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Synthesis, antimicrobial and antimalarial activity of 1, 4-benzothiazepine and pyrazoline derivatives incorporating carbazole moiety

V. A. Kadnor[1], S. N. Shelke[2*]

[1]*Department of Chemistry, A. C. S. College Satral, Rahuri, Ahmednagar, MS 413711, India*
[2]*Department of Chemistry, S. S. G. M. College, Kopargaon, Ahmednagar, MS 423601, India*

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A series of carbazole-based 1, 4-benzothiazepine and pyrazoline derivatives were synthesized and the structures of the newly synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. All new derivatives **4(a-f)** and **5(a-e)** were screened for their *in vitro* antimicrobial activity, and also for their antimalarial activity. Compounds **4a, 4b, 4d, 5a, 5b** and **5c** exhibited promising antimicrobial and antimalarial activities as compared to positive control. Notably, compounds **4a, 4b** and **4d** showed excellent antifungal activity against *Penicillium sp.* comparable to that of a standard drug.

Key words: Carbazole, 1, 4-Benzothiazepine, Pyrazoline, Antimicrobial and antimalarial activities

INTRODUCTION

A large number of natural and synthetic carbazole derivatives have been reported to exhibit diverse biological activities such as antimicrobial [1, 2], antiviral [3], antimalarial [4] and potential application as pharmacological agents [5, 6]. Recently carbazole-substituted chalcone and its urea derivatives have been reported to exhibit antimicrobial, radical scavenger, cancer chemopreventive and polyphenol oxidase enzyme activities [7, 8]. Chalcones are also key precursors in the synthesis of many biologically important heterocyclic compounds such as benzothiazepines and pyrazolines.

Thiazepines belong to the important class of heterocyclic compounds for the synthesis of pharmaceutical agents, as well as biologically active compounds [9]. Benzothiazepines play an important role in drug discovery, as they show bioactivities such as anticonvulsant [10], endogenous natriuretic factors [11], potential central nervous system agents [12], antibiotics [13], antimicrobials [14], antihypertensive [15], antidiabetic [16] and cytotoxic agents [17]. Novel carbazole assembled 1, 4-thiazepine derivatives have been reported, which not only have significant antioxidant activities, but also exhibit remarkably selective cytotoxicity to carcinoma cell line HCT 116 [18]. Pyrazolines and their derivatives have been found to possess a wide spectrum of biological activities such as antimicrobial [19-22], antimalarial [23, 24], anti-inflammatory [25] and antioxidant [26]. 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1*H*-2-pyrazolines are reported as a novel class of anti-inflammatory and antioxidant agents [27], thus literature survey reveals that carbazole is a useful starting material for pharmacologically important products.

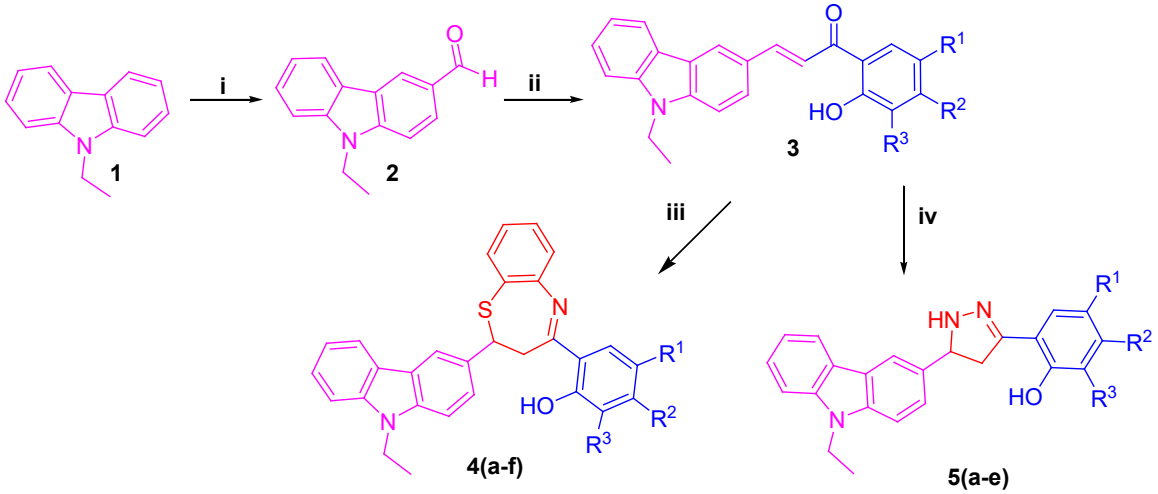
Therefore, in continuation of our efforts to synthesize biologically active heterocyclic compounds [28, 29], herein we report the synthesis of carbazole-containing 1, 4-benzothiazepine and pyrazoline derivatives with their antimicrobial and antimalarial activities.

RESULTS AND DISCUSSION

Chemistry

In view of the emerging biological importance of carbazole, we synthesized a series of carbazole chalcones and its corresponding 1,4-benzothiazepine and pyrazoline derivatives from 3-formyl-9-ethyl carbazole **2** as shown in scheme 1 on the hope of obtaining more antimicrobial and antimalarial agents. Thus, the starting compound 3-formyl-9-ethylcarbazole **2** was prepared by Vilsmeier-Haack formylation of carbazole **1**. 3-formyl-9-ethylcarbazole **2** was obtained by Claisen-Schmidt condensation with various substituted 2-hydroxyacetophenones in ethanolic potassium hydroxide afforded carbazole chalcones **3.** The 1, 4-benzothiazepine **4(a-f)** derivatives were synthesized by Michael addition of 2-aminothiophenol to carbazole chalcones **3** in acetic acid and ethanol. Carbazole pyrazolines **5(a-e)** were prepared from the compounds **3** on treatment with hydrazine hydrate in ethanol and acetic acid, the reaction most likely takes place through the intervention of an appropriate α,β-unsaturated hydrazone, which instantly cyclizes to give a pyrazoline ring, at reflux temperature cyclizing agent is acetic acid.

* To whom all correspondence should be sent:
E-mail: snshelke@yahoo.co.in



Scheme 1. Reagents and conditions: (**i**) DMF, $POCl_3$, 80ºC, 4 h (**ii**) Substituted 2-hydroxyacetophenones, KOH, EtOH, rt., 24-36 h. (**iii**) 2-Aminothiophenol, AcOH, EtOH, Reflux, 8 h. (**iv**) $NH_2NH_2.H_2O$, EtOH, AcOH, reflux 6 h.

The structures of **4(a-f)** and **5(a-e)** were confirmed by FT-IR, ^1H and ^{13}C NMR, and mass spectroscopic technique. For example, the infrared spectra of compounds **4(a-f)** showed characteristic signals at 1688 and 3350 cm^{-1} for C=N and hydroxyl group absorption, respectively. In general, three thiazepine protons of carbazole-based benzothiazepines showed similar patterns of signals in the ^1H NMR spectra. They displayed a doublet of a doublet at C_{17} for two protons and a triplet at C_{16} for one proton. The methine proton at C_{16} of the thiazepine nucleus resonates at around δ 3.25 ppm as a triplet with coupling constant (*J*) of nearly 12.6 Hz. This signal is observed as a triplet instead of a doublet of a doublet because two *J*-values accidentally are the same and two inner lines of the quartet occur at the same point, appearing as a single line of double intensity [30]. The two methylene protons at C_{17} displayed two signals as a doublet of doublet at around δ 3.45 ppm with coupling constants of nearly 9.5 Hz and 3.8 Hz and a doublet of doublet at around δ 5.16 ppm with coupling constants of nearly 9.4 Hz and 3.9 Hz. The ^{13}C NMR spectrum of compounds **4(a-f)** showed aromatic carbon signals in the region of δ 108.68-157.89 ppm. In the mass spectrum in all cases, peaks corresponding to molecular ions were observed which confirmed their molecular weights.

IR spectra of the compounds **5(a-e)** revealed a characteristic strong intensity band due to –OH and –NH stretching at 3668 and 3205 cm^{-1} respectively, while a pyrazoline –C=N band was observed around 1614 cm^{-1}. The ^1H NMR spectrum of these compounds exhibited an ABX pattern for the presence of two diastereotopic protons at C_{17} and one single proton at the C_{16} position. Asymmetric -CH proton displayed a triplet at δ 5.12 ppm with *J*=10.8 Hz, whereas the pro-chiral methylene (CH_2) protons appeared as two characteristic doublets of a doublet at δ 3.16 and 3.66 ppm with *J*=10.8 and 5.7 Hz which indicates the magnetic non-equivalence of the two protons. According to the high resolution mass spectrum (HRMS) of the representative compound **5a** calculated for $C_{23}H_{20}ON_3Cl_2$ $(M+H)^+$ ws 424.0981, found 424.0978.

Antibacterial and antifungal evaluation

The synthesized carbazole-assembled 1,4-benzothiazepine **4(a-f)** and pyrazoline **5(a-e)** derivatives were tested for their *in vitro* antimicrobial activity against two gram negative (*Escherichia coli, Pseudomonas putida*), two gram positive (*Bacillus subtilis, Streptococcus lactis*) bacterial strains and three (*Aspergillus niger, Penicillium sp., Candida albicans*) fungal strains using ampicillin and greseofulvin as standard drugs, respectively. The inhibition zone diameters were measured in millimeters (mm) and minimal inhibitory concentration (MIC) was expressed as µg/mL of all synthesized compounds, the results obtained are enclosed in Table 1. Among the synthesized compounds, **4a**, **4b**, **4d, 5a**, **5b** and **5c** could effectively inhibit the growth of most tested bacterial and fungal strains with considerable MIC (µg/mL) values. Carbazole-tethered 1,4-benzothiazepines **4(a-f)**, three derivatives **4a, 4b** and **4d** exhibited a significant activity against *P. putida* with MIC values of 50, 40 and 45 µg/mL, respectively as compared with positive control. Three compounds **4c, 4e** and **4f** also displayed moderate antibacterial activities (65-100 µg/mL) against all evaluated bacterial strains. Notably, compounds **4a**, **4b** and **4d** gave remarkable broader antifungal bioactive spectrum with MIC values in the range of 40-45 µg/mL against *Penicillium sp.* while two compounds **4c** and **4e** had satisfying activities against all screened fungal strains with considerable MIC values. It was found that

carbazole pyrazolines **5(a-e),** compounds **5a, 5b** and **5c** showed strong activities (45-65µg/mL) against gram positive *B. subtilis* and gram negative *P. putida* bacteria, while compounds **5d** and **5e** showed good activities (70-110 µg/mL) against all four bacterial strains as compared with standard drug ampicillin. As for antifungal activities, compound **5a** exhibited significant activity against *Penicillium sp*. and *C. albicans* with MIC values of 55 and 60 µg/mL, respectively, while **5b**, **5c**, **5d** and **5e** showed moderate activities (70-100 µg/mL) against all tested fungal strains compared to that of standard drug greseofulvin.

Antimalarial activity

The synthesized compounds **4** and **5** were also screened for their *in vitro* antimalarial activity against *Plasmodium falciparum* strain using chloroquine and quinine as reference drugs. The mean IC_{50} (µg/mL) values of the test compounds against the test microbe are presented in Table 2. The results revealed that the majority of the synthesized compounds showed significant degrees of inhibition against *P. falciparum* as compared with positive control quinine than that of chloroquine. Carbazole benzothiazepine derivatives **4(a-f)**, **4a** and **4b** showed moderate growth inhibition activities with IC_{50} values of 0.75 and 0.80 µg/mL as compared with standard drug quinine, while compounds **4c**, **4d**, **4e** and **4f** showed the lowest inhibition activities against *P. falciparum* comparable to that of reference compounds. The carbazole-pyrazoline derivatives **5(a-e)**, compound **5a** exhibited a good antimalarial spectrum with IC_{50} value of 0.56 µg/mL as compared with standard drug quinine, the remaining four compounds **5b**, **5c**, **5d** and **5e** showed considerable inhibition activities with IC_{50} values in the range of 0.76-1.25 µg/mL.

CONCLUSION

As structure-activity relationships (SAR) of all compounds were taken into account, it was observed that compounds **4a, 4b**, **4d**, **5a**, **5b** and **5c** having electron withdrawing groups like chloro and bromo substituents on the phenyl ring showed excellent potential of antibacterial and antifungal activities. The antimalarial evaluation of **4(a-f)** and **5(a-e)** revealed that, as the electronegativity nature of the substituents attached to an aromatic ring decreased, activity also decreased. Two derivatives **4c** and **5d** containing electron releasing methyl and electron withdrawing chlorine group attached to phenyl ring were able to display moderate growth inhibitory activity against all tested microorganisms. In addition, carbazole derivatives **4e** and **5e** containing methyl and methoxy group on the phenyl ring also inhibited the growth of the tested bacterial and fungal strains. Furthermore, compound **4f** without substituent in the phenyl ring showed the lowest activities against all tested bacterial, fungal and antimalarial strains. In general, all synthesized compounds **4** and **5** exhibited only moderate antimalarial activity IC_{50} values ranging 0.56-1.25 µg/mL. High potency and promising antimicrobial and antimalarial activity of the newly synthesized compounds **4(a-f)** and **5(a-e)** suggest that these compounds could serve as good leads for further optimization and development.

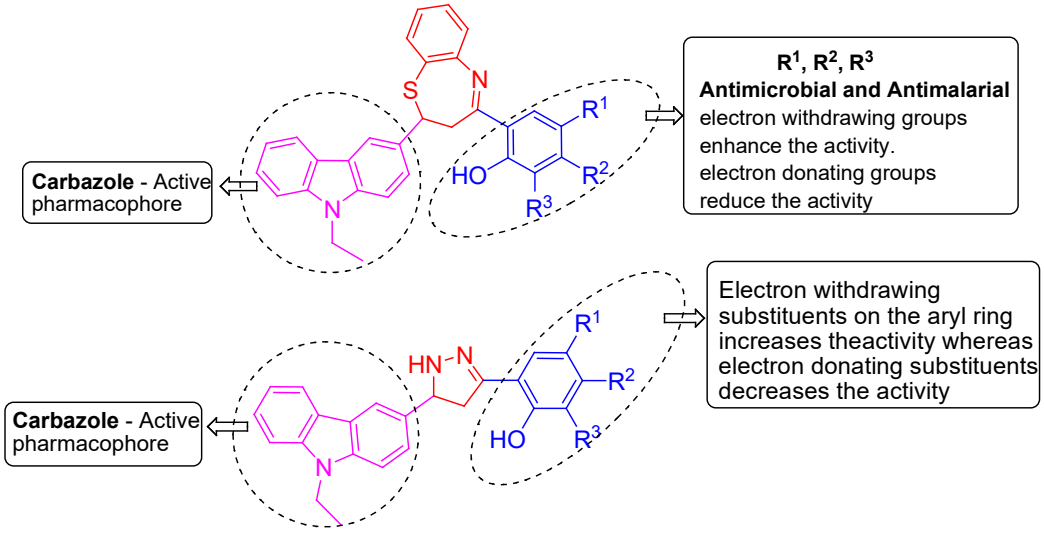


Figure 1. The structure - activity relationship in the target compounds

Table 1. Antimicrobial activities of the synthesized compounds **4** and **5** against pathological organisms expressed as: [a]inhibition zone diameter in millimeters (mm) and [b]MIC (µg/mL, between brackets)

Compounds	Microorganisms						
	Gram negative bacteria		Gram positive bacteria		Fungi		
	E. coli	*P. putida*	*B. subtilis*	*S. lactis*	*A. niger*	*Penicillium sp.*	*C. albicans*
4a	16(50)	18(50)	15(100)	20(110)	17(80)	14(45)	12(100)
4b	16(65)	17(40)	16(80)	16(80)	18(100)	14(40)	12(130)
4c	17(80)	16(65)	15(80)	19(100)	17(65)	13(100)	11(100)
4d	17(50)	18(45)	16(100)	15(110)	17(100)	14(40)	12(80)
4e	15(80)	14(80)	12(65)	17(80)	14(80)	11(80)	11(100)
4f	14(100)	13(80)	13(100)	13(100)	12(65)	12(80)	10(80)
5a	15(80)	16(45)	17(50)	18(45)	17(80)	12(55)	12(60)
5b	14(80)	16(50)	17(50)	16(50)	16(100)	12(70)	12(80)
5c	14(100)	15(65)	16(45)	14(65)	16(100)	11(90)	11(90)
5d	15(90)	14(80)	16(70)	18(80)	13(90)	11(100)	11(80)
5e	11(110)	12(100)	11(100)	15(80)	12(100)	09(100)	11(80)
Ampicillin	24(25)	20(25)	19(25)	22(25)	…	…	…
Greseofulvin	…	…	…	…	24(25)	14(25)	14(25)
Control (1% DMSO)	NA	NA	NA	NA	NA	NA	NA

[a]Inhibition zone diameters were measured for stock solutions (100 µg/mL). NA - No activity
[b]Minimal inhibitory concentration (MIC) values. 1% DMSO was used as control.

Table 2. Substitution pattern and *in vitro* antimalarial activity of the target compounds **4** and **5**

Compounds	R^1	R^2	R^3	*P. falciparum* Mean IC_{50} (µg/mL)
4a	Cl	H	Cl	0.75
4b	Cl	H	H	0.80
4c	Cl	CH_3	H	0.85
4d	Br	H	H	0.90
4e	CH_3	H	H	1.10
4f	H	H	H	1.30
5a	Cl	Cl	H	0.56
5b	Cl	H	H	0.76
5c	Br	H	H	0.88
5d	Cl	CH_3	H	1.20
5e	H	OCH_3	H	1.25
Quinine	---	---	---	0.268
Chloroquine	---	---	---	0.020

EXPERIMENTAL

The recorded melting points were determined in an open capillary and are uncorrected. IR spectra were recorded on a Perkin Elmer Fourier-transform infrared (FTIR) spectrophotometer with ATR. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II (400 MHz) and Bruker (125 MHz) spectrometer respectively, using TMS as internal standard. Mass spectra were recorded on a Waters, Q-Tof micromass spectrometer and high-resolution mass spectra (ESI) were recorded on a Thermo scientific Q-Exactive, Accela 1250 pump. The thin layer chromatography (TLC) was carried out on precoated silica gel aluminum plates to check compounds purity. Starting compound 9-ethyl-9*H*-carbazole is of Sigma Aldrich make.

In vitro antimicrobial assay

The antimicrobial activity was evaluated by the agar well diffusion method [31]. The activity was determined by measuring the diameter of inhibition zone (in mm). The samples of the tested compound concentrations (50 µL, 1mg /mL) were loaded into wells on the plates. All solutions were prepared in DMSO, and pure DMSO was loaded as a control. The plates were incubated at 37 ºC for 1-5 days and then were examined for the formation of inhibition zone. Each inhibition zone was measured three times to get an average value. The test was performed three times for each bacterium culture [32].

Minimal inhibitory concentration (MIC) measurement

The potato dextrose broths and microorganism susceptibility tests in nutrient media were used for the determination of MIC. Tested compounds stock 1000 µg/mL solutions, ampicillin and greseofulvin were prepared in DMSO followed by dilutions to 250-25 µg/mL concentrations. Inoculated microorganism suspensions were incubated at 37°C for 1-5 days for MIC determination.

Antimalarial activity

A stock solution of 5 mg/mL of each of the test samples, as well as standards was prepared in DMSO and subsequent dilutions were prepared with the culture medium. The diluted samples in 20 µL volumes were added to the test wells so as to obtain final concentrations (at five-fold dilutions) ranging between 0.4 and 100 µg/mL in duplicate well containing parasitized cell preparation. The *in vitro* antimalarial assay was carried out in 96 well plates according to the micro assay protocol with minor modifications [33]. The cultures of *P. falciparum* strain were maintained in a medium of RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat-inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8-1.5% at 3% haematocrit in a total volume of 200 µL of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining [34] to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O^{+ve}). The culture plates were incubated at 37°C in a candle jar. After 36-40 h of incubation, thin blood smears from each well were prepared stained with JSB stain. The slides were microscopically observed to record maturation of the ring stage parasites into trophozoites and schizonts in the presence of different concentrations of the test agents. The test concentrations which inhibited the complete maturation in to schizonts were recorded as the minimum inhibitory concentrations (MIC). Chloroquine and quinine were used as the reference drugs.

General procedure for the synthesis of 3-formyl-9-ethylcarbazole(2)

9-ethyl carbazole **1** (1.95 g, 10 mmol) was dissolved in dry DMF (20 mL) under anhydrous conditions. It was cooled to 0°C, and $POCl_3$ (1.89 mL) was added dropwise and stirring continued for 4 h at 80°C. Completion of reaction was monitored by TLC. The reaction mass was poured over crushed ice, neutralized with $NaHCO_3$, the white colored precipitate was filtered off and purified through recrystallization using ethyl alcohol to afford compound **2.**

General procedure for the synthesis of benzothiazepine derivatives 4(a-f)

Chalcone **3** (2 mmol) was dissolved in a minimum quantity of ethanol. To this, 2-aminothiophenol (2 mmol) was added and the resulting reaction mixture was refluxed at 60–70 °C for 3 h. Then, the mixture was acidified with 5–6 drops of glacial acetic acid and heating was continued for further 4–5 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and poured over crushed ice. The obtained solid was filtered and purified by recrystallization from methanol to afford compounds **4(a-f).**

*2,4-Dichloro-6-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4yl)phenol **(4a)**:* Light yellow colored solid; Yield (69%); R_f = 0.54 (6% ethylacetate in n-hexane); m. p. 222-223°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3559 (OH), 2976 (CH), 1593 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.42 (t, 3H, *J*=6.4 Hz, CH_3), 3.25 (t, 1H, *J*=12.5 Hz, thiazepine ring), 3.41 (dd, 1H, *J*=9.5 & 3.8 Hz, thiazepine ring), 4.40 (q, 2H, *J*=6.7 Hz, N-CH_2), 5.36 (dd, 1H, *J*=9.2 & 3.8 Hz, thiazepine ring), 7.22-7.30 (m, 2H, Ar-H), 7.35-7.42 (m, 3H, Ar-H), 7.48-7.55 (m, 4H, Ar-H), 7.70- 8.05 (m, 4H, Ar-H), 15.92 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.84, 37.68, 60.99, 108.68, 108.86, 117.85, 119.08, 119.54, 120.49, 122.40, 122.63, 122.97, 123.84, 124.00, 125.34, 125.74, 126.08, 126.62, 127.22, 130.09, 133.12, 133.56, 135.44, 139.71, 140.42, 147.48, 157.89, 172.25; MS (*m/z*): 517 $(M+H)^+$.

4-Chloro-2-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)phenol ***(4b)***: Light yellow colored solid; Yield (70%); R_f = 0.52 (6% ethylacetate in n-hexane); m. p. 226-228°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3376 (OH), 3055 (CH), 1611(C=N); ^{1}H NMR ($CDCl_3$) δ / ppm: 1.43 (t, 3H, *J*=7.0 Hz, CH_3), 3.24 (t, 1H, *J*=12.6 Hz, thiazepine ring), 3.42 (dd, 1H, *J*=8.8 & 4.5 Hz, thiazepine ring), 4.37 (q, 2H, *J*=7.0 Hz, N-CH_2), 5.32 (dd, 1H, *J*=8.2 & 4.4 Hz, thiazepine ring), 7.03 (m, 1H, Ar-H), 7.22-7.28 (m, 3H, Ar-H), 7.32-7.37 (m, 3H, Ar-H), 7.40-7.46 (m, 2H, Ar-H), 7.51-7.53 (m, 2H, Ar-H), 7.69-8.06 (m, 3H, Ar-H), 14.63 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 14.19, 37.75, 60.80, 108.16, 108.60, 108.83, 109.25, 110.47, 119.80, 120.02, 120.76, 121.23, 122.10, 123.08, 123.31, 124.94, 126.04, 126.82, 127.48, 133.30, 137.03, 138.44, 140.98, 141.49, 142.39, 144.25, 145.45, 168.95, 169.25, 175.02; MS (*m/z*): 483 $(M+H)^+$.

4-Chloro-2-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)-5-methylphenol ***(4c)***: Light yellow colored solid; Yield (68%); R_f = 0.58 (6% ethylacetate in n-hexane); m. p. 196-197°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3550 (OH), 2935 (CH), 1688 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm: 1.48 (t, 3H, *J*=7.0 Hz, CH_3), 2.35 (s, 3H, Ar-CH_3), 3.20 (t, 1H, *J*=12.6 Hz, thiazepine ring), 3.44 (dd, 1H, *J*=8.8 & 4.5 Hz, thiazepine ring), 4.39 (q, 2H, *J*=7.0 Hz, N-CH_2), 5.30 (dd, 1H, *J*=7.8 & 4.4 Hz, thiazepine ring), 7.05 (m, 1H, Ar-H), 7.20-7.29 (m, 2H, Ar-H), 7.33-7.42 (m, 3H, Ar-H), 7.40-7.48 (m, 2H, Ar-H), 7.55-7.65 (m, 2H, Ar-H), 7.73-7.85 (m, 1H, Ar-H), 8.05-8,10 (m, 2H, Ar-H), 14.60 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.74, 37.50, 60.85, 108.66, 108.83, 117.77, 119.25, 119.58, 120.39, 122.55, 122.65, 122.90, 123.80, 124.10, 125.25, 125.72, 126.23, 126.68, 127.49, 130.08, 133.17, 133.47, 135.49, 139.75, 140.48, 147.40, 157.85, 170.85; MS (*m/z*): 497 $(M+H)^+$.

4-Bromo-2-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)phenol ***(4d)***: Light yellow colored solid; Yield (71%); R_f = 0.50 (6% ethylacetate in n-hexane); m. p. 188-189°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3545 (OH), 2935 (CH), 1688 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm: 1.45 (t, 3H, *J*=7.3 Hz, CH_3), 3.28 (t, 1H, *J*=12.3 Hz, thiazepine ring), 3.45 (dd, 1H, *J*=8.9 & 4.2 Hz, thiazepine ring), 4.32 (q, 2H, *J*=7.1 Hz, N-CH_2), 5.30 (dd, 1H, *J*=8.7 & 4.3 Hz, thiazepine ring), 7.05 (m, 1H, Ar-H), 7.20-7.29 (m, 3H, Ar-H), 7.34-7.38 (m, 3H, Ar-H), 7.43-7.49 (m, 2H, Ar-H), 7.55-7.63 (m, 2H, Ar-H), 7.70-8.09 (m, 3H, Ar-H), 14.33 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.79, 37.59, 60.89, 108.62, 108.81, 117.88, 119.07, 119.52, 120.44, 122.47, 122.68, 122.92, 123.89, 124.09, 125.37, 125.72, 126.06, 126.63, 127.21, 130.08, 133.28, 133.59, 135.49, 139.72, 140.48, 147.41, 157.75, 172.85; MS (*m/z*): 527 $(M+H)^+$.

2-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)-4-methylphenol ***(4e)***: Light yellow colored solid; Yield (69%); R_f = 0.56 (6% ethylacetate in n-hexane); m. p. 215-216°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3363 (OH), 2973 (CH), 1594 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm: 1.44 (t, 3H, *J*=7.0 Hz, CH_3), 1.55 (s, 3H, Ar-CH_3), 3.23 (t, 1H, *J*=12.6 Hz, thiazepine ring), 3.42 (dd, 1H, *J*=8.8 & 4.5 Hz, thiazepine ring), 4.37 (q, 2H, *J*=7.0 Hz, N-CH_2), 5. 33 (dd, 1H, *J*=7.8 & 4.4 Hz, thiazepine ring), 7.01 (m, 1H, Ar-H), 7.25-7.27 (m, 3H, Ar-H), 7.32-7.40 (m, 3H, Ar-H), 7.42-7.44 (m, 2H, Ar-H), 7.45-7.51 (m, 2H, Ar-H), 7.53-7.70 (m, 1H, Ar-H), 8.02-8.06 (m, 2H, Ar-H), 14.59 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.78, 37.58, 60.87, 108.65, 108.80, 117.87, 119.04, 119.58, 120.49, 122.44, 122.67, 122.96, 123.88, 124.06, 125.39, 125.70, 126.03, 126.69, 127.29, 130.09, 133.27, 133.57, 135.48, 139.70, 140.44, 147.43, 157.72, 172.80; MS (*m/z*): 463 $(M+H)^+$.

2-((E)-2-(9-ethyl-9H-carbazol-3-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)phenol ***(4f)***: Light yellow colored solid; Yield (68%); R_f = 0.55 (6% ethylacetate in n-hexane); m. p. 226-227°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3555 (OH), 2935 (CH), 1688 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm: 1.28 (t, 3H, *J*=7.2 Hz, CH_3), 3.12 (t, 1H, *J*=12.8 Hz, thiazepine ring), 3.65 (dd, 1H, *J*=9.4 & 3.7 Hz, thiazepine ring), 4.41 (q, 2H, *J*=6.8 Hz, N-CH_2), 5. 45 (dd, 1H, *J*=9.4 & 3.9 Hz, thiazepine ring), 6.95-6.99 (m, 2H, Ar-H), 7.18 (m, 1H, Ar-H), 7.29 (m, 1H, Ar-H), 7.38-7.45 (m, 4H, Ar-H), 7.53-7.64 (m, 4H, Ar-H), 7.91 (m, 3H, Ar-H), 14.32 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.74, 37.48, 60.92, 108.58, 108.83, 117.81, 119.06, 119.53, 120.45, 122.42, 122.67, 122.90, 123.80, 124.08, 125.35, 125.74, 126.07, 126.65, 127.20, 130.07, 133.10, 133.57, 135.46, 139.73, 140.47, 147.42, 157.80, 172.23; MS (*m/z*): 449 $(M+H)^+$.

General procedure for the synthesis of pyrazoline derivatives 5(a-e)

Chalcone **3** (2 mmol) was dissolved in ethanol (15 mL) under stirring. To this reaction mixture, 0.5 mL of hydrazine hydrate and 0.2 mL of acetic acid was added. The reaction mixture was heated at reflux temperature for 6 h. Completion of reaction was monitored by TLC. The reaction mixture was cooled to room temperature. Then slowly 15 mL of cold water were added to the flask, the white solid obtained was washed with cold water several times. The crude compounds were recrystallized from ethanol to afford the target compounds **5(a-e)**.

*4,5-Dichloro-2-(5-(9-ethyl-9H-carbazol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (**5a**):* White colored solid; Yield (65%); R_f = 0.48 (6% ethylacetate in n-hexane); m. p. 155-156°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3668 (OH), 3205 (NH), 3051 (CH), 1614 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.36 (t, 3H, *J*=7.5 Hz, CH_3), 3.16 (dd, 1H, *J*=10.7 Hz & 5.9 Hz, pyrazoline ring), 3.65 (dd, 1H, *J*=10.7 & 5.9 Hz, pyrazoline ring), 4.43 (q, 2H, *J*=7.5 Hz, N-CH_2), 5.12 (t, 1H, *J*=10.7 Hz, pyrazoline ring), 7.15-7.35 (m, 3H, Ar-H), 7.40-7.55 (m, 4H, Ar-H), 8.01 (m, 2H, Ar-H), 8.13 (m, 1H, NH), 12.02 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.82, 37.67, 41.70, 63.62, 108.67, 109.03, 118.25, 118.65, 119.10, 120.53, 122.05, 122.54, 123.22, 123.58, 123.77, 125.48, 126.08, 129.85, 131.72, 139.76, 140.76, 152.26; HRMS (ESI): calculated for $C_{23}H_{20}ON_3Cl_2$ $(M+H)^+$ 424.0981, found 424.0978.

*4-Chloro-2-(5-(9-ethyl-9H-carbazol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (**5b**):* White colored solid; Yield (69%); R_f = 0.52 (6% ethylacetate in n-hexane); m. p. 141-142°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3363 (OH), 3055 (NH), 2950 (CH), 1593 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.35 (t, 3H, *J*=8.5 Hz, CH_3), 3.18 (dd, 1H, *J*=12.5 Hz & 6.5 Hz, pyrazoline ring), 3.69 (dd, 1H, *J*=12.5 & 6.5 Hz, pyrazoline ring), 4.45 (q, 2H, *J*=8.5 Hz, N-CH_2), 5.12 (t, 1H, *J*=12.5 Hz, pyrazoline ring), 7.16-7.29 (m, 2H, Ar-H), 7.41-7.48 (m, 3H, Ar-H), 7.50-7.55 (m, 2H, Ar-H), 8.10-8.23 (m, 4H, Ar-H, NH), 9.70 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.73, 37.98, 41.93, 62.96, 99.07, 108.61, 109.06, 111.14, 117.71, 118.38, 119.54, 120.78, 122.63, 123.74, 126.06, 126.57, 127.26, 128.89, 131.43, 148.96, 154.79, 155.45, HRMS (ESI): calculated for $C_{23}H_{21}ON_3Cl$ $(M+H)^+$ 390.11856, found 390.11876.

*4-Bromo-2-(5-(9-ethyl-9H-carbazol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (**5c**):* White colored solid; Yield (71%); R_f = 0.60 (6% ethylacetate in n-hexane); m. p. 183-184°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3655 (OH), 3225 (NH), 3065 (CH), 1635 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.40 (t, 3H, *J*=7.5 Hz, CH_3), 3.12 (dd, 1H, *J*=10.7 Hz & 5.9 Hz, pyrazoline ring), 3.61 (dd, 1H, *J*=10.7 & 5.9 Hz, pyrazoline ring), 4.44 (q, 2H, *J*=7.5 Hz, N-CH_2), 5.14 (t, 1H, *J*=10.7 Hz, pyrazoline ring), 7.11-7.35 (m, 4H, Ar-H), 7.48-7.75 (m, 4H, Ar-H), 8.10(m, 2H, Ar-H), 8.18 (m, 1H, NH), 12.10 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 14.82, 37.55, 41.68, 63.65, 108.77, 109.23, 118.25, 118.89, 119.63, 120.68, 122.45, 122.83, 123.26, 123.78, 123.89, 125.69, 126.28, 129.79, 131.80, 139.76, 140.68, 154.10; HRMS (ESI): calculated for $C_{23}H_{21}ON_3Br$ $(M+H)^+$ 434.0478, found 434.0485.

*4-Chloro-2-(5-(9-ethyl-9H-carbazol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl)-5-methylphenol (**5d**):* White colored solid; Yield (67%); R_f = 0.46 (6% ethylacetate in n-hexane); m. p. 138-139°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3650 (OH), 3238 (NH), 3029 (CH), 1650 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.39 (t, 3H, *J*=7.5 Hz, CH_3), 2.30 (s, 3H, Ar-CH_3), 3.16 (dd, 1H, *J*=10.8 Hz & 5.9 Hz, pyrazoline ring), 3.67 (dd, 1H, *J*=10.8 & 5.9 Hz, pyrazoline ring), 4.45 (q, 2H, *J*=7.5 Hz, N-CH_2), 5.12 (t, 1H, *J*=10.8 Hz, pyrazoline ring), 7.24-7.40 (m, 3H, Ar-H), 7.51-7.75 (m, 4H, Ar-H), 8.10 (m, 2H, Ar-H), 8.25 (m, 1H, NH), 12.15 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.95, 37.55, 41.68, 63.69, 108.80, 109.23, 117.29, 118.78, 119.12, 120.68, 122.65, 122.83, 123.90, 124.80, 124.95, 125.69, 126.78, 128.79, 131.80, 139.72, 140.68, 155.25; HRMS (ESI): calculated for $C_{24}H_{23}ON_3Cl$ $(M+H)^+$ 403.12514, found 403.12516.

*2-(5-(9-Ethyl-9H-carbazol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl)-5-methoxyphenol (**5e**):* White colored solid; Yield (68%); R_f = 0.55 (6% ethylacetate in n-hexane); m. p. 121-122°C; IR $\tilde{\upsilon}_{max}$/ cm^{-1}: 3325 (OH), 3056 (NH), 2973 (CH), 1678 (C=N); ^{1}H NMR ($CDCl_3$) δ / ppm : 1.36 (t, 3H, *J*=8.8 Hz, CH_3), 3.18 (dd, 1H, *J*=13.3 Hz & 7.4 Hz, pyrazoline ring), 3.33 (s, 3H, Ar-OCH_3), 3.65 (dd, 1H, *J*=13.3 & 7.4 Hz, pyrazoline ring), 4.43 (q, 2H, *J*=8.8 Hz, N-CH_2), 5.14 (t, 1H, *J*=13.3 Hz, pyrazoline ring), 7.16-7.21 (m, 2H, Ar-H), 7.33-7.45 (m, 3H, Ar-H), 7.47-7.55 (m, 3H, Ar-H), 8.04-8.10 (m, 2H, ArH), 8.13 (s, 1H, NH), 12.00 (s, 1H, Ar-OH); ^{13}C NMR ($CDCl_3$) δ / ppm: 13.96, 37.98, 44.77, 63.41, 68.08, 107.94, 110.03, 117.48, 120.24, 121.22, 122.10, 123.30, 123.76, 125.39, 125.61, 126.57, 127.00, 128.47, 128.90, 129.35, 140.53, 141.95, 149.41, 157.31; HRMS (ESI): calculated for $C_{24}H_{24}O_2N_3$ $(M+H)^+$ 386. 1904, found 386.1908.

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View supporting data **here**

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