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RESEARCH ARTICLE

Synthesis, antimicrobial activity and cytotoxicity of some new carbazole derivatives

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Abstract

In this work, some *N*-(9-Ethyl-9*H*-carbazole-3-yl)-2-(phenoxy)acetamide derivatives were synthesised and evaluated for their antimicrobial activity and cytotoxicity. The structural elucidation of the compounds was performed by IR, ^{1}H-NMR, ^{13}C-NMR and FAB^{+}-MS spectral data and elemental analyses. The title compounds were obtained by reacting 2-chloro-*N*-(9-ethyl-9*H*-carbazole-3-yl)acetamide with some substituted phenols. The synthesised compounds were investigated for their antibacterial and antifungal activities against *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Candida albicans*. The compounds *N*-(9-Ethyl-9*H*-carbazole-3-yl)-2-(4-ethylphenoxy)acetamide (**2c**) and *N*-(9-Ethyl-9*H*-carbazole-3-yl)-2-(quinolin-8-yloxy)acetamide (**2n**) showed notable antimicrobial activity. The compounds were also studied for their cytotoxic effects using MTT assay, and it was seen that **2n** had the lowest cytotoxic activity against NIH/3T3 cells.

Keywords: Carbazole, phenol, antibacterial activity, antifungal activity, cytotoxicity

Introduction

The alarming rates of emerging and reemerging microbial threats coupled with the rapid development of multi-drug-resistant microbial pathogens are major escalating concerns to the public health, particularly during the past decades[1,2]. Despite a large number of antibiotics and chemotherapeutics available for medical use, at the same time, the emergence of old and new antibiotic resistance created revealed a substantial medical need for new classes of antimicrobial agents[3]. There is no doubt that the existing arsenal of antimicrobial agents we have in hand for the treatment of infectious diseases is insufficient to protect us over the long term[4–7]. Thus there is a need to search for new and efficacious antimicrobial agents[8,9]. Nevertheless, there is a continuing effort among the scientists especially in the pharmaceutical industry to develop new antimicrobial agents for the treatment of resistant infections[10].

The tricyclic carbazole nucleus is an important type of nitrogen-containing aromatic heterocyclic compound and possesses desirable electronic and charge-transport properties, as well as large p-conjugated system, and the various functional groups are easily introduced into the structurally rigid carbazole ring. These characteristics result in the extensive potential applications of carbazole-based derivatives in the field of chemistry and medicinal chemistry[11].

Carbazole and its derivatives are a considerable structural unit which have been isolated from different sources such as some genera of higher plants, blue green algae, actinomycetes and filamentous fungi[12–14]. It is known that natural origin carbazoles especially for those complex carbazoles fusing with a heterocyclic fragment show well-known pharmacological activities[15,16]. Accordingly, to the present, a large number of natural and synthetic carbazole derivatives have been reported to exhibit diverse biological activities such as antituberculosis[17], antiproliferative[14], antibacterial[18,19], antiviral[20], antifungal[21,22], antitumour[23], anti-inflammatory[24], antioxidant[12] and antihistaminic activities[25].

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It is known that the phenol and phenol derivatives are in use as potential antimicrobial agent[26]. Several studies demonstrated the antimicrobial activity of phenols and/or phenolic compounds making them a good alternative to antibiotics and chemical preservatives[27]. Also the alkoxy and aryloxy moiety, which is containing phenol residue, has low toxicity and active against the growth of various yeasts and bacteria[28–30].

In the view of these observations, we designed and synthesised carbazole-based aryloxy compounds as potential antimicrobial agent.

Experimental

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co. All melting points (m.p.) were determined by Electrothermal 9100 digital melting point apparatus and are uncorrected. Spectroscopic data were recorded with the following instruments: ^{1}H-NMR, Bruker 400 MHz spectrometer; ^{13}C-NMR, Bruker 100 MHz spectrometer; and MS-FAB, VG Quattro Mass spectrometer. Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer.

General procedure for the synthesis of the compounds

2-Chloro-N-(9-ethyl-9H-carbazole-3-yl)acetamide (1)

9-Ethyl-9*H*-carbazole-3-amine (0.05 mol) and triethylamine (0.06 mol) were dissolved in THF with a constant stirring at 0–5°C, then chloroacetyl chloride (0.06 mol) was added dropwise gradually to this solution. The reaction mixture thus obtained was further agitated for 1 h at room temperature. After the solvent was evaporated to dryness, the solid was filtered and washed with water.

N-(9-Ethyl-9H-carbazole-3-yl)-2-(substituted phenoxy) acetamide derivatives (2a-n)

A mixture of 2-chloro-*N*-(9-ethyl-9*H*-carbazole-3-yl) acetamide **(1)** (1.65 mmol, 0.5 g), the appropriate phenol derivatives (1.98 mmol) and K_2CO_3 (1.98 mmol, 0.3 g) in acetonitrile was refluxed for 6 hours. The cooled mixture was filtered and recrystallised from alcohol. Some characteristics of the compounds are given in Table 1.

N-(9-Ethyl-9H-carbazole-3-yl)-2-(o-tolyloxy)acetamide (2a)

IR (KBr) ν_{max} (cm^{-1}): 3332 (amide N-H), 3050 (aromatic C-H), 2922, 2850 (aliphatic C-H), 1683 (amide C=O), 1604, 1556, 1442 (C=C), 1000–1300 (C-N), 1245 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t J=6 Hz, CH_3), 2.29 (3H, s, Ar-CH_3), 4.43 (2H, q J=7 Hz, N-CH_2), 4.75 (2H, s, O-CH_2), 6.91–7.60 (9H, m, Ar-H), 8.07 (H, d, J=8 Hz, carbazole C_5-H), 8.44 (H, s, carbazole C_4-H), 10.04 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.65 (CH_3), 16.16 (CH_3), 36.95 (CH_2), 67.52 (CH_2), 108.98 (CH), 109.16 (CH), 111.50 (CH), 111.88 (CH), 118.57 (CH), 119.39 (CH), 120.19 (CH), 120.85 (CH), 125.78 (CH), 130.23 (2C), 130.58 (2CH), 136.39 (2C), 139.96 (2C), 156.10 (C), 166.27 (C).

For $C_{23}H_{22}N_2O_2$ calculated: 77.07% C, 6.19% H, 7.82% N; found: 77.02% C, 6.18% H, 7.80% N.

MS (FAB) $[M+1]^+$: m/z 359

N-(9-Ethyl-9H-carbazole-3-yl)-2-(m-tolyloxy)acetamide (2b)

IR (KBr) ν_{max} (cm^{-1}): 3299 (amide N-H), 3030 (aromatic C-H), 2904 (aliphatic C-H), 1665 (amide C=O), 1610, 1557 (C=C), 1000–1300 (C-N), 1247 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 2.31 (3H, s, Ar-CH_3), 4.43 (2H, q, J=7 Hz, N-CH_2), 4.71 (2H, s, O-CH_2), 6.85–7.65 (9H, m, Ar-H), 8.07 (H, d, J=8 Hz, carbazole C_5-H), 8.44 (H, s, carbazole C_4-H), 10.06 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.65 (CH_3), 21.10 (CH_3), 36.95 (CH_2), 67.20 (CH_2), 108.94 (CH), 109.16 (CH), 111.62 (CH), 115.47 (CH), 118.57 (3CH), 121.80 (C), 125.78 (C), 129.22 (2CH), 130.17 (2C), 136.42 (CH), 138.97 (2C), 139.96 (CH), 157.88 (C), 166.20 (C).

For $C_{23}H_{22}N_2O_2$ calculated: 77.07% C, 6.19% H, 7.82% N; found: 77.04% C, 6.19% H, 7.82% N.

MS (FAB) $[M+1]^+$: m/z 359

Table 1. Some characteristics of the synthesised compounds.

Compound	Ar	Yield (%)	Melting point (°C)	Molecular formula	Molecular weight
2a	2-Methylphenyl	70	146	$C_{23}H_{22}N_2O_2$	358
2b	3-Methylphenyl	72	173	$C_{23}H_{22}N_2O_2$	358
2c	4-Ethylphenyl	75	166	$C_{24}H_{24}N_2O_2$	372
2d	2-Chlorophenyl	80	159	$C_{22}H_{19}ClN_2O_2$	378.5
2e	4-Chlorophenyl	81	186	$C_{22}H_{19}ClN_2O_2$	378.5
2f	2-Nitrophenyl	73	198	$C_{22}H_{19}N_3O_4$	389
2g	3-Nitrophenyl	75	178	$C_{22}H_{19}N_3O_4$	389
2h	4-Nitrophenyl	80	175	$C_{22}H_{19}N_3O_4$	389
2i	2,3-Dimethylphenyl	76	165	$C_{24}H_{24}N_2O_2$	372
2j	3,4-Dimethylphenyl	72	191	$C_{24}H_{24}N_2O_2$	372
2k	2,4-Dimethylphenyl	74	146	$C_{24}H_{24}N_2O_2$	372
2l	3,5-Dimethylphenyl	75	178	$C_{24}H_{24}N_2O_2$	372
2m	1,1′-Biphenyl]-4-yl	70	183	$C_{28}H_{24}N_2O_2$	420
2n	Quinolin-8-yl	71	85	$C_{25}H_{21}N_3O_2$	395

N-(9-Ethyl-9H-carbazole-3-yl)-2-(4-ethylphenoxy) acetamide (2c)

IR (KBr) ν_{max} (cm^{-1}): 3354 (amide N-H), 3042, 3015 (aromatic C-H), 2987, 2889 (aliphatic C-H), 1691 (amide C=O), 1554, 1486 (C=C), 1000–1300 (C-N), 1230 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.15 (3H, t, J=7.4, Hz, C-CH_2CH_3) 1.31 (3H, t, J=7.1 Hz, CH_3), 2.31 (2H, q, J=7 Hz C-CH_2), 4.43 (2H, q, J=7.2 Hz, N-CH_2), 4.71 (2H, s, O-CH_2), 6.96–7.65 (9H, m, Ar-H), 8.07 (H, d, J=7.5 Hz, carbazole C_5-H), 8.44 (H, s, carbazole C_4-H), 10.06 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.82 (CH_3), 16.8 (CH_3), 28.0 (CH_2), 37.09 (CH_2), 68.35 (CH_2), 109.63 (CH), 111.51 (2CH), 115.72 (CH), 119.75 (CH), 120.94 (CH), 121.76 (2CH), 123.78 (C), 124.02 (CH), 128.81 (C), 132.42 (2CH), 134.34 (C), 137.17 (C), 141.63 (2C), 156.75 (C), 167.77 (C).

For $C_{24}H_{24}N_2O_2$ calculated: 77.39% C, 6.49% H, 7.52% N; found: 77.39% C, 6.47% H, 7.56% N.

MS (FAB) $[M+1]^+$: m/z 373

N-(9-Ethyl-9H-carbazole-3-yl)-2-(2-chlorophenoxy) acetamide (2d)

IR (KBr) ν_{max} (cm^{-1}): 3413 (amide N-H), 3012 (aromatic C-H), 2989, 2876 (aliphatic C-H), 1698 (amide C=O), 1610, 1564 (C=C), 1000–1300 (C-N), 1218 (C-O-C),

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=6 Hz, CH_3), 4.43 (2H, q, J=6 Hz, N-CH_2), 4.88 (2H, s, O-CH_2), 7.01–7.60 (9H, m, Ar-H), 8.08 (H, d, J=8 Hz, carbazole C_5-H), 8.45 (H, s, carbazole C_4-H), 10.14 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.65 (CH_3), 36.95 (CH_2), 67.80 (CH_2), 109.06 (2CH), 109.16 (CH), 111.59 (CH), 114.11 (2C), 118.58 (CH), 120.20 (2C), 122.07 (CH), 125.81 (2CH), 128.25 (CH), 130.06 (CH), 130.22 (C), 136.38 (C), 139.97 (CH), 153.51 (C), 165.50 (C).

For $C_{22}H_{19}ClN_2O_2$ calculated: 69.75% C, 5.05% H, 7.39% N; found: 70.02% C, 5.03% H, 7.41% N.

MS (FAB) $[M+1]^+$: m/z 379

N-(9-Ethyl-9H-carbazole-3-yl)-2-(4-chlorophenoxy) acetamide (2e)

IR (KBr) ν_{max} (cm^{-1}): 3249 (amide N-H), 3079 (aromatic C-H), 2908 (aliphatic C-H), 1683 (amide C=O), 1612, 1594 (C=C), 1000–1300 (C-N), 1274 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 4.43 (2H, q, J=7 Hz, N-CH_2), 4.75 (2H, s, O-CH_2), 7.09–7.61 (9H, m, Ar-H), 8.07 (H, d, J=8 Hz, carbazole C_5-H), 8.43 (H, s, carbazole C_4-H), 10.11 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.65 (CH_3), 36.95 (CH_2), 67.42 (CH_2), 108.95 (CH), 109.16 (2CH), 112.05 (2C), 116.53 (C), 118.58 (CH), 120.17 (2C), 124.84 (C), 129.24 (2CH), 130.10 (CH), 136.44 (2CH), 139.96 (2CH), 156.77 (C), 165.83 (C).

For $C_{22}H_{19}ClN_2O_2$ calculated: 69.75% C, 5.05% H, 7.39% N; found: 69.78% C, 5.06% H, 7.40% N.

MS (FAB) $[M+1]^+$: m/z 379

N-(9-Ethyl-9H-carbazole-3-yl)-2-(2-nitrophenoxy) acetamide (2f)

IR (KBr) ν_{max} (cm^{-1}): 3317 (amide N-H), 3100 (aromatic C-H), 2980 (aliphatic C-H), 1687 (amide C=O), 1525, 1442 (C=C), 1000–1300 (C-N), 1239 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.30 (3H, t, J=8 Hz, CH_3), 4.42 (2H, q, J=8 Hz, N-CH_2), 4.99 (2H, s, O-CH_2), 7.18–7.96 (9H, m, Ar-H), 8.08 (H, d, J=8 Hz, carbazole C_5-H), 8.44 (H, s, carbazole C_4-H), 10.10 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.64 (CH_3), 36.95 (CH_2), 67.87 (CH_2), 109.11 (CH), 109.17 (C), 111.55 (C), 115.44 (2C), 118.61 (2C), 121.20 (CH), 125.26 (CH), 125.84 (2CH), 130.05 (2CH), 134.57 (CH), 136.42 (CH), 139.46 (CH), 139.98 (CH), 150.92 (C), 165.04 (C).

For $C_{22}H_{19}N_3O_4$ calculated: 67.86% C, 4.92% H, 10.79% N; found: 67.85% C, 4.90% H, 10.75% N.

MS (FAB) $[M+1]^+$: m/z 390

N-(9-Ethyl-9H-carbazole-3-yl)-2-(3-nitrophenoxy) acetamide (2g)

IR (KBr) ν_{max} (cm^{-1}): 3440 (amide N-H), 3012 (aromatic C-H), 2980 (aliphatic C-H), 1695 (amide C=O), 1602, 1543, 1551 (C=C), 1000–1300 (C-N), 1245 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 4.43 (2H, q, J=8 Hz, N-CH_2), 4.92 (2H, s, O-CH_2), 7.19–7.89 (9H, m, Ar-H), 8.08 (H, d, J=8 Hz, carbazole C_5-H), 8.43 (H, s, carbazole C_4-H), 10.20 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.66 (CH_3), 36.96 (CH_2), 67.46 (CH_2), 108.99 (CH), 109.17 (CH), 109.52 (2CH), 112.11 (C), 116.01 (C), 118.60 (CH), 120.18 (2C), 121.91 (2C), 125.81 (CH), 130.02 (CH), 130.72 (CH), 136.48 (CH), 139.97 (CH), 148.62 (CH), 158.43 (C), 165.47 (C).

For $C_{22}H_{19}N_3O_4$ calculated: 67.86% C, 4.92% H, 10.79% N; found: 67.81% C, 4.90% H, 10.78% N.

MS (FAB) $[M+1]^+$: m/z 390

N-(9-Ethyl-9H-carbazole-3-yl)-2-(4-nitrophenoxy) acetamide (2h)

IR (KBr) ν_{max} (cm^{-1}): 3298 (amide N-H), 3017 (aromatic C-H), 2980, 2875 (aliphatic C-H), 1669 (amide C=O), 1556, 1510 (C=C), 1000–1300 (C-N), 1236 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 4.43 (2H, q, J=8 Hz, N-CH_2), 4.95 (H, s, O-CH_2), 7.19–8.42 (11H, m, Ar-H), 10.27 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.66 (CH_3), 43.61 (CH_2), 67.42 (CH_2), 109.00 (CH), 109.09 (CH), 109.18 (CH), 111.60 (CH), 115.35 (C), 118.61 (C), 119.44 (CH), 121.86 (CH), 125.81 (CH), 130.05 (CH), 130.29 (CH), 136.45 (CH), 139.97 (2C), 141.22 (2C), 163.21 (CH), 164.21 (C), 165.16 (C).

For $C_{22}H_{19}N_3O_4$ calculated: 67.86% C, 4.92% H, 10.79% N; found: 67.82% C, 4.89% H, 10.80% N.

MS (FAB) $[M+1]^+$: m/z 390

N-(9-Ethyl-9H-carbazole-3-yl)-2-(2,3-dimethylphenoxy) acetamide (2i)

IR (KBr) ν_{max} (cm^{-1}): 3356 (amide N-H), 3059 (aromatic C-H), 2932, 2879 (aliphatic C-H), 1689 (amide

C=O), 1601, 1557, 1444 (C=C), 1000–1300 (C-N), 1215 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 2.16 (3H, s, Ar-CH_3) 2.21 (3H, s, Ar-CH_3), 4.42 (2H, q, J=8 Hz, N-CH_2), 4.66 (2H, s, O-CH_2), 6.97–7.76 (8H, m, Ar-H), 8.04 (H, d, J=8.2 Hz, carbazole C_5-H), 8.42 (H, s, carbazole C_4-H), 10.02 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.56 (CH_3), 18.82 (CH_3), 19.65 (CH_3), 35.54 (CH_2), 67.12 (CH_2), 108.92 (CH), 110.16 (CH), 115.18 (2C), 113.51 (2C), 117.26 (CH), 117.512 (CH), 121.18 (CH), 126.78 (CH), 128.67 (C), 132.54 (CH), 135.40 (2C), 137.58 (CH), 140.94 (2CH), 155.45 (C), 166.34 (C).

For $C_{24}H_{24}N_2O_2$ calculated: 77.39% C, 6.49% H, 7.52% N; found: 77.34% C, 6.48% H, 7.56% N.

MS (FAB) $[M+1]^+$: m/z 373

N-(9-Ethyl-9H-carbazole-3-yl)-2-(3,4-dimethylphenoxy) acetamide (2j)

IR (KBr) ν_{max} (cm^{-1}): 3346 (amide N-H), 3013 (aromatic C-H), 2984, 2750 (aliphatic C-H), 1687 (amide C=O), 1650, 1556 (C=C), 1000–1300 (C-N), 1254 (C-O-C),

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 2.16 (3H, s, Ar-CH_3) 2.21 (3H, s, Ar-CH_3), 4.43 (2H, q, J=8 Hz, N-CH_2), 4.66 (2H, s, O-CH_2), 6.77–7.65 (8H, m, Ar-H), 8.07 (H, d, J=8.1 Hz, carbazole C_5-H), 8.43 (H, s, carbazole C_4-H), 10.02 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.66 (CH_3), 18.42 (CH_3), 19.65 (CH_3), 36.94 (CH_2), 67.32 (CH_2), 108.93 (CH), 109.16 (CH), 111.58 (2C), 112.03 (2C), 116.16 (CH), 118.57 (CH), 120.17 (CH), 125.78 (CH), 128.67 (C), 130.15 (CH), 136.40 (2C), 137.28 (CH), 139.94 (2CH), 155.94 (C), 166.34 (C).

For $C_{24}H_{24}N_2O_2$ calculated: 77.39% C, 6.49% H, 7.52% N; found: 77.35% C, 6.50% H, 7.53% N.

MS (FAB) $[M+1]^+$: m/z 373

N-(9-Ethyl-9H-carbazole-3-yl)-2-(2,4-dimethylphenoxy) acetamide (2k)

IR (KBr) ν_{max} (cm^{-1}): 3381 (amide N-H), 3059 (aromatic C-H), 2927, 2874 (aliphatic C-H), 1694 (amide C=O), 1600, 1556, 1448 (C=C), 1000–1300 (C-N), 1224 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8 Hz, CH_3), 2.21 (3H, s, Ar-CH_3) 2.26 (3H, s, Ar-CH_3), 4.43 (2H, q, J=8.3 Hz, N-CH_2), 4.70 (2H, s, O-CH_2), 6.83–7.59 (8 H, m, Ar-H), 8.07 (H, d, J=8 Hz, carbazole C_5-H), 8.43 (H, s, carbazole C_4-H), 10.01 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.66 (CH_3), 16.11 (CH_3), 20.05 (CH_3), 36.94 (CH_2), 67.71 (CH_2), 108.97 (C), 109.16 (C), 111.53 (C), 111.87 (CH), 118.57 (2C), 119.39 (2C), 120.19 (CH), 121.96 (CH), 125.78 (CH), 129.49 (CH), 130.22 (CH), 131.29 (2CH), 136.37 (CH), 139.94 (CH), 154.01 (C), 166.40 (C).

For $C_{24}H_{24}N_2O_2$ calculated: 77.39% C, 6.49% H, 7.52% N; found: 77.40% C, 6.51% H, 7.47% N.

MS (FAB) $[M+1]^+$: m/z 373

N-(9-Ethyl-9H-carbazole-3-yl)-2-(3,5-dimethylphenoxy) acetamide (2l)

IR (KBr) ν_{max} (cm^{-1}): 3412 (amide N-H), 3053 (aromatic C-H), 2962, 2858 (aliphatic C-H), 1635 (amide C=O), 1504, 1482 (C=C), 1000–1300 (C-N), 1287 (C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8.2 Hz, CH_3), 2.26 (6H, s, 2CH_3), 4.43 (2H, q, J=8.1 Hz, N-CH_2), 4.67 (2H, s, O-CH_2), 6.64–7.65 (8H, m, Ar-H), 8.07 (H, d, J=8 Hz, carbazole C_5-H), 8.43 (H, s, carbazole C_4-H), 10.02 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.66 (CH_3), 21.05 (2CH_3), 36.95 (CH_2), 67.17 (CH_2), 108.94 (CH), 109.16 (CH), 112.05 (CH), 112.46 (CH), 118.57 (2C), 119.54 (CH), 120.16 (C), 122.77 (C), 125.78 (C), 130.17 (2CH), 136.42 (2CH), 138.62 (CH), 139.96 (2C), 157.89 (C), 166.25 (C).

For $C_{24}H_{24}N_2O_2$ calculated: 77.39% C, 6.49% H, 7.52% N; found: 77.34% C, 6.46% H, 7.49% N.

MS (FAB) $[M+1]^+$: m/z 373

N-(9-Ethyl-9H-carbazole-3-yl)-2-([1,1′-biphenyl]-4-yloxy) acetamide (2m)

IR (KBr) ν_{max} (cm^{-1}): 3346 (amide N-H), 3084 (aromatic C-H), 2978, 2880 (aliphatic C-H), 1673 (amide C=O), 1650, 1589, 1442 (C=C), 1000–1300 (C-N), 1238 (C-O-C),

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=7.2 Hz, CH_3), 4.44 (2H, q, J=7.6 Hz, N-CH_2), 4.80 (2H, s, O-CH_2), 7.14–7.67 (14H, m, Ar-H), 8.09 (H, d, J=7.8 Hz, carbazole C_5-H), 8.46 (H, s, carbazole C_4-H), 10.20 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 14.24 (CH_3), 37.95 (CH_2), 67.87 (CH_2), 109.11 (CH), 109.17 (CH), 112.55 (C), 116.44 (2C), 118.61 (2C), 121.20 (CH), 123.26 (CH), 125.84 (2CH), 127.05 (2CH), 128.57 (CH), 129.42 (CH), 130.46 (CH), 131.98 (CH), 134.92 (C), 137.04 (C), 141.04 (2CH), 141.27 (2CH), 158.96 (C), 167.72 (C).

For $C_{28}H_{24}N_2O_2$ calculated: 79.98% C, 5.75% H, 6.66% N; found: 79.96% C, 5.76% H, 6.68% N.

MS (FAB) $[M+1]^+$: m/z 421

N-(9-Ethyl-9H-carbazole-3-yl)-2-(quinolin-8-yloxy) acetamide (2n)

IR (KBr) ν_{max} (cm^{-1}): 3411 (amide N-H), 3056 (aromatic C-H), 2987, 2850 (aliphatic C-H), 1659(amide C=O), 1652, 1587, 1498 (C=C), 1000–1300 (C-N), 1247(C-O-C).

^{1}H NMR (400 MHz, DMSO-d_6): 1.31 (3H, t, J=8, CH_3), 4.42 (2H, q, J=8.3 Hz, N-CH_2), 5.01 (2H, s, O-CH_2), 7.19–9.04 (13H, m, Ar-H), 10.65 (H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 13.65 (CH_3), 18.52 (CH_2), 36.95 (CH_2), 56.00 (CH), 70.12 (CH), 109.13 (CH), 111.40 (C), 113.06 (C), 118.58 (2C), 118.93 (2C), 120.25 (CH), 121.39 (CH), 125.83 (CH), 129.18 (CH), 130.24 (2CH), 136.24 (CH), 136.39 (CH), 139.97 (CH), 140.05 (CH), 149.55 (C), 154.21 (C), 166.40 (C).

For $C_{25}H_{21}N_3O_2$ calculated: 75.93% C, 5.35% H, 10.63% N; found: 75.92% C, 5.36% H, 10.60% N.

MS (FAB) $[M+1]^+$: m/z 396

Antimicrobial activity

The antimicrobial activities of compounds **(2a-n)** were tested using the microbroth dilution method[31]. Tested

microorganism strains were *Micrococcus luteus* (NRLL B-4375), *Bacillus subtilis* (NRS-744), *P. aeroginosa* (ATCC-254992), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (ATCC-25922), *Listeria monocytogenes* (ATCC-7644) and *Candida albicans* (ATCC-22019). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethylsulfoxide. Dilution series using sterile distilled water were prepared from 0.65–4 mg/mL to 0.000633–0.0039 mg/mL in micro test tubes that were transferred to 96-well microtitre plates. Overnight-grown bacterial and *C. albicans* suspensions in double-strength Mueller–Hinton broth were standardised to 10^8 CFU/mL using McFarland No: 0.5 standard solutions. 100 µL of each microorganism suspension were then added into the wells. The last well chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18–24 h, antimicrobial activity was detected by spraying of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining. Streptomycin was used as standard antibacterial agent, whereas ketoconazole was used as an antifungal agent.

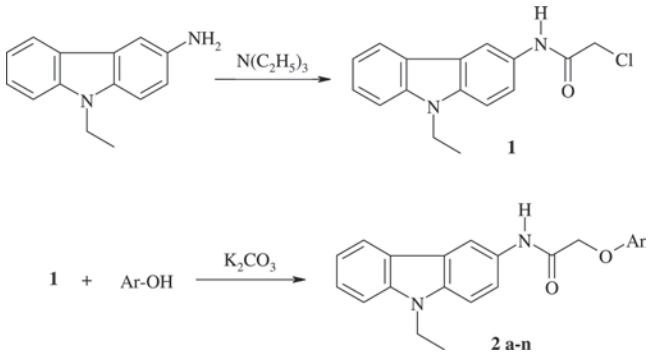
Cytotoxicity

The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[32,33]. Mouse embryonic fibroblast (NIH/3T3) cells were cultured in 96-well flat-bottom plates at 37°C for 24 h (2×10^4 cells per well). All of the compounds were dissolved in dimethyl sulfoxide (DMSO) individually and added to culture wells at varying concentrations (0.5–500 µg/mL); the highest final DMSO concentration was under 0.1%. After 24-hour drug incubation at 37°C, 20 µl MTT solution (5 mg/mL MTT powder in PBS) was added to each well. Then 3-hour incubation period was maintained in the same conditions. Purple formazan was occurred at the end of the process which is the reduction product of MTT agent by the mitochondrial dehydrogenase enzyme of intact cells. Formazan crystals were dissolved in 100 µL DMSO, and the absorbance was read by ELISA reader (OD_{570nm}). The percentage of viable cells was calculated based on the medium control.

Result, discussion and conclusion

Chemistry

In this study, a series of carbazole-based compounds were synthesised via an easy, convenient and efficient synthetic route. The synthesis of the compounds is shown in Scheme 1. To obtain final compounds, 3-amino-9-ethylcarbazole was reacted with chloroacetyl chloride to produce 2-chloro-*N-(*9-ethyl-9H-carbazole-3-yl)acetamide



Scheme 1. The synthetic protocol of the compounds (2a-n).

(1) which was then reacted with some phenol derivatives to get *N*-(9-ethyl-9H-carbazole-3-yl)-2-(substituted phenoxy)acetamide derivatives **(2a-n)**.

The structure elucidation of the compounds was determined by IR, ^{1}H-NMR, ^{13}C-NMR, FAB$^+$-MS spectral data and elemental analyses results.

In the IR spectra of all compounds, characteristic amide function was observed in the region 1659–1698 cm^{-1} because of the amide C=O vibration. In addition, the amide N-H vibration of the compounds were seen at 3249–3440 cm^{-1} region as expected.

The ^{1}H-NMR spectral data were also consistent with the assigned structures. In the 400 MHz ^{1}H-NMR spectrum of compounds, the O-CH_2 protons resonated at 4.6–5.01 ppm as a singlet and also N-H protons were observed at 10.01–10.65 ppm. For ethyl substitution, CH_3 protons were observed at about 1.31 ppm as triplet and CH_2 protons at 4.42–4.43 ppm as quartet. In aromatic region, the signal of the carbazole C_4-H and carbazole C_5-H protons was observed much further from upfield at about 8.06–8.46 ppm[34] and the other characteristic aromatic protons were observed at expected regions.

In the ^{13}C-NMR spectra of the compounds, the signal of characteristic carbonyl carbon appeared at 166.27 ppm and O-CH_2 at 67.52 ppm, respectively.

In the MS spectra, the electron-spraying technique with positive polarity mode was applied and M+1 peaks were detected as base peak.

All compounds gave satisfactory elemental analysis results.

Antimicrobial activity and toxicity

Antimicrobial activity was investigated by finding minimum inhibitory concentration (MIC) of the synthesised compounds against *S. aureus*, *L. monocytogenes*, *E. coli*, *Pseudomonas aeruginosa*, *M. luteus*, *B. subtilis* and *C. albicans* comparing with streptomycin and ketoconazole as standard drug. The MIC value of the compounds and control drugs are summarised in Table 2.

The MIC values were generally within the range of 31.25–500 µg/mL. Most of the compounds showed significant antifungal activity against *C. albicans.* In consideration of synthesised compounds' MIC values with

Table 2. Antimicrobial activities of the compounds (μg/mL).

Compound	A	B	C	D	E	F	G
2a	187.5	93.75	93.75	93.75	187.5	187.5	187.5
2b	250	125	125	125	250	250	250
2c	81.25	40.625	40.625	40.625	81.25	81.25	81.25
2d	500	250	250	250	500	500	500
2e	500	250	250	250	500	500	500
2f	500	250	250	250	250	500	250
2g	281.25	140.625	140.625	140.625	281.25	281.25	281.25
2h	393.75	196.875	196.875	196.875	196.875	393.75	393.75
2i	500	250	250	250	250	500	500
2j	393.75	196.875	196.875	196.875	196.875	393.75	393.75
2k	337.5	168.75	168.75	168.75	337.5	337.5	337.5
2l	500	250	250	250	500	500	500
2m	500	250	250	250	250	500	500
2n	31.25	250	250	250	250	31.25	125
Reference 1	31.25	7.81	31.25	125	15.625	15.625	–
Reference 2	–	–	–	–	–	–	250

Reference 1: Sytreptomycin; Reference 2: Ketoconazole.
A: *Staphylococcus aureus* (NRRL B-767), B: *Listeria monocytogenes* (ATCC-7644), C: *Escherichia coli* (ATCC-25922), D: *P. aeroginosa* (ATCC-254992), E: *Micrococcus luteus* (NRLL B-4375), F: *Bacillus subtilis* (NRS-744), G: *Candida albicans* (ATCC-22019).

standard drug, compounds **2a, 2c and 2n** had more and **2g** had less antifungal activity than did ketoconazole and also **2b and 2f** had similar antifungal activity to ketoconazole.

It was also observed that all compounds had antimicrobial activity against all tested bacteria according to standard drug streptomycin. Especially, compounds **2c** and **2n** are highly active against all of the evaluating bacterial strains. Compound **2n,** which includes quinolinoxy moiety, exhibited equipotent activity to standard drug with an MIC value of 31.25 μg/mL against *S. aureus.*

Compounds **2a, 2c and 2b** had satisfying activity against *P. aeruginosa*. Compound **2c** was the most effective against this bacteria with an MIC value of 40.25 μg/mL, whereas streptomycin had an MIC value of 125 μg/mL.

Compounds were also studied for their cytotoxic properties using MTT assay. The IC_{50} (μg/mL) values of the compounds against NIH/3T3 cells are shown in Table 3. The biological study indicated that compounds **2a, 2c, 2e, 2j and 2l** possessed the highest cytotoxicity, whereas the other compounds exhibited moderate cytotoxicity except **2n**. The IC_{50} concentration of compound **2n** was 300 μg/mL, which was greater than all MIC values observed against all tested bacteria and fungi for this compound.

In comparison of the results of cytotoxicity and antimicrobial activity tests, it can be claimed that compound **2n** possibly has antimicrobial activity not because of its general toxicity but because of its selective antimicrobial effect. The recognition of alkoxyquinoline's antimicrobial activity supports this result[35].

In conclusion, all synthesised compounds can be potential antimicrobial agent against different tested microorganisms.

Table 3. *In vitro* cytotoxicity of the compounds.

Compound	IC_{50} (μg/mL)[a]
2a	15.7 ± 0.6
2b	120.7 ± 22.0
2c	11.0 ± 1.7
2d	76.7 ± 25.2
2e	20.1 ± 0.2
2f	78.9 ± 2.5
2g	76.0 ± 8.7
2h	74.7 ± 8.1
2i	122.7 ± 4.6
2j	16.7 ± 1.2
2k	37.5 ± 13.0
2l	23.7 ± 7.5
2m	65.0 + 5.0
2n	300 + 50

[a]Cytotoxicity of the compounds to mouse fibroblast (NIH/3T3) cell line. Incubation for 24 hours. IC_{50} is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± SD of triplicate determinations.

Declaration of interest

The authors report no conflicts of interest.

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