Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 818739, 7 pages
http://dx.doi.org/10.1155/2013/818739

*Research Article*

# Synthesis, Antibacterial, and Antifungal Activities of Novel Pyridazino Carbazoles

**Ghazala Yaqub,[1] Abdul Hannan,[2] Erum Akbar,[3] Muhammad Usman,[2] Almas Hamid,[1] Zubi Sadiq,[4] and Mehwish Iqbal[3]**

[1] *Department of Environmental Sciences, Kinnaird College for Women, Lahore 54000, Pakistan*
[2] *Department of Microbiology, University of Health Sciences, Lahore 54000, Pakistan*
[3] *Department of Chemistry, Lahore College for Women University, Lahore 54000, Pakistan*
[4] *Department of Chemistry (Girls), College of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

Correspondence should be addressed to Ghazala Yaqub; ghazala_yaqub@yahoo.com

Received 30 September 2012; Revised 25 November 2012; Accepted 25 November 2012

Academic Editor: Naoki Toyooka



Strategy to synthesize novel carbazole, that is, 2,3-dihydro-2-phenyl-6*H*-pyridazino[4, 5-*b*]carbazole-1,4-dione (**2**), is developed as key reaction. This novel compound showed ideal reactivity toward different functional groups like methyl, carboxylic, nitro, piperazine, and amine; thus series of its derivatives are synthesized sequentially with the aim to fuel up the carbazole compounds with new versatile derivatives. All compounds were investigated for their activity against bacteria (MRSA and *S. typhi*) and fungi (*Candida albicans*). Among tested compounds **3**, **6**, and **8** exhibited pronounced antibacterial activities while **2**, **5**, and **9** also showed moderate activities. **2**, **3**, **5**, **7**, and **9** showed stronger antifungal activity against *Candida albicans* comparable to positive control.

## 1. Introduction

Spread of drug-resistant bacteria has badly affected the efficiency of many known antibacterial agents [1] while the emergence of antifungal infections in immune compromised population has also significantly increased over past few decades [2, 3]. Thus novel antibacterial and antifungal agents with various mechanisms of action and antimicrobial activities are needed for the effective control of these clinically important infections. Carbazoles are one of the predominant antimicrobial agents [4]. During last few decades a large number of carbazoles have been isolated or synthesized exhibiting tremendous antibacterial activities against broad spectrum bacteria, that is, *S. aureus*, *E. coli*, *B. Subtilis*, *S. typhi*, and so forth and fungi, that is, *C. neoformans, C. albicans*, and so forth [5].

Carbazoles are among exclusive types of *N*-containing aromatic heterocycles possessing desirable electronic and charge-transport properties, as well as large *pi*-conjugated system [6]. The structurally rigid carbazolyl ring is also found friendly towards the introduction of various functional groups. Carbazole nucleus has always stimulated the endeavors to find new pathways for its synthesis and its novel derivatives because of extensive photophysical, photochemical, and biological properties [7] especially their amazing pharmacological profile and their role as drug molecule [8]. To date, numerous researchers make efforts to develop efficient synthetic avenues to carbazole and its well-modified derivatives, which are well documented [9, 10]. Based on the documented facts which highlighted the incredible potential applications of carbazole-based derivatives in the field of chemistry, the work undertaken here relates to the synthesis of some novel carbazoles (Figure 1) and evaluation of antimicrobial properties of synthesized carbazoles especially against bacteria which have acquired resistance against known antibiotics.

## 2. Experimental

*2.1. General.* $^{1}$H and $^{13}$C NMR spectral data were recorded using Varian-Inova-500 NMR spectrometer in $CD_3OD$ or DMSO-$d_6$. Chemical shifts ($\delta$) are reported in ppm ($\delta$) with tetramethylsilane (TMS) as an internal reference. Signals are

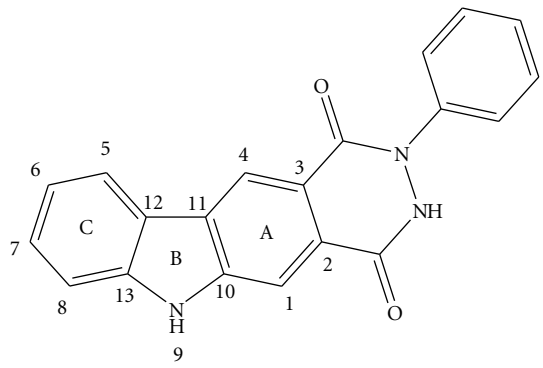



Figure 1

described as s (singlet), d (doublet), t (triplet), dd (doublet of doublet), m (multiple), and q (quartet), and coupling constants ($J$ values) are given in Hz. Mass spectra (MS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. FTIR spectra were recorded using FTIR spectrophotometer (JASCO-4100). Reaction monitoring by thin layer chromatography was carried out using Merck silica gel 60 F254 (0.2 mm) plates. Solvent used for TLC was the mixture of hexane and ethyl acetate in 7 : 3 ratio, respectively. To visualize the compounds, TLC plates were examined under UV light or by staining with cerium ammonium molybdate. Commercially available reagents were purchased from Fluka, Aldrich, Acros Organic, and Alfa Aesar. All reagents were recrystallized, and solvents were made anhydrous before use in reactions.

*2.2. Synthesis of 2,3-Dihydro-2-phenyl-6H-pyridazino[4, 5-b]carbazole-1,4-dione (***2**). To the carbazole dicarboxylic acid (**1**) (2.55 gm, 0.01 mol) was added 4 mL of 8% phenylhydrazine and 6 mL of ethyleneglycol and the reaction mixture was heated at 150°C with continuous stirring for some time to dissolve the reaction contents. Water produced was removed from reaction mixture by distillation and then reaction contents were strongly heated up to 250°C to close newly formed heterocyclic ring. At the end, reaction mixture was cooled in ice bath, filtered, and washed with methanol. The pure white colored crystals of **2** (2.01 gm, 70%) were obtained after recrystallization in acetone with m.p. 213°C (Scheme 1).

Anal Calcd for $C_{20}H_{13}N_3O_2$: C, 73.38; H, 4.00; N, 12.84% found: C, 73.27; H, 4.10; N, 12.80%; IR (KBr, $\nu$max, $cm^{-1}$) 3418, 3040, 1604. $^1$H NMR (DMSO) $\delta$: 6.30 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 7.7 Hz, 1H), 6.94 (dd, $J$ = 8.0, 8.2 Hz, 1H), 7.00 (dd, $J$ = 7.2, 7.7 Hz, 1H), 7.12 (dd, $J$ = 7.4, 7.3 Hz, 1H), 7.38 (d, $J$ = 7.3 Hz, 1H), 7.49 (d, $J$ = 7.3 Hz, 1H), 8.0 (s, 1H), 8.19 (s, 1H), 8.40 (s, 1H), 9.8 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 107.2, 110.2, 112.0, 114.3, 119.3, 119.5, 119.8, 120.8, 121.3, 123.5, 124.2, 126.4, 130.1, 133.0, 137.5, 139.1, 164.0, 169.1. MS m/z: 327.10 ($MH^+$).

*2.3. Synthesis of 2,3-Dihydro-9-nitro-2-phenyl-6H-pyridazino [4, 5-b]carbazole-1,4-dione (***3**). To the stirring solution of compound **2** (3.27 gm, 0.01 mol) in 25 mL methanol was added a suspension of cupric nitrate (1.86 gm, 0.01 mol) in 10 mL acetic anhydride with keeping the temperature of the reaction contents at 0°C. The reaction mixture was further stirred at the same temperature for 3 hours under nitrogen. The deposited precipitates were collected by filtration and washed with methanol to obtain yellowish solid compound **3** (2.52 gm, 68%) having melting point 176°C.

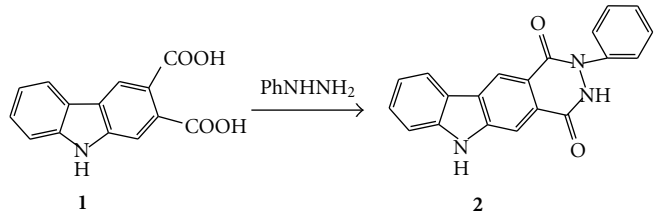
Anal Calcd for $C_{20}H_{12}N_4O_4$: C, 64.52; H, 3.25; N, 15.05% found the following: C, 63.99; H, 3.24; N, 15.10%; IR (KBr, $\nu$max, $cm^{-1}$): 3398 (NH), 3033, 1680 (C=O), 1570, 1360 ($NO_2$). $^1$H NMR (DMSO) $\delta$: 6.30 (d, $J$ = 8.0 Hz, 1H, phenyl proton), 6.67 (d, $J$ = 7.7 Hz, 1H, phenyl proton). 7.12 (dd, $J$ = 7.4, 7.3 Hz, 1H, phenyl proton), 7.60 (d, $J$ = 8.0 Hz, 1H, H-7), 8.0 (s, 1H, O=CNNH), 8.10 (d, $J$ = 7.7 Hz, 1H, H-8), 8.29 (s, 1H, H-1), 8.44 (s, 1H, H-4), 8.55 (s, H, H-5), 9.8 (s, 1H, NH). $^{13}$CNMR (DMSO) $\delta$: 107.2, 110.2, 112.2, 113.2, 114.3, 115.6, 119.3, 119.5, 123.5, 124.3, 126.4, 130.1, 133.2, 137.5, 139.1, 141.6, 164.1, 169.2. MS m/z: 372.09 ($MH^+$).

*2.4. Synthesis of 2,3-Dihydro-2-phenyl-9-(piperazin-1-yl)-6H-pyridazino[4, 5-b]carbazole-1,4-dione (***4**). To the compound **2** (3.27 gm, 0.01 mol) in 180 mL DCM piperazine (2.58 gm, 0.03 mol) (dissolved in 80 mL DCM) was added dropwise (2.58 gm, 0.03 mol) in 80 mL DCM. The reaction mixture was stirred at room temperature for 35 min (monitored by TLC) and then extracted with ammonia buffer. The aqueous was washed with diethylether to remove the excess of piperazine. The aqueous phase was collected and lyophilized. Pure colorless crystals of **4** (3.0 gm, 73%) were obtained after recrystallization from methanol having m.p. 222°C.

Anal Calcd for $C_{24}H_{21}N_5O_2$: C, 70.05; H, 5.14; N, 17.00% found the following: C, 70.15; H, 5.35; N, 17.19%; IR (KBr, $\nu$max, $cm^{-1}$): 3408, 3080, 1730, 1718, 1425. $^1$H NMR (DMSO) $\delta$: 2.0 (m, 1H), 2.47 (d, $J$ = 8.0 Hz, 3H), 2.78 (q, $J$ = 7.4, 7.3 Hz, 2H), 2.83 (s, 3H), 3.49 (t, $J$ = 7.3, 7.2 Hz, 2H), 6.26 (d, $J$ = 8.0 Hz, 1H), 6.30 (d, $J$ = 7.7 Hz, 1H). 6.67 (d, $J$ = 7.4, 7.3 Hz, 1H), 6.90 (s, 1H), 7.12 (dd, $J$ = 7.7, 7.9 Hz, 1H), 7.22 (d, $J$ = 7.3, 1H), 8.0 (s, 1H), 8.20 (s, 1H), 8.40 (s, 1H), 9.8 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 35.9, 37.8, 48.7, 59.5, 102.7, 107.3, 107.9, 110.4, 112.0, 113.2, 119.4, 119.6, 123.5, 124.2, 126.5, 130.1, 133.0, 137.5, 137.7, 164.1, 169.3. MS m/z: 413.19 ($MH^+$).

*2.5. Synthesis of 9-(N-methyl-N-(2-(methylamino)ethyl) amino)-2,3-dihydro-2-phenyl-6H-pyridazino[4, 5-b]carbazole-1,4-dione (***5**). To the compound **2** (3.27 gm, 0.01 mol) in 180 mL DCM, *N*,*N*-dimethyl ethane-1,2- diamine (2.64 gm, 0.03 mmol) (dissolved in 80 mL DCM) was added dropwise (2.64 gm, 0.03 mmol) in 80 mL DCM. The reaction mixture was stirred for 45 min at room temperature, and the contents were extracted with ammonia buffer afterwards. The aqueous phase was collected and extracted with diethylether to remove the excess of amine. The aqueous phase after collection was lyophilized to reveal the slightly brownish solid product **5** with melting point 216°C (2.84 gm, 69%) that was recrystallized in methanol.

Anal Calcd for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 16.99% found the following: C, 70.10; H, 5.71; N, 17.00%; IR (KBr, $\nu$max, $cm^{-1}$): 3212, 3080, 1760, 1050. $^1$H NMR




1 2

Scheme 1: Novel pyridazino carbazole.

(DMSO) $\delta$: 2.0 (m, 1H), 2.78 (t, $J$ = 7.4, 7.3 Hz, 2H), 3.49 (t, $J$ = 7.2, 7.4 Hz 2H), 6.39 (d, $J$ = 8.0 Hz, 1H), 6.30 (d, $J$ = 7.7 Hz, 1H). 6.66 (d, $J$ = 7.4 Hz, 1H), 6.90 (s, 1H), 7.12 (dd, $J$ = 7.7, 8.0 Hz, 1H), 7.26 (d, $J$ = 7.3 1H), 2.83 (s, 1H), 8.19 (s, 1H), 8.40 (s, 1H), 9.8 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 45.9, 52.2, 102.7, 105.3, 107.2, 110.2, 112.2, 113.2, 119.4, 119.6, 123.5, 124.2, 126.3, 129.9, 133.2, 137.5, 139.2, 139.7, 164.0, 169.3. MS m/z: 411.17 ($MH^+$).

*2.6. Synthesis of 9-(dimethylamino)-2,3-dihydro-2-phenyl-6H-pyridazino[4, 5-b]carbazole-1,4-dione (***6**). In the round bottom flask, compound **2** (3.27 gm, 0.01 mol) was added followed by 20 mL of methanol. Then this solution was treated with methanolic solution of dimethylamine (0.72 gm, 0.016 mol) dropwise while keeping the contents of round bottom flask in ice. After the addition of DMA the reaction mixture was stirred at room temperature. The reaction progress was monitored by TLC until its completion. After 4 hours, the crude product was filtered and recrystallized with methanol to get yellowish product **6** with melting point 198°C (2.52 gm, 68%).

Anal Calcd for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.93; N, 15.13% found the following: C,72.01; H, 5.01; N, 15.34%; IR (KBr, $\nu$max, $cm^{-1}$): 3350, 3100, 1630, 1468. $^1$H NMR ($CD_3OD$) $\delta$: 2.83 (s, 3H), 6.39 (d, $J$ = 8.0 Hz, 1H), 6.30 (d, $J$ = 7.7 Hz, 1H). 6.68 (d, $J$ = 7.4 Hz, 1H), 6.78 (s, 1H), 7.12 (dd, $J$ = 7.7, 7.9 Hz, 1H), 7.27 (d, $J$ = 7.3, 1H), 8.0 (s, 1H), 8.19 (s, 1H), 8.40 (s, 1H) 9.8 (s, 1H). $^{13}$CNMR ($CD_3OD$) $\delta$: 40.3, 102.7, 105.3, 107.2, 110.3, 112.2, 113.2, 119.3, 119.5, 123.5, 124.2, 126.3, 129.2, 130.1, 133.0, 137.3, 137.7, 139.3, 164.0, and 169.3. MS m/z: 370.14 ($MH^+$).

*2.7. Synthesis of 2,3-dihydro-8,9-dimethyl-2-phenyl-6H-pyridazino[4, 5-b]carbazole-1,4-dione (***7**). Compound **2** (3.27 gm, 0.01 mol) and methyl iodide (3.12 gm, 0.02 mol) were refluxed in carbon tetrachloride for six hours. Then the reaction mixture was cooled in ice bath, and the crude product was filtered, washed with methanol, and recrystallized from acetone to get colorless crystals of **7** having melting point 265°C (2.83 gm, 80%).

Anal Calcd for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.84; N, 11.98% found the following: C,74.01; H, 4.90; N, 12.21%; IR (KBr, $\nu$max, $cm^{-1}$): 3418, 3013, 2880, 1751. $^1$H NMR (DMSO) $\delta$: 2.35 (s, 3H), 2.40 (s, 3H), 6.30 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 7.7 Hz, 1H), 7.00 (s, 1H), 7.13 (dd, $J$ = 7.4, 7.3 Hz, 1H), 7.23 (s, 1H), 8.0 (s, 1H), 8.19 (s, 1H), 8.39 (s, 1H), 9.8 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 17.8, 18.1, 107.2, 110.2, 111.2, 113.5, 119.3, 119.5, 120.8, 123.5, 124.2, 126.4, 130.1, 131.6, 133.3, 137.5, 139.1, 164.0, and 169.1. MS m/z: 355.39 ($MH^+$).

*2.8. Synthesis of 2,3,4,6-Tetrahydro-1,4-dioxo-2-phenyl-1H-pyridazino[4, 5-b]carbazole-8,9-dicarboxylic acid (***8**). Product **7** (3.55 gm, 0.01 mol) and excess of potassium ferricyanide solution in 0.5 M sodium hydroxide with few drops of pyridine were refluxed for five hours at 200°C. The reaction mixture was cooled in ice bath to obtain solid product. After filtration, it was washed with methanol and recrystallized in acetone to afford white crystalline product **8** having melting point 232°C (3.44 gm, 83%).

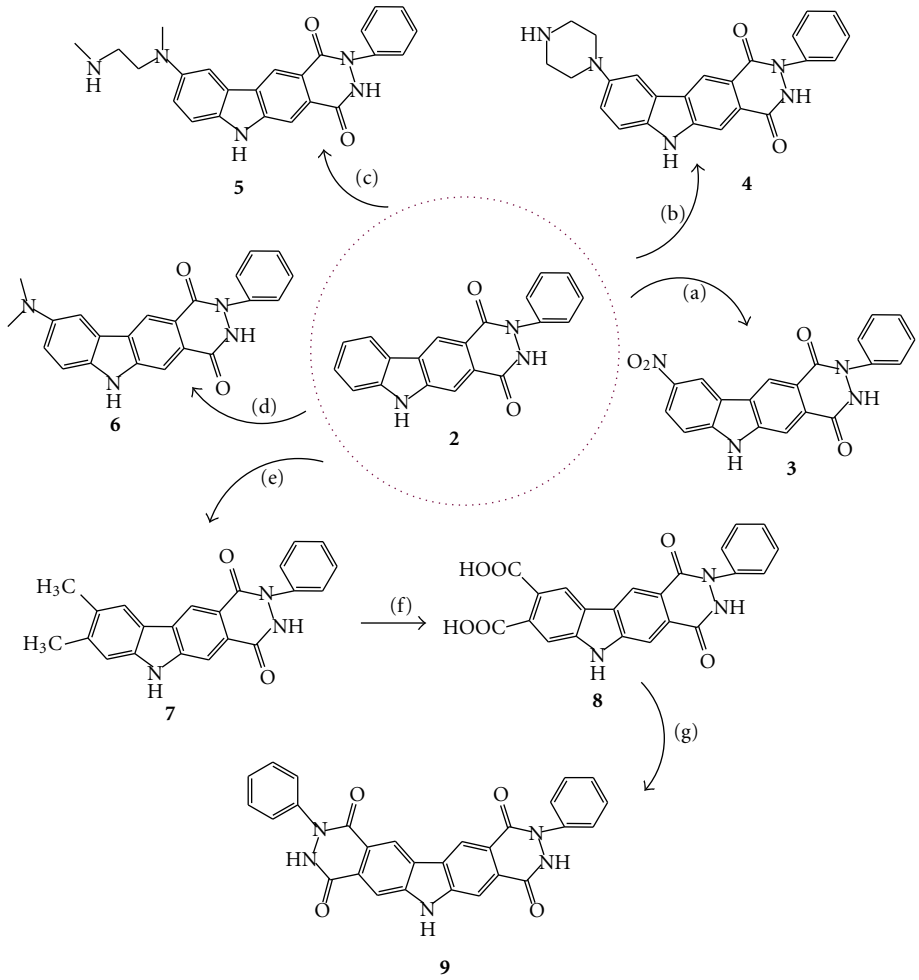
Anal Calcd for $C_{22}H_{13}N_3O_6$: C, 63.62; H, 3.16; N, 10.14% found the following: C, 64.61; H, 3.79; N, 11.01%; IR (KBr, $\nu$max, $cm^{-1}$): 3375, 3320, 2980, 1698. $^1$H NMR (DMSO) $\delta$: 6.30 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 7.7 Hz, 1H), 7.13 (dd, $J$ = 7.4, 7.3 Hz, 1H), 8.0 (s, 1H), 8.19 (s, 1H), 8.40 (s, 1H), 8.45 (s, 1H), 8.52 (s, 1H), 9.8 (s, 1H), 10.8 (s, 1H), 10.5 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 107.2, 110.2, 112.0, 113.2, 119.3, 119.5, 121.3, 123.2, 123.5, 124.2, 126.4, 126.9, 130.1, 133.0, 137.4, 139.2, 164.0, 169.1 and 169.4. MS m/z: 415.08 ($MH^+$).

*2.9. Synthesis of Pyridazino Carbazole (***9**). To the compound **8** (4.15 gm, 0.01 mol) was added 4 mL of 8% phenyl hydrazine and 6 mL of ethylene glycol and then reaction mixture heated at 150°C for some time to dissolve the contents. Water produced was removed by distillation and then strongly heated up to 250°C to close newly formed heterocyclic ring. Reaction vessel was cooled in ice bath, filtered, and washed with methanol. The white crystals of compound **9** having melting point 289°C (3.55 gm, 73%) were obtained after recrystallization from acetone.

Anal Calcd for $C_{28}H_{17}N_5O_4$: C, 68.99; H, 3.52; N, 14.38% found the following: C, 69.12; H, 3.69; N, 15.00%; IR (KBr, $\nu$max, $cm^{-1}$): 3418, 3390, 3040, 1609, 720. $^1$H NMR (DMSO) $\delta$: 6.66 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 7.7 Hz, 1H), 7.13 (dd, $J$ = 7.4, 7.3 Hz, 1H), 8.0 (s, 1H), 8.19 (s, 1H), 8.40 (s, 1H), 9.8 (s, 1H). $^{13}$CNMR (DMSO) $\delta$: 110.3, 113.2, 119.3, 119.5, 120.8, 123.5, 126.4, 130.1, 132.5, 137.5, 164.0, 169.1. MS m/z: 487.13 ($MH^+$).

*2.10. Antimicrobial Studies*

*2.10.1. Procedure.* Antimicrobial activity of all synthesized carbazoles was determined in terms of MIC to check the




Scheme 2: Approaches to novel carbazoles. Reaction condition and reagents: (a) cupric nitrate in acetic anhydride; (b) Piperazine, DCM; (c) *N*,*N*-dimethyl ethane-1, 2-diamine, DCM (d), dimethyl amine in methanol (e) methyl iodide in CCl4; (f) potassium ferricyanide solution in sodium hydroxide; (g) phenyl hydrazine in ethylene.

lowest concentration of the compounds at which bacterial growth was completely inhibited. MIC was determined against thirty-four clinical isolates of *Salmonella typhi* (gram negative bacteria), multiresistant *Staphylococcus aureus* (MRSA, gram positive bacteria) and *Candida albicans* (fungi) including the ATCC reference strains. The method was adopted from Zhang work on antimicrobial activity of carbazoles [6] with few modifications according to working lab conditions.

*2.10.2. Preparation of Compounds Incorporated Muller-Hinton Agar Plates.* Muller-Hinton agar (38 gm) was dissolved in 1 liter of distilled water. Then 33 flasks were taken for one compound (as eleven concentrations for each compound in triplicates were to be prepared). 20 mL of Muller-Hinton agar was poured in each flask and autoclaved. After autoclaving for 15 min at 120°C the agar was allowed to cool in water bath up to the temperature of 50°C. Different concentrations of each compound, that is, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, and 2048 $\mu$g/mL, were prepared to determine an approximate MIC of synthesized compounds. The respective concentration of each compound was added in labeled flask. The flask was shaken well to mix the compound thoroughly and poured into 90 mm Petri dish. The plates were allowed to solidify at room temperature and then stored in refrigerator. At the time of use these plates were placed in hot air oven at 55°C for about five minutes to permit the evaporation of surface moisture if present.

*2.10.3. Procedure for MIC.* The microorganism strains from microbank were refreshed on blood agar plates. Bacterial suspensions with turbidity equivalent to 0.5 McFarland standards were made for each strain in tryptic soya broth. It was then further diluted to one tenth with normal saline. Then in the labeled wells of multi-inoculator grid 600 $\mu$L of each bacterial strain dilution was added in the respective well.

Compound incorporated plates were taken out of the refrigerator, dried in hot air oven (55°C) for about five minutes, and inoculated with multipoint multi-inoculator (Mast Diagnostic, UK). Two control plates were also set: one

with Muller-Hinton agar without any compound inoculated at the start of inoculation with all strains to confirm the sterility of the cultures. Second control plate was having Muller-Hinton agar medium without inoculation. Plates were then incubated at 37°C for 18–24 hours and were observed for the growth inhibition by unaided eye. Potato dextrose agar was used for antifungal screening rather than Muller-Hinton agar [11].

## 3. Results and Discussion

*3.1. Synthesis and Characterization.* Carbazole and its derivatives are privileged natural product motifs that are dowered with enormous properties and exclusive biological profile [12, 13]. The current work emphasizes the synthesis of novel carbazoles to fuel up the class of these versatile compounds with some novel derivatives.

In one of our previous work we have reported the successful synthesis of regioselective 2,3-dicarboxylic acid derivative of carbazole [14]. While initiating the current work we found **1** very reactive towards phenyl hydrazine resulting in the formation of the amazing pyridazino carbazole, that is, 2,3-dihydro-2-phenyl-6*H*-pyridazino [4,5-*b*] carbazole-1,4-dione **2**. It is more likely to have phenyl substituted amide group at position 3 of carbazole ring as this position is much more reactive and accepts activating phenyl group readily. The other regioisomer of **2** was in negligible amount and separated on TLC cards to get single spotted pure product. Once having stable **2** in hand, a series of its novel derivatives **3**–**9** were synthesized and characterized. Subsequent product **2** was traditionally nitrated by cupric nitrate in acetic anhydride under inert atmosphere to get the **3** in an appreciable yield 68%. Successive synthesis of **4** and **5** was conducted smoothly by stirring the product **2** with piperazine and *N,N*-dimethylethane-1,2-diamine, respectively. The excess of piperazine and *N,N*-dimethylethane-1,2-diamine from their reactions was removed by extraction with diethyl ether, and the isolated products **4** and **5** were revealed in 73% and 69% yield. The reaction of compound **2** went through smoothly with dimethyl amine in methanol just by stirring at room temperature under nitrogen for about four hours to yield **6** as isolated product. The most interesting aspect of this proposed reaction scheme was the successive synthesis of compound **9** subsequently from **7** and **8**. It was found that the product **2** gives its dimethyl derivative **7** as single compound when it was refluxed for six hours with methyl iodide in presence of carbon tetrachloride keeping the mole ratio 1 : 2 (product **2**: methyl iodide). Product **7** was further considered for the treatment with potassium ferricyanide in sodium hydroxide to convert the two methyl groups to carboxylic groups. This reaction successfully afforded the product **8** in 75% yield. The pyridazine ring formation was repeated with this dicarboxylic product **8** with phenyl hydrazine. Reaction works even faster and smoother to reveal the product **9** in 73% yield. Spectral data, that is, elemental analysis, IR, $^1$H NMR, $^{13}$CNMR, MS, was supporting successful formation of **2**. Absorption observed in IR spectrum was at 3418 (NH), 3040 (aromatic), and 1604 cm$^1$ (C=O bond). The mass spectrum base peak for the prepared compound was revealed at 327.10 ($MH^+$) proving the successful formation of **2**. As expected no carboxylic protons were found for position 2 and 3 as they were replaced due to the formation of new ring. The singlet at $\delta$ 8.0 (for the proton of O=C–N–H), $\delta$ 6.30, 6.67, and 7.12 (benzene protons) was found in addition to the carbazole ring signals which clearly vote for successful fabrication of **2** (Scheme 2).

IR spectrum of **3** showed the absorption at 3398 cm$^{-1}$ for N–H bond, 3033 cm$^{-1}$ showing aromatic group, 1680 cm$^{-1}$ for C=O group, 1360 and 1570 cm$^{-1}$ showing occurrence of C–$NO_2$ group. Due to $NO_2$ group at position 6, it was observed that $^1$H NMR signals shift towards downfield region. H-5 appeared as singlet at $\delta$ 8.55 due to substitution at H-6. Two doublets at 7.60 and 8.10 ppm correspond to H-7 and H-8. In mass spectrum base peak appeared at 372.09 ($MH^+$). In IR spectrum of **4**, absorption at 3408 (NH), 3080 (aromatic), 1730 (C=O), and 1425 cm$^{-1}$ (N–C bond) was observed. Proton NMR data was quite interesting in case of piperazine substitution to pyridazino carbazole. The base peak in mass spectrum at 413.19 ($MH^+$) is for compound **4**. In IR spectrum of **5** peak found at 3212, 3080, and 1760 cm$^{-1}$ was related to NH, aromatic, and C=O groups, respectively, while a sharp and long peak at 1473 was for C–H ($CH_2$–$CH_2$) bond. In NMR spectrum, singlet at $\delta$ 2.83 was for $CH_3$ protons (attached with Ar–N–$CH_3$). Two triplets at $\delta$ 2.78 and 3.49 were due to the two –$CH_2$ groups (Ar–$NCH_3$–$CH_2$–$CH_2$–$NHCH_3$), respectively, of *N,N*-dimethyl ethane-1, 2-diamine. The signal of aliphatic NH proton appeared at $\delta$ 2.0 as multiplet. In IR spectrum of **6** peak at 3350, 3100, and 1630 cm$^{-1}$ corresponded to N–H, aromatic, and C=O group. A strong peak at 1468 cm$^{-1}$ was for C–H bond (–$CH_3$). Singlet for two methyl groups appeared at 2.83 ppm. The mass spectrum base peak at 370.14 ($MH^+$) was in support of the synthesized compound. IR spectrum of **7** showed sharp peaks at 3418 cm$^{-1}$ (N–H), 3013 cm$^{-1}$ (aromatic), 2880 cm$^{-1}$ (C–H of $CH_3$), and 751 cm$^{-1}$ (disubstitution to aromatic ring). $^1$H NMR spectra show all of the characteristic peaks of pyridazino carbazole with some exceptions. Two doublet of doublets which was seen in case of pyridazino carbazole due to protons of position **6** and **7** was no more observed in this spectrum. Neighboring protons of **5** and **8** appeared at $\delta$ 6.94 and 7.38 as singlets. The peak for methyl groups appeared at $\delta$ 2.35 and 2.40. Mass spectrum showed the base peak at 355.39 ($MH^+$) for **7.** IR spectrum of **8** gave characteristic peaks at 2980 and 1698 cm$^{-1}$ and showed the presence of OH (COOH) and C=O groups. In NMR spectrum, singlets for protons of position 5 and 8 were at $\delta$ 8.45 and 8.52. Signals for two 1 H singlets of COOH were at $\delta$ 10.5 and 10.8. Base peak for mass spectrum was at 415.08 ($MH^+$). IR spectrum of **9** showed peaks at 3418 (N–H), 3040 (aromatic group), and 1604 cm$^{-1}$ (C=O bond). NMR spectrum showed that molecule behaves as one unit due to having same substitution on both sides. Singlet for protons of position 1 and 7 at $\delta$ 8.19 and one singlet for protons of position 4 and 5 at $\delta$ 8.40 were observed. Structure **9** was also confirmed from mass spectrum whose base peak was at

Table 1: Antimicrobial activities of compound **2**–**9** in terms of minimum inhibitory concentration ($\mu$g/mL).

| Tested compounds | MRSA | | *S. typhi* | | *C. albicans* | |
|---|---|---|---|---|---|---|
| | $MIC_{50}$ | $MIC_{100}$ | $MIC_{50}$ | $MIC_{100}$ | $MIC_{50}$ | $MIC_{100}$ |
| **2** | 256 | >512 | 512 | >512 | 16–32 | 64 |
| **3** | 8 | 12 | 512 | >512 | 16 | 32 |
| **4** | 512 | >512 | 256 | >512 | 128 | 512 |
| **5** | 128 | 512 | 256 | 512 | 16 | 64 |
| **6** | 128 | 256 | 14 | 32 | 512 | >512 |
| **7** | >512 | >512 | 256 | 512 | 32 | 64 |
| **8** | 4 | 12 | >512 | >512 | 256 | 512 |
| **9** | 512 | >512 | 128 | 256 | 16 | 32 |
| Ciprofloxacin | >512 | >512 | >512 | >512 | — | — |
| Ketoconazole | — | — | — | — | 14 | 32 |

DMSO: negative control.

487.13 ($MH^+$). In short, we make effort to synthesize some new carbazole derivatives with effective synthetic protocols and novelty. We found product **2** to be very reactive toward many functionals which strongly assist its further role in design and development process of carbazoles.

## 4. Antimicrobial Studies

All newly synthesized carbazole derivatives were analyzed for their antibacterial and antifungal activity. Minimum inhibitory concentration (MIC) of each of the derivatives was determined using standard agar dilution method. Thirty-four clinical isolates comprising of multiresistant *Staphylococcus aureus* ($n = 34$), *Salmonella typhi* ($n = 34$), and *C. albicans* ($n = 34$) were used for antimicrobial assay including reference ATCC strains. ATCC reference strains were used to monitor the quality control among the synthesized compounds **3** (having nitrosubstitution) and **8** (having carboxylic substitution) that showed excellent activity against multiresistant *Staphylococcus aureus*. Rest of the compounds also showed moderately good activity against MRSA. Compound **6** (having dimethyl amine substitution) was strongly active against *S. typhi*. All synthesized compounds were better antifungal agents as compared to antibacterials. Compounds **2**, **3**, **5**, and **7** demonstrated profound antifungal activity while **4** and **8** also possessed moderately good activity (Table 1).

## 5. Conclusion

The present research work emphasized different synthetic approaches to new derivatives of carbazoles. The interest in synthesizing these carbazoles lies in the prospect of obtaining new biologically active substances having potential to act as future antibiotics. The molecules with basic carbazole skeleton are already known to possess versatile biological and medicinal activities. Novel carbazoles reported here showed moderate-to-excellent antimicrobial activities against multiresistant *Staphylococcus aureus* (gram positive), *Salmonella typhi* (gram negative), and *Candida albicans* (fungi). Among the novel carbazoles, **3** and **8** were effective against multiresistant *Staphylococcus aureus* (bacteria that acquired resistance against many well-known antibiotics and are responsible for a range of difficult-to-treat infections in humans) and thus showed their strong potential to be part of one of the future antibiotics against drug-resistant bacteria. Compound **6** was effectively active against *Salmonella typhi*, bacteria responsible for another life-threatening human disease, typhoid fever, and compounds **3**, **5**, **7**, and **9** were excellently active against *Candida albicans*, a fungi responsible for genital and oral infections in humans. Novelty of the synthesized compounds, the highly efficient synthetic protocols, and the tremendous activities shown by some of the synthesized compounds largely assist the drug and development process and their potential to act as antifungal agents and potent antibiotics against-drug resistant bacteria.

## Acknowledgments

The authors would like to thank Mrs. Sughra Bibi, Higher Education Commission Pakistan, School of Chemistry, University of Wollongong, and Department of Microbiology, University of Health Sciences.

## References

[1] A. H. C. Uttley, C. H. Collins, J. Naidoo, and R. C. George, "Vancomycin-resistant enterococci," *The Lancet*, vol. 1, no. 8575-8576, pp. 57–58, 1988.

[2] R. Fox, K. R. Neal, C. L. S. Leen, M. E. Ellis, and B. K. Mandal, "Fluconazole resistant candida in AIDS," *Journal of Infection*, vol. 22, no. 2, pp. 201–204, 1991.

[3] M. A. Pfaller, M. Bale, B. Buschelman et al., "Selection of candidate quality control isolates and tentative quality control ranges for in vitro susceptibility testing of yeast isolates by National Committee for Clinical Laboratory Standards proposed standard methods," *Journal of Clinical Microbiology*, vol. 32, no. 7, pp. 1650–1653, 1994.

[4] W. Gu and S. Wang, "Synthesis and antimicrobial activities of novel 1*H*-dibenzo[a,c]carbazoles from dehydroabietic acid," *European Journal of Medicinal Chemistry*, vol. 45, no. 10, pp. 4692–4696, 2010.

[5] A. E. Martin and K. J. Prasad, "Synthesis and characterization of carbazole derivatives and their antimicrobial studies," *Acta Pharmaceutica*, vol. 56, no. 1, pp. 79–86, 2006.

[6] F. F. Zhang, L. L. Gan, and C. H. Zhou, "Synthesis, antibacterial and antifungal activities of some carbazole derivatives," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 6, pp. 1881–1884, 2010.

[7] A. Chakraborty, C. Saha, G. Podder, B. K. Chowdhury, and P. Bhattacharyya, "Carbazole alkaloid with antimicrobial activity from Clausena heptaphylla," *Phytochemistry*, vol. 38, no. 3, pp. 787–789, 1995.

[8] N. Haider, "Pyridazine-fused carbazoles: synthesis, reactivity, and antitumor activity," *Journal of Heterocyclic Chemistry*, vol. 39, no. 3, pp. 511–521, 2002.

[9] H. J. Knölker and K. R. Reddy, "Isolation and synthesis of biologically active carbazole alkaloids," *Chemical Reviews*, vol. 102, no. 11, pp. 4303–4428, 2002.

[10] G. Yaqub, E. Akbar, M. A. Rehman, and B. Mateen, "Review on advancements in synthesis of carbazoles," *Asian Journal of Chemistry*, vol. 21, no. 4, pp. 2485–2520, 2009.

[11] G. Yaqub, A. Hannan, M. Usman, E. Akbar, and B. Mateen, "In vitro antifungal and antibacterial activity of carbazoles," *Asian Journal of Chemistry*, vol. 24, no. 11, pp. 5121–5123, 2012.

[12] N. Ty, G. Dupeyre, G. G. Chabot et al., "Structure-activity relationships of indole compounds derived from combretastatin A4: synthesis and biological screening of 5-phenylpyrrolo[3,4-a] carbazole-1,3-diones as potential antivascular agents," *European Journal of Medicinal Chemistry*, vol. 45, no. 9, pp. 3726–3739, 2010.

[13] Y. Li, H. Zou, J. Gong et al., "Efficient synthesis of maleimides and carbazoles via $Zn(OTf)_2$-catalyzed tandem annulations of isonitriles and allenic esters," *Organic Letters*, vol. 9, no. 20, pp. 4057–4060, 2007.

[14] G. Yaqub, E. Akbar, Z. Sadiq, M. Iqbal, and B. Mateen, "Efficient and simple strategy to synthesize carbazoles," *International Journal of Syntheses and Characterization*, vol. 3, no. 1, pp. 21–22, 2010.