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Synthesis, characterization and biological evaluation of some newer carbazole derivatives

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Abstract: A series of novel 5-[(9*H*-carbazol-9-yl)methyl]-*N*-[(substituted phenyl)(piperazin-1-yl)methyl]-1,3,4-oxadiazol-2-amines (**4a**–**o**) derivatives was synthesized by starting with carbazole, which on reaction with ethyl chloroacetate yielded ethyl 2-(9*H*-carbazole-9-yl)acetate (**1**). Compound **1** on reaction with semicarbazide followed by cyclisation with sulphuric acid gave 5-((9*H*-carbazole-9-yl)-1,3,4-oxadiazol-2-amine (**3**), which through Mannich reaction with piperazine and a variety of aromatic aldehydes in the presence of acetic acid yielded the titled compounds (**4a**–**o**). The structures of compounds were characterized by UV, FT-IR, ^{1}H-NMR and MS spectral studies, and by elemental analysis. All the derivatives were evaluated for their antibacterial, antifungal and anticancer activities. Among the tested compounds, **4a**, **4d**, **4e** and **4n** exhibited significant antibacterial and antifungal activity, while the compounds **4a**, **4d**, **4k** and **4n** were found to be active on the human breast cancer cell line MCF7.

Keywords: Mannich reaction; antibacterial activity; antifungal activity; MCF7; synthesis; spectroscopy.

INTRODUCTION

Carbazole derivatives are well known for their pharmacological activities. It is evident from the literature that the derivatives of carbazole moiety possess a wide spectrum of pharmacological activities, such as antibacterial,[1–3] antifungal,[4,5] antitumour, antineoplastic,[6–10] anticonvulsant,[11] antioxidant,[12] antidiabetic,[13] antipsychotic[14] and larvicidal activity.[15]

Various heteroannulated carbazole derivatives have drawn attention because of their natural occurrence and the broad spectrum of biological activity associated with these compounds. The carbazole moiety is a frequent moiety of numerous drugs, such as olivacine, ondansetron, rimcazole, stauroapirone, carbazolol, carvedilol, carprofen, cacotheline, rebaccamycin, ellipticine and various

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naturally occurring carbazole alkaloids.[16] Carbazole derivatives have documented consistent advances in the design of novel antipsychotic, neuroleptic and anticonvulsant agents.[17] Furthermore, various congeners of oxadiazole, thiadiazole, azetidinone and thiazolidinone have also been reported to exhibit potential antimicrobial, anticancer, antipsychotic, antidepressant and anticonvulsant activity.[18–21] In view of broad biological activity of carbazole derivatives, in this study it was planned to synthesize new carbazole derivatives and by incorporation of new pharmacophores, such as oxadiazole at position 9 of carbazole nucleus, with the hope of obtaining better pharmacologically active drugs as anticancer and antimicrobial agents. In the same direction, a one pot method was developed to synthesize a series of 5-[(9*H*-carbazol-9-yl)methyl]-*N*-[(substituted--phenyl)(piperazin-1-yl)methyl]-1,3,4-oxadiazol-2-amine derivatives (**4a**–**o**) as Mannich bases.

EXPERIMENTAL

The purity of all the newly synthesized compounds were checked by TLC on silica gel--protected aluminium sheets (type 60 F_{254}, Merck) and the spots were detected by exposure to iodine vapour and a UV-lamp at λ 254 nm. The melting points were determined in open capillary tubes and are uncorrected. The infrared (FT-IR) spectra were recorded on a 470--Shimadzu infrared spectrophotometer using the KBr pressed pellet technique and the result are expressed in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 instrument using $CDCl_3$ as a solvent. The chemical shifts, δ, are given in ppm downfield from the internal standard tetramethylsilane (TMS). The splitting patterns are designated as follows; *s*: singlet; *d*: doublet and *m*: multiplet. The mass spectra were obtained on a Shimadzu 2010A LC-MS spectrometer. Elemental analyses were realised on an Elemental Vario EL III Carlo Erba 1108 instrument and the obtained values were within ±0.04 % of the theoretical values.

*Synthesis of ethyl 2-(9*H*-carbazol-9-yl)acetate (**1**)*

To a solution of carbazole (2.01 g, 0.012 mol) in 18 mL of dry acetone, ethyl chloroacetate (1.472 g, 0.012 mol) was added dropwise in the presence of anhydrous potassium carbonate (0.09 g) and the resultant mixture refluxed for 22 h. Then the mixture was cooled and the thus obtained solid was filtered, dried and recrystallized from methanol to give compound **1**.[17] Yield: 59.44 %; m.p.: 240–241 °C

*Synthesis of 1-[2-(9*H*-carbazol-9-yl)acetyl]semicarbazide (**2**)*

Compound **1** (2.53 g, 0.01 mol) was dissolved in 90 mL of acetone (solution A) and semicarbazide (0.62 g, 0.01 mol) was dissolved in 20 mL of water (solution B). Solution B was poured into solution A and the mixture was refluxed for 28 h. On cooling, the solid product that separated out was filtered, dried and recrystallized from methanol to give compound **2**.[17] Yield: 70.40 %; m.p.: 184–185 °C.

*Synthesis of 5-[(9*H*-carbazol-9-yl)methyl]-1,3,4-oxadiazol-2-amine (**3**)*

A solution of compound **2** (2.83 g, 0.01 mol) with 25 mL of conc. H_2SO_4 was kept overnight at room temperature, then the reaction mixture was poured into ice-cold water, neutralized with ammonia and extracted with ether. The ethereal solution was distilled off and the product obtained was recrystallized from acetone to give compound **3**.[17] Yield: 65.9 %; m.p.: 161–162 °C.

*General synthetic procedure for the synthesis of 5-[(9*H*-carbazol-9-yl)methyl]*-N-*[(substituted phenyl)(piperazin-1-yl)methyl]-1,3,4-oxadiazol-2-amines (**4a**–**o**)*

A mixture of equimolar quantity (0.01 mol) of compound (**3**), derivative of aromatic aldehydes and piperazine along with few drops of glacial acetic acid was refluxed in 15 mL of methanol for 8–13 h. The hot solution was poured onto crushed ice and the thus obtained solid mass was filtered, dried and recrystallized from acetone to give compounds **4a**–**o**.

Antibacterial activity

The antibacterial activities of the newly synthesized compounds were tested by the disc diffusion method on nutrient agar medium against the bacterial strains *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633 (Gram-positive), and *Escherichia coli* ATCC 35210 and *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative).

In the disc diffusion method, a paper disc (6 mm in diameter) was impregnated with the test compounds dissolved in DMSO at concentrations of 25, 50 and 100 μg mL^{-1}. A disc impregnated with DMSO was used as the solvent control because of the free solubility of the test compounds. The nutrient agar medium in Petri dishes was inoculated with bacterial strains and the discs impregnated with the solutions of different concentrations of the compounds were placed over it. The plates were incubated at 35 °C for 24 h. The zone of inhibition indicating the inhibited growth of microorganism around the discs was observed. Ciprofloxacin was used as the standard (50 μg mL^{-1}) to compare the efficacy of tested compounds. Each testing was performed in triplicate. The results were interpreted in terms of diameter (mm) of the zone of inhibition.

Antifungal activity

The antifungal activity of the newly synthesized compounds was tested on the fungal strains *Candida albicans* ATCC 10261 and *Aspergillus niger* ATCC 9643 using the paper disc diffusion method by using agar medium.

The procedure for the activity testing was similar to that described above for the antibacterial testing and the same concentrations of the tested compounds were employed. The results were recorded and reported after incubation for 48 h at 25 °C for the fungal strains and fluconazole was used as the standard (50 μg mL^{-1}). The zones of inhibition indicating the inhibited growth of microorganism by the prepared compounds around the discs was observed and reported. Each experiment was performed in triplicate.

Anticancer activity

The newly synthesized compounds were evaluated for their anticancer activity by determining the percentage control growth of human breast cancer cell lines MCF7 by the *in vitro* sulforhodamine B assay (SRB assay) method.[22] The cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10 % foetal bovine serum and 2 mM L-glutamine. The tested compounds were solubilised in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing the test article at a concentration of 10^{-3} μg ml^{-1}. Additional three 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 μL of these different drug dilutions were added to the appropriate microtitre wells already containing 90 μL of medium, resulting in the required final drug concentrations, *i.e.*, 10, 20, 40 and 80 μg mL^{-1}. The absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The percent growth was calculated on a plate-by-plate basis for the test wells relative to the control wells.

The percent growth was expressed as the ratio of the average absorbance of the test well to the average absorbance of the control wells×100. Using the six absorbance measurements (time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)), the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: $((T_i - T_z)/(C - T_z))\times100$ for concentrations for which $T_i \geq T_z$ ($T_i - T_z$ is positive or zero) or $((T_i - T_z)/T_z)\times100$ for concentrations for which $T_i < T_z$ ($T_i - T_z$ is negative). The dose response parameters, *i.e.*, growth inhibition of 50 % (GI_{50}), total growth inhibition (*TGI*) and killing of 50 % of the cells (LC_{50}), were calculated for each test substance.

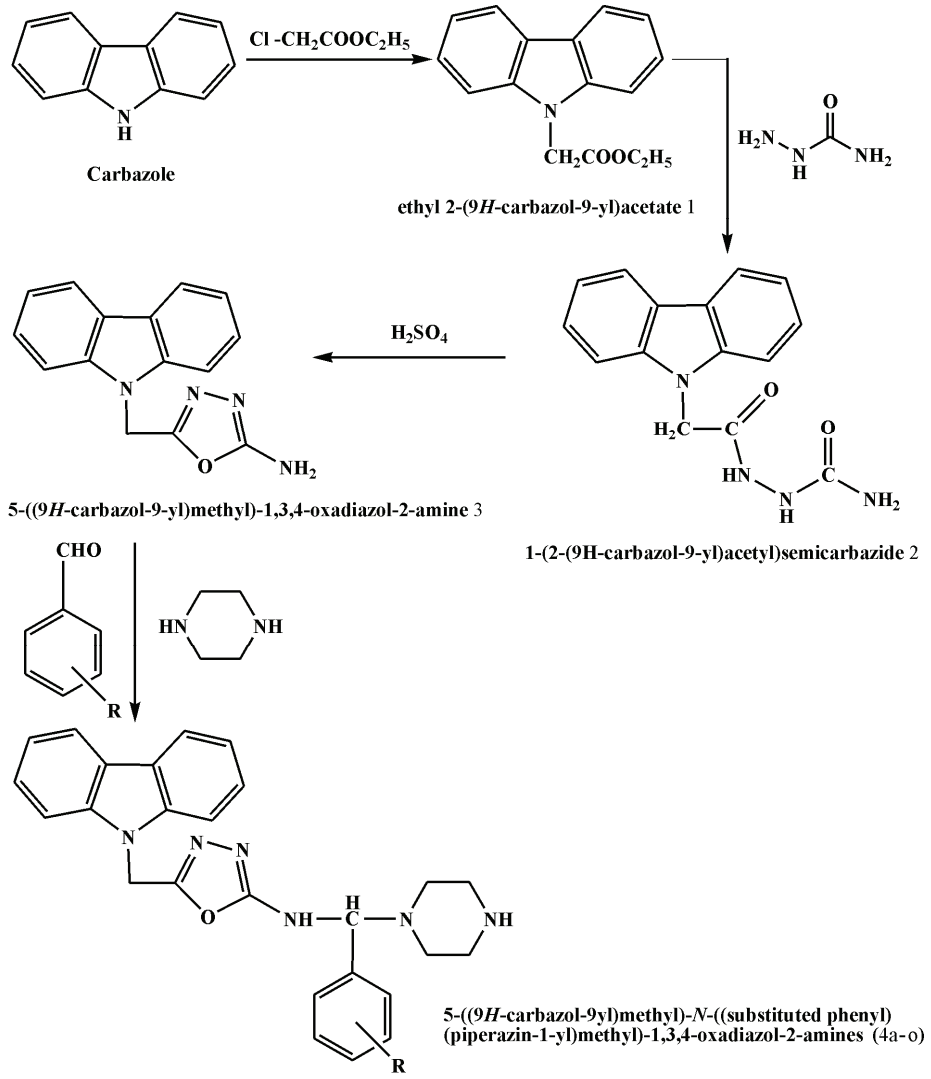
RESULTS AND DISCUSSION

The reaction sequences leading to different carbazole derivatives are outlined in Scheme 1. The carbazole derivatives were synthesized by incorporating new pharmacophores, such as oxadiazole, at position 9 of the carbazole nucleus by a conventional method in which carbazole was taken as a starting material to produce the corresponding 5-[(9*H*-carbazol-9-yl)methyl]-*N*-[(substituted-phenyl)(piperazin-1-yl)methyl]-1,3,4-oxadiazol-2-amines (**4a**–**o**). Synthesized compounds were identified based on their physical parameters, *i.e.*, solubility, melting point, chromatographic methods (TLC). The data is given in Table I and spectroscopic methods (UV, IR, ^{1}H-NMR, MS and elemental analysis are given in the Supplementary material to this paper. The ^{1}H-NMR spectra showed a peak between δ 6.00–7.00 ppm, which was assigned to the N–H (aliphatic) proton. A peak characteristic of –CH– appeared between δ 4.00–5.00 ppm. The peaks at δ 6.00–8.00 ppm showed the presence of aromatic protons. In the FT-IR spectra, a peak characteristic of N–H (aliphatic) appeared at 3200–3450 cm^{-1} and a peak for C=N was observed at 1500–1600 cm^{-1}.

Pharmacological screening

All the newly synthesized compounds **4a**–**o** were screened for their antibacterial and antifungal activity (Table II). The results revealed that among the synthesized compounds tested, **4a**, **4d** and **4n** were found to be more potent against all the bacterial and fungal strains at a concentration 50 μg mL^{-1}

Anticancer screening. All the synthesized compounds **4a**–**o** were evaluated for their anticancer activity against the human breast cancer cell line MCF7 by the SRB assay The *in vitro* anticancer study was realised at the Tata Memorial Centre, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi Mumbai, India. The obtained data are given in Table III. Among all the synthesized compounds tested, **4a**, **4d**, **4k**, **4m** and **4n** were found to be most active against human breast cancer cell lines. It was found that for a large variety of polycyclic compounds, basic moieties attached to the ring system not only improve the solubility under physiological conditions, but also lead to an increase in anticancer activity.[1] In the present study, it was established that presence of an electronegative atom on the benzene ring makes MCF7 cell lines



Scheme 1. Synthesis of carbazole derivatives **4a**–**o**.

TABLE I. Physical data of the synthesized compounds **4a**–**o**

Compound	R	Yield, %	Reaction time, h	M.p. range, °C	R_f value[a]
4a	*p*-Nitro	82.55	4.0	236–237	0.73
4b	*p*-Hydroxy	79.69	5.0	208–209	0.62
4c	3,4,5-Trimethoxy	61.5	4.3	184–185	0.69
4d	*p*-Chloro	73.22	6.0	221–222	0.75
4e	*p*-(Dimethylamino)	53.78	5.5	202–203	0.59
4f	*o*-Nitro	63.96	4.4	225–226	0.65
4g	*m*-Hydroxy	72.71	5.2	213–214	0.56

TABLE I. Continued

Compound	R	Yield, %	Reaction time, h	M.p. range, °C	R_f value[a]
4h	*m*-Nitro	69.98	6.8	229–230	0.71
4i	*o*-Hydroxy	59.33	5.0	218–219	0.60
4j	*m*-Chloro	67.07	4.4	231–232	0.67
4k	*o*-Chloro	56.45	6.2	237–238	0.66
4l	*m*-Methoxy	52.54	4.5	192–193	0.77
4m	*p*-Methoxy	58.75	7.0	189–190	0.79
4n	*p*-Fluoro	55.0	6.0	234–235	0.54
4o	H	51.23	5.2	176–177	0.63

[a]Solvent system: benzene:chloroform:methanol (4:3:2)

TABLE II. Antibacterial and antifungal activity of compounds **4a**–**o**; diameter of zone of inhibition at 50 μg mL^{-1}, mm (mean±*SD* (*n* = 3))

Compound	Bacteria				Fungi	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
4a	16.2±0.3	16.1±0.3	22.0±0.2	24.0±0.1	16.3±0.3	11.1±0.2
4b	12.6±0.1	14.6±0.1	20.2±0.1	19.5±0.2	13.1±0.1	9.9±0.1
4c	10.1±0.3	12.8±0.3	17.1±0.1	17.1±0.2	10.1±0.4	8.1±0.5
4d	15.6±0.1	16.2±0.1	21.8±0.1	24.1±0.1	16.1±0.1	11.7±0.2
4e	14.1±0.3	15.9±0.1	22.3±0.5	22.7±0.3	15.3±0.2	10.5±0.2
4f	13.6±0.2	14.8±0.2	21.6±0.2	23.0±0.4	14.9±0.1	10.2±0.1
4g	11.3±0.1	13.6±0.2	19.4±0.2	18.0±0.1	12.2±0.3	9.5±0.4
4h	15.4±0.1	15.2±0.1	21.9±0.2	22.4±0.1	15.1±0.4	10.9±0.1
4i	10.8±0.2	11.4±0.1	18.6±0.1	19.1±0.4	14.9±0.2	10.3±0.3
4j	14.3±0.3	13.7±0.2	20.8±0.1	23.7±0.2	15.6±0.3	10.8±0.4
4k	13.5±0.1	12.4±0.3	21.0±0.3	21.5±0.3	15.1±0.1	10.4±0.3
4l	10.1±0.4	8.8±0.1	15.8±0.1	15.6±0.1	8.9±0.4	7.1±0.2
4m	9.9±0.1	10.3±0.1	16.7±0.3	17.2±0.2	9.5±0.1	8.3±0.1
4n	16.8±0.2	16.5±0.1	22.6±0.1	23.6±0.1	15.9±0.3	11.4±0.4
4o	8.2±0.1	9.9±0.3	14.5±0.1	15.9±0.2	8.1±0.2	5.8±0.1
Ciprofloxacin[a]	15.5±0.2	17.8±0.6	22.2±0.4	26.5±0.5	–	–
Fluconazole[a]	–	–	–	–	16.9±0.5	11.8±0.3

[a]Standards – ciprofloxacin and fluconazole at 50 μg mL^{-1} concentrations. Statistical analysis of the data was performed by one way ANOVA

TABLE III. Anticancer activity of compounds **4a**–**o** against human breast cancer cell line MCF7

Compound	Control growth %	Drug concentration μg mL^{-1}			Drug concentration, μg mL^{-1}		
	10	20	40	80	GI_{50}[a]	*TGI*	LC_{50}
4a	40.4	27.4	10.3	2.1	<10	34.4	60.6
4b	58.5	45.3	38.2	30.8	<20	>80	>80
4c	52.4	40.9	34.7	29.2	<20	>80	>80
4d	43.7	19.1	10.0	2.8	<10	35.2	60.2
4e	59.6	41.5	30.2	22.6	<20	>80	>80

TABLE III. Continued

Compound	Control growth %	Drug concentration μg mL^{-1}			Drug concentration, μg mL^{-1}		
	10	20	40	80	GI_{50}[a]	*TGI*	LC_{50}
4f	53.4	38.7	27.2	13.3	<20	>80	>80
4g	61.6	50.2	41.9	36.0	<40	>80	>80
4h	51.9	33.7	28.5	16.4	<20	68.4	>80
4i	58.2	49.3	37.0	31.3	<20	>80	>80
4j	55.8	38.5	27.6	18.1	<20	72.9	>80
4k	46.9	31.5	11.8	3.4	<10	26.5	53.6
4l	50.6	37.3	29.7	20.2	<20	>80	>80
4m	49.2	34.6	18.3	13.9	<10	60.3	>80
4n	35.4	11.3	8.4	1.2	<10	16.5	35.6
4o	66.4	53.7	45.2	39.4	<40	>80	>80
ADR[b]	0.3	–10.7	–33.6	–59.7	<10	11.6	23.2

[a]A GI_{50} value of < 10 μg mL^{-1} is considered to demonstrate activity in the case of a pure compound; [b]standard – adriamycin (ADR), positive control compound

more susceptible towards the compound. The results revealed that the substitution is more effective at position 4 than at position 2.

SUPPLEMENTARY MATERIAL

Analytic and spectral data for the prepared compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И ИСПИТИВАЊЕ БИОЛОШКЕ АКТИВНОСТИ НОВИХ ДЕРИВАТА КАРБАЗОЛА

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Синтетисана је серија нових деривата 5-[(9*H*-карбазол-9-ил)метил]-*N*-[(супституисани фенил)(пиперазин-1-ил)метил]-1,3,4-оксадиазол-2-амина (**4a**–**o**), полазећи од карбазола. У реакцији карбазола и етил-хлорацетата добијен је етил-2-(9*H*-карбазол-9-ил)ацетат (**1**), који у реакцији са семикарбазолом и након циклизације у присуству сумпорне киселине даје 5-(9*H*-карбазол-9-ил)-1,3,4-оксадиазол-2-амин (**3**). Амин **3** у Маниковој реакцији са пиперазином и различитим ароматичним алдехидима, у присуству сирћетне киселине, даје циљане деривате **4a**–**o**. Једињења су окарактерисана UV, FT-IR, ^{1}H-NMR, MS спектрима и елементалном анализом. Испитане су антибактеријске, антифунгална и антиканцерска активност свих добијених дервата. Деривати **4a**, **4d**, **4e** и **4n** погазују значајну антибактеријску и антифунгалну активност, док деривати **4a**, **4d**, **4k** и **4n** показују активност и према хуманим ћелијским линијама рака дојке MCF7.

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