Design, Synthesis and Computational Studies of Novel Carbazole N-phenylacetamide Hybrids as Potent Antibacterial, Anti-inflammatory, and Antioxidant Agents

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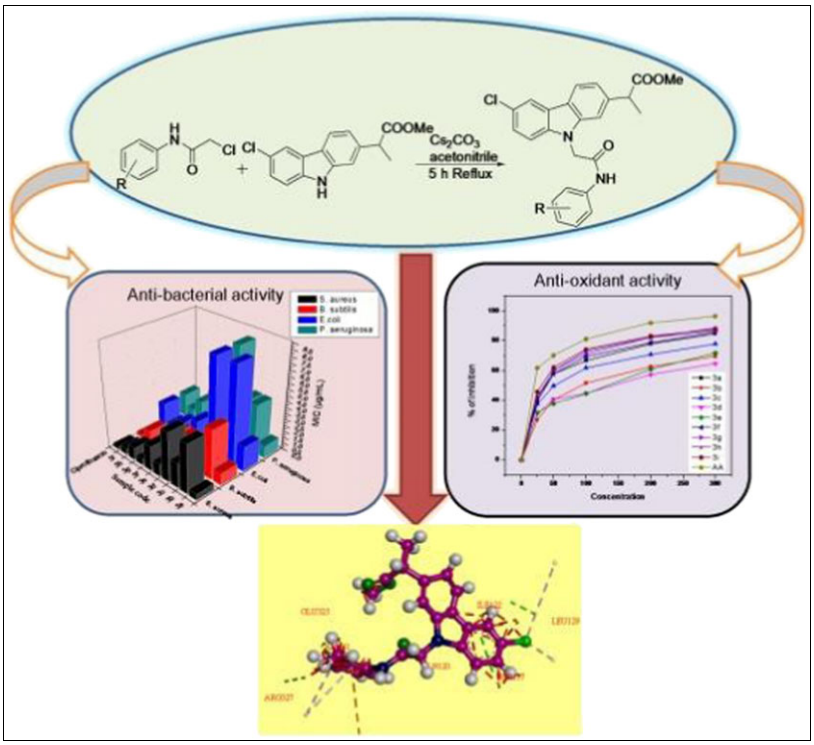


The present study deals with the synthesis of N-phenylacetamide-functionalized carbazole derivatives and their antibacterial, anti-inflammatory, and antioxidant assays. *In vitro* antibacterial studies of synthesized compounds shows prominent activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. In addition, *in silico* molecular docking studies corroborated that the methyl substituent (**3g**), (**3h**), and (**3i**) showed promising activity with lower ΔG (kcal/mol) values. This study envisages that these compounds can serve as a new leading template in the chemotherapy of various bacterial ailments.

INTRODUCTION

Bacterial infectious diseases have become a serious cause of death worldwide [1]. In recent decades, problems of multidrug-resistant microorganisms have reached an alarming level in many countries around the world. Resistance to a number of antimicrobial agents among the variety of clinically significant species of bacteria is becoming an increasingly important global problem. For instance, growing drug resistance among gram-positive bacteria such as *Enterococci*, *Streptococci*, and *Staphylococci* are major health rely [2]. This need has fortified the researchers for the improvement of the drug to combat these diseases. However, most of the antibacterial medicines reported in the literature do not have much efficacy for drug-resistant bacteria [3–5]. Therefore, there is an urgent requirement for the invention of latest drugs enriched with antibacterial susceptibility. In this regard, various carbazole derivatives have been designed and synthesized. In our previous work [6], we demonstrated

that coumarin-carprofen scaffolds (**1**) could be considered as possible antimicrobial agents and also notable bioactivity of amide linkage has prompted us to incorporate substituted N-phenylacetamide with carbazole to evaluate their antibacterial activity.

Furthermore, the inflammation is a multifactorial process as well as very general sign of many chronic diseases. Inflammation is a typical protective response of living tissues towards infection and injuries caused by physical and chemical or microbial agents [7] and are related to many disorders such as asthma, psoriasis, and rheumatoid arthritis which require repeated or prolonged medication. Antecedently, this disease was treated with a plant or plant-derived formulations, which suffered long-time period treatment. Since two decades, a number of medicines have been discovered, and still, the problem persists. Recently, "antioxidant research" has fascinated numerous scientists because of its widespread applications in the administration and improvement of a variety of human ailments [8,9]. The development of reactive oxygen species (ROS) is the normal function of aerobic organisms that can ordinarily protect themselves against these profoundly receptive species utilizing the biocatalysts such as glutathione peroxidase and superoxide dismutase, and naturally available promising antioxidants like ascorbic acid, β-carotene, and α-tocopherol [10]. Conversely, in pathophysiological conditions, a vast generation of ROS overwhelms the regular antioxidant protective mechanisms. An excessive level of free radicals and ROS play a fundamental part to trigger the various diseases composing of atherogenesis, inflammation, carcinogenesis, aging in aerobic microorganisms, and drug-associated toxicity [11–13]. Currently, some of the carbazole scaffolds are used as antioxidants to cure these diseases.

Carprofen is one of the most popular nonsteroidal anti-inflammatory drugs. It is a carbazole substituted with propionic acid class, which has been used widely in human medicine, especially for post-surgery inflammation. Many of the oxygenated tri-cyclic carbazole alkaloids like O-demethylmurrayanine (**2**), clausine D (**3**), and murrayanine (**4**) has shown promising anti-inflammatory activity [14]. Recently, Angelo D. Favia *et al*. have also reported that carprofen (**5**) as a multitarget fatty acid amide hydrolase/cyclooxygenase hit [15]. The aforementioned compounds are mentioned in (Fig. 1).

The amide linkage in the organic moiety unveils a variety of applications in the medicinal field [16]. Most of the compounds embraced with amide functional group are responsible for exhibiting antimicrobial [17–19], antioxidant [20–22], and anti-inflammatory [23,24] properties. Amide group has gained a prominent role in drug discovery as the incorporation of this functionality is responsible for the higher binding affinity and improved

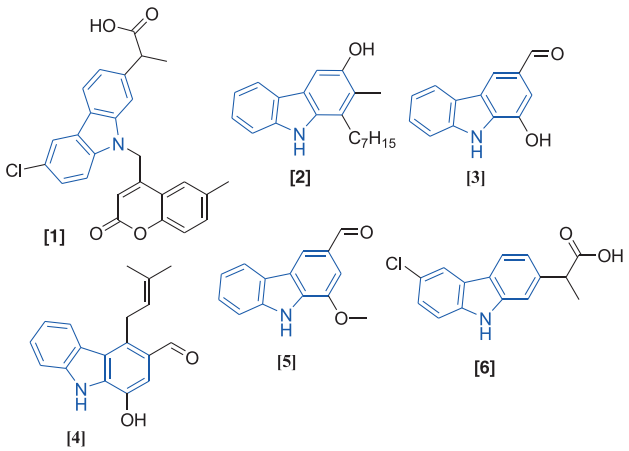


Figure 1. Structure of some potent carbazole derivatives exhibiting anti-inflammatory and antibacterial activity. [Color figure can be viewed at wileyonlinelibrary.com]

bioavailability. Currently, available antibiotic and anti-inflammatory drugs like Penicillin G (**1**), chloramphenicol (**2**), cefpiramide (**3**), loracarbef (**4**), and paracetamol (**5**) contain amide linkage (Fig. 2). Therefore, the development of new and more efficacious drugs against infectious diseases and inflammation is crucially needed with the insignificant amount of side effects.

Influenced by the intrinsic biological significance of carbazole and acetamide group, we have introduced pharmacophoric moieties into N-phenylacetamide to obtain the title compounds. It was conceptualized that these two efficacious pharmacophores such as carbazole and N-phenylacetamide if clubbed together would fructify specific molecular pattern which might be likely to exhibit fascinating pharmacological properties in animal models. Consequently, the title compounds **3(a–i)** are obtained by the condensation of substituted 2-chloro-*N*-phenylacetamide with carbazole. It is predicted that this combined effect would possibly procreate a synergetic effect by intensifying the pharmacological activity of the title compound. The theoretical interaction model is as mentioned in Figure 3. Thus, to the best of our knowledge, the synthesized compound in this study has not been reported hitherto (Scheme 2). The preliminary biological screening of title compounds has been tested for their biological properties, viz., antibacterial, antioxidant, and anti-inflammatory activities. In corroborated with the above *in vitro* biological activity followed by *in silico* molecular docking studies also have been carried out.

RESULT AND DISCUSSION

Chemistry. In continuation of our previous work on carprofen [6], we have been synthesized the methyl 2-(6-chloro-9-(2-oxo-2-(phenylamino)ethyl)-9*H*-carbazol-2-yl)

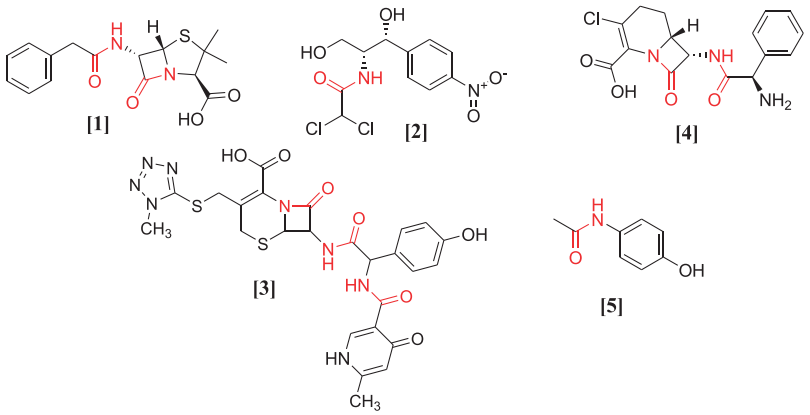


Figure 2. Structure of some antibiotic (**1**, **2**, **3**, and **4**) and analgesic (**5**) drugs containing amide groups. [Color figure can be viewed at wileyonlinelibrary.com]

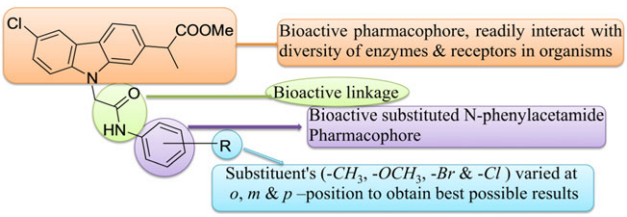
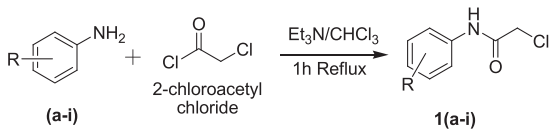


Figure 3. Design strategy to enhance antibacterial susceptibility (hypothetical interaction model). [Color figure can be viewed at wileyonlinelibrary.com]

propanoate derivatives **3**(**a**–**i**), and the synthetic route is shown in Scheme 2. The methyl 2-(6-chloro-9*H*-carbazol-2-yl)propanoate **2** have been synthesized by the esterification of the carprofen in acidic methanol yields the methyl ester **2**. The homogeneous mixture of carprofen (1.055 g) and H_2SO_4 (0.1 mL) in methanol was stirred at ambient temperature for about 12 h. After the completion of the reaction, methanol is evaporated by using rota vapor and the residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate ($NaHCO_3$) and brine solution. Separated organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo to give methyl 2-(6-chloro-9*H*-carbazol-2-yl)propanoate. The synthesis of compounds **1**(**a**–**i**) is summarized in Scheme 1.

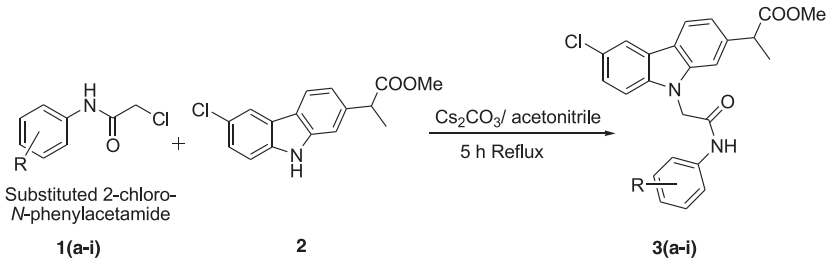
Spectral analysis of compound 3a. Characterization of the synthesized compounds has been carried out by using spectroscopic techniques such as FTIR, ^1H and ^{13}C NMR, mass, and elemental analysis. The experimental section demonstrates the physical and elemental analysis of the compounds. The presented spectral data of the compounds **3**(**a**–**i**) are witnessed to be in accordance with the assigned structures of the compounds. The IR spectrum of compound **3a** exhibited characteristic bands at 3418.29 cm^{-1} for -NH group of amide and the two stretching band at 1676.18 and 1654.54 cm^{-1} due to the carbonyl group of ester and amide, respectively. The molecular ion peak at 450$[M]^+$ in the GC-MS spectrum confirms the expected structure for compound **3a**. And further, the formation of the product was approved by ^1H NMR spectrum. Here the -NH of the amide group is resonated at 8.30 ppm as singlet, methylene linker C_{10}-H (N-CH_2) resonated at 4.46 ppm as singlet and C_{15}-H of carbazole resonated as a quartet at 3.79 ppm. A sharp singlet peak is discovered for the methoxy protons at 3.69 ppm which corresponds to the C_{18}-H of the carbazole and the methyl protons (C_{16}-H_3) resonated as a doublet at 1.56 ppm. Whereas C_4-OCH_3 appeared as singlet at 3.76 ppm. Aromatic protons resonated as multiplets between 7.15 and 7.35 ppm. The additional evidence that supports the structure of the compound **3a** is contributed by ^{13}C NMR. The carbonyl carbon of ester (C_{17}) and amide (C_9) resonates at 178.53 ppm and 172.85 ppm, respectively, the methoxy carbon of C_{18} and C_7 resonated at 45.61 and 53.81 ppm and carbon C_{10} (N-CH_2) and C_{15} resonates at 55.59 ppm and 31.05 ppm,

Scheme 1. Synthesis of different substituted 2-chloro-*N*-phenylacetamide **1**(**a–i**).



R = (a) p-OCH_3, (b) p-Cl, (c) o-Cl, (d) m-Cl, (e) p-Br, (f) m-Br , (g) 2,6-$DiCH_3$, (h) p-CH_3, (i) o-CH_3.

Scheme 2. Synthetic pathway of methyl 2-(6-chloro-9-(2-oxo-2-(phenylamino)ethyl)-9*H*-carbazol-2-yl)propanoate derivatives **3(a–i)**.



R = (a) p-OCH_3, (b) p-Cl, (c) o-Cl, (d) m-Cl, (e) p-Br, (f) m-Br , (g) 2,6-$DiCH_3$, (h) p-CH_3, (i) o-CH_3.

respectively. The methyl carbon (C_{16}-CH_3) resonates at 18.71 ppm. Signals for the remaining aromatic carbons have been shown in between 109.78 and 156.69 ppm which are in accordance with the predicted values. The numbering of the compound **3a** is represented in (Fig. 4).

***In vitro* antibacterial activity.** All the synthesized compounds are evaluated against *Staphylococcus aereus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. All these compounds exhibit excellent results with minimum inhibitory concentration (MIC) ranges from 0.25–8.0 µg/mL. As a result, compounds **3a**, **3g**, **3h**, and **3i** have shown more significant antibacterial activity. However, the MIC value of **3a**, **3g**, and **3i** was observed to be 0.50 µg/mL for *S. aereus*, and compounds **3g** and **3i** have shown MIC of 0.25 and 0.50 µg/mL for *E. coli*, respectively, which is more significant than standard ciprofloxacin. The MIC of compound **3h** was found to be 0.25 for *B. subtilis*. However, **3d** and **3b** were discovered to be less reactive for *E. coli* with a MIC of 8.0 µg/mL, whereas remaining compounds **3b–f** exhibited moderate activity. Ciprofloxacin served as control and has shown the MIC of 0.25, 0.25, 2.0, and 2.0 µg/mL for *S. aereus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively. The detailed account of the antibacterial activity of all the compounds is tabulated in the Table 1 and plotted in (Fig. 5).

Preliminary structure–activity relationship. A few key features regarding structural requirements for these title compounds **3(a–i)** to exert their antibacterial activity may be determined. Our initial strategy was to recognize the key sub unity required for activity such as, carbazole, amide linkage and the essential substituent's like -CH_3, -OCH_3 (electron donating groups), -Cl and -Br (electron withdrawing) groups were varied at *ortho*, *para*, and *meta* position of the phenyl acetamide ring to get the optimum results.

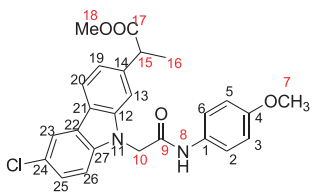


Figure 4. The structure of the compound **3a** for ^{1}H and ^{13}C NMR. [Color figure can be viewed at wileyonlinelibrary.com]

Structure–activity relationship studies revealed that the insertion of amide group to carbazole significantly improved the antibacterial activities of the compounds. The results revealed that aromatic substituent noticeably influenced the antibacterial activities of the carbazole derivatives. Compounds **3g**, **3h**, and **3i**, with methyl substituent in the phenyl ring, were most active against all the test bacteria when compared with the compounds containing electron-withdrawing groups. Specifically, the carbazole derivatives containing the -OCH_3 group which has shown excellent activity. However, halogen substituted derivatives have also been shown moderate activity.

***In vitro* anti-inflammatory activity.** The compounds **3(a–i)** have been evaluated for their anti-inflammatory susceptibility. The outcome of the anti-inflammatory activity of the tested compounds was comparable to the Diclofenac sodium and carprofen as a standard drug at the concentration of 100 µg/mL. A noticeable difference was observed in protein denaturation. Among all tested compounds, inhibition activity of compounds **3a**, **3b**, **3g**, and **3i** have shown potent anti-inflammatory activity, whereas remaining compounds **3c**, **3d**, **3e**, **3f**, and **3h** showed moderate inhibitory activity Table 2. Compounds **3a**, **3b**, **3g**, and **3i** exhibited an inhibition of heat-induced protein denaturation 88.23%, 84.82%, 80.81%, and 70.86%, respectively, remaining compounds **3c**, **3d**, **3e**, **3f**, and **3h** have shown 54.33%, 45.10%, 65.31%, 39.64%, and 64.44% of inhibition, respectively.

Free radical scavenging activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) exhibits the greatest absorbance at 517 nm in methanol. It is one of the most commonly used methods in the examination of antioxidant activity of the synthesized compounds. The scavenging ability of compounds **3(a–i)**

Table 1

In vitro antibacterial assay.

Compounds code	Minimum inhibitory concentration (MIC) (µg/mL)			
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	**0.5**	**1.0**	**2.0**	**1.0**
3b	4.0	4.0	8.0	4.0
3c	2.0	2.0	2.0	4.0
3d	4.0	2.0	8.0	8.0
3e	**1.0**	**2.0**	**1.0**	**2.0**
3f	2.0	2.0	2.0	4.0
3g	**0.5**	**1.0**	**0.25**	**1.0**
3h	**1.0**	**0.25**	**1.0**	**2.0**
3i	**0.5**	**1.0**	**0.5**	**1.0**
Ciprofloxacin	0.25	0.25	2.0	2.0

MIC values of a more potent compounds, PDB ID: MBR and highest docking score are shown in bold values.

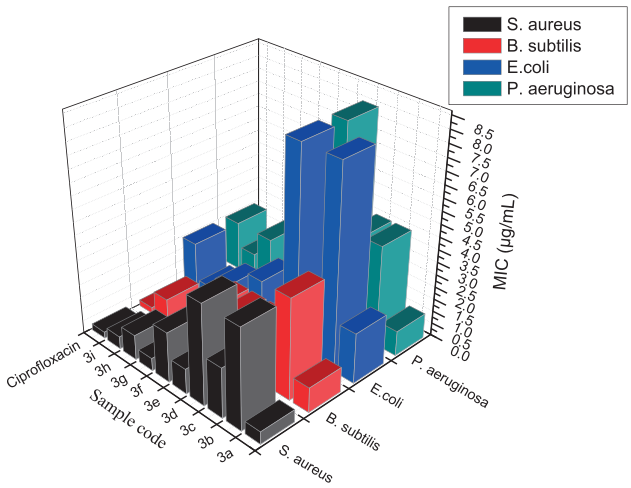


Figure 5. Graphical representation of *in vitro* antibacterial activity of compounds (**3a**–**i**). [Color figure can be viewed at wileyonlinelibrary.com]

Table 2

In vitro anti-inflammatory assay.

Sl no.	Concentration	Treatment	(%) Inhibition
1	100 µg	Diclofenac sodium	94.4633 ± 0.13051
2	100 µg	Carprofen	98.0833 ± 0.30022
3	100 µg	**3a**	88.2333 ± 0.11504
4	100 µg	**3b**	84.8267 ± 0.07506
5	100 µg	**3c**	54.3321 ± 0.15592
6	100 µg	**3d**	45.1000 ± 0.09539
7	100 µg	**3e**	65.3167 ± 0.10017
8	100 µg	**3f**	39.6467 ± 0. 11240
9	100 µg	**3g**	80.8167 ± 0. 07506
10	100 µg	**3h**	64.4444 ± 0.21297
11	100 µg	**3i**	70.8667 ± 0.15535

against DPPH radical is shown in (Fig. 6). The results reveal that, among all the compounds, **3h** and **3i** have shown higher activity with low absorbance value. The IC_{50} value of the **3h** and **3i** was discovered to be 29.83 and 27.23 µg/mL, respectively, whereas the remaining derivatives have shown moderate free radical scavenging activity.

Molecular docking. The computational study of the newly synthesized compounds has been evaluated as per our previous work [25]. PDB code: 2MBR have been used for an antibacterial molecular docking study. The antibacterial molecular docking results were assisted in the wet investigation. The interaction mechanism between enzyme and compounds **3(a–i)** was comprehended by employing the Hex molecular docking software. The best conformation has been chosen for the further investigation, as a result of its better binding affinity and minimum molecular energy.

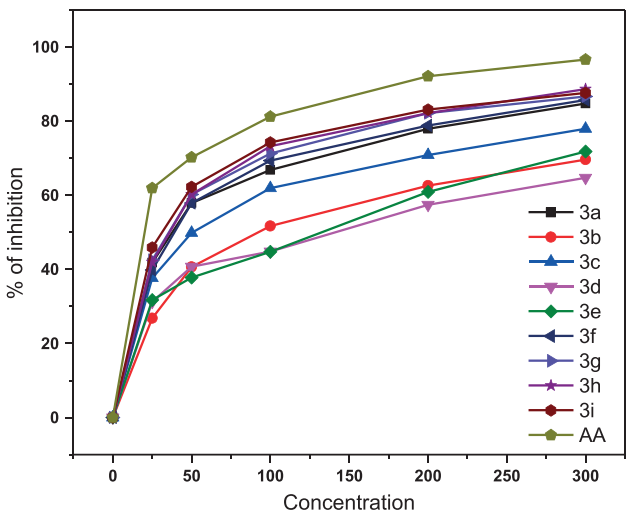


Figure 6. Graphical representation of 2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging assay of compound (**3a**–**i**). [Color figure can be viewed at wileyonlinelibrary.com]

The obtained docking results are tabulated in Table 3. All the synthesized compounds **3(a–i)** showed an interesting binding mode with nominal binding energy to the receptor 2MBR. The docking results reveal that the docked compounds (**3g**, **3i**, and **3h**) exhibit extremely least docking scores of 305.25, 303.43, and 281.31 kcal/mol, respectively, than the reference standard ciprofloxacin (237.66 kcal/mol) and possess a greater inhibiting capability towards the *E. coli* MurB enzyme receptor, by interacting with active site of the receptor. All these derivatives have shown better binding interaction within the vicinity of receptors through hydrogen bonds, alkyl, pi-alkyl, Vander walls, and other interactions with a variety of amino acids of the receptor, with this inhibits the bacterial pathogenicity property of the receptor. The interaction of compounds **3g**, **3i**, and **3h** with amino acids of 2MBR are depicted in (Figs. 7–9).

The binding energy of all the newly synthesized compounds has shown prominent binding interactions with *E. coli* MurB enzyme receptor by the key amino acid residues are ASN51, GLN120, GLU325, GLU334, LEU107, LEU104, ILE122, VAL132, LEU178, LEU53, VAL326, ARG327, TRP89, PHE328, VAL52, VAL326, and ILE110. The hydrophobic and hydrophilic spheres are used to recognize the potential ligand binding sites in each possible position. Subsequently, from the molecular docking results, it concluded that the synthesized compounds as a potent antimicrobial agent.

Table 3

Antimicrobial docking scores.

Entry	PDB ID	ΔG (kcal/mol)
3a	2MBR	−275.38
3b	2MBR	−269.16
3c	2MBR	−275.72
3d	2MBR	−275.32
3e	2MBR	−279.10
3f	2MBR	−276.42
3g	**2MBR**	**−305.25**
3h	**2MBR**	**−281.31**
3i	**2MBR**	**−303.43**
Ciprofloxacin (STD)	2MBR	−237.66

***In silico* toxicology.** Investigating the toxicity of the targeted compounds is an important step in drug designing and is required to recognize its adverse consequence on human and environment. The toxicity of the synthesized compounds was predicted by PROTOX web server. However, these computational methods are a fast and inexpensive alternative to animal experiments. PROTOX, a web server predicts rodent oral toxicity [26] based on the analysis of known drug candidates and their toxicity associated with a chemical structure or toxic fragments. It compares the structural similarity of the loaded molecule to a database of known toxic compounds (with their median LD50) and identifies the toxic fragments of the compound. Along with that server also identifies possible toxicity targets.

As per the *in silico* toxicology, all the newly synthesized compounds have shown median lethal dose (LD_{50}) values ranging from 500 to 2000 mg/kg. Compounds **3f**, **3g**, and **3i** showed LD_{50} values 500 mg/kg, compounds **3c** and **3a** showed 800 and 1000 mg/kg, respectively. Remaining compounds **3b**, **3d**, **3e**, and **3h** have shown LD_{50} values 2000 mg/kg. All the targeted compounds belong to toxicity class of 4 and none of them have toxicity fragments. The predicted results of all the compounds are tabulated in Table 4.

Experimental. Analytical grade chloroform, Chloroacetyl chloride, acetonitrile, and TEA have been obtained from Sigma Aldrich and utilized as received. An open capillary method was used to determine melting points on a Buchi equipment and are uncorrected. Nicolet 5700 was used to record infrared spectra using KBr pellets. ^{1}H and ^{13}C nuclear magnetic resonance spectra of the compounds have been recorded on JEOL 400 MHz spectrometer using deuterated chloroform as solvent and TMS was chosen as an internal standard, and chemical shifts are expressed in part per million (ppm). The mass

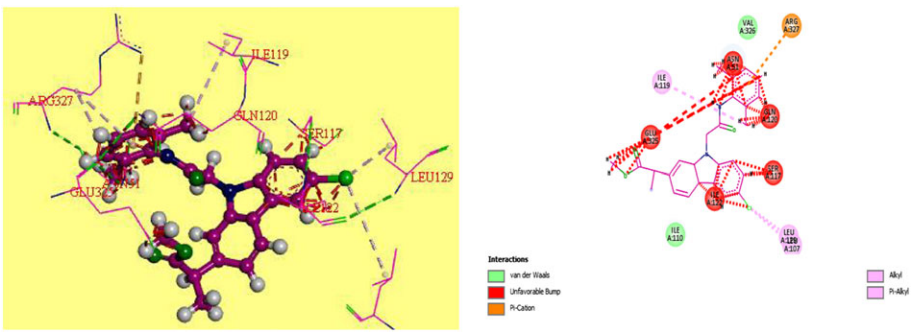


Figure 7. Interaction of compound **3g** with amino acids of 2MBR (a) 3D-structure compound (ball and stick model Oxygen-red, Nitrogen-blue) protein receptor (stick-model) (b) 2D-structure. [Color figure can be viewed at wileyonlinelibrary.com]

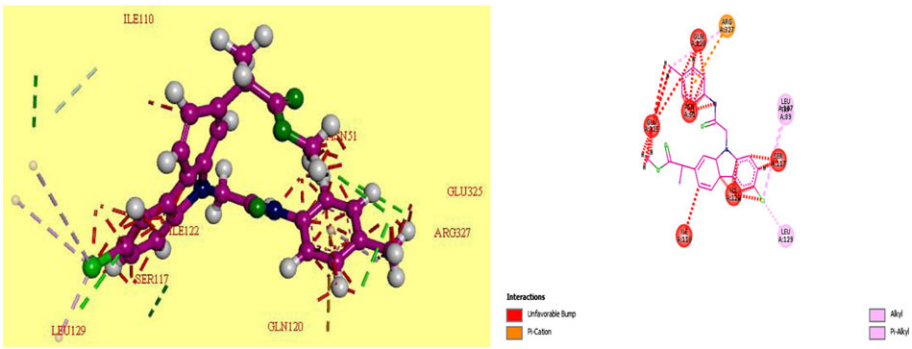


Figure 8. Interaction of compound **3h** with amino acids of 2MBR (a) 3D-structure compound (ball and stick model Oxygen-red, Nitrogen-blue) protein receptor (stick-model) (b) 2D-structure. [Color figure can be viewed at wileyonlinelibrary.com]

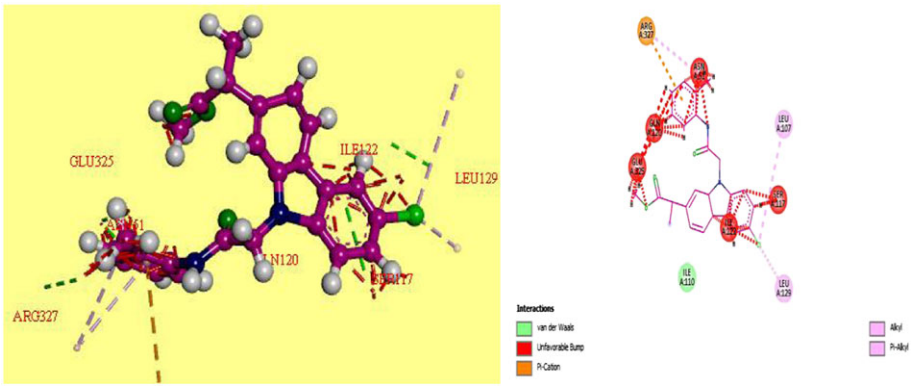


Figure 9. Interaction of compound **3i** with amino acids of 2MBR (a) 3D-structure compound (ball and stick model Oxygen-red, Nitrogen-blue) protein receptor (stick-model) (b) 2D-structure. [Color figure can be viewed at wileyonlinelibrary.com]

Table 4

Oral toxicity prediction results of Carbazole N-phenylacetamide hybrids molecules.

Sample code	Predicted LD_{50} (mg/kg)	Predicted toxicity class	Average similarity (%)	Prediction accuracy (%)	Toxic fragments
3a	1000	4	54.04	67.38	Nil
3b	2000	4	52.07	67.38	Nil
3c	800	4	51.73	67.38	Nil
3d	2000	4	51.65	67.38	Nil
3e	2000	4	50.1	67.38	Nil
3f	500	4	49.18	54.26	Nil
3g	500	4	51.36	67.38	Nil
3h	2000	4	53.31	67.38	Nil
3i	500	4	51.37	67.38	Nil

spectra of the synthesized compounds were recorded using Shimadzu GCMSQP2010S. The elemental analysis was performed by using Hereaus CHN rapid analyzer.

Synthesis of substituted 2-chloro-*N*-phenylacetamide. Various 2-chloro-*N*-phenylacetamide **1(a–i)** was brought about by the condensation of substituted anilines with chloroacetyl chloride in presence of triethylamine (Et_3N), using chloroform as a solvent. To a solution of substituted aniline (1.0 molar equiv) in chloroform, Et_3N was added to a catalytic amount. Chloroacetyl chloride (1.0 molar equiv) was added dropwise in cold condition with stirring. The reaction was refluxed for 1 h after complete addition of chloroacetyl chloride and completion of the reaction was monitored by using thin-layer chromatography (TLC). After being cooled, quenched in ice water, the precipitate was filtered and washed with water. The schematic route is mentioned in (Scheme 1).

Synthesis of methyl 2-(6-chloro-9-(2-oxo-2-(phenylamino) ethyl)-9*H*-carbazol-2-yl)propanoate derivatives. The substituted methyl 2-(6-chloro-9-(2-oxo-2-(phenylamino) ethyl)-9*H*-carbazol-2-yl)propanoate have been synthesized by condensation of substituted 2-chloro-*N*-phenylacetamide **1(a–i)** (3 to 5 equiv) with methyl 2-(6-chloro-9*H*-carbazol-2-yl)propanoate (**2**) (0.05 M) in anhydrous Cs_2CO_3 (5 equiv) using acetonitrile as the solvent [15] and refluxed for about 5 h. The reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool, the blend was filtered. The filtrate was quenched in water and extracted using Ethyl acetate. Separated organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo and purified by using column chromatography (Cy/EtOAc) to yield the title compound (Scheme 2).

Computational studies. *In silico* study was carried out using molecular modeling package version 8.2 (Hex). The homology modeling of the compounds **3(a–i)** have been evaluated against Crystal structure of *E. coli* MurB enzyme under PDB ID: 2MBR was retrieved from the Brookhaven Protein Database (PDB: http://www.rcsb.org/pdb). Two dimensional and three-dimensional energy-minimized conformations of the ligand was performed in Hex 3D Ultra 8.2, respectively, and the confirmation was analyzed by using Acceryl Discovery Studio 3.1 Client; and ciprofloxacin drug is used as standard for docking studies. The molecular structures of the newly synthesized compounds were drawn in Chem Draw ultra. Three-dimensional optimization was performed by utilizing Chem Draw 3D ultra software and saved as PDB file. The docking results can be deciphered as interaction energy. Higher the negative E total value implies stronger the interaction between ligand and receptor. This strong interaction leads to the inhibition of receptor action.

***In vitro* antibacterial activity.** To evaluate the antimicrobial activity of the newly synthesized compounds, National Committee on Clinical Laboratory Standards Macrodilution broth technique was carried out. The MIC value was resolved as the minimum dose of the synthesized compounds that introverted the growth of the test microbes. In brief, the different concentrations (0.125–128 μg/mL) of the **3(a–i)** were added to the sterile Mueller Hinton Broth medium tubes. The tubes were inoculated with 1 mL lag phase cultures of the *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. The inoculated tubes have been incubated at 37°C for 24 h followed by observing the inhibition of the bacterial growth. The MIC was determined as the minimum concentration of the **3(a–i)** containing tube showing 1no noticeable growth of the test microorganisms. Ciprofloxacin was utilized as a standard.

***In vitro* anti-inflammatory activity (egg albumin denaturation method).** *In vitro* anti-inflammatory susceptibility of the newly synthesized compounds **3(a–i)** was performed against denaturation of egg albumin method [27]. The reaction mixture consisting of 2.0 mL of known concentration of compounds **3(a–i)** (100 μg/mL) or diclofenac sodium (100 μg/mL) and 2.8 mL of phosphate buffered saline (PBS, pH 6.4) was mixed with 2.0 mL of egg albumin and incubated at 27°C for about 15 min. Denaturation was induced by placing the mixtures at 70°C in a water bath for about 10 min. Further, the reaction mixtures were allowed to cool at room temperature and the absorbance was recorded at 660 nm (SHIMADZU, UV-1800 Spectrophotometer), milli-q water was used as a blank and Diclofenac sodium was used as a standard drug. All the trials were performed in triplicates. The subsequent formula has been used to calculate the percentage of inhibition of the protein denaturation.

$$\%\text{inhibition} = \frac{(A_c\text{-}A_t)}{A_c} \times 100$$

where A_c is absorbance of control and A_t is the absorbance of test sample.

2,2-Diphenyl-1-picryl-hydrazyl-hydrate radical scavenging assay. The radical-scavenging activity of the **3(a–i)** was determined using the stable radical DPPH, according to Blois method. Briefly, Aliquots (20–100 μL) of stock solution were made up to 3 mL of methanol. Each test solutions was added by 1 mL of DPPH (0.0001 mol in methanol) solution and the blend was incubated for about 30 min in dark conditions. The absorbance was measured at 517 nm using suitable blank. Ascorbic acid was utilized as a reference standard/positive control. The scavenging activity of the samples was calculated using the following equation:

$$\%\text{inhibition} = \frac{(A_c\text{-}A_t)}{A_c} \times 100$$

where A_c is absorbance of control and A_t is the absorbance of the test sample.

CONCLUSION

Herein, we have described a facile synthesis of methyl2-(6-chloro-9-(2-oxo-2-(phenylamino)ethyl)-9*H*-carbazol-2-yl) propanoate derivatives **3(a–i)**, under conventional method in excellent yields. *N*-substituted carbazoles exhibited potent antibacterial activity against *S. aereus*, *B. subtilis*, *E. coli* and *P. aeruginosa*, anti-inflammatory, and antioxidant activity. Molecular docking study revealed that the compounds **3g**, **3i**, and **3h** have shown extremely lower E-total values. Among all the

tested compounds, **3a**, **3e**, **3g**, **3h**, and **3i** exhibited antibacterial activity with MICs ranging from 2.0 to 0.25 μg/mL. Compounds **3a**, **3b**, **3g**, and **3i** showed excellent anti-inflammatory activity. Compound **3f** showed excellent antioxidant activity and remaining derivatives have shown moderate activity. These results will help succeeding generation in design and discovery of potent antimicrobial drugs taking into account of this work.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined with open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz Spectrometer using $CDCl_3$ as solvent and TMS as an internal standard and chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analysis was carried out using Hereaus CHN rapid analyzer. Purity of the compound was checked by TLC. All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.

General procedure for the synthesis of title compounds. The substituted methyl 2-(6-chloro-9-(2-oxo-2-(phenylamino) ethyl)-9*H*-carbazol-2-yl)propanoate were synthesized by condensation of substituted 2-chloro-*N*-phenylacetamide **1**(**a**–**c**) (3 to 5 equiv) with methyl 2-(6-chloro-9*H*-carbazol-2-yl) propanoate (**2**) (0.05 M) in anhydrous Cs_2CO_3 (5 equiv) using acetonitrile as the solvent [15]. The mixture was refluxed for about 5 h. After completion of the reaction being cooled to room temperature, the mixture was filtered. EtOAc and H_2O were added to the filtrate. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography(Cy/EtOAc) to provide the title compound.

Methyl 2-(6-chloro-9-(2-(4-methoxyphenylamino)-2-oxoethyl)-9H-carbazol-2-yl)propanoate (3a). This compound is obtained as a light green color and Yield 84%; m.p: 210–212°C; IR (KBr) cm^{-1} 3418.29 (-NH) 1676.18 (C=O ester) and 1654.54 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 8.30 (s, 1H, -NH), 7.15–7.35 (m, 10H, Ar-H), 4.46 (s, 2H, -NCH_2), 3.79 (q, 1H, C_{15}-H), 3.76 (s, 3H, C_4-OCH_3), 3.69 (s, 3H, C_{18}-H ester), 1.56 (d, 3H, C_{16}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.7, 31.0, 45.6, 53.81, 55.5, 109.8, 111.7, 114.8, 119.6, 120.0, 120.6, 124.2, 124.9, 125.9, 126.6, 128.4, 134.2, 137.3, 137.4, 140.4, 156.6, 172.8, 178.5,EI-MS: $M^{+\cdot}$ *m/z* 450; *Anal*. Calcd for $C_{25}H_{23}ClN_2O_4$ (%), Calcd: C, 66.59; H, 5.14; N, 6.21; found: C, 66.58; H, 5.13; N, 6.19;

Methyl 2-(6-chloro-9-(2-(4-chlorophenylamino)-2-oxoethyl)-9H-carbazol-2-yl)propanoate (3b). As buff color powder, Yield 87%; m.p: 221–225°C; IR (KBr) cm^{-1} 3415.93 (-NH) 1682.21 (C=O ester) and 1659.33 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 9.06 (s, 1H, -NH), 7.77–8.61 (m, 10H, Ar-H), 5.75 (s, 2H, -NCH_2), 3.59 (q, 1H, C_{14}-H), 2.66 (s, 3H, C_{17}-H ester), 1.50 (d, 3H, C_{15}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.8, 45.7, 53.9, 55.7, 107.1, 109.9, 110.0, 114.9, 119.8, 120.1, 120.8, 124.4, 125.1, 126.0, 126.7, 128.6, 134.4, 137.4, 138.9, 140.6, 170.2, 175.4, EI-MS: $M^{+\cdot}$ *m/z* 455; *Anal*. Calcd for $C_{24}H_{20}Cl_2N_2O_3$ (%), Calcd: C, 63.31; H, 4.43; N, 6.15; found: C, 63.30; H, 4.41; N, 6.14;

Methyl 2-(6-chloro-9-(2-(2-chlorophenylamino)-2-oxoethyl)-9H-carbazol-2-yl)propanoate (3c). As light yellow powder, Yield 77%; m.p: 230–232°C; IR (KBr) cm^{-1} 3416.25 (-NH) 1677.29 (C=O ester) and 1660.01 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 9.86 (s, 1H, -NH), 7.82–8.72 (m, 10H, Ar-H), 5.82 (s, 2H, -NCH_2), 3.66 (q, 1H, C_{14}-H), 2.72 (s, 3H, C_{17}-H ester), 1.56 (d, 3H, C_{15}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.5, 45.1, 51.9, 57.2, 109.7, 112.5, 117.9, 119.5, 123.2, 123.4, 123.5, 123.9, 124.2, 124.9, 125.4, 127.5, 128.9, 129.2, 131.3, 135.8, 137.7, 140.1, 169.5, 174.9, EI-MS: $M^{+\cdot}$ *m/z* 455; *Anal*. Calcd for $C_{24}H_{20}Cl_2N_2O_3$ (%), Calcd: C, 63.31; H, 4.43; N, 6.15; found: C, 63.28; H, 4.42; N, 6.13;

Methyl 2-(6-chloro-9-(2-(3-chlorophenylamino)-2-oxoethyl)-9H-carbazol-2-yl)propanoate (3d). This compound is obtained as buff color and Yield 87%; m.p: 215–217°C; IR (KBr) cm^{-1} 3417.36 (-NH) 1678.32 (C=O ester) and 1659.26 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 8.72 (s, 1H, -NH), 7.50–8.43 (m, 10H, Ar-H), 5.52 (s, 2H, -NCH_2), 3.35 (q, 1H, C_{14}-H), 2.42 (s, 3H, C_{17}-H ester), 1.51 (d, 3H, C_{15}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.7, 45.7, 52.9, 55.2, 109.7, 112.5, 117.5, 117.9, 118.50, 119.50, 122.8, 123.2, 123.4, 123.5, 123.9, 125.4, 130.3, 133.0, 135.8, 137.7, 140.1, 140.1, 169.5, 174.9, EI-MS: $M^{+\cdot}$ *m/z* 455; *Anal*. Calcd for $C_{24}H_{20}Cl_2N_2O_3$ (%), Calcd: C, 63.31; H, 4.43; N, 6.15; found: C, 63.29; H, 4.41; N, 6.14;

Methyl 2-(9-(2-(4-bromophenylamino)-2-oxoethyl)-6-chloro-9H-carbazol-2-yl)propanoate (3e). As a light brown color powder, Yield 71%; m.p: 198–200°C; 3416.11 (-NH) 1688.37 (C=O ester) and 1657.17 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 8.70 (s, 1H, -NH), 7.19–8.30 (m, 10H, Ar-H), 5.94 (s, 2H, -NCH_2), 3.75 (q, 1H, C_{14}-H), 2.53 (s, 3H, C_{17}-H ester), 1.50 (d, 3H, C_{15}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.5, 45.1, 51.9, 57.2, 109.7, 112.5, 115.2, 117.9, 119.5, 120.7, 123.2, 123.4, 123.5, 123.9, 125.4, 130.6, 135.8, 137.7, 138.4, 140.1, 169.5, 174.9, EI-MS: $M^{+\cdot}$ *m/z* 499; *Anal*. Calcd for $C_{24}H_{20}BrClN_2O_3$ (%), Calcd: C, 57.68; H, 4.03; N, 5.61; found: C, 57.66; H, 4.02; N, 5.60;

Methyl 2-(9-(2-(3-bromophenylamino)-2-oxoethyl)-6-chloro-9H-carbazol-2-yl)propanoate (3f). As a brown color solid, Yield 80%; m.p: 220–222°C; IR (KBr) cm^{-1} 3419.12 (-NH) 1681.52 (C=O ester) and 1659.31 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 8.62 (s, 1H, -NH), 7.19–8.30 (m, 10H, Ar-H), 5.90 (s, 2H, -NCH_2), 3.62 (q, 1H, C_{14}-H), 2.58 (s, 3H, C_{17}-H ester), 1.51 (d, 3H, C_{15}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.5, 45.1, 51.9, 57.2, 109.7, 112.5, 117.9, 118.1, 119.5, 121.6, 122.05, 123.2, 123.4, 123.5, 123.9, 125.4, 126.0, 131.2, 135.8, 137.7, 140.1, 141.1, 169.5, 174.9, EI-MS: $M^{+\cdot}$ *m/z* 499; *Anal*. Calcd for $C_{24}H_{20}BrClN_2O_3$ (%), Calcd: C, 57.68; H, 4.03; N, 5.61; found: C, 57.67; H, 4.03; N, 5.60;

Methyl 2-(6-chloro-9-(2-(2,6-dimethylphenylamino)-2-oxoethyl)-9H-carbazol-2-yl)propanoate (3g). As a colorless powder, Yield 69%; m.p: 247–249°C; IR (KBr) cm^{-1} 3416.23 (-NH) 1677.35 (C=O ester) and 1650.48 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 9.43 (s, 1H, -NH), 7.02–7.24 (m, 9H, Ar-H), 5.81 (s, 2H, -NCH_2), 4.37 (q, 1H,

C_{16}-H), 3.94 (s, 3H, C_{19}-H ester), 2.21 (s, 6H, C_2- CH_3 & C_6-CH_3), 1.49 (d, 3H, C_{17}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.1, 18.5, 45.4, 52.0, 54.4, 109.3, 112.7, 119.0, 121.9, 123.1, 123.5, 123.8, 124.2, 125.2, 127.2, 128.1, 133.8, 135.4, 137.2, 138.8, 140.0, 167.8, 174.2, EI-MS: $M^{+\cdot}$ *m/z* 448; *Anal*. Calcd for $C_{26}H_{25}ClN_2O_3$ (%), Calcd: C, 69.56; H, 5.61; N, 6.24; found: C, 69.55; H, 5.60; N, 6.22;

Methyl 2-(9-(2-(p-toluidino)-2-oxoethyl)-6-chloro-9H-carbazol-2-yl)propanoate (3h). As a light yellow, Yield 70%; m.p: 254–256°C; IR (KBr) cm^{-1} 3418.33 (-NH) 1675.19 (C=O ester) and 1653.85 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 8.86 (s, 1H, -NH), 7.57–8.40 (m, 10H, Ar-H), 5.55 (s, 2H, -NCH_2), 3.39 (q, 1H, C_{15}-H), 2.46 (s, 3H, C_{18}-H ester), 2.04 (s, 3H, C_4- CH_3), 1.53 (d, 3H, C_{16}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 18.0, 20.2, 45.0, 51.6, 54.0, 108.9, 112.2, 118.5, 121.5, 122.7, 123.1, 123.4, 123.8, 124.8, 126.8, 127.7, 133.3, 135.0, 136.8, 138.4, 140.0, 167.3, 175.0, EI-MS: $M^{+\cdot}$ *m/z* 434; *Anal*. Calcd for $C_{25}H_{23}ClN_2O_3$ (%), Calcd: C, 69.04; H, 5.33; N, 6.44; found: C, 69.03; H, 5.31; N, 6.42;

Methyl 2-(9-(2-(o-toluidino)-2-oxoethyl)-6-chloro-9H-carbazol-2-yl)propanoate (3i). As a light buff, Yield 83%; m. p: 195–197°C; IR (KBr) cm^{-1} 3414.23 (-NH) 1677.82 (C=O ester) and 1656.12 (C=O amide); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ (ppm): 9.46 (s, 1H, -NH), 7.04–8.00 (m, 10H, Ar-H), 5.84 (s, 2H, -NCH_2), 4.40 (q, 1H, C_{15}-H), 3.97 (s, 3H, C_{18}-H ester), 2.24 (s, 3H, C_2- CH_3), 1.52 (d, 3H, C_{16}-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 17.9, 18.3, 45.2, 51.8, 54.2, 109.1, 112.5, 118.8, 121.7, 122.9, 123.3, 123.6, 124.0, 125.0, 127.0, 127.9, 133.6, 135.2, 137.0, 138.6, 139.8, 167.5, 174.0, EI-MS: $M^{+\cdot}$ *m/z* 434; *Anal*. Calcd for $C_{25}H_{23}ClN_2O_3$ (%), Calcd: C, 69.04; H, 5.33; N, 6.44; found: C, 69.03; H, 5.32; N, 6.42.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.