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Synthesis and biological evaluation of novel carbazole hybrids as promising antimicrobial agents

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Abstract

Two series of carbazole analogs of 8-methoxy-*N*-substituted-9*H*-carbazole-3-carboxamides (series-1) and carbazolyl substituted rhodanines (series-2) were synthesized through facile synthetic routes. All the final compounds from these two series were evaluated for their preliminary *in vitro* antifungal and antibacterial activity against four fungal (*Candida albicans, Cryptococcus neoformans, Cryptococcus tropicalis* and *Aspergillus niger*) and four bacterial (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) strains, respectively. Among the tested compounds, **6d**, **6e** and **6f** (series-1) displayed promising antifungal and antibacterial activity, especially against *C. neoformans* and *S. aureus.* In addition, compound **6f** displayed notable antimicrobial activity (MIC: 6.25 µg/mL) against clinical isolates of *C. albicans* and *C. neoformans* (MIC: 12.5 µg/mL). From the second series, **15f**, **15g**, **15i** and **16f** exhibited significant antifungal and antibacterial activity, especially against *C. neoformans* and *S. aureus*. The most active compound **15i** displayed a prominent antimicrobial activity against *C. neoformans* (MIC: 3.125 µg/mL) and *S. aureus* (MIC: 1.56 µg/mL), respectively.

Keywords: Carbazole, Antifungal, Antibacterial, Clinical isolate of *S. aureus*.

Introduction

It is well documented that the pathogenic microorganisms (bacteria, parasites, viruses and fungi) are rapidly developing resistance, and their associated infections can range in severity from asymptomatic to life-threatening diseases. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections, thus posing a grave concern to human kind and challenges to the medical community worldwide. In recent years, there has been rapid rise in the frequency and spectrum of antimicrobial-resistant infections particularly in hospitals settings and the community at large. This has resulted in higher morbidity and mortality, with an overall increase in healthcare costs.[1] Across the globe, there has been a constant rise in common infections such as urinary tract infections, pneumonia, and blood stream infections due to the antibiotic resistance. A large percentage of hospital-acquired infections are caused by extremely resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), *S. epidermis* and vancomycin-resistant *Enterococcus* (VRE), or multidrug-resistant Gram-negative bacteria, which are responsible for an alarming crisis of ever-increasing significance.[2–4] In recent years, the occurrence of antifungal resistance has grown to be a major health concern in clinical practice as the morbidity and mortality linked to invasive fungal diseases remains very high despite of the availability of newer antifungal and therapeutic strategies. In the past two decades, complications due to fungal infections have been recognized as a major cause of morbidity and mortality, especially in immune compromised patients including those affected by tuberculosis, HIV-1, organ-transplants, diabetes and cancer.[5–7] In addition, an increase in the incidence of fungal infections often follows the frequent use of antibacterial and cytotoxic drugs. The three most common human pathogens are *C. albicans* (mortality rate: 20-40%), *C. neoformans* (mortality rate: 20-70%) and *A. fumigatus* (mortality rate: 50-90%).[8,9] This situation has stimulated an urgent need to develop more effective and novel antimicrobial agents to overcome the drug resistance and improve the antimicrobial potency.

The chemistry of heterocyclics has contributed immensly to the drug discovery and more than 95% of the drugs in the market are heterocyclic compounds. Carbazole is one such versatile heterocyclic scaffold, obtained either from natural or by synthesis.[10] The synthesis of novel carbazole derivatives and the investigation of their chemical and biological behaviour have gained importance in recent decades for their biological, medicinal and photoelectrical applications.[11,12] Although carbazole is a rigid moiety, but can easily be functionalised to develop novel bioactive molecules. This has resulted in potential applications of carbazole-based derivatives as industrial and pharmaceutical products. Many

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recent studies have reported that carbazole derivatives exhibit a variety of biological activities such as antimicrobial,[13] anticancer,[14] anti-inflammatory,[15] antimalarial,[16] antiviral,[17] antitubercular,[18] antipsychotic and anticonvulsant[19] (*Figure 1*).

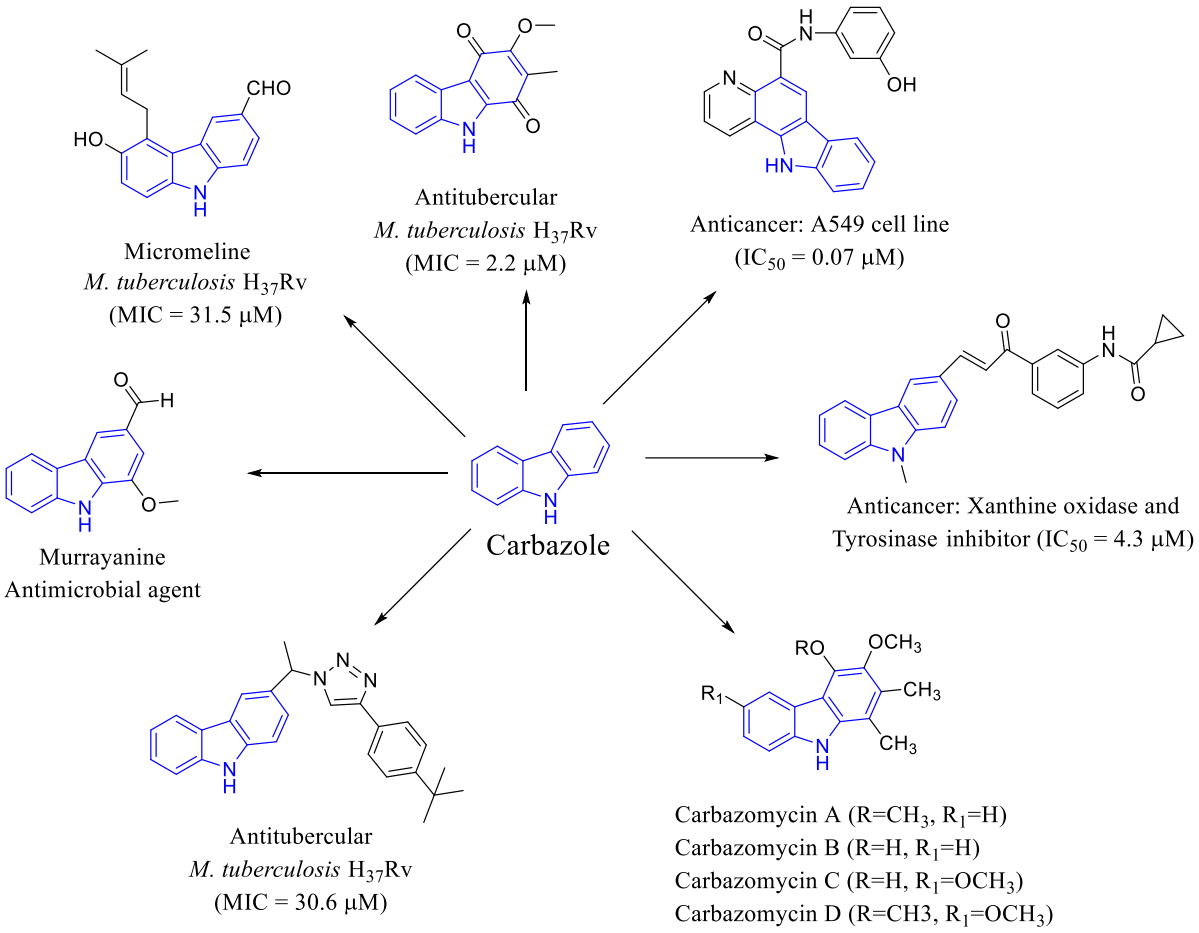

Figure 1. Potential carbazole analogs with their reported pharmacological activity.

In our present work, we report thirty four novel compounds consisting of 8-methoxy-9*H*-carbazole-3-carboxamides (**4a-g** and **6a-i**), carbazolyl-rhodanine hybrids (**15a-l** and **16a-f**) along with their antifungal and antibacterial activities.

Results and Discussion

Chemistry

The synthesis of a novel series of 8-methoxy-9*H*-carbazole-3-carboxamide derivatives (**4a-g** and **6a-i**) and carbazole tethered rhodanine derivatives (**15a-l** and **16a-f**) was achieved through versatile synthetic routes (*Scheme 1* and *2*).

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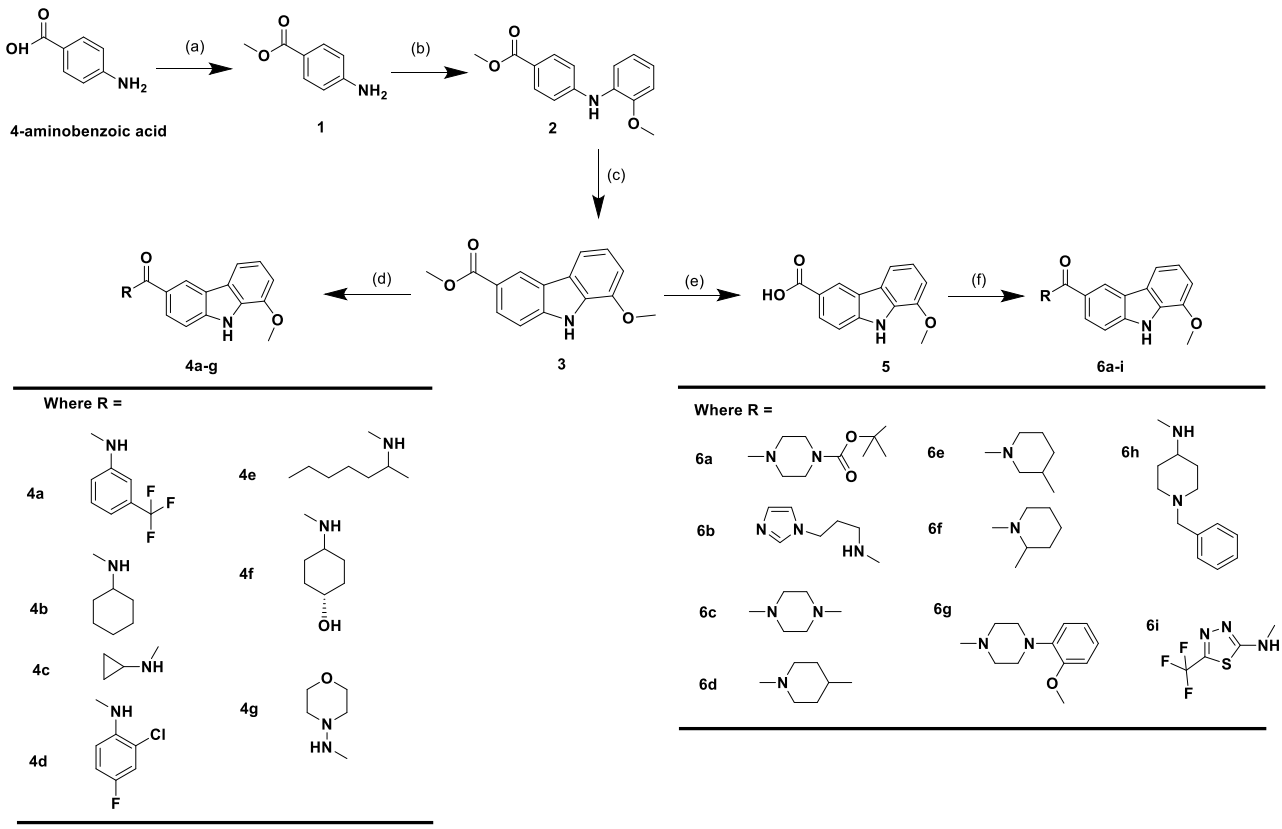

Scheme 1 Synthetic outline of a novel series of 8-methoxy-*N*-substituted-9*H*-carbazole-3-carboxamide derivatives (**4a-g**) and (**6a-i**). **Reagents and Conditions**: (a) MeOH, Conc. H_2SO_4, Stir, 60 °C, 12 h; (b) 1.1 Equiv of 2-iodoanisole, 7 mol % $Pd(OAc)_4$, 8 mol % rac-BINAP, 1.4 Equiv of Cs_2CO_3, dry toluene, stir, 110 °C, 36 h; (c) 10 mol % $Pd(OAc)_4$, 2.5 Equiv of $Cu(OAc)_2$, HOAc, stir, 117 °C, 48 h; (d) 1.2 Equiv of R-NH_2, 2M Trimethylaluminium solution in toluene, reflux, 1-5 h; (e) 2.5 Equiv of Aq. NaOH, MeOH, stir, 50°C, 12 h; (f) primary and/or secondary amines, 1.5 Equiv of HATU, 1.5 Eq. of TEA, stir, RT, 24 h.

It is clear from the synthetic scheme 1 that two unique final steps were performed in obtaining the target compounds which have structural variations at the C(3) atom of the carboxamide bond on the carbazole scaffold. The starting material **1** (methyl ester of 4-amino benzoic acid) was prepared by an esterification reaction of 4-amino benzoic acid in the presence of absolute methanol and a few drops of Conc. H_2SO_4 under reflux for 12 h. This was subjected to Buchwald-Hartwig coupling reaction with 2-iodo anisole under the influence of palladium acetate and racemic BINAP catalyst to afford methyl 4-(2-methoxy-phenylamino) benzoate (**2**). The compound **2** was then treated with a trace amount of palladium acetate and copper acetate as catalysts in the presence of glacial acetic acid to yield methyl 8-methoxy-9*H*-carbazole-3-carboxylate **3** through a carbon-carbon bond formation. Compound **3** was further reacted with appropriately substituted aryl and alkyl amines in the presence of 2 M trimethylaluminium solution in toluene, which gave the desired 8-methoxy-*N*-substituted-9*H*-carbazole-3-carboxamides **4a-g**. In addition, alkaline hydrolysis of **3** in the presence of sodium hydroxide and methanol resulted 8-methoxy-9*H*-carbazole-3-carboxylic acid **5**. 8-Methoxy-*N*-substituted-9*H*-carbazole-3-carboxamides **6a-i** were synthesized by

reacting compound **5** with appropriately substituted primary and secondary amines in the presence of coupling reagent like HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium3-oxid hexafluorophosphate) in THF/DMF mixture. The progress of the reaction and the purity of the products were established by TLC and other chromatographic methods.

The structures of key intermediate **3** and its corresponding carboxamide derivatives (**4a-g** and **6a-i**) were established on the basis of their physicochemical and spectral data (FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS). All the newly synthesized compounds showed acceptable results of their anticipated structures, which are summarized in the experimental section. In general, the IR spectrum of compound **3** presented typical absorption bands around 3265 cm^{-1} for N-H and 1696 cm^{-1} for C=O groups. This was further substantiated by the ^{1}H-NMR spectrum of **3**, which revealed the presence of a singlet at δ 8.80 ppm (4th proton), δ 8.48 ppm (N-H proton), δ 4.02 ppm (acetate protons), and δ 3.97 ppm for methoxyl protons confirming the carbon-carbon bond formation. IR spectra of the compounds **4a-g** and **6a-i** showed moderately strong bands around 3398-3164 cm^{-1} and 1699-1611 cm^{-1}, which are characteristic of the N-H and amide C=O groups, respectively. The ^{1}H-NMR spectra (400 MHz) recorded in DMSO-d_6 displayed some charecteristic singlet signals at around δ 13.59-11.55 ppm for N-H proton of the carbazole ring, δ 11.89-8.36 ppm for N-H proton of amide group at the 3rd position of the carbazole, and δ 9.10-8.12 ppm for an aromatic proton at the 4th position of the carbazole nucleus. The distinctive singlet at around δ 4.02-3.87 ppm specified the presence of the methoxyl group on the C(8) position of the carbazole ring. These findings were further substantiated from the ^{13}C-NMR spectra of the compounds with the characteristic signals appearing at around δ 170.36-170.07 ppm for the carbonyl carbon of the amide and δ 55.50-52.10 ppm for the methoxy group attached to C(3) and C(8) positions of the carbazole, respectively. The prominent signals appeared around δ 120.9-120.45 ppm indicated the presence of the CF_3 group in **4a** and **6i**, while various aromatic carbons resonated around δ 149.7-110.5 ppm and the heterocyclic aliphatic carbons resonated around δ 48.2-21.3 ppm. In addition, the formation of novel titled compounds was also confirmed based on the high resolution mass spectra (HRMS) which were in agreement with their expected molecular weights.

Carbazole tethered rhodanine derivatives (**15a-l** and **16a-f**) were prepared (*Scheme 2*) according to our earlier reported method[20].

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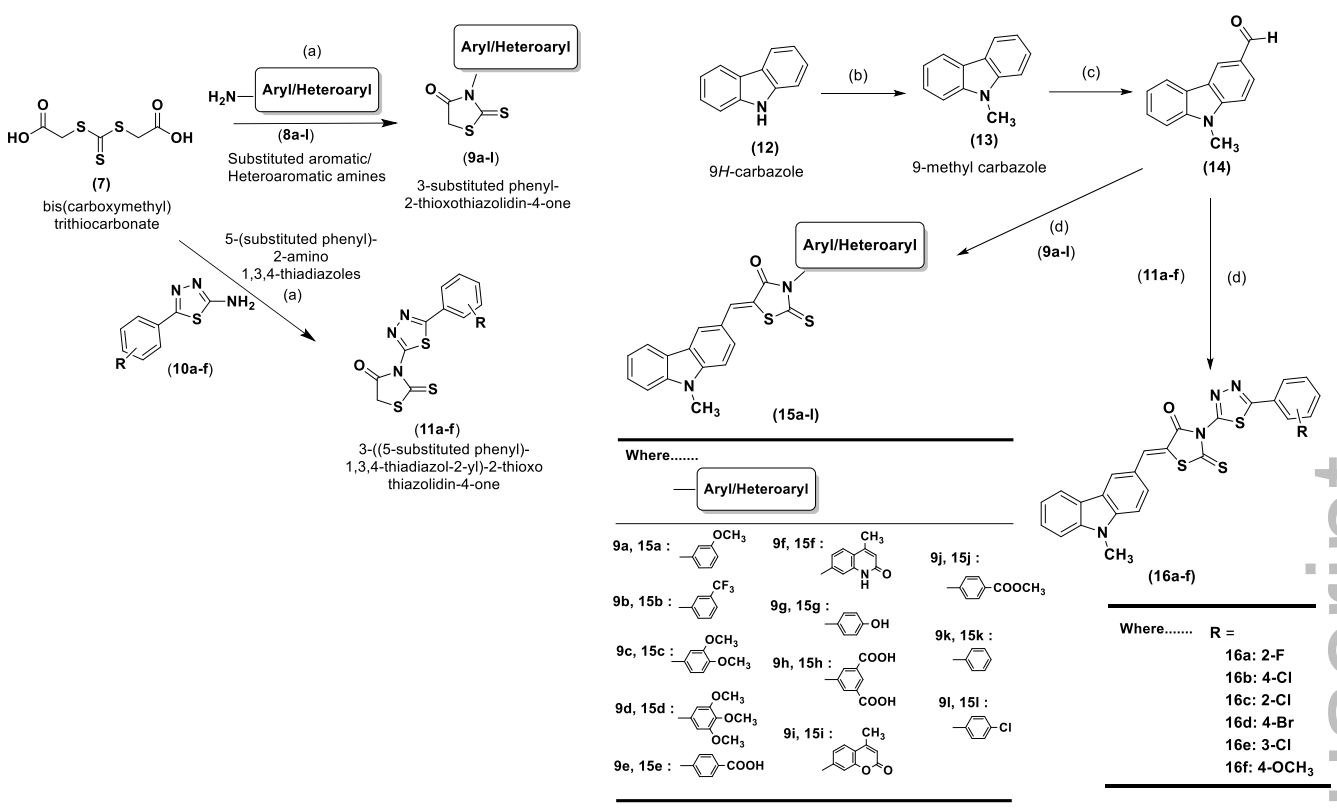

Scheme 2 Synthetic outline of carbazolyl-rhodanines (**15a-l** and **16a-f**)[20]. **Reagents and conditions**: (a) bis(carboxymethyl) trithiocarbonate, substituted aromatic/heteroaromatic amines (**8a-l** and **10a-f**), H_2O, reflux, 100 °C, 12-19 h; (b) DMF, NaH, CH_3I, stir, RT, 5 h; (c) $POCl_3$, DMF, 0 °C, stir, 90 °C, 6 h, Na_2CO_3; (d) Compound **14**, Rhodanine derivatives (**9a-l** and **11a-f**), piperidine, ethanol, microwave irradiation, 30 mins, 80 ºC, 150 psi.

Biological evaluation

Antifungal activity

All the synthesized compounds from the two series namely; 8-methoxy-*N*-substituted-9*H*-carbazole-3-carboxamide analogues (**4a-g** and **6a-i**) and carbazolyl substituted rhodanines (**15a-l** and **16a-f**) were evaluated for their *in vitro* antifungal activity against a panel of fungi: *Candida albicans* (ATCC90028), *Cryptococcus neoformans* (ATCC6603) and its clinical isolate, *Cryptococcus tropicalis* (ATCC66029) and *Aspergillus niger* (ATCC16404). Amphotericin B was used as the reference drug and the screening results (MIC values) are summarized in *Table 1*. A careful analysis of the screening data in *Table 1* revealed that the compounds from both the series were predominantly more active against *C. neoformans* (MIC = 3.125 to 50 µg/mL) and its clinical isolate (MIC = 6.25 to 50 µg/mL). The best active compounds against *C. neoformans* (CN) were **15f** and **15i** (MIC = 3.125 µg/mL), followed by **6f**, **15g**, and **16f** with MIC values at 6.25 µg/mL each and subsequently **6d** and **6e** displaying MIC at 12.5 µg/mL, respectively. All the remaining compounds from these two series

displayed moderate to low activity with MIC's 25.0 to 50.0 µg/mL. In addition the most active compounds from the above-mentioned series were further evaluated against the clinical isolates of *C. neoformans* and interestingly four carbazolyl substituted rhodanine hybrids **15f**, **15g**, **15i**, and **16f** displayed significant activity at MIC = 6.25 µg/mL, while from the 9*H*-carbazole-3-carboxamide analogues, only **6f** indicated a moderate activity (MIC= 12.5 µg/mL). From *Table 1*, It was also observed that few of the carbazolyl substituted rhodanine hybrids presented good activity against *Candida albicans* (CA), with **15f** displaying the best activity at MIC = 6.25 µg/mL, followed by **15g**, **15i**, and **16f** at MIC = 12.5 µg/mL. However, against *Cryptococcus tropicalis* (CT) and *Aspergillus niger* (AN), poor activity (MIC = 25.0 to 50.0 µg/mL) was displayed by both the series. Over all from the activity data, it was concluded that the best antifungal compound was **15f** followed by **15i**.

Antibacterial activity

It is well documented from literature reports that natural alkaloid carbazole and its synthetic analogues have displayed notable antibacterial activity.[21] Hence, we decided to evaluate these compounds against a panel of Gram positive (*S. aureus*, *B. subtilis*) and Gram negative (*E. coli*, *P. aeruginosa*) bacterial strains. The antibacterial activity was carried out in a MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL) with amoxicillin as a reference standard.[22] The *in vitro* antibacterial screening of the compounds are summarized in *Table 1*. From both the series, it was witnessed that **6d**, **6f**, **15a**, **15c**, **15d**, **15f**, **15g**, **15i** and **16f** displayed notable antibacterial activity specifically against *S. aureus* (MIC = 1.56 to 12.5 µg/mL). Interestingly, compound **15i** presented the highest activity at MIC = 1.56 µg/mL followed by **15c**, **15f**, and **16f** (MIC = 3.125 µg/mL). In addition, compounds **6f**, **15a**, **15d**, and **15g** presented good activity at MIC of 6.25 µg/mL and **15d** displayed a worthwhile activity at MIC of 12.5 µg/mL. The remaining compounds from both the series presented moderate activity with MIC's 25 to 50 µg/mL. Further, the most active compounds against *S. aureus* were also evaluated against its clinical isolates. These compounds displayed significant antibacterial activity with **15i** indicating the best activity at MIC = 3.125 µg/mL, followed by **6f**, **15c**, **15f**, **15g**, and **16f** at MIC = 6.25 µg/mL and **15d** at MIC = 12.5 µg/mL. Against *B. subtilis* strain, seven compounds (**15a**, **5c**, **15d**, **15f**, **15g**, **15i**, and **16f**) demonstrated moderate inhibition (MIC = 12.5 µg/mL). In the case of *E. coli*, three compounds (**15g**, **15i**, and **16f**) exhibited good antibacterial activity (MIC = 6.25 µg/mL), while four compounds (**15a**, **15c**, **15d**, and **15f**)

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displayed moderate activity (MIC = 12.5 µg/mL). Similarly against *P. aeruginosa*, **15d** and **15i** exhibited moderate activity at MIC = 12.5 µg/mL, while rest of the compounds presented poor activity. Overall, it was quite evident from *Table 1* that **15i** was the most active compound exhibiting a broad spectrum of antifungal and antibacterial activity.

Table 1. Antifungal and antimicrobial activity[a] of final compounds (**4a-4g**, **6a-6i**, **15a-15l** and **16a-16f**).

Comp	CA	CN	CN (Clinical Isolate)[b]	CT	AN	SA	SA (Clinical Isolate)[b]	BS	EC	PA
4a	50	25	ND	50	50	25	ND	100	100	50
4b	50	25	ND	50	100	50	ND	100	100	50
4c	50	25	ND	25	50	50	ND	100	25	50
4d	25	25	ND	50	100	25	ND	100	50	100
4e	50	25	ND	50	50	25	ND	100	100	50
4f	50	25	ND	50	50	25	ND	100	100	50
4g	50	25	ND	50	50	25	ND	50	25	50
6a	50	25	ND	50	50	25	ND	100	100	100
6b	50	25	ND	50	100	25	ND	100	100	50
6c	50	25	ND	25	100	25	ND	100	100	50
6d	50	**12.5**	25	50	50	**12.5**	50	100	100	50
6e	50	**12.5**	50	50	50	25	50	100	100	50
6f	25	**6.25**	**12.5**	50	50	**6.25**	**6.25**	50	50	50
6g	50	25	ND	50	100	25	ND	100	100	50
6h	50	25	ND	50	50	25	ND	50	25	50
6i	50	50	ND	50	50	25	ND	50	50	50
15a	25	25	ND	25	25	**6.25**	**12.5**	**12.5**	**12.5**	25
15b	100	25	ND	100	100	50	ND	100	50	50
15c	25	50	ND	100	25	**3.125**	**6.25**	**12.5**	**12.5**	25
15d	25	25	ND	50	100	**6.25**	**12.5**	**12.5**	**12.5**	**12.5**
15e	25	25	ND	50	50	25	ND	25	25	25
15f	**6.25**	**3.125**	**6.25**	25	25	**3.125**	**6.25**	**12.5**	**12.5**	25

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15g	**12.5**	**6.25**	**6.25**	50	100	**6.25**	**6.25**	**12.5**	**6.25**	50
15h	100	50	ND	50	100	25	ND	100	100	50
15i	**12.5**	**3.125**	**6.25**	25	25	**1.56**	**3.125**	**12.5**	**6.25**	**12.5**
15j	50	25	ND	50	50	25	ND	100	100	50
15k	25	25	ND	50	50	25	ND	50	50	50
15l	50	25	ND	50	50	25	ND	100	100	100
16a	50	25	ND	50	100	25	ND	100	100	50
16b	50	25	ND	25	100	25	ND	100	100	50
16c	50	100	ND	50	50	100	ND	100	100	50
16d	50	50	ND	50	50	25	ND	50	25	50
16e	50	25	ND	50	50	25	ND	100	100	50
16f	**12.5**	**6.25**	**6.25**	25	25	**3.125**	**6.25**	**12.5**	**6.25**	25
Amphotericin	1.2	25	1.2	1.95	-	-	-	-	-	-
Amoxicillin	-	-	-	-	<0.39	<0.39	<0.39	<0.39	-	-

[a]Values in MIC (µg/mL); [b]Clinical isolates are well-stored and characterized clinical isolates obtained from Department of Microbiology, Inkosi Albert Luthuli Hospital, Durban, South Africa. CA: *C. albicans*; CN: *C. neoformans*; CT: *C. tropicalis*; AN: *A. niger*; SA: *S. aureus*; BS: *B. subtilis*; EC: *E. coli*; PA: *P. aeruginosa*; ND: Not determined.

Structure-activity relationship (SAR) analysis

In general, a close inspection of the biological screening results revealed that the antimicrobial activity of carbazole hybrids was considerably affected by the nature of substitutions on the carbazole nucleus. Of the various substituents, nitrogen containing heterocyclic systems such as piperidinyl and pyrimidinyl on the carbazole nucleus exerted a significant influence on the biological activity in the first series of compounds. Specifically, 2-methylpiperidinyl carboxamide derivative **6f** (*Figure 2*) demonstrate better antifungal and antibacterial activity than its isomeric (3-methyl or 4-methyl) piperidinyl carboxamides (**6d** and **6e)** thereby confirming the crucial role of steric bulkiness for the antimicrobial action. Increasing the nucleophilicity of the substituents by incorporating additional nitrogen atoms in the ring system (compounds **6g-i**) detrimental on the antifungal or antibacterial activity.

In the second series of compounds bearing various substitutions at position-3 on the rhodanine, which is conjugated to 9-methylcarbazole through an arylidine linkage, we envisaged to study the impact of some aromatic/hetero-aromatic groups towards the

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antimicrobial activity. Specifically, we have investigated the effect of bioisosteres such as 2-quinolone (**15f**) and coumarin (**15i**) systems towards antifungal or antibacterial activity and obtained potent inhibitory profiles against the respective microbial strains including clinical isolates. Captivatingly, coumarin ring substituted rhodanine-tethered carbazole derivative **15i** showed highly potent inhibition of *S. aureus* and its clinical isolates leading to the discovery novel therapeutic application for the rhodanine-hybridized carbazoles. Replacement of aromatic groups on rhodanine moiety with thiadiazole-conjugated aromatic systems (**16a-f**) exerted no additional improvement to the activity profile, with an exception of compound **16f** bearing *para*-methoxy group. It was also observed that the compounds with electron donating groups such as methoxy (OCH_3) and hydroxy (OH) on the phenyl ring greatly contributed to the antifungal and antibacterial activity for four compounds (**15a**, **15c**, **15d** and **15g**). However, electron withdrawing substituents like halogens (F, Cl, Br and CF_3) decreased the antibacterial activity profile. Hence, the rational SAR analysis of these carbazole hybrids unveiled the significance of bulky groups and electron-donating groups in imparting the antifungal and antibacterial activity, respectively (*Figure 2*).

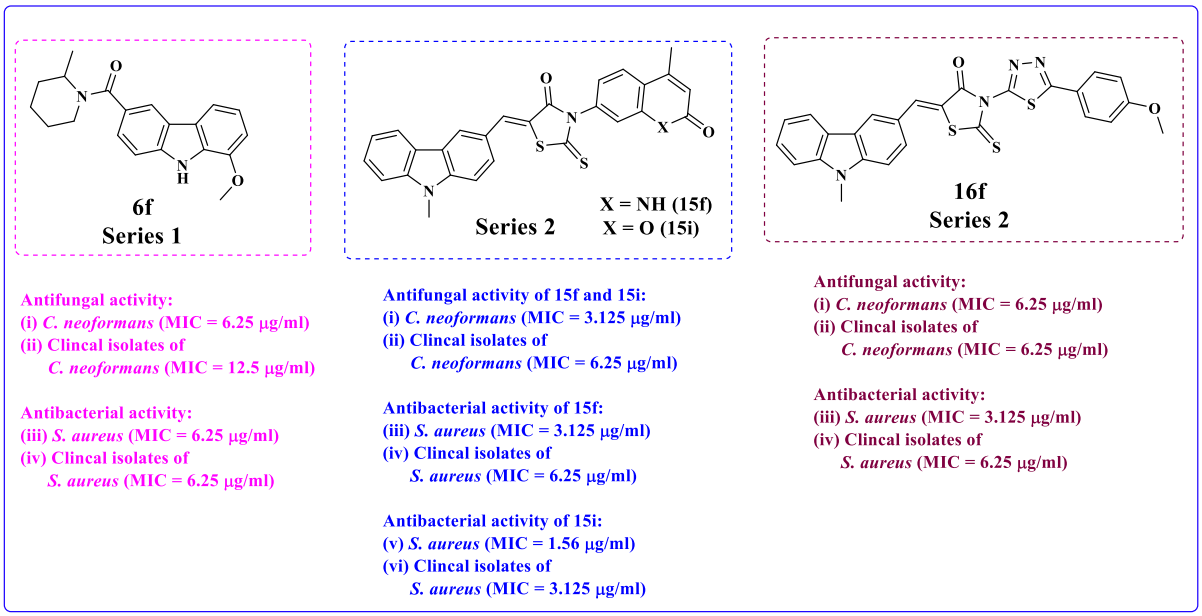

Figure 2. Structure-activity relationship (SAR) analysis of the most active compounds.

Conclusions

In summary, we have synthesized novel carbazole hybrids and evaluated for preliminary *in vitro* antimicrobial activities. Interestingly, three compounds **6d**, **6e** and **6f** from the first

series displayed promising antifungal (*C. neoformans*) and antibacterial (*S. aureus*) activities. From the second series, **15f**, **15g**, **15i** and **16f** exhibited significant activities against the same microbial strains. Amongst all the screened compounds, **15i** was found to be a potent antimicrobial against the clinical isolates of *C. neoformans* and *S. aureus* and can be considered as a lead compound. These exciting results will assist the scientific community to develop safer yet potential carbazole-based antimicrobial agents.

Experimental

Materials and Instrumentation

All reagents and fine chemicals were purchased from Sigma–Aldrich and Merck Millipore, South Africa. Solvents except the laboratory grade reagent were dried and purified according to the literature, when necessary. The progress of the reactions was 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 vapors as visualizing agent. Thermo Fisher Scientific (IA9000, Essex, Great Britain) digital melting point apparatus was employed for the melting point determination of the synthesized compounds and were uncorrected. Bruker Alpha FT-IR Spectrometer (Billerica, MA, USA) was used for the FT-IR analysis employing ATR technique. ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were recorded on Bruker AVANCE 400 (Bruker, Rheinstetten/Karlsruhe, Germany) using deuteriated solvents such as $CDCl_3$ and/or DMSO-d_6. Chemical shift values were reported in δ ppm units with respect to TMS as an internal standard. Autospec mass spectrometer under the electron impact at 70 eV was used for HRMS analysis.

Chemistry

*Synthesis of methyl 4-amino benzoate (**1**)*

To a constantly stirred solution of 4-amino benzoic acid (5 g, 1 Equiv) in 50 ml of MeOH, 3-4 drops of conc. sulphuric acid were added. The stirring was further continued at 60 ˚C for 12 h. After completing the reaction (monitored by TLC), the excess of MeOH was removed under reduced vacuum. The reaction mixture was poured onto crushed ice and extracted successively with 50 ml of DCM (3 times). The combined organic layer was washed with saturated solution of sodium bicarbonate and dried over anhydrous Na_2SO_4, filtered, and the

organic layer was concentrated *in vacuo* to yield pale brown crystalline solid. Yield: 94% (5.2 g), M.P.; 108-112 ºC; ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.85 (d, 2H, *J* = 8.60 Hz, ArH), 6.63 (d, 2H, *J* = 8.52 Hz, ArH), 4.03 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3).

*Synthesis of methyl-4-(2-methoxyphenylamino)benzoate (**2**)*[23]

A mixture 7 mol% Pd $(OAc)_4$, 8 mol% rac-BINAP and Cs_2CO_3 (1.4 Equiv) were cautiously added to a constantly stirred solution of the compound **1** (5 g, 1 Equiv) and 2-iodo anisole (1.1 Equiv) dissolved in 25 ml of dry toluene. The reaction mixture was further stirred at 110 ˚C for 36 h. After completing the reaction (monitored by TLC), the reaction mass was allowed to attain room temperature, poured onto ice-cold water and extracted successively with 50 ml of ethyl acetate (3 times). The combined ethyl acetate fraction was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain crude compound which was further purified by column chromatography (silica gel) using 5% ethyl acetate in hexane as the mobile phase to afford the compound **2** as a colorless liquid. Yield: 97% (5.31 g). IR (ATR, ν_{max}, cm^{-1}): 3398, (N-H), 3064 (Ar-H), 2946 (C-H), 1699 (C=O), 1519 (C=C), 1238 (Ar-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.93 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.38 (t, 2H, *J* = 7.7 Hz, ArH), 7.08 (d, 2H, *J* = 8.72 Hz, ArH), 6.95-7.02 (m, 2H, Ar-H), 6.37 (s, 1H, N-H), 3.88 (s, 3H, $COOCH_3$), 3.87 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 167.2 (C=O), 149.7, 147.7, 131.5, 130.7, 122.4, 120.9, 117.9, 115.3, 111.0, 55.8 ($COOCH_3$), 51.9 (OCH_3).

*Synthesis of methyl-8-methoxy-9H-carbazole-3-carboxylate (**3**)*[23]

A mixture of 10 mol% Pd $(OAc)_4$ and $Cu(OAc)_2$ (2.5 Equiv) was added to a constantly stirred solution of compound **2** (5 g, 1 Equiv) dissolved in 25 ml glacial acetic acid. The reaction mass was further stirred for 48 h at 117 ˚C and was monitored by TLC. The reaction mixture was added in ice cold water and basified with saturated solution of sodium bicarbonate (alkaline to litmus). The inorganic solid collected was filtered over celite bed and the filtrate was extracted successively with 50 ml of DCM (3 times) and dried over anhydrous Na_2SO_4. The combined organic layer was concentrated *in vacuo* and purified by column chromatography using 6% ethyl acetate in hexane as the mobile phase to obtain compound **3** as a white crystalline solid. Yield: 52% (2.61 g), M.P.; 136-138 ºC; IR (ATR, ν_{max}, cm^{-1}): 3265 (N-H), 3066 (Ar-H), 2930, 2857 (C-H), 1696 (C=O), 1508 (C=C), 1238 (Ar-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.80 (s, 1H, H-4 of carbazole), 8.48 (s, 1H, N-H), 8.11 (dd, 1H, *J* = 8.56 Hz, ArH), 7.73 (d, 1H, *J* = 7.9 Hz, ArH), 7.46 (d, 1H, *J* = 8.56 Hz, ArH), 7.19 (t, 1H, *J* = 7.9 Hz, ArH), 6.95 (d, 1H, *J* = 7.8 Hz, ArH), 4.02 (s, 3H, $COOCH_3$), 3.97 (s, 3H,

OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 168.07 (C=O), 145.9, 142.0, 130.4, 127.5, 124.5, 123.6, 121.6, 120.9, 113.2, 110.6, 106.8, 55.8 ($COOCH_3$), 52.1 (OCH_3).

General procedure for the synthesis of 8-methoxy-N-substituted-9H-carbazole-3-carboxamide (**4a**-**g**)[24]

Compound **3** (1 Equiv) and the amines (1.2 Equiv) were dissolved in dry toluene, to which 2M solution of trimethylaluminium in toluene (1 ml) was slowly added in a drop-wise manner. The resulting solution was stirred at 80 °C for 1-5 h. After completing the reaction (monitored by TLC), the mass was cooled and dumped in cold water, extracted successively with 10 ml of ethyl acetate (3 times) and dried over anhydrous Na_2SO_4. The combined organic layer was concentrated *in vacuo* and purified by trituration with diethyl ether and *n*-pentane to obtain the desired compounds **4a**-**g**. The physicochemical and spectroscopic data of newly synthesized compounds are provided below.

*8-Methoxy-N-(3-(trifluoromethyl) phenyl)-9H-carbazole-3-carboxamide (**4a**)* Yield: 80%, M.P.; 274-276 °C. IR (ATR, ν_{max}, cm^{-1}): 3370 (N-H), 3203, 3008 (Ar-H), 2937, 2908, 2835 (C-H), 1645 (C=O), 1596, 1578, 1507 (C=C), 1440, 1400, 1324, 1256, 1225 (Ar-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.72 (s, 1H, NH), 10.52 (s, 1H, CO-NH), 8.80 (s, 1H, H-4 of carbazole), 8.30 (s, 1H, ArH), 8.12 (d, 1H, *J* = 8.32 Hz, ArH), 8.04 (dd, 1H, *J* = 8.57 Hz, ArH), 7.79 (d, 1H, *J* = 7.76 Hz, ArH), 7.63-7.59 (m, 2H, *J* = 6.76 Hz, ArH), 7.44 (d, 1H, *J* = 7.72 Hz, ArH), 7.17 (t, 1H, *J* = 7.80 Hz, ArH), 7.06 (d, 1H, *J* = 7.80 Hz, ArH), 4.01 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 166.93 (C=O), 145.2, 141.07, 139.9, 129.72, 129.23, 125.03, 124.86, 124.15, 123.07, 121.61, 120.10, 119.4, 118.92, 115.67, 112.15, 110.45, 106.34, 78.73, 54.90 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{21}H_{15}F_3N_2O_2$ $[M+H]^+$: 385.1223 found 385.1226.

*N-cyclohexyl-8-methoxy-9H-carbazole-3-carboxamide (**4b**)* Yield: 63%, M.P.; 218-220 °C; IR (ATR, ν_{max}, cm^{-1}): 3405 (NH), 3252, 3072, 3011 (Ar-H), 2928, 2853 (C-H), 1621 (C=O), 1579, 1547, 1504 (C=C), 1457, 1395, 1325, 1237 (Ar-O-C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.55 (s, 1H, NH), 8.62 (s, 1H, CO-NH), 8.11 (d, 1H, *J* = 7.92 Hz, H-4 of carbazole), 7.89 (dd, 1H, *J* = 8.56 Hz, ArH), 7.72 (d, 1H, *J* = 7.76 Hz, ArH), 7.47 (d, 1H, *J* = 8.52 Hz, ArH), 7.12 (t, 1H, *J* = 7.82 Hz, ArH), 7.02 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.81 (q, 1H), 1.87 (d, 2H), 1.76 (t, 2H), 1.63 (d, 1H), 1.34 (q, 4H), 1.15 (q, 1H); ^{13}C-NMR

(100 MHz, DMSO-d_6, δ ppm): 165.89 (C=O), 145.74, 141.07, 130.14, 125.41, 123.70, 122.0, 119.83, 12.61, 110.54, 106.61,55.41 (OCH_3), 48.27, 32.63, 25.34; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{22}N_2O_2[M+H]^+$: 323.1819 found 323.1819.

*N-cyclopropyl-8-methoxy-9H-carbazole-3-carboxamide (**4c**)* Yield: 73%, M.P.; 246-248 °C; IR (ATR, ν_{max}, cm^{-1}): 3164 (NH_2), 3071, 3032 (Ar-H), 2933, 2838 (C-H), 1611 (C=O), 1597, 1579, 1506 (C=C), 1450, 1408, 1362, 1322, 1277, 1259, 1236 (Ar-O-C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.57 (s, 1H, NH), 8.58 (s, 1H, H-4 of carbazole), 8.36 (d, 1H, *J* = 4Hz, CO-NH), 7.84 (dd, 1H, *J* = 8.52 Hz, ArH), 7.71 (d, 1H, *J* = 7.76 Hz, ArH), 7.46 (d, 1H, *J* = 8.52 Hz, ArH), 7.12 (t, 1H, *J* = 7.82 Hz, ArH), 7.02 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 2.91-2.85 (m, 1H), 0.73-0.68 (m, 2H), 0.62-0.58 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.11(C=O), 145.75, 141.14, 130.16, 125.03, 123.68, 122.03, 119.80, 112.58, 110.62, 106.66, 55.42 (OCH_3), 23.11; HRMS (ESI+) m/z: calculated for $C_{17}H_{16}N_2O_2$ $[M+H]^+$: 281.1692 found 281.1694.

*N-(2-chloro-4-fluorophenyl)-8-methoxy-9H-carbazole-3-carboxamide (**4d**)* Yield: 41%, M.P.; 238-240 °C. IR (ATR, ν_{max}, cm^{-1}): 3437, 3372 (NH), 3009 (Ar-H), 2932, 2910, 2831 (C-H), 1662 (C=O), 1578, 1528 (C=C), 1486, 1459, 1399, 1302, 1290, 1237 (Ar-O-C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.70 (s, 1H, NH), 10.02 (s, 1H, CO-NH), 8.81 (s, 1H, H-4 of carbazole), 8.02 (dd, 1H, *J* = 8.57 Hz, ArH), 7.76 (d, 1H, *J* = 7.72 Hz, ArH), 7.61 (d, 2H, *J* = 8.88 Hz, ArH), 7.57-7.54 (m, 1H, ArH), 7.26 (d, 1H, *J* = 8.65 Hz, ArH), 7.15 (t, 1H, *J* = 7.8 Hz, ArH), 7.06 (d, 1H, *J* = 7.80 Hz, ArH), 4.01 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 165.88 (C=O), 158.14, 145.61, 141.42, 132.13, 130.47, 129.79, 125.14, 123.94, 122.02, 120.45, 116.56, 114.42, 112.50, 110.76, 106.68, 70.57, 55.27 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{20}H_{14}ClFN_2O_2$ $[M+H]^+$: 369.0871 found 369.0871.

*N-(heptan-2-yl)-8-methoxy-9H-carbazole-3-carboxamide (**4e**)* Yield: 68%, M.P.; 140-142 °C. IR (ATR, ν_{max}, cm^{-1}) : 3392, 3235 (NH), 3065 (Ar-H), 2948, 2929, 2852 (C-H), 1624 (C=O), 1579, 1545, 1506 (C=C), 1458, 1396, 1321, 1256, 1241 (Ar-O-C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm) : 11.55 (s, 1H, NH), 8.61 (s, 1H, CO-NH), 8.06 (d, 1H, *J* = 8.16 Hz, H-4 of carbazole), 7.90 (d, 1H, *J* = 8.40 Hz, ArH), 7.71 (d, 1H, *J* = 7.72 Hz, ArH), 7.46 (d, 1H, *J* = 8.48 Hz, ArH), 7.14 (t, 1H, *J* = 7.76 Hz, ArH), 7.02 (d, 1H, *J* = 7.76 Hz, ArH), 4.05 (t, 1H), 3.99 (s, 3H, OCH_3), 1.56-1.49 (m, 2H), 1.27 (s, 8H), 1.15 (t, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 166.08 (C=O), 145.73, 141.05, 130.14, 125.46, 123.68, 122.01, 119.76,

119.70, 112.58, 110.54, 106.61, 55.40 (OCH_3), 44.71, 36.08, 31.22, 25.58, 22.05, 20.93, 13.91; HRMS (ESI+) *m/z*: calculated for $C_{21}H_{26}N_2O_2$ $[M+H]^+$: 339.2146 found 339.2146.

*N-(4-hydroxycyclohexyl)-8-methoxy-9H-carbazole-3-carboxamide (**4f**)* Yield: 68%, M.P.; 252-254 °C. IR (ATR, ν_{max},cm^{-1}): 3432 (OH), 3266 (NH), 2955, 2929, 2856 (C-H), 1621 (C=O), 1580, 1540, 1505 (C=C), 1472, 1456, 1396, 1352, 1330, 1241 (Ar-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.55 (s, 1H, NH), 8.61 (s, 1H, CO-NH), 8.09 (d, 1H, *J* = 7.76 Hz, H-4 of carbazole), 7.89 (d, 1H, *J* = 8.48 Hz, ArH), 7.71 (d, 1H, *J* = 7.76 Hz, ArH), 7.46 (d, 1H, *J* = 8.52 Hz, ArH), 7.14 (t, 1H, *J* = 7.82 Hz, ArH), 7.02 (d, 1H, *J* = 7.80 Hz, ArH), 4.55 (d, 1H), 3.99 (s, 3H, OCH_3), 3.79-3.73 (m, 1H), 3.44 (s, 1H, OH), 1.86 (t, 1H), 1.41 (q, 4H), 1.27 (q, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm); 166.09 (C=O), 145.73, 141.06, 130.13, 125.32, 123.68, 121.99, 119.81, 112.58, 110.55, 106.61, 68.42 (C-OH), 55.40 (OCH_3), 47.84, 34.32, 30.49; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{22}N_2O_3[M+H]^+$: 338.1630 found 338.1644.

*8-Methoxy-N-morpholino-9H-carbazole-3-carboxamide (**4g**)* Yield: 39%, M.P.; 270-272 °C. IR (ATR, ν_{max}, cm^{-1}): 3270 (NH), 3194, 3039 (Ar-H), 2975, 2955, 2914, 2889, 2859, 2841 (C-H), 1622 (C=O), 1581, 1538, 1507 (C=C), 1465, 1428, 1408, 1366, 1326, 1313, 1296, 1240 (Ar-O-C); ^{1}H -NMR (400 MHz, DMSO-d_6, δ ppm): 11.59 (s, 1H, NH), 9.44 (s, 1H, CO-NH), 8.56 (s, 1H, H-4 of carbazole), 7.82 (dd, 1H, *J* = 8.52 Hz, ArH), 7.73 (d, 1H, *J* = 7.80 Hz, ArH), 7.48 (d, 1H, *J* = 8.52 Hz, ArH), 7.15 (t, 1H, *J* = 7.84 Hz, ArH), 7.03 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.69 (s, 4H), 2.94 (t, 4H); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 164.92 (C=O), 145.73, 141.19, 130.14, 124.96, 123.59, 122.0, 119.90, 119.80, 112.62, 110.71, 106.69, 66.09 (C-O), 55.4 (C-N), 54.54 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{18}H_{19}N_3O_3Na$ $[M+H]^+$: 348.1324 found 348.1330.

*Synthesis of 8-methoxy-9H-carbazole-3-carboxylic acid (**5**)*

An aqueous solution of NaOH (2.5 Equiv) was slowly added to a constantly stirred solution of **3** (1 g, 1 Equiv) in MeOH. The reaction mixture was stirred at 50 °C for 12 h. After completion of reaction (monitored by TLC), the mixture was cooled and acidified with dil. HCl, thus separated solid was filtered and purified by recrystallization to yield compound **4** as a white solid. Yield: 95% (0.900 g). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 12.48 (s, 1H, COOH), 11.72 (s, 1H, NH), 8.72 (s, 1H, H-4 of carbazole), 7.96 (dd, 2H, *J* = 8.52 Hz, ArH), 7.80 (d, 1H, *J* = 7.72 Hz, ArH), 7.51 (d, 1H, *J* = 8.52 Hz, ArH), 7.16 (t, 1H, *J* = 7.80 Hz,

ArH), 7.04 (d, 1H, *J* = 7.80 Hz, ArH), 4.00 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 168.07 (C=O), 145.73, 142.22, 130.20, 126.71, 123.61, 122.63, 121.02, 120.08, 121.91, 110.96, 106.93, 55.47 (OCH_3).

General procedure for the synthesis of 8-methoxy-N-substituted-9H-carbazole-3-carboxamide (**6a-i**)[25]

A mixture of compound **4** (1 Equiv), primary/secondary amines (1.5 Equiv), HATU (1.5 Equiv) and TEA (1.5 Equiv) were dissolved in DMF: THF (1:5). The resultant reaction mass was stirred at room temperature for 24 h. After completing the reaction (monitored by TLC), it was cooled and dumped in cold water, extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The organic layer was concentrated *in vacuo* and purified through silica gel column using a mobile phase of 5% MeOH in DCM to obtain the desired target compounds (**6a-i**). The physicochemical and spectroscopic data of these compounds are provided below.

tert-Butyl-4-(8-methoxy-9H-carbazole-3-carbonyl)piperazine-1-carboxylate ***(6a)*** Yield: 51%, M.P.; 203-205 °C. IR (ATR, ν_{max}, cm^{-1}): 3286 (NH), 3208 (Ar-H), 2980, 2914, 2862, 2840 (C=C), 1671 (C=O), 1627, 1580, 1540, 1508 (C-H), 1454, 1427, 1404, 1364, 1323, 1284, 1240 (Ar-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.54 (s, 1H, NH), 8.19 (s, 1H, H-4 of carbazole), 7.76 (d, 1H, *J* = 7.72 Hz, ArH), 7.50 (d, 1H, *J* = 8.28 Hz, ArH), 7.44 (d, 1H, *J* = 8.36 Hz, ArH), 7.10 (t, 1H, *J* = 7.80 Hz, ArH), 7.02 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.54 (s, 4H), 3.41 (s, 4H), 1.41 (s, 9H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 169.93 (C=O), 153.39, 145.2, 139.7, 129.64, 125.23, 124.43, 122.97, 121.6, 119.59, 112.49, 110.5, 106.21, 78.59, 54.95 (OCH_3), 27.57; HRMS (ESI+) *m/z*: calculated for $C_{23}H_{27}N_3O_4[M+Na]^+$: 432.1899 found 432.1895.

N-(3-(1H-imidazol-1-yl)propyl)-8-methoxy-9H-carbazole-3-carboxamide ***(6b)*** Yield: 48%, M.P.; 188-190 °C; IR (ATR, ν_{max}, cm^{-1}): 3390 (NH), 3260, 3065 (Ar-H), 2924, 2857 (C=C), 1671 (C=O), 1579, 1542, 1508 (C-H), 1458, 1406, 1365, 1325, 1263, 1240 (Ar-O-C), 1169, 1095, 1072, 1021, 955, 841, 786; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.60 (s, 1H, NH), 8.63 (s, 1H, H-4 of carbazole), 8.48 (t, 1H, *J* = 5.26 Hz, CO-NH), 7.90 (dd, 1H, *J* = 8.53 Hz, ArH), 7.86 (s, 1H, ArH), 7.74 (d, 1H, *J* = 7.76 Hz, ArH), 7.50 (d, 1H, *J* = 8.48 Hz, ArH), 7.32 (s, 1H, ArH), 7.16 (t, 1H, *J* = 7.80 Hz, ArH), 7.04 (d, 1H, *J* = 7.80 Hz, ArH), 7.00 (s, 1H, ArH), 4.09 (t, 2H, *J* = 6.86 Hz), 4.01 (s, 3H, OCH_3), 3.31 (q, 2H *J* = 6.23 Hz), 2.03 (t, 2H, *J* =

6.78 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 167.06 (C=O), 145.74, 141.16, 137.12, 130.16, 127.43, 125.06, 123.64, 122.06, 119.85, 112.57, 110.66, 106.67, 55.40 (OCH_3), 45.68, 44.14, 40.12, 36.48, 30.84; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{20}N_4O_2$ $[M+H]^+$: 348.1586 found 348.1589.

(8-Methoxy-9H-carbazol-3-yl)(4-methylpiperazin-1-yl)methanone ***(6c)*** Yield 28%, M.P.; 166-167 °C. IR (ATR, $\nu_{max,}$ cm^{-1}): 3392 (NH), 3248, 3000 (Ar-H), 2924, 2856 (C=C), 1671 (C=O), 1579, 1542 (C-H), 1457, 1427, 1406, 1365, 1322, 1239 (Ar-O-C), 1168, 1134, 1094, 1072, 1022, 999, 842, 785, 733, 703; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.53 (s, 1H, NH), 8.16 (s, 1H, H-4 of carbazole), 7.76 (d, 1H, *J* = 7.76 Hz, ArH), 7.50 (t, 1H, *J* = 6.66 Hz, ArH), 7.41 (d, 1H, *J* = 8.32 Hz, ArH), 7.10 (t, 1H, *J* = 7.78 Hz, ArH), 7.02 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.57 (s, 4H), 2.39 (s, 4H), 2.24 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 170 (C=O), 145.65, 140.04, 130.06, 125.72, 124.72, 123.39, 122.08, 119.86, 119.53, 112.91, 110.90, 106.62, 55.38 (OCH_3), 54.46, 40.12; HRMS (ESI+) *m/z*: calculated for $C_{19}H_{21}N_3O_2$[M+H]$^+$: 324.1779 found 324.1779.

(8-Methoxy-9H-carbazol-3-yl)(4-methylpiperidin-1-yl)methanone ***(6d)*** Yield: 37%, M.P.; 181-182 °C. IR (ATR, ν_{max}, cm^{-1}): 3393 (NH), 3233, 3066 (Ar-H), 2952, 2923, 2862 (C=C), 1654 (C=O), 1580, 1534 (C-H), 1508, 1458, 1407, 1364, 1320, 1263, 1238 (Ar-O), 1171, 1092, 1071, 1022, 975, 837, 785, 746; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.50 (s, 1H, NH), 8.13 (s, 1H, H-4 of carbazole), 7.75 (d, 1H, *J* = 7.76 Hz, ArH), 7.49 (d, 1H, *J* = 8.32 Hz, ArH), 7.37 (dd, 1H, *J* = 8.2 Hz, ArH), 7.12 (t, 1H, *J* = 7.82 Hz, ArH), 7.01 (d, 1H, *J* = 7.76 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.09 (q, 1H), 2.92 (s, 1H), 2.86 (d, 3H), 2.84 (s, 1H), 1.66 (t, 4H), 1.10 (q, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6 , δ ppm): 170.07 (C=O), 161.94, 145.67, 139.92, 130.06, 126.6, 124.53, 123.42, 122.08, 119.55, 112.91, 110.87, 106.59, 55.39 (OCH_3), 48.16, 33.91, 32.19, 30.56, 29.70, 21.65; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{22}N_2O_2$[M+Na]$^+$: 345.1579 found 345.1570.

(8-Methoxy-9H-carbazol-3-yl)(3-methylpiperidin-1-yl)methanone ***(6e)*** Yield: 42%, M.P.; 183-184 °C. IR (ATR, ν_{max},cm^{-1}): 3395 (NH), 3231, 3001 (Ar-H), 2945, 2922, 2861 (C=C), 1654 (C=O), 1591, 1579, 1543, 1508 (C-H), 1459, 1440, 1405, 1365, 1318, 1260, 1238 (Ar-O), 1173, 1119, 1093, 1023, 968, 838, 788, 750, 731; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.51 (s, 1H, NH), 8.12 (s, 1H, H-4 of carbazole), 7.75 (d, 1H, *J* = 7.72 Hz, ArH), 7.49 (d, 1H, *J* = 8.32 Hz, ArH), 7.36 (dd, 1H, *J* = 8.16 Hz, ArH), 7.12 (t, 1H, *J* = 7.78 Hz, Ar-H),

7.01 (d, 1H, *J* = 7.76 Hz, ArH), 3.99 (s, 3H, OCH_3), 2.92 (s, 1H), 1.80 (d, 1H), 1.62 (s, 1H), 1.46 (d, 1H), 1.12-1.21 (m, 2H), 0.84 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 170.03 (C=O), 145.66, 139.89, 130.04, 126.59, 124.49, 123.41, 122.07, 119.55, 112.88, 110.85, 106.58, 55.39 (OCH_3), 32.60, 18.79; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{22}N_2O_2[M+Na]^+$: 345.1579 found 345.1570.

(8-Methoxy-9H-carbazol-3-yl)(2-methylpiperidin-1-yl)methanone ***(6f)*** Yield: 40%, M.P.; 201-203 °C. IR (ATR, ν_{max}, cm^{-1}): 3421 (NH), 3251, 3000 (Ar-H), 2949, 2927, 2863 (C=C), 1654 (C=O), 1596, 1579, 1508 (C-H), 1458, 1432, 1365, 1318, 1260, 1239 (Ar-O-C), 1172, 1119, 1099, 1022, 1009, 887, 840, 787, 767, 747; ^{1}H-NMR (400 Hz, DMSO-d_6, δ ppm): 11.49 (s, 1H, NH), 8.10 (s, 1H, H-4 of carbazole), 7.76 (d, 1H, *J* = 7.76 Hz, ArH), 7.49 (d, 1H, *J* = 8.32 Hz, ArH), 7.34 (dd, 1H, *J* = 8.37 Hz, ArH), 7.11 (t, 1H, *J* = 7.80 Hz, ArH), 7.01 (d, 1H, *J* = 7.76 Hz, ArH), 4.47 (s, 1H), 3.99 (s, 3H, OCH_3), 3.01 (t, 1H), 1.63 (q, 4H), 1.40-1.53 (m, 2H), 1.21 (d, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 170.36 (C=O), 145.67, 139.82, 130.06, 127.17, 124.09, 123.43, 122.16, 119.49, 112.93, 110.94, 106.58, 55.4 (OCH_3), 29.91, 25.64, 18.54, 15.83; HRMS (ESI+) *m/z*: calculated for $C_{20}H_{22}N_2O_2[M+Na]^+$: 345.1579 found 345.1570.

(8-Methoxy-9H-carbazol-3-yl)(4-(2-methoxyphenyl)piperazin-1-yl)methanone ***(6g)*** Yield: 33%, M.P.; 214-216 °C. IR (ATR, ν_{max}, cm^{-1}): 3421 (NH), 3251, 3058, 3001 (Ar-H), 2927, 2858, 2832 (C=C), 1636 (C=O), 1596, 1580, 1508 (C-H), 1458, 1406, 1366, 1319, 1294, 1263, 1238 (Ar-O), 1172, 1116, 1101, 1014, 946, 893, 843, 816, 786, 748; ^{1}H-NMR (400 MHz, D MSO-d_6, δ ppm): 11.54 (s, 1H, NH), 8.21 (s, 1H, H-4 of carbazole), 7.76 (d, 1H, *J* = 7.73 Hz, ArH), 7.50 (d, 1H, *J* = 8.28 Hz, ArH), 7.45 (d, 1H, *J* = 8.16 Hz, ArH), 7.13 (t, 2H, *J* = 7.76 Hz, ArH), 7.01 (d, 1H, *J* = 7.80 Hz, ArH), 6.95 (d, 1H, *J* = 8.00 Hz, ArH), 6.91 (d, 1H, *J* = 7.40 Hz, ArH), 6.88 (d, 1H, *J* = 7.08 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.72 (s, 4H), 3.00 (s, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 170.13 (C=O), 152.04, 145.69, 140.82, 130.11, 125.90, 124.92, 123.46, 122.90, 122.13, 120.82, 120.01, 119.59, 118.40, 112.99, 111.91, 110.97, 106.68, 55.43 (OCH_3), 50.41 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{25}H_{25}N_3O_3[M+Na]^+$: 438.1794 found 438.1794.

N-(1-benzylpiperidin-4-yl)-8-methoxy-9H-carbazole-3-carboxamide ***(6h)*** Yield: 33%, M.P.; 232-234 °C. IR (ATR, ν_{max}, cm^{-1}): 3388 (NH), 3251 (Ar-H), 2930, 2859, 2842, 2800 (C=C), 1672 (C=O), 1622, 1579, 1539, 1506 (C-H), 1455, 1405, 1365, 1326, 1239 (Ar-O), 1169,

1100, 1071, 1022, 839, 785, 732; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.57 (s, 1H, NH), 8.62 (s, 1H, H-4 of carbazole), 8.20 (s, 1H, CO-NH), 7.89 (d, 1H, *J* = 8.44 Hz, ArH), 7.71 (d, 1H, *J* = 7.72 Hz, ArH), 7.46 (d, 1H, *J* = 8.52 Hz, ArH), 7.36 (s, 5H, ArH), 7.13 (t, 1H, *J* = 7.78 Hz, ArH), 7.01 (d, 1H, *J* = 7.80 Hz, ArH), 3.99 (s, 3H, OCH_3), 3.87 (s, 1H), 3.56 (s, 2H), 3.08 (d, 1H, *J* = 6.92 Hz), 2.92 (s, 2H), 1.86 (s, 2H), 1.68 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 145.73 (C=O), 141.11, 130.14, 128.24, 125.21, 123.66, 122.0, 119.88, 112.59, 110.57, 106.64, 55.4 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{26}H_{27}N_3O_2[M+H]^+$: 414.2103 found 414.2104.

8-Methoxy-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)-9H-carbazole-3-carboxamide ***(6i)*** Yield: 15%, M.P.; 279-281 °C. IR (ATR, ν_{max}, cm^{-1}): 3390 (NH), 3262, 3065, 3000 (Ar-H), 2925, 2856 (C=C), 1671 (C=O), 1654, 1624, 1579, 1542, 1508, 1458, 1406, 1365, 1326, 1263, 1240 (Ar-O), 1169, 1096, 1072, 1021, 842, 786, 767, 741; ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 13.59 (s, 1H, NH), 11.89 (s, 1H, CO-NH), 9.10 (s, 1H, H-4 of carbazole), 8.19 (dd, 1H, *J* = 8.72 Hz, ArH), 7.76 (d, 1H *J* = 7.76 Hz, ArH), 7.59 (d, 1H, *J* = 8.64 Hz, ArH), 7.20 (t, 1H, *J* = 7.84 Hz, ArH), 7.07 (d, 1H, *J* = 7.84 Hz, ArH), 4.02 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 145.8 (C=O), 142.51, 130.31, 126.06, 123.61, 122.43, 120.48, 112.72, 111.36, 107.22, 55.5 (OCH_3); HRMS (ESI+) *m/z*: calculated for $C_{17}H_{11}F_3N_4O_2S$ $[M+H]^+$: 393.0695 found 393.0698.

Synthesis and spectral characterization of second series of compounds *3-(substitutedphenyl)-5-((9-methyl-9H-carbazol-6-yl) methylene)-2-thioxothiazolidin-4-ones* (**15a-l**) and *2-(9-methyl-9H-carbazol-6-yl) methylene)-4-(5-substitutedphenyl-1,3,4-thiadiazol-2-yl)-5-thioxo-thiazolidin-4-ones* (**16a-f**) were reported in our recently published article [20].

Biological evaluation

The synthesized final compounds (series-1: **4a-g**; **6a-i**) and (series-2: **15a-l** and **16a-f**) were assessed for their antimicrobial activity against a panel of bacterial and fungal strains by following MIC assay method using resazurin dye.[26] The protocols corresponding to microorganisms, preparations of medium, solutions, inoculums, and standard assay by broth micro-dilution method employed in this work were based on our earlier published paper.[22] The antifungal and antibacterial activity on the clinical isolates was carried out at the Department of Microbiology, Inkosi Albert Luthuli Hospital, Durban, South Africa.

Supporting Information

Spectral images of FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS are provided in the Supporting Information Section.

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Author Contribution Statement

Mahamadhanif S. Shaikh and Mahesh B. Palkar synthesized the compounds. Balakumar Chandrasekaran, Ashish M. Kanhed and Parvesh Singh contributed to the data analysis and manuscript drafting. Afsana Kajee, Koleka P. Mlisana, Meenu Ghai, and Mavela Cleopus Mahlalela contributed to the biological experiments and data analysis. Rajshekhar Karpoormath designed the experiments and supervised the project.

Conflict of interest: Authors declare that they have no conflict of interest.

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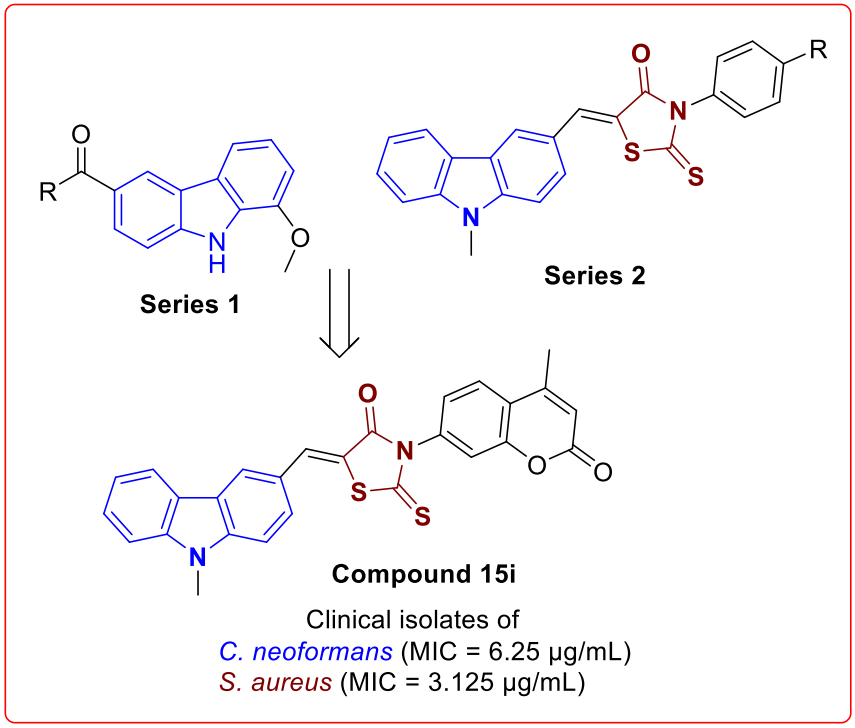
Graphical Illustration



Novel carbazole analogs were synthesized and characterized by spectral methods. Antimicrobial evaluation was conducted for these hybrids against four bacterial, four fungal and a couple of clinical isolates. The lead compound **15i** displayed a prominent antimicrobial activity against the clinical isolates of *C. neoformans* (MIC = 6.25 µg/mL) and *S. aureus* (MIC = 3.125 µg/mL) which explored the potential of carbazole-based antimicrobial agents.