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A new class of arylsulfonamide-based 3-acetyl-2-methyl-4-phenylquinolines and in vitro evaluation of their antioxidant, antifungal, and antibacterial activities

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Abstract A series of new arylsulfonamide-based 3-acetyl-2-methyl-4-phenylquinolines were synthesized. Initially, 1-(2-methyl-6-nitro-4-phenylquinolin-3-yl)ethanone was synthesized and its nitro function reduced using zinc dust and ammonium chloride, leading to formation of 1-(6-amino-2-methyl-4-phenylquinolin-3-yl)ethanone, which in turn was converted to the corresponding arylsulfonamides by treatment with various substituted sulfonyl chlorides. The synthesized derivatives were evaluated for antibacterial activity against a series of Gram-positive and Gram-negative bacteria and for antifungal activity against *Candida* strains, *Aspergillus niger* (*A. niger*), and *Issatchenkia hanoiensis* (*I. hanoiensis*). Compound **5a** showed prominent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity with half-maximal effective concentration (EC_{50}) of 9.78 ± 1.68 μg mL^{-1}. Compound **5f** exhibited considerable antifungal activity [minimum inhibitory concentration (MIC) of 0.89 μg mL^{-1}] against *A. niger* MTCC1344. Compounds **5e**, **f** showed excellent antibacterial activity (MIC 4.68 μg mL^{-1}) against *Klebsiella planticola* MTCC2277.

Keywords Arylsulfonamides · Synthesis · Antioxidant · Antifungal · Antibacterial activity

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Introduction

Sulfonamide-based organic compounds have attracted considerable interest due to their broad range of applications as heterocycles as well as in medicinal chemistry. Development of sulfonamides is an interesting and developing area of medicinal chemistry. The original antibacterial sulfonamides are synthetic antimicrobial agents containing sulfonamide group. Antibacterial agents with sulfonamide structure, such as sulfadiazine and hydrochlorothiazide, have been used therapeutically for many decades [1]. They are preferred due to their ease of administration and wide spectrum of antimicrobial activity [2–5]. Antibacterial sulfonamides act as competitive inhibitors of dihydropteroate synthase (DHPS), an enzyme involved in folate synthesis. Sulfonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Sulfonamide derivatives are widely used to treat various conditions, including gastrointestinal and urinary tract infections. Sulfonamides are used to treat allergies and cough, and act as antifungal and antimalarial agents. They possess numerous medicinal activities such as antibacterial [2–5], anti-carbonic anhydrase [6, 7], antiglaucoma [7, 8], antithyroid [8], high-ceiling diuretic [9], antiinflammatory [10], Alzheimer disease [11], and hypoglycemia [12]. Some of the drugs with aryl sulphoamide moiety available in the market is included as Fig. 1.

Recently, a series of arylsulfonamides were reported as potent inhibitors of various types of cancer cell at very low concentrations [13–15]. The drug *N*-aryl benzenesulfonamide has been used against the *C. albicans* MTCC227 fungal pathogen. Sulfamethoxazole and sulfonyl urea are well-known antibacterial drugs (Fig. 1). On the other hand, 3-acetyl-4-phenylquinolines are frequently used for design and synthesis of compounds with various pharmacological properties, such as antibacterial [5–19], antifungal [19, 20], antimalarial [21, 22], antiinflammatory [23], anticancer [24], and antitubercular activity [21]. These diverse biological activities of quinolines and arylsulfonamides prompted us to attempt synthesis of a

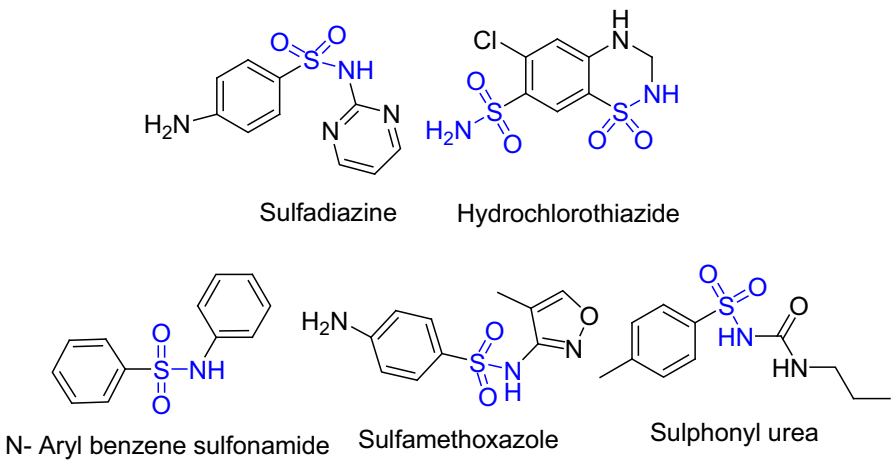


Fig. 1 Previously reported bioactive sulfonamide drugs

series of new compounds having both 3-acetyl-4-phenylquinoline and arylsulfonamide moieties in a single molecule.

In the present work, we initially synthesized 6-nitro-3-acetyl-2-methyl-4-phenylquinoline (**2**) and reduced it using ammonium chloride and zinc dust to obtain 6-amino-3-acetyl-2-methyl-4-phenylquinoline (**3**), which in turn was converted to its corresponding arylsulfonamides (**5a**–**f**).

Experimental

Materials and methods

Melting point (m.p.) values reported herein were recorded in open capillary tubes using Elchem microprocessor-based DT apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using Bruker 400 MHz and 500 MHz spectrometers with tetramethylsilane (TMS) as internal reference. Chemical shift values are reported in parts per million (δ, ppm) from internal standard TMS. High-resolution mass spectra were recorded using a Bruker MaXis HR-MS (ESI-Q-TOF–MS) instrument. All reagents were purchased from Aldrich and used as received. Solvents were removed under vacuum. Organic extracts were dried with anhydrous Na_2SO_4. Silica gel $60F_{254}$ aluminum sheets were used for analytical thin-layer chromatography (TLC). Visualization of spots on TLC plates was effected by ultraviolet (UV) illumination, exposure to iodine vapor, and heating the plates dipped in $KMnO_4$ stain. Silica gel with 230–400 mesh size was used for purification by column chromatography.

*General procedure for synthesis of compounds **2** and **3***

A mixture of 2-amino-5-nitro-benzophenone (0.1 g, 0.0005 M, **1**) with acetyl acetone and catalytic amount of *ortho*-phosphoric acid was stirred in ethanol for 12 h. After reaction completion, the crude product was poured onto ice-water; the obtained product **2** was filtered and dried. The obtained product **2** was further reduced with zinc dust (0.17 g, 0.0026 M) in presence of ammonium chloride (0.16 g, 0.0026 M) in dioxane/water (1:1) for 1 h with stirring. Reaction mixture was filtered to remove excess zinc and purified by column chromatography (basic alumina) using ethylacetate/hexane (4:6) as eluent.

1-(2-methyl-6-nitro-4-phenylquinolin-3-yl)ethanone (**2**) Yellow solid, yield 95%, m.p.: 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.58 (d, $J = 2.40$ Hz, 1H), 8.49 (dd, $J = 2.40$, 9.20 Hz, 1H), 8.20 (d, $J = 9.20$ Hz, 1H), 7.59 (dd, $J = 2.40$, 5.00 Hz, 3H), 7.37 (dd, $J = 2.00$, 4.40 Hz, 2H), 2.74 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 204.52, 157.97, 149.58, 145.71, 145.65, 136.30, 133.63, 130.80, 129.93, 129.27, 124.42, 123.62, 123.19, 31.71, 24.26.

1-(6-amino-2-methyl-4-phenylquinolin-3-yl)ethanone (**3**) Reddish-brown solid, yield 98%, m.p.: 179–181 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.88 (d, $J = 8.80$ Hz, 1H), 7.49 (d, $J = 4.80$ Hz, 3H), 7.34 (d, $J = 2.80$ Hz, 2H), 7.15 (dd, $J = 2.00$, 8.80 Hz, 1H), 6.66 (d, $J = 2.40$ Hz, 1H), 3.85 (s, 2H), 2.62 (s, 3H), 1.98

(s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 206.24, 149.30, 144.73, 142.67, 141.63, 135.75, 135.12, 130.03, 129.16, 128.68, 128.65, 126.46, 123.79, 121.92, 106.20, 67.10, 31.99, 23.40.

*General procedure for synthesis of sulfonamides **5a–f***

To solution of 1-(6-amino-2-methyl-4-phenylquinolin-3-yl)ethanone (**3**) (0.1 g, 0.0003 M) in tetrahydrofuran (THF, 5 mL), N,N-Diisopropylethylamine (DIEPA, 0.3 mL, 0.0009 M) and 2-nitrobenzenesulfonyl chloride (**4a**, 0.09 g, 0.0003 M) were added. The reaction mixture was refluxed for 5 h. After reaction completion, the crude product was extracted with ethyl acetate, concentrated, and dried. The obtained crude product **5** was purified through column chromatography (silica gel) using mixture of ethylacetate/hexane (7:3) as eluent. This procedure was repeated with **4b–f** to obtain corresponding sulfonamides **5b–f**.

N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-2-nitrosulfonamide (**5a**) Brown solid, yield 82%, m.p.: 206–208 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.99 (d, $J = 8.80$ Hz, 1H), 7.81 (dd, $J = 1.20$, 8.20 Hz, 1H), 7.68 (d, $J = 8.00$ Hz, 2H), 7.55 (d, $J = 2.40$ Hz, 1H), 7.53–7.50 (m, 4H), 7.45 (s, 1H), 7.36 (d, $J = 2.40$ Hz, 1H), 7.21 (dd, $J = 1.60$, 7.80 Hz, 2H), 2.65 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 205.32, 153.88, 148.13, 145.71, 143.45, 135.44, 134.48, 134.09, 133.72, 132.55, 132.06, 131.87, 130.57, 129.82, 129.32, 128.90, 125.88, 125.37, 118.52, 31.85, 23.80. HRMS-ESI (*m/z*) calcd. for $C_{24}H_{19}N_3O_5S$ $[M + H]^+ = 462.1124$, found = 462.1148.

N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-3-nitrosulfonamide (**5b**) Brown solid, yield 72%, m.p.: 210–212 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.35 (d, $J = 1.50$ Hz, 2H), 8.34 (d, $J = 2.00$ Hz, 2H), 8.10 (d, $J = 2.00$ Hz, 2H), 8.09 (d, $J = 2.00$ Hz, 2H), 7.43–7.36 (m, 2H), 7.17 (dd, $J = 1.50$ Hz, 8.00 Hz, 2H), 7.03 (d, $J = 2.00$ Hz, 1H), 2.72 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 204.50, 156.61, 150.90, 147.69, 144.02, 143.92, 135.84, 133.70, 131.34, 131.28, 130.99, 129.97, 129.58, 129.53, 128.91, 125.13, 124.35, 31.75, 24.06. HRMS-ESI (*m/z*) calcd. for $C_{24}H_{19}N_3O_5S$ $[M + H]^+ = 462.1124$, found = 462.1148.

N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-4-bromosulfonamide (**5c**) Pale-yellow solid, yield 87%, m.p.: 215–217 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.29 (d, $J = 0.80$ Hz, 1H), 6.91 (d, $J = 8.80$ Hz, 2H), 6.82 (dd, $J = 1.60$, 6.80 Hz, 2H), 6.71 (dd, $J = 2.80$, 6.60 Hz, 2H), 6.67 (dd, $J = 7.60$, 9.40 Hz, 2H), 6.57 (dd, $J = 2.40$, 8.80 Hz, 1H), 6.33 (dd, $J = 1.20$, 8.00 Hz, 2H), 6.28 (d, $J = 2.00$ Hz, 1H), 2.66 (s, 3H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 204.88, 156.04, 147.55, 144.15, 137.94, 135.56, 133.83, 132.47, 131.91, 131.72, 131.25, 130.76, 129.94, 129.74, 129.64, 129.45, 128.97, 127.82, 125.00, 31.81, 24.04. HRMS-ESI (*m/z*) calcd. for $C_{24}H_{19}BrN_2O_3S$ $[M + H]^+ = 495.0378$, found = 495.0380.

N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-4-nitrosulfonamide (**5d**) Brown solid, yield 80%, m.p.: 235–237 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ ppm 8.57 (dd, $J = 3.20$, 8.80 Hz, 2H), 8.25–8.19 (m, 3H), 8.09 (d, $J = 6.80$ Hz, 2H), 7.92–7.87 (m,

3H), 7.55 (dd, $J = 1.60, 7.60$ Hz, 3H), 2.93 (s, 3H), 2.32 (s, 3H).^{13}C NMR (100 MHz, d_6-DMSO): δ ppm 205.05, 152.50, 149.92, 145.35, 135.46, 135.22, 134.84, 129.99, 129.83, 129.10, 128.77, 128.59, 125.31, 124.64, 124.16, 114.97, 31.90, 23.35. HRMS-ESI (*m/z*) calcd. for $C_{24}H_{19}N_3O_5S$ $[M + H]^+ = 462.1124$, found = 462.1148.

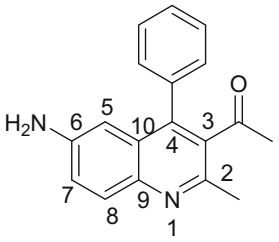
N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-4-methylbenzenesulfonamide (**5e**) Yellow solid, yield 75%, m.p.: 192–194 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.03 (d, $J = 8.80$ Hz, 1H), 7.72 (d, $J = 8.40$ Hz, 3H), 7.44 (t, $J = 1.20$ Hz, 1H), 7.42 (s, 1H), 7.41 (d, $J = 1.60$ Hz, 1H), 7.35 (dd, $J = 2.40, 8.80$ Hz, 1H), 7.24 (d, $J = 2.40$ Hz, 1H), 7.19–7.14 (m, 4H), 2.68 (s, 3H), 2.45 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 205.07, 155.59, 147.52, 145.05, 144.13, 135.38, 134.15, 132.44, 132.42, 130.30, 129.85, 129.80, 129.62, 129.11, 128.73, 128.50, 124.93, 31.78, 24.01, 21.75. HRMS-ESI (*m/z*) calcd. for $C_{25}H_{22}N_2O_3S$ $[M + H]^+ = 431.1429$, found = 431.1428.

N-(3-acetyl-2-methyl-4-phenylquinolin-6-yl)-benzenesulfonamide (**5f**) Light-brown solid, yield 88%, m.p.: 186–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.06 (d, $J = 8.40$ Hz, 1H), 7.86 (d, $J = 7.60$ Hz, 2H), 7.65 (t, $J = 7.20$ Hz, 2H), 7.48 (t, $J = 7.60$ Hz, 3H), 7.43 (d, $J = 7.43$ Hz, 2H), 7.40 (d, $J = 4.40$ Hz, 1H), 7.15 (d, $J = 8.40$ Hz, 2H), 2.70 (s, 3H), 1.96 (s, 3H), 1.71 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 205.07, 155.77, 147.55, 144.16, 139.17, 135.43, 134.04, 132.33, 132.18, 130.46, 129.86, 129.80, 129.21, 129.07, 128.86, 128.49, 124.96, 31.79, 24.04. HRMS-ESI (*m/z*) calcd. for $C_{24}H_{20}N_2O_3S$ $[M + H]^+ = 417.1273$, found = 417.1219.

Spectral characterization

The newly synthesized compounds **3**, **5a–f** were characterized using ^{1}H NMR, ^{13}C NMR, H–H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and HR-MS (ESI); the data are included in "Experimental" section. All newly derived target molecules **5a–f** were subjected to in vitro antioxidant, antifungal, and antibacterial evaluations; the results are discussed in "Biological evaluations" section.

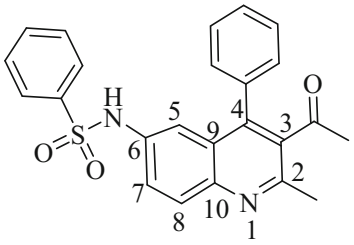
Spectral characterization of compound 3



Structutre of Compound **3**

The melting point and ^{1}H and ^{13}C NMR spectral data of compound **2** were found to be in accord with literature. Formation of compound **2** with nitro function was inferred based on the absorption peaks at 3419 and 3348 cm^{-1} in the IR spectrum, whose disappearance confirmed conversion of **2** into **3**. Compound **3** was further characterized by ^{1}H NMR, ^{13}C NMR, H–H COSY, heteronuclear single quantum coherence (HSQC), and HR-MS (ESI) spectral analysis. The ^{1}H NMR spectrum of compound **3** exhibited the following chemical shifts: δ 7.88 d [$J = 9.0$ Hz, one proton]; 7.49 m [for 3 protons]; 7.34 m [2 protons]; 7.15 dd [9.0 Hz and 2.4 Hz, one proton]; 6.66 d [2.4 Hz, one proton]; 2.61 s [3 protons]; 1.90 s [3 protons]. Examination of the H–H COSY spectrum of the molecule revealed singlets at δ 1.98 and 2.61 integrating for three protons, each due to the two methyl groups of the molecule. The relatively upfield signal δ 1.98 ppm is due to the methyl at C-2, and the relatively downfield signal δ 2.61 ppm is due to the methyl of acetyl group at C-3. The broad signal at δ 3.86 ppm is due to the protons of amino group at C-6. The signal at δ 7.49 ppm integrating for three protons is coupled with δ 7.34 ppm, which integrates for two protons. The signal at δ 7.49 ppm is due to the *meta* and *para* protons of phenyl at C-4, whereas the other signal at δ 7.34 ppm was assigned to *ortho* protons of the same phenyl ring. The signal at δ 7.15 ppm [$J = 9.0$ and 2.4 Hz, 1 proton] coupled with the signals at δ 6.66 [d, $J = 2.4$ Hz, 1 proton] and 7.88 [d, $J = 9.0$ Hz, 1 proton] and was hence assigned to proton at C-7. The signal at δ 6.66 ppm was assigned to proton at C-5, whereas the other signal at δ 7.88 ppm was assigned to C-8 proton. The related carbon chemical shift values were assigned based on the ^{13}C NMR and HSQC spectra. The ^{13}C NMR spectrum exhibited the following chemical shift values: δ 23.39, 31.99, 106.21, 121.93, 126.47, 128.09, 128.65, 141.65, 142.66, 144.74, and 206.22. The upfield signal at δ 23.39 was assigned to methyl carbon at C-2, and the other signal at δ 31.99 ppm to the methyl of acetyl group at C-3. The extreme downfield signal at δ 206.22 ppm was assigned to carbonyl carbon of acetyl group at C-3. Proton-bearing carbons were identified using the HSQC spectrum. The signal at δ 106.22 ppm was assigned to the carbon at C-5. Carbons C-7 and C-8 were identified at δ 121.93 and 128.65 ppm, respectively. Similarly, the *ortho*, *meta*, and *para* carbons of phenyl group at C-4 were identified at δ 128.68, 126.47, and 128.09 ppm, respectively. The signals at δ 130.03, 131.10, 135.13, 135.76, 141.65, 142.66, and 144.77 ppm are due to non-proton-bearing carbons C-2, C-3, C-4, C-6, C-9, C-10, and *ipso* positions. Formation of compound **3** was also supported by the *m*/*z* value of 277 observed in the mass spectrum.

Spectral characterization of compound 5f



Structure of compound **5f**

Compound **5f** was also further characterized by ^{1}H NMR, ^{13}C NMR, H–H COSY, HSQC, and HR-MS (ESI) spectral analysis. The ^{1}H NMR spectrum of compound **5f** exhibited the following chemical shifts: δ 8.07 d [J = 8.80 Hz, 1 proton], 7.87 d [J = 8.00 Hz, 2 protons], 7.65 t [J = 7.20 Hz, 2 protons], 7.49 t [J = 7.60 Hz, 3 protons], 7.43 d [J = 7.20 Hz, 1 protons], 7.40 d [J = 4.40 Hz, 1 proton], 7.15 d [J = 7.60 Hz, 3 protons]. Examination of the H–H COSY spectrum of the molecule revealed singlets at 2.70 ppm and 1.96 ppm integrating for three protons, each due to the methyl groups of the molecule, and one more singlet at 1.72 ppm due to the one –NH proton. The relatively upfield signal at δ 1.96 ppm is due to the methyl at C-2, and the relatively downfield signal δ 2.70 ppm is due to the methyl of acetyl group at C-3. The broad signal at δ 1.72 ppm is due to the one proton of secondary amine at C-6. The signal at δ 8.06 ppm [d, J = 8.8 Hz] which integrates for one proton coupled with δ 7.40 ppm [d, J = 4.4 Hz] which integrates for one proton. The signal at δ 7.40 ppm actually appeared as doublet of doublet (compared with molecule **3**), appearing as doublet since it merged with another signal at δ 7.43 ppm. Close examination of the H–H COSY spectrum revealed that the signal at δ 7.40 ppm exhibited correlation with δ 8.06 ppm and δ 7.15 ppm and was hence assigned to proton at C-7. The other signals at δ 8.06 ppm and δ 7.15 ppm were assigned to protons at C-8 and C-5, respectively. The signal at δ 7.15 ppm, also not a well-defined doublet, integrates for three protons; close examination of the H–H COSY and HSQC spectra revealed that the C-5 proton and two more aryl protons merged in this signal and appeared as a single signal (in HSQC, two carbons were found to be in correlation with this particular proton signal). The two phenyl rings at C-4, C-6 (as a part of sulfonamides) are responsible for the other aromatic signals. The H–H COSY spectrum revealed that the triplet signal at δ 7.65 ppm correlated with the signals at δ 7.87 and δ 7.49 and was hence assigned to *meta* protons of the aryl at C-6. Its related *ortho* and *para* protons were found to resonate at δ 7.87 and δ 7.49 ppm. The signal at δ 7.49 ppm integrated to three protons, and the HSQC spectrum revealed two carbons for this particular proton signal. This signal was found to correlate with those at δ 7.43 and δ 7.15 ppm, hence the remaining two protons are due to *meta* protons of other aryl substituent placed at C-4. Its coupling partners *ortho* and *meta* protons appeared at δ 7.43 and δ 7.15 ppm. The related

carbon chemical shift values were assigned based on the ^{13}C NMR and HSQC spectra. The ^{13}C NMR spectrum exhibited the following chemical shift values: δ 24.04, 31.79, 124.96, 128.49, 128.86, 129.07, 129.21, 129.80, 129.86, 130.46, 132.18, 132.33, 134.04, 135.43, 139.17, 144.16, 147.55, 155.77, and 205.07. Comparison of the distortionless enhancement by polarization transfer (DEPT)-135 spectrum with the normal broadband decoupled ^{13}C NMR spectrum revealed that the peaks observed for the following chemical shifts were due to non-proton-bearing carbons: δ 124.96, 132.18, 135.43, 139.17, 144.16, 147.55, 155.77, and 205.07. The extreme downfield signal at δ 205.07 ppm was assigned to carbonyl carbon of acetyl group at C-3. The other signals at δ 124.96, 132.18, 135.43, 139.17, 144.16, 147.55 (*ipso* of aryl at C-4), and 155.77 (*ipso* of arylsulfonamide at C-6) are due to carbons C-2, C-3, C-6, C-9, C-10 and *ipso* carbons of two aryl rings. The HSQC spectrum revealed that the upfield signal at δ 24.04 could be assigned to methyl carbon of acetyl group at C-3 and the other signal at δ 31.79 ppm to the methyl group at C-2. Proton-bearing carbons were identified using the DEPT-135 and HSQC spectra. Carbons C-5, C-7, and C-8 were identified to resonate at δ 129.86, 132.33, and 130.46, respectively. Similarly, the *ortho*, *meta*, and *para* carbons of phenyl group at C-4 were identified at δ 129.80, 129.21, and 128.86 ppm, respectively. The signals at 128.49, 129.07, and 134.04 ppm were assigned to *ortho*, *meta*, and *para* carbons of arylsulfonamide group at C-6. Formation of compound **5f** was also supported by the *m*/*z* value observed at 417.1219 in the mass spectrum.

Biological evaluations

In vitro antioxidant activity by DPPH free radical scavenging method

The antioxidant activity of the compounds was assayed by measuring scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. In this experiment, different concentrations (10, 20, 40, 80, and 200 μg mL^{-1}) of each compound were prepared in methanol. Then, working solutions were mixed with 100 μL 160 μM methanol solution of DPPH. The reaction mixture was thoroughly mixed for 2 min. The samples were then incubated for 30 min in the dark at 35 °C. After incubating for 30 min, the absorbance of the samples was measured spectroscopically at 517 nm using a TRIAD multimode reader. In this experiment, ascorbic acid was used as positive control and methanol solution as negative control. The radical scavenging potential is expressed as an EC_{50} value, representing the compound concentration at which 50% of the DPPH radicals were scavenged. All tests were performed in triplicate, and values are presented as means. The ability of the compound to scavenge the formation of DPPH free radicals was measured in a concentration-dependent manner and the DPPH scavenging activity calculated in terms of the effective concentration.

In vitro antifungal activity by broth dilution method

The antifungal activity of the compounds was determined by broth dilution method using *p*-iodonitrotetrazolium (INT) dye reduction in 96-well plates [2]. The test fungal

pathogens used in this study were *C. albicans* MTCC227, *C. parapsilosis* MTCC6510, *A. niger* MTCC1344, *C. glabrata* MTCC3984, *I. hanoiensis* MTCC4755, and *C. aaseri* MTCC1907. The test fungal strains [10^7 colony-forming units (CFU)/mL] were inoculated in 100 μL potato dextrose broth, and the test cultures were stored at 4 °C for further experiments. The purity of the test cultures was verified by culturing on nutrient agar medium. In a 96-well plate, fungal test cultures were inoculated with final concentration of approximately 10^7 CFU/mL in final volume of 100 μL per well. To each fungal suspension, compound was added at different concentrations of 300, 150, 75, 37.5, 18.75, 9.37, 4.68, 2.34, 1.17, and 0.58 μg mL^{-1}. In positive control experiments, micanazole (Sigma) was added to each fungal test culture, while potato dextrose broth medium alone was used as negative control. The fungal pathogens were incubated along with test compound for 24 h at 30 °C. After 24 h, 40 mL INT test reagent containing 0.02% 20 mg INT in 100 mL 40% dimethylformamide (DMF) was added to each well. The samples were further incubated for 2 h at 37 °C. Reduction of INT dye was monitored spectrometrically by measuring samples at 450 nm using a TRIAD multimode reader. The percentage of fungal pathogen growth inhibition was calculated using the formula

$$\%\ \text{of fungal pathogen growth inhibition} = (1 - A_C/A_0) \times 100,$$

where A_C is the INT absorbance of wells with compound at concentration C and A_0 represents the INT absorbance of the negative control without compound. The minimum inhibitory concentration (MIC, μg mL^{-1}) of the samples represents the lowest concentration of the compound with >95% decrease in fungal growth. All experiments were performed in triplicates, and the results are expressed as the average of three independent experiments.

In vitro antibacterial activity by microdilution method

All the newly synthesized compounds were subjected to antibacterial assay against pathogenic bacteria using microdilution method [1]. The indicator strains used were *Staphylococcus aureus* MTCC737, *K. planticola* MTCC2277, *Bacillus cereus* MTCC430, *S. aureus* MTCC2940, *Escherichia coli* MTCC1687, and *Pseudomonas aeruginosa* MTCC424. The test bacterial strains were grown in nutrient broth medium, and prepared inoculums were stored at 4 °C for further use. The dilutions of the bacterial inoculums were cultured on nutrient agar medium to check for presence of contamination. The bacterial suspension was adjusted with sterile saline to final concentration of approximately 10^7 CFU/mL in final volume of 100 μL per well in 96-well plate. The bacterial pathogens were inoculated in Müller–Hinton broth along with different concentrations of compounds including 300, 150, 75, 37.5, 18.75, 9.37, 4.68, 2.34, 1.17, and 0.58 μg mL^{-1}. Neomycin (Sigma) was used as positive control, and Müller–Hinton culture medium alone as negative control. The bacterial pathogens were incubated with compounds for 24 h. After incubating for 24 h, 40 μL INT dye solution was added to each well. INT dye solution was prepared by dissolving 0.02% 20 mg INT in 100 mL 40% dimethylformamide. The samples were further incubated for 2 h. The samples were then read

spectrometrically at 450 nm using a TRIAD multimode reader, which measures the reduction of INT dye by bacterial pathogens. The percentage bacterial growth inhibition was calculated using the formula

$$\% \text{ of bacterial growth inhibition} = (1 - A_C/A_0) \times 100,$$

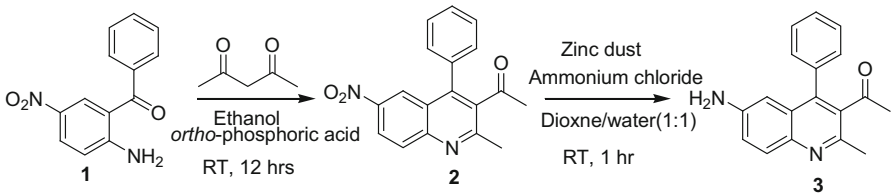
where A_C represents the INT absorbance of the wells with compound at concentration C and A_0 represents the INT absorbance of the negative control without compound. Furthermore, the minimum inhibitory concentration (MIC, μg mL^{-1}) value representing the lowest concentration of test compound which exhibited significant decrease (>95%) in bacterial viability was determined. All experiments were performed in triplicates, and the results are expressed as the average of three independent experiments.

Results and discussion

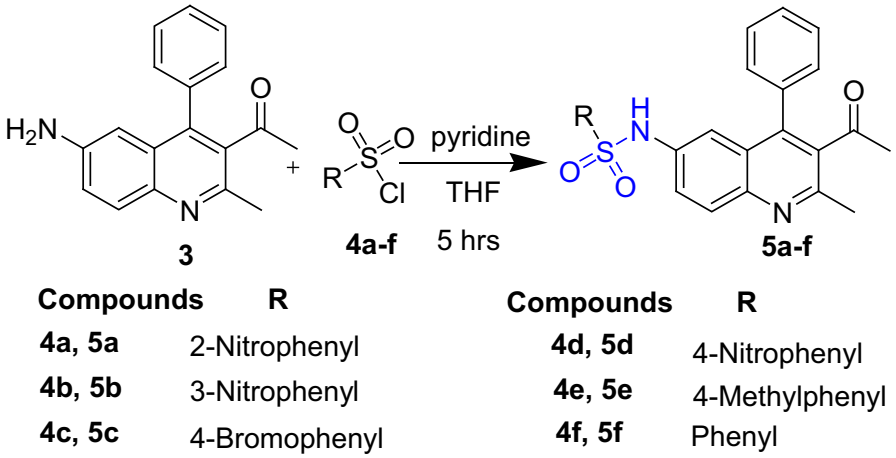
Chemistry

A series of arylsulfonamide-based 3-acetyl-2-methyl-4-phenylquinolines were synthesized from 3-acetyl-2-methyl-4-phenylquinoline in presence of base. In the first step, nitro-substituted aminobenzophenone was converted to 1-(2-methyl-6-nitro-4-phenylquinolin-3-yl)ethanone **2** (Scheme 1) in ethanol in presence of *ortho*-phosphoric acid (catalyst). The obtained compound **2** was further reduced in presence of ammonium chloride and zinc dust in dioxane/water (1:1) to form corresponding 1-(6-amino-2-methyl-4-phenylquinolin-3-yl)ethanone **3** (Scheme 1), which in turn reacted with various sulfonyl chlorides (**4a–f**) to form the products **5a–f** (Scheme 2). Various bases such as Et_3N [7], DIEPA [5], pyridine [4], K_2CO_3 [7], KOH [10], and NaOH [8] were employed as catalyst in the conversion of **3** to **5a–f** with better yields observed with pyridine, which was hence considered a suitable base to form the sulfonamides (Table 1).

We employed DIEPA as base as it also offered similar, only slightly lower yield. Considering cost and availability, we considered pyridine to be a suitable base for effective formation of **5a–f**. All synthesized compounds showed lower yield when K_2CO_3 was used as base (Scheme 2). All these newly synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR, DEPT-135, H–H COSY, HSQC and HRMS (ESI); the data are included in "Experimental" section. All the newly



Scheme 1 General procedure for synthesis of 1-(6-amino-2-methyl-4-phenylquinolin-3-yl)ethanone **3**



Scheme 2 General procedure for synthesis of 3-acetyl-4-phenylquinoline-based sulfonamide derivatives **5a–f**

Table 1 Optimization for reaction in Scheme 2

S. no.	R^1	R^2	Pdt	Solvent	Base	Yield (%)	S. no.	R^1	R^2	Pdt	Solvent	Base	Yield (%)
1	**3**	**4a**	**5a**	THF	Et_3N	66	19	**3**	**4d**	**5d**	THF	Et_3N	69
2	**3**	**4a**	**5a**	THF	KOH	45	20	**3**	**4d**	**5d**	THF	KOH	49
3	**3**	**4a**	**5a**	THF	K_2CO_3	10	21	**3**	**4d**	**5d**	THF	K_2CO_3	15
4	**3**	**4a**	**5a**	THF	DIEPA	92	22	**3**	**4d**	**5d**	THF	DIEPA	94
5	**3**	**4a**	**5a**	THF	Pyridine	98	23	**3**	**4d**	**5d**	THF	Pyridine	99
6	**3**	**4a**	**5a**	THF	NaOH	38	24	**3**	**4d**	**5d**	THF	NaOH	30
7	**3**	**4b**	**5b**	THF	Et_3N	60	25	**3**	**4e**	**5e**	THF	Et_3N	61
8	**3**	**4b**	**5b**	THF	KOH	40	26	**3**	**4e**	**5e**	THF	KOH	44
9	**3**	**4b**	**5b**	THF	K_2CO_3	08	27	**3**	**4e**	**5e**	THF	K_2CO_3	11
10	**3**	**4b**	**5b**	THF	DIEPA	90	28	**3**	**4e**	**5e**	THF	DIEPA	90
11	**3**	**4b**	**5b**	THF	Pyridine	95	29	**3**	**4e**	**5e**	THF	Pyridine	99
12	**3**	**4b**	**5b**	THF	NaOH	31	30	**3**	**4e**	**5e**	THF	NaOH	31
13	**3**	**4c**	**5c**	THF	Et_3N	59	31	**3**	**4f**	**5f**	THF	Et_3N	60
14	**3**	**4c**	**5c**	THF	KOH	39	32	**3**	**4f**	**5f**	THF	KOH	44
15	**3**	**4c**	**5c**	THF	K_2CO_3	06	33	**3**	**4f**	**5f**	THF	K_2CO_3	13
16	**3**	**4c**	**5c**	THF	DIEPA	89	34	**3**	**4f**	**5f**	THF	DIEPA	93
17	**3**	**4c**	**5c**	THF	Pyridine	96	35	**3**	**4f**	**5f**	THF	Pyridine	95
18	**3**	**4c**	**5c**	THF	NaOH	29	36	**3**	**4f**	**5f**	THF	NaOH	30

derived compounds **3**, **5a–f** were subjected to in vitro antioxidant, antifungal, and antibacterial evaluation; the results are discussed in "Biological evaluations" section.

Biological evaluations

In vitro antioxidant activity by DPPH free radical scavenging method

The antioxidant activity results for the compounds are summarized in Table 2, indicating that compounds **5a** ($EC_{50} = 9.78$ μg mL^{-1}) and **5b** ($EC_{50} = 15.78$ μg mL^{-1}) exhibited DPPH free radical scavenging activity as effective as ascorbic acid ($EC_{50} = 17.95$ μg mL^{-1}). Furthermore, compounds **5a**, **d**, **f**, **e**, **3** exhibited moderate DPPH free radical scavenging activity with EC_{50} values ranging between 24.78 and 1.55 μg mL^{-1}. These results clearly demonstrate that the compounds possess the ability to scavenge DPPH free radical and hence exhibit antioxidant activity in cell-free system.

In vitro antifungal activity

The antifungal activity of the compounds is presented in terms of MIC in Table 3. These minimum inhibitory concentration values demonstrate that the compounds exhibited good to moderate antifungal activity against the tested fungal pathogens. Among the compounds, **5f** and **3** showed the highest antifungal activity with MIC values ranging between 0.89 and 4.68 μg mL^{-1} against *C. albicans* test pathogen, as compared with positive control (micanazole, MIC 9.37 μg mL^{-1}). In the case of *C. parapsilosis*, promising antifungal activity was observed for compounds **5f**, **c**, **3** with MIC values from 1.67 to 2.34 μg mL^{-1}. In the case of *A. niger*, compound **5f** showed good antifungal activity as compared with positive control micanazole with MIC value of 0.89 μg mL^{-1}. In the case of *C. glabrata*, compounds **5f**, **3** exhibited good antifungal activity with MIC values ranging from 1.67 to 2.34 μg mL^{-1}, respectively. In the case of *I. hanoiensis*, compounds **5f**, **3** showed good antifungal activity with MIC values ranging between 2.34 and 4.68 μg mL^{-1}, respectively. In the case of *C. aaseri*, compounds **5c**, **3** showed good antifungal activity with MIC values from 2.34 to 1.67 μg mL^{-1}, respectively, as compared with standard micanazole (MIC 4.68 μg mL^{-1}). These results demonstrate that the compounds

Table 2 DPPH free radical scavenging activity of compounds

Compound	EC_{50}, concentration to scavenge 50% of DPPH free radicals (μg mL^{-1})
3	21.09 ± 1.55
5a	9.78 ± 1.68
5b	15.78 ± 1.78
5c	28.9 ± 1.95
5d	23.57 ± 1.26
5e	24.67 ± 1.80
5f	24.78 ± 1.85

Ascorbic acid, standard: 17.95 ± 0.95

Table 3 Antifungal activity of compounds

Compound	Ca MTCC227	Cp MTCC6510	An MTCC1344	Cg MGTC3984	Ih MTCC4755	Ca MTCC1907
3	75	2.34	1.67	2.34	4.68	1.67
5a	2.34	18.75	4.68	9.37	37.5	75
5b	–	–	–	–	–	–
5c	4.68	2.34	–	–	–	2.34
5d	2.34	9.37	18.75	18.75	75	18.75
5e	–	–	–	–	–	–
5f	2.34	1.67	0.89	1.67	2.34	75
Micanazole	9.37	9.37	9.37	9.37	9.37	4.68

– no activity; micanazole standard

Ca = *C. albicans*; Cp = *C. parapsilosis*; An = *A. niger*; Cg = *C. glabrata*; Ih = *I. hanoiensis*; Ca = *C. aaseri*

possess good antifungal activity against the fungal pathogens. Among all the compounds, **5f** exhibited considerable fungal activity (MIC 0.89 μg mL^{-1}) against *A. niger* MTCC134. The other compounds such as **5a–e** showed lower antifungal activity than **5f** due to substitution on benzene ring, whereas compound **5f** has no such substitution and hence exhibiting better activity.

In vitro antibacterial activity

The compounds exhibited promising broad-spectrum antibacterial activity against the tested Gram-positive and Gram-negative bacterial pathogens. The antibacterial activity of the compounds is presented in terms of minimum inhibitory concentration in Table 4. From these MIC values, it is observed that, compared with positive control neomycin (MIC 18.75 μg mL^{-1}), compounds **5c**, **e** showed promising antibacterial activity against *S. aureus* test strain with MIC ranging

Table 4 Antibacterial activity of compounds

Compounds	Sa MTCC737	Kp MTCC2277	Bc MTCC430	Sa MTCC2940	Ec MTCC1687	Pa MTCC424
3	18.75	–	4.68	4.68	2.34	2.34
5a	75	37.5	18.75	–	–	
5b	18.75	–	150	–	75	–
5c	9.37	–	18.75	9.37	–	18.75
5d	18.75	–	–	18.75	–	–
5e	9.37	4.68	18.75	9.37	–	–
5f	150	4.68	–	–	–	300
Neomycin	18.75	18.75	18.75	18.75	18.75	18.75

– no activity; neomycin standard

Sa = *S. aureus*; Kp = *K. planiticola*; Bc = *B. cereus*; Ec = *E. coli*; Pa = *P. aeruginosa*

between 9.37 μg mL^{-1}. In the case of *K. planticola* strain, compounds **5e**, **f** exhibited the highest antibacterial activity with minimum MIC value (4.68 μg mL^{-1}) compared with the positive control. Compound **3** showed promising antibacterial activity in the case of test strain *B. cereus* with MIC ranging between 4.68 μg mL^{-1}. Similarly, in the case of *S. aureus* compounds **5c**, **e**, **3** exhibited good antibacterial activity with minimum MIC values (4.68–9.37 μg mL^{-1}) in comparison with neomycin standard. Furthermore, it was observed that compound **3** showed promising antibacterial activity compared with neomycin standard against *E. coli* bacterial pathogen. In the case of *P. aeruginosa*, compared with standard neomycin (MIC 18.75 μg mL^{-1}), compound **3** exhibited the highest antibacterial activity with MIC of 2.34 μg mL^{-1}. Overall, moderate to minimum antibacterial activity was observed for the compounds, with MIC values ranging from 2.34 to 300 μg mL^{-1} against various bacterial pathogens. These data clearly suggest that the compounds exhibit promising broad-spectrum antibacterial activity against test bacterial pathogens. Compounds **5e**, **f** showed excellent antibacterial activity towards *K. planticola* MTCC2277 (MIC value 4.68 μg mL^{-1}), whereas other compounds such as **5a–d** showed low antibacterial activity due to halo and nitro substitution on benzene ring, whereas compounds **5e**, **f** have no such substitution and hence with better activity.

Conclusions

We synthesized arylsulfonamide-based 3-acetyl-4-phenylquinolines from 3-acetyl-4-phenylquinoline and subjected all newly synthesized compounds to in vitro antioxidant, antifungal, and antibacterial evaluation to determine their efficacy and identify potent lead molecules.

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References

1. W.B. Susan, F. Colin, Biochem. Pharmacol. **28**, 751–756 (1979)
2. A.K. Gadad, C.S. Mahajanshetti, S. Nimbalkar, A. Raichurkar, Eur. J. Med. Chem. **39**, 853–857 (2000)
3. V.S. Misra, V.K. Saxena, R.J. Srivastava, Ind. Chem. Soc. **59**, 781–785 (1982)
4. J.S. Brzozowski, F. Saczewski, A. Innocenti, C.T. Supran, Eur. J. Med. Chem. **45**, 2396–2404 (2010)
5. G. Renzi, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. **10**, 673–676 (2000)
6. T.H. Maren, Physiol. Rev. **47**, 595–781 (1967)
7. J. Drew, Sciences **287**, 1960–1964 (2000)
8. J.J. Li, G.D. Anderson, E.G. Burton, J.N. Cogburn, J.T. Collins, D.J. Garland, S.A. Gregory, H.-C. Huang, P.C. Isakson, J. Med. Chem. **38**, 4570–4578 (1995)

9. H. Fuwa, K. Hiromoto, Y. Takahashi, S. Yokoshima, T. Kan, T. Fukuyama, T.I. Taisuke, T.N. Hideaki, Bioorg. Med. Chem. Lett. **16**, 4184–4189 (2006)
10. A. Andreani, M. Rambaldi, A. Locatelli, A.M. Isetta, Eur. J. Med. Chem. **26**, 335–337 (1991)
11. I.M. Khazi, C.S. Mahajanshetti, A.K. Gadad, A.D. Tarnalli, C.M. Sultanpur, A. Forsch, Drug Res. **46**, 949–952 (1996)
12. H. Elo, Spectrosc. Lett. **22**, 123–130 (1989)
13. A.K. Gadad, I.M. Khazi, C.S. Mahajanshetti, J. Heterocycl. Chem. **2**, 125–128 (1992)
14. M.A. Daly, C.P. Hopkinson, G.D. Meakins, A.J. Raybould, J. Chem. Soc. **1**, 855–860 (1991)
15. N.S. El-Sayed, E.R. El-Bendary, S.M.El-Ashry, M.M. El-Kerdawy, Eur. J. Med. Chem. **46**, 3714–3720 (2011)
16. A. Christian, G.N. Montalbetti, F. Virginie, Tetrahedron **61**, 10827–10852 (2005)
17. M. Singh, P.A. Narshinha, Synthesis **44**, 3797–3804 (2012)
18. A. Adrianus, C.H. Vanden, N.P. Daniele, L. Heitman, A. Goblyos, P. Adriaan, I.J. Zerman, J. Med. Chem. **47**, 663–672 (2004)
19. S. Sarveswari, V. Vijayakumar, Arab. J. Chem. **9**, S35–S40 (2016)
20. V. Struki, B. Bartolec, T. Portada, I. Dilovic, I. Halasz, D. Margetic, Chem. Commun. **48**, 12100–12102 (2012)
21. V. Duraipandiyan, S. Ignacimuthu, J. Ethnopharmacol. **123**, 494–498 (2009)
22. T. Mahalakshmi, P. Lavanya, K.M. Kumar, V. Vijayakumar, S. Sarveswari, A. Anand, R. Sudha, J. Biomol. Struct. Dyn. **33**, 961–977 (2015)
23. A. Andrenani, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, G. Lenaz, R. Fato, C. Bergamini, G. Farruggia, J. Med. Chem. **48**, 3085–3089 (2005)
24. A. Dorn, S.R. Vippagunta, H. Matile, C. Jaquet, L.J. Vennerstrom, G.R. Ridley. Biochem. Pharmacol. **55**, 727–736 (1998)