, Ar-H), 7.79 (s, 1H, H-5 of thiazole), 7.75-7.72 (dd, 1H, *J* = 8.72-8.88 Hz, Ar-H), 7.65-7.63 (d, 1H, *J* = 8.64 Hz, Ar-H), 7.61-7.59 (d, 1H, *J* = 8.32 Hz, Ar-H), 7.51-7.47 (t, 1H, *J* = 7.54 Hz, Ar-H), 7.40-7.38 (d, 1H, *J* = 8.76 Hz, Ar-H), 7.22-7.26 (t, 1H, *J* = 7.52 Hz, Ar-H), 3.89 (s, 3 H, *N*-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 167.86 (C=O),

158.27, 151.22, 143.88 (CH=N), 141.24, 136.56, 133.57, 130.58, 126.10, 125.27, 123.54, 122.08, 121.86, 120.40, 119.51, 118.06, 116.32, 111.08, 109.69 (C-5 of thiazole), 29.11 (*N*-CH_3); HR ESI *m/z*: calculated for $C_{26}H_{17}BrN_4O_2S$ $[M+Na]^+$: 528.0256 found 551.0253

5.2 *In-vitro* anti-mycobacterial evaluation

The *in-vitro* anti-mycobacterial activity of synthesized compounds **6** (**a-o**) was carried out at Infectious Disease Research Institute (IDRI) within the National Institute of Allergy and Infectious Diseases (NIAID) screening program, Bethesda, MD, USA. The activity was assessed against *M. tuberculosis* $H_{37}Rv$ grown under aerobic conditions by using a dual read-out ($OD_{590}$and fluorescence) assay procedure[23-25]. Test compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of 2%. The highest concentration of compound was 200μM and compounds were soluble in DMSO at 10 μM. For compounds with limited solubility, the highest concentration was 50X less than the stock concentration *e.g.* 100 μM for 5 mM DMSO stock, 20 μM for 1 μM DMSO stock. For potent compounds, assays were repeated at lower starting concentrations. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (100 μM Rifampicin) and maximum growth (DMSO only), as well as a rifampicin dose response curve. Plates were inoculated with M. tuberculosis and incubated for 5 days: growth was measured by OD590and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy4 plate reader. Growth was calculated separately for OD590 and RFU. MIC was calculated on the basis of 10-point dose response curve which was plotted as % growth. The MIC was defined as the minimum concentration at which growth was completely inhibited and was calculated from the inflection point of the fitted curve to the lower asymptote (zero growth). In addition dose response curves were generated using the Levenberg-Marquardt algorithm and

the concentrations that resulted in 50% and 90% inhibition of growth were determined (IC_{50} and IC_{90}respectively).

5.3 Cytotoxicity studies: MTT assay

Mammalian VERO cells were cultured in Dulbecco Modified Eagle Medium (DMEM) containing 2 μM Na_2CO_3 supplemented with 10% (v/v) fetal bovine serum (FBS). The cells were incubated at 37 °C under 5% CO_2 and 95% air in a humidified atmosphere until confluent and then diluted with phosphate-buffered saline to 106 cells/mL. Stock solutions were prepared in dimethyl sulfoxide (DMSO) and further dilutions were made with fresh culture medium. The concentration of DMSO in the final culture medium was 1%, which had no effect on the cell viability. In a transparent 96-well plate, serially diluted stock solutions were placed at 37 °C for 72 h then the medium was removed and monolayer was washed twice with 100 μL of warm Hanks' balanced salt solution (HBSS). 100 μL of warm medium and 20 μL of freshly made MTS-PMS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and phenyl methasulfazone] (Promega) were added to each well, plates were incubated for 3 h and absorbance was determined at 490 nm using a plate reader. The same experimental conditions were provided for all compounds and analysis was repeated three times for each cell line. [29]

Acknowledgment

We are thankful to Department of Pharmaceutical Chemistry, Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa for their constant support, encouragement, and financial assistance. Authors are grateful to National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA for anti-

mycobacterial screening, Mr. Dilip Jagjivan (UKZN, South Africa) for his assistance with the NMR experiments.

References

1 D.E. Snider, M. Raviglione, A. Kochi, B. Bloom, Tuberculosis: Pathogenesis, Protection and Control: *Global Burden of Tuberculosis, ASM Press*, Washington, DC, 1994.

2 P. Nunn, B. Williams, K. Floyd, C .Dye, G. Elzinga, M. Raviglione, *Nat. Rev. Immunol*, 2005, 5, 819.

3 T. R. Frieden, T. R. Sterling, S. S. Munsiff, C. J. Watt, C.Dye,*Lancet*,2003, 362 , 887

4 Global Tuberculosis Report, *World Health Organization*, 2012, 25.

5 Y. L. Janin, *Bioorg. Med. Chem,* 2007, 15, 2479.

6 T. Kaneko, C. Cooper, K. Mdluli, *Future Med. Chem*, 2011, 3, 1373.

7 U. Sharma, Expert Opin. *Drug Discovery*,2011, 6, 1171.

8 S.P.Reddy, L.Pulipati, P.Yogeeswari, D.Sriram,N. Jain, B.Sridhar, R. Murthy, T.AnjanaDevi, S. V.Kalivendi, S.Kantevari, *J. Med. Chem*, 2012, 55, 3911.

9 L. P. Ormerod, *Br. Med. Bull,* 2005, 73/74, 17.

10 Y. H. Taufiq-Yap, T. H. Peh, G. C Ee, M. Rahmani, M. A.Sukari, A. M.Ali, R.Muse, *Nat. Prod. Res*, 2007, 21, 810.

11 T. Choi, R. Czerwonka, W. Frohner, M. Krahl, K. Reddy, S. Franzblau, H.-J. Knolker, *Chem. Med. Che,*2006, 1, 812.

12 S. Prado, Y. L. Janin, B. Saint-Joanis, P. Brodin, S. Michel, M. Koch, S. T.Cole, F. Tillequin, P. Bost, *Bioorg. Med. Chem*, 2007, 20, 2177.

13 A. Termentzi, I. Khouri, T. Gaslonde, S. Prado, B. Saint-Joanis, F.Bardou, E. P. Amanatiadou, I. S. Vizirianakis, J. Kordulakova, M. Jackson, R. Brosch, Y. L. Janin, M. Daffé , F. Tillequin, S. Michel, *Eur. J. Med. Chem*, 2010, 45, 5833.

14 T. A. Choi, R. Czerwonka, R. Forke, A.Jager, J.Knoll, M. P.Krahl, T.Krause, K. R. Reddy,S. G .Franzblau,H.J. Knolker, *Med. Chem. Res*, 2008, 17, 374.

15 S. Kantevari, T.Yempala, G. Surineni, B. Sridhar, P. Yogeeswari, D. Sriram, *Eur. J. Med. Chem,*2011, 46, 4827.

16 H. Kitagawa, T. Ozawa, S. Takahata, M. Iida, J. Saito, M. Yamada, *J. Med. Chem*, 2007, 50, 4710.

17 B. Villemagne, M. Flipo, N. Blondiaux, C. Crauste, S. Malaquin, F. Leroux, C. Piveteau, V. Villeret, P. Brodin,B. O. Villoutreix, O. Sperandio, S. H. Soror, A. Wohlkönig, R. Wintjens, B Deprez, A. R. Baulard, N. Willand, *J. Med. Che,* 2014, 57, 4876.

18 M. B. Palkar, M. N.Noolvi, V. S.Maddi, M.Ghatole,L. V. G. Nargund, *Med. Chem. Res,* 2012, 21, 1313.

19 S. Jaju, M. B Palkar, V. S. Maddi, P. M. Ronad, S. N.Mamledesai, M.Ghatole, Arch der Pharmazie, 2009, 342, 723.

20 H. M., Patel, M. N. Noolvi, N. S.Sethi,A. K.Gadad, S. S.Cameotra, *Arabian J. Chem.* DOI: 10.1016/j.arabjc.2013.01.001

21 A. K. Gadad, M. N. Noolvi, R. V. Karpoormath, *Bioorg. Med. Chem*, 2004, 12, 5651.

22 A. Arshad, H.Osman, M. C. Bagley, C. K. Lam, M. Suriyati, S.M. Anis, *Eur. J. Med. Chem,* 2011, 46, 3788.

23 J. Ollinger, M. A.Bailey, G. C.Moraski, A. Casey, S. Florio, T. Alling, M. J. Miller, *Parish, T. PLOS One*, 2013, 8, e60531.

24 A. Zelmer, P. Carroll, N. Andreu, K. Hagens, J. Mahlo, N. Redinger, B. D. Robertson, S. Wiles, T. H. Ward, T. Parish, J. Ripoll, G. J. Bancroft, U. E. Schaible, *J Antimicrob. Chemother*, 2012, 67, 1948.

25 R. Lambert, J. Pearson., *J. Appl. Microbiol*, 2000, 88, 788.

26 B. P. Bandgar, L. K. Adsul, H. V.Chavan, S. S.Jalde, S. N. Shringare, R. Shaikh, R. J Meshram, R. N. Gacche, V. Masand, *Bioorg. Med. Chem. Lett*, 2012, 22, 5839.

27 a) I. Moreno, I. Tellitu, E. Domínguez, R. SanMartín, *Eur. J. Org. Chem*. 2002, 2126. b) S. P Aletti, H. S. Yathirajan, B. Narayana, B. K. Sarojini, *Med Chem Res*, 2014, 23, 259.

28 D. M. Rotstein, D. J. Kertesz, K. M.Walker, D. C.Swinney, *J. Med. Che,*. 1992, 35, 2819.

29 K. Falzari, Z .Zhu, D. Pan, H. Liu, P. Hongmanee, S. G. Franzblau, *Antimicrobial Agents and Chemotherapy*, 2005, 49,1447

Table 1 Antimycobacterial activity and cytotoxicity data of a novel series of thiazolyl substituted carbazole hydrazine analogues (**3**, **6a-o**).

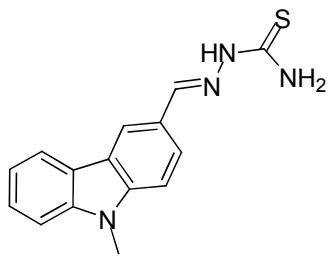
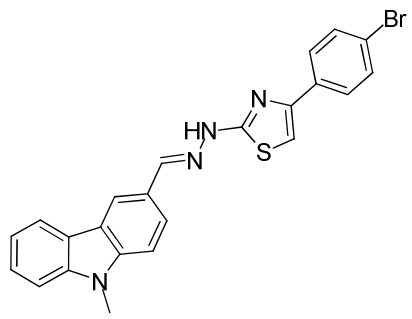
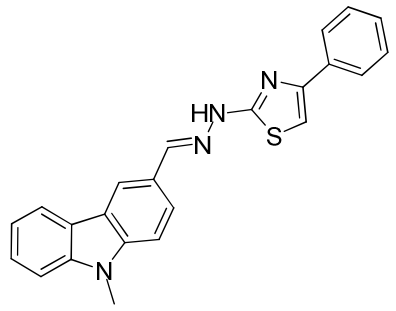
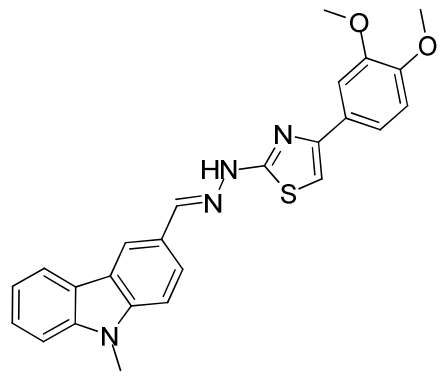
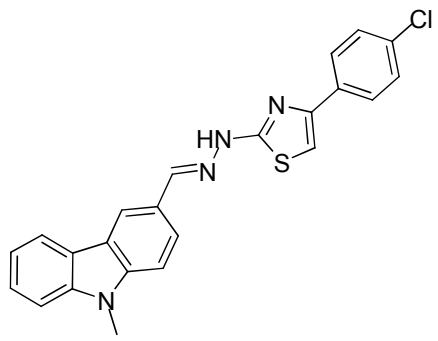
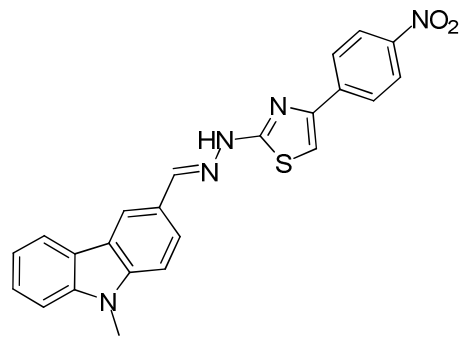
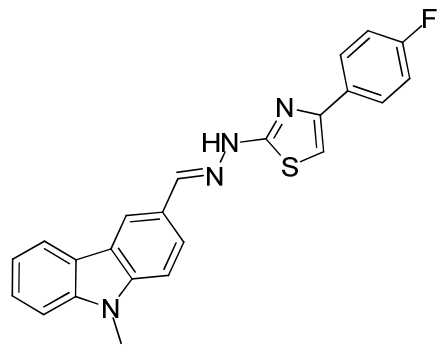
Compound Code	Structure	MIC (μM) [a]	IC_{50} (μM) [b]	IC_{90} (μM) [c]	Cytotoxicity IC_{50} (μM) [d]
3		ND	**1.6**	**5.9**	202.4
6a		>200	110	160	393.0
6b		**32**	**35**	**62**	321.5
6c		**21**	**28**	>50	220.8
6d		>200	>200	>200	389.6
6e		>200	>200	>200	421.5
6f		>200	61	170	268.5

Table 1 Continue

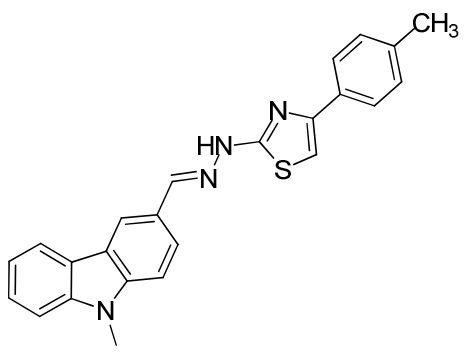
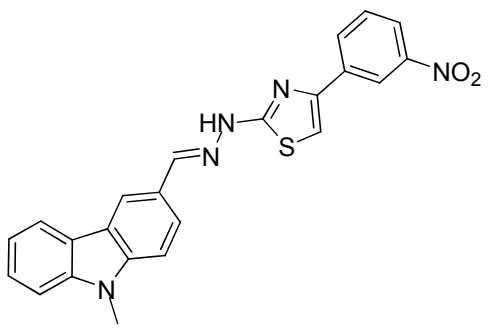
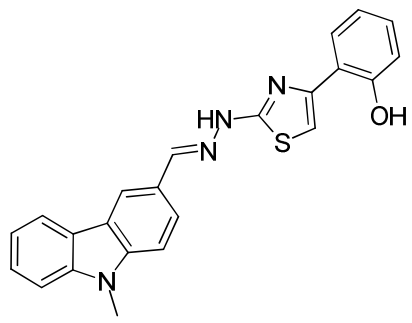
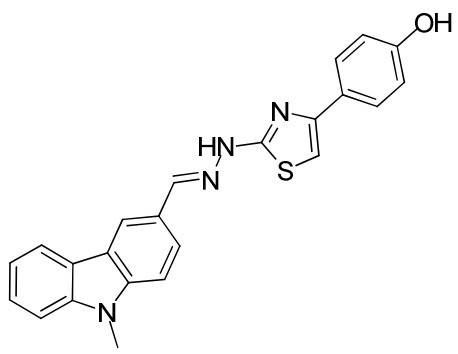
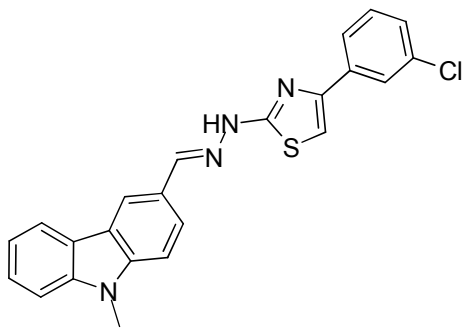
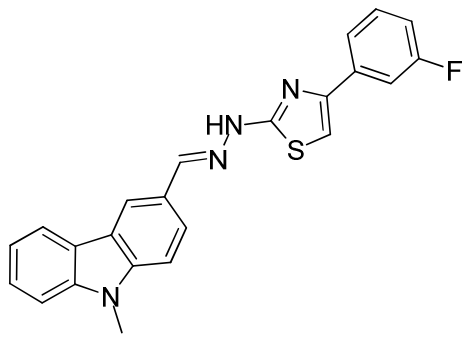
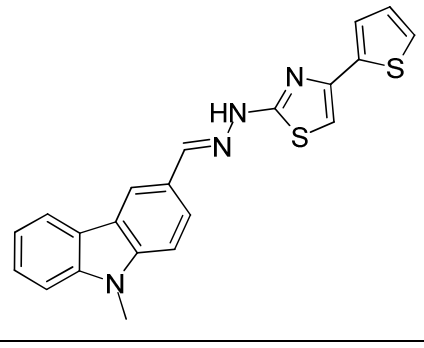
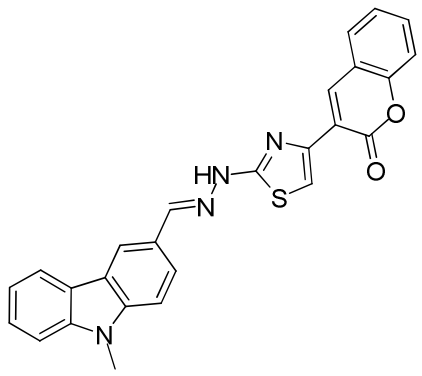
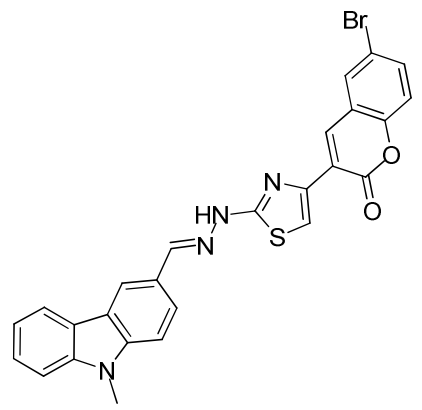
Compound Code	Structure	MIC (μM) [a]	IC_{50} (μM) [b]	IC_{90} (μM) [c]	Cytotoxicity IC_{50} (μM) [d]
6g		ND	ND	ND	ND
6h		>200	>200	>200	306.0
6i		**31**	**39**	>50	440.8
6j		**37**	**41**	>200	271.1
6k		>200	>200	>200	259.9
6l		>200	>200	>200	248.5
6m		>200	>50	>50	210.2

Table 1 Continue

Compound Code	Structure	MIC (μM) [a]	IC_{50} (μM) [b]	IC_{90} (μM) [c]	Cytotoxicity IC_{50} (μM) [d]
6n		>200	>25	>25	399.2
6o		>200	>200	>200	401.7
Rifampicin	-	0.0067	0.0037	0.007	--

[a] MIC is minimum inhibitory concentration at which *M. tuberculosis* $H_{37}Rv$ growth was completely inhibited; [b] IC_{50} value is the concentration at which growth is inhibited by 50%; [c] IC_{90} value is the concentration at which growth is inhibited by 90%; [d] cytotoxicity activity was determined on mammalian Vero cell line; ND = Not Determined.

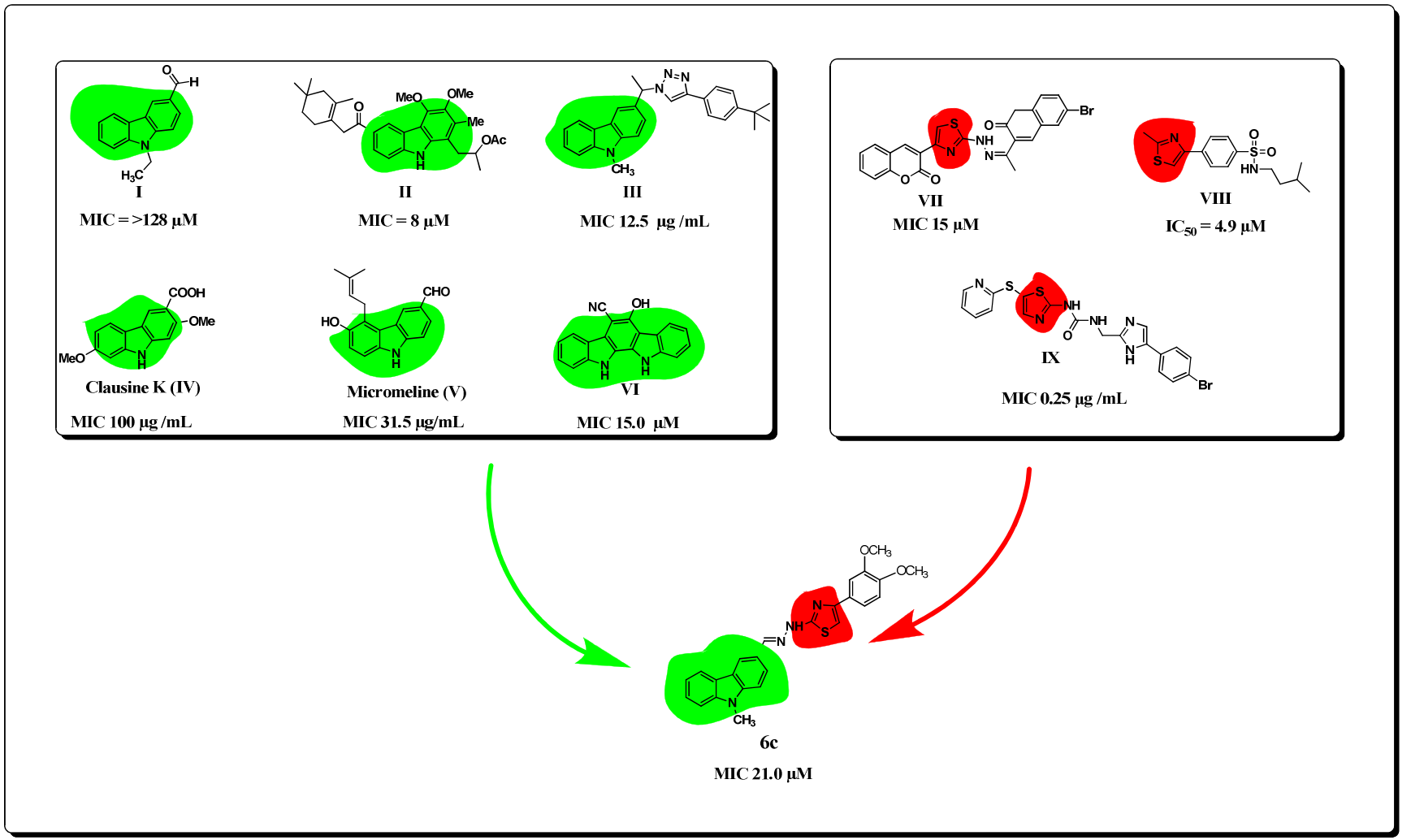
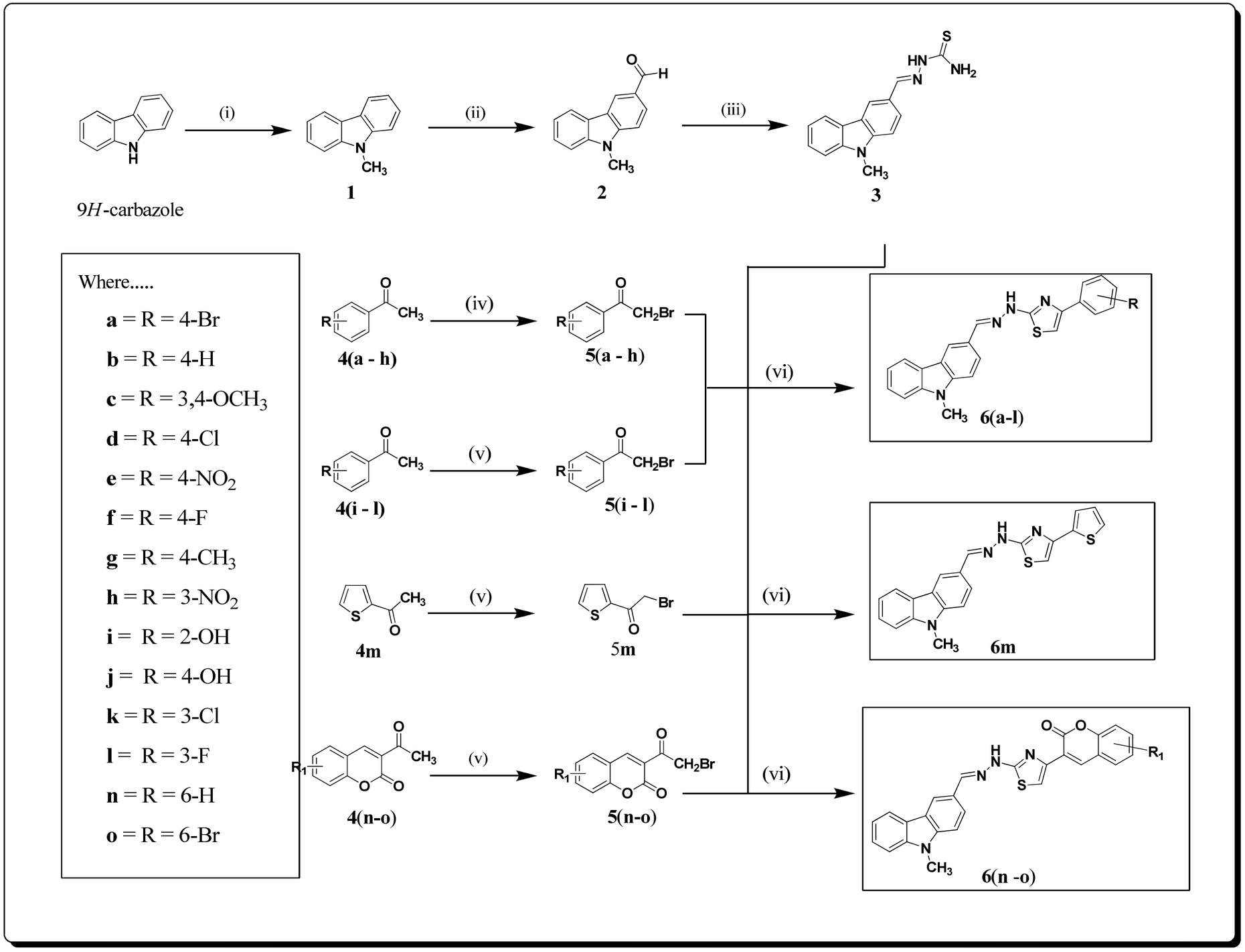


Fig. 1 The literature reported derivatives of carbazoles and thiazoles along with their anti-mycobacterial activities[11-17]. Compound **6c**, exhibited most promising anti-mycobacterial compound among the synthesized compounds.



Scheme 1 Synthetic outline of a novel series of thiazolyl substituted carbazole hydrazine analogues **6**(**a**-**o**).

Reagents and conditions: i) DMF, NaH, CH_3I, RT, 3h; ii) $POCl_3$, DMF, 4h reflux; iii) thiosemicarbazide, AcOH, methanol, reflux , 2h; iv) Br_2, $CHCl_3$/AcOH, RT; v) $CuBr_2$, EtOAc, $CHCl_3$, reflux,12 h, vi) Compound **3**, ∝-bromoaromatic/heteroaromatic ketones (**a**-**o**), methanol, reflux, 3h.