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Research Article

Regioselective Synthesis of Dispiro Pyrrolizidines as Potent Antimicrobial Agents for Human Pathogens

N. Sirisha and R. Raghunathan

Department of Organic Chemistry, University of Madras, Guindy Campus, Chennai 600 025, India

Correspondence should be addressed to R. Raghunathan; ragharaghunathan@yahoo.com

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Synthesis of a series of novel dispiro pyrrolizidines has been accomplished by 1,3-dipolar cycloaddition reaction of azomethine ylide generated from secondary amino acids and diketones with bischalcones. These compounds were evaluated for their antibacterial activity. Most of the synthetic compounds exhibited good antibacterial activity against microorganisms.

1. Introduction

1,3-dipolar cycloaddition is one of the important tools for the construction of five membered heterocycles [1], and many important natural products have been synthesized by this method [2, 3]. Pyrrolidine-based natural products are very useful in preventing and treating rheumatoid arthritis, asthma, and allergies and also possess anti-influenza virus and anticonvulsant activities [4]. The azomethine ylide represents one of the most reactive and versatile classes of 1,3-dipoles and is readily trapped by a range of dipolarophiles forming substituted pyrrolidines [5]. Spiro compounds represent an important class of naturally occurring substances characterized by highly pronounced biological properties [6, 7].

Chalcones belong to the broad class of compounds present in almost all vascular plants, not only in their terrestrial parts but also in roots, as well as in flakes and seeds. Chalcones are precursors of flavonoids and play a crucial role in their biosynthesis [8, 9]. Depending on the substitution pattern on the two aromatic rings, a wide range of pharmacological activities has been identified for various chalcones [10, 11]. These include among others cytotoxic, antiprotozoal [12], antibacterial, antifungal [13], and antitumor activities [14, 15]. More recently, there has been strong interest in the antimalarial activity of chalcones and bischalcones [16].

As a part of the ongoing research program on the synthesis of complex novel spiro heterocycles [17], herein we report for the first time an expeditious protocol for the synthesis of novel dispiro pyrrolizidines through 1,3-dipolar cycloaddition reaction of azomethine ylide generated from various diketones and secondary amino acids with bischalcones derived from terephthalaldehyde and substituted acetophenones as dipolarophiles. The synthesized compounds were screened for antimicrobial activity, and the results are presented in this paper.

2. Materials and Methods

*2.1. General Procedure for the Synthesis of Bischalcones **3a–c**.* A solution of substituted acetophenone (2 equiv) and terephthalaldehyde (1 equiv) in methanolic solution of NaOH (60%) was stirred for 20 h at room temperature. The solution was poured into ice-cold water at pH 2 (pH adjusted by HCl). The solid separated was dissolved in CH_2Cl_2, washed with saturated solution of $NaHCO_3$, and evaporated to dryness. The residue was purified by column chromatography using hexane, ethyl acetate mixture (9 : 1) as eluent. The compound was recrystallized in chloroform.

*(2E,2′E)-3,3′-(1,4-Phenylene)bis(1-phenylprop-2-en-1-one) (**3a**).* Pale yellow solid, yield 81%; m.p. 158°C; IR, 1653, 1610, 1541. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.18 (s, 6H), 6.83 (d, J = 16 Hz, 2H), 7.24–7.51 (m, 8H), 7.75 (d, 4H), 8.02 (d, J = 16 Hz, 2H); ^{13}C NMR (75 MHz); ppm 122.51, 128.08, 129.32, 131.14, 136.78,

138.45, 142.82, 192.37. MS (EI); m/z = 339.1. Anal. Calc. for $C_{24}H_{18}O_2$: C, 85.17; H. 5.3; found: C. 85.5; H 5.73.

*(2E,2′E)-3,3′-(1,4-Phenylene)bis(1-p-tolylprop-2-en-1-one) (**3b**).* Pale yellow solid, yield 75%; m.p. 178°C; IR, 1653, 1610, 1541. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.9 (d, J = 15 Hz, 2H), 7.25–7.49 (m, 10H), 7.6 (d, 4H), 7.92 (d, J = 15 Hz, 2H); ^{13}C NMR (75 MHz); ppm 31.22, 123.02, 128.51, 128.68, 132.94, 136.86, 138.05, 143.53, 194.4. MS (EI); m/z = 367.4. $C_{26}H_{22}O_2$: C, 85.22; H, 6.05; found: C. 85.41; H 5.94.

*(2E,2′E)-3,3′-(1,4-phenylene)bis(1-(2-hydroxyphenyl)prop-2-en-1-one) (**3c**).* Pale yellow solid, yield 83%; m.p. 190°C; IR, 1653, 1610, 1541. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.72 (d, J = 14 Hz, 2H), 7.15–7.32 (m, 8H), 7.5 (d, 4H), 7.89 (d, J = 14 Hz, 2H), 11.68 (s, 2H); ^{13}C NMR (75 MHz); ppm 124.21, 127.34, 128.61, 129.52, 133–91, 138.11, 139.17, 144.92, 196.35. MS (EI); m/z = 371.4. Anal. Calc. for $C_{24}H_{18}O_4$: C, 77.82; H, 4.90; found: C. 77.95; H 5.01.

*2.2. General Procedure for Synthesis of Cycloadducts, **6a–c** and **8a–c**.* To a solution of acenaphthenequinone **5**/isatin **7** (2 eq), proline **4** (3 eq) in dry toluene (15 mL) bischalcones **3a–c** (1eq) was added. The solution was refluxed until the completion of the reaction (5–7 h) as evidenced by TLC analysis. The solvent was removed in vacuo, and the crude product was subjected to column chromatography on silica gel (100–200 mesh) using petroleum ether/ethyl acetate (8 : 2) as eluent.

*(1R,1′R,2′R,7a′S)-2′-Benzoyl-1′-(4-((1S,1′S,2′S,7a′R)-2′-benzoyl-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizin]-2-one (**6a**).* Yellow solid, yield: 66%. m.p: 172°C. IR (KBr): 1724, 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.73–1.92 (m, 4H), 1.96 (m, 2H), 2.38 (m, 2H), 2.63 (m, 2H), 3.93 (m, 2H), 4.24 (m, 2H), 4.94 (d, 2H J = 11.4 Hz), 5.25 (m, 2H), 6.73–7.83 (m, 26H). ^{13}C NMR (75 MHz); ppm 26.87, 30.39, 48.80, 53.09, 64.52, 72.13, 77.13. 121.73, 124.32, 125.12, 127.28, 127.39, 127.71, 127.87, 128.16, 128.52, 128.67, 130.30, 131.49, 132.19, 134.88, 137.07, 138.62, 141.75, 198.33, 206.41. MS (EI); m/z = 809.5 (M^+). Anal. Calcd for: $C_{56}H_{44}N_2O_4$: C, 83.14; H, 5.48; N, 3.46; found; C, 83.23; H, 5.43; N, 3.50.

*(1R,1′R,2′R,7a′S)-2′-(4-Methylbenzoyl)-1′-(4-((1S,1′S,2′S,7a′R)-2′-(4-methylbenzoyl)-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′,7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizin]-2-one (**6b**).* Yellow solid, yield: 69%. m.p: 158°C. IR (KBr): 1737, 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.72–1.79 (m, 4H), 1.82–1.88 (m, 2H), 1.96 (s, 6H), 2.38 (m, 2H), 2.65 (m, 2H), 3.94 (m, 2H), 4.19–4.26 (m, 2H), 4.91 (d, 2H J = 11.10 Hz), 5.22 (m, 2H), 6.56–7.84 (m, 24H). ^{13}C NMR (75 MHz); ppm 20.20, 25.77, 29.27, 47.80, 52.19, 63.18, 71.02, 76.44, 120.68, 123.35, 124.02, 126.56, 126.85, 127.41, 127.45, 129.32, 130.43, 130.51, 133.63, 133.95, 137.60, 140.79, 141.90, 196.86, 205.33. MS (EI); m/z = 837.3 (M^+). Anal. Calcd for: $C_{58}H_{48}N_2O_4$: C, 83.23; H, 5.78; N, 3.35; found; C, 83.34; H, 5.69; N, 3.29.

*(1R,1′R,2′R,7a′S)-2′-(2-Hydroxybenzoyl)-1′-(4-((1S,1′S,2′S,7a′R)-2′-(2-hydroxybenzoyl)-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′,7′,7a′-hexahydro-2H-spiro[acenaphthylene-1,3′-pyrrolizin]-2-one (**6c**).* Yellow solid, yield: 71%. m.p: 134°C. IR (KBr): 3038, 1727, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.73–1.91 (m, 4H), 1.94–2.01 (m, 2H), 2.27 (m, 2H), 2.54 (m, 2H), 3.79 (m, 2H), 4.08 (m, 2H), 4.82 (d, J = 11.4 Hz, 2H), 5.19 (m, 2H), 6.59–7.83 (m, 24H). 12.17 (s, 2H). ^{13}C NMR (75 MHz); ppm 26.09, 29.38, 48.10, 53.37, 63.98, 71.69, 76.22, 119.92, 120.34, 121.79, 121.98, 122.37, 123.78, 125.47, 126.76, 128.98, 129.15, 131.18, 132.75, 136.20, 140.81, 141.82, 197.18, 205.43 MS (EI); m/z = 841.2 (M^+). Anal. Calcd for: $C_{56}H_{44}N_2O_6$: C, 79.98; H, 5.27; N, 3.33; O, 11.42; found; C, 80.09; H, 5.31; N, 3.24.

*(2′R,3R)-2′-Benzoyl-1′-(4-((2′S,3S)-2′-benzoyl-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizin]-2-one (**8a**).* Yellow solid, yield: 61%. m.p: 152°C. IR (KBr): 1712, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.49–1.57 (m, 4H), 1.70–1.77 (m, 2H), 1.89 (m, 2H), 2.01 (m, 2H), 2.19 (m, 2H), 2.54 (m, 2H), 3.55 (d, J = 11.10 Hz, 2H), 4.18 (m, 2H), 6.41–7.66 (m, 22H), 8.10 (s, 2H). ^{13}C NMR (75 MHz); ppm 25.12, 26.39, 31.41, 42.03, 52.75, 62.19, 71.17, 120.93, 122.76, 125.53, 126.92, 127.52, 128.81, 129.08, 129.93, 131.61, 136.90, 140.08, 141.17, 181.33, 199.81. MS (EI); m/z = 739.2 (M^+). Anal. Calcd for: $C_{48}H_{42}N_2O_4$: C, 78.03; H, 5.73; N, 7.58; found; C, 78.12; H, 5.81; N, 7.42.

*(2′R,3R)-2′-(4-Methylbenzoyl)-1′-(4-((2′S,3S)-2′-(4-methylbenzoyl)-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′,7′,7a′-hexa hydrospiro[indoline-3,3′-pyrrolizin]-2-one (**8b**).* Yellow solid, yield: 64%. m.p: 192°C. IR (KBr): 1712, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.65–1.72 (m, 4H), 1.78 (m, 2H), 1.96 (m, 2H), 2.01 (S, 6H), 2.48 (m, 2H), 3.81 (m, 2H), 4.04 (m, 2H), 4.85 (d, J = 11.10 Hz, 2H), 5.18 (m, 2H), 6.52–7.41 (m, 20H), 8.32 (s, 2H). ^{13}C NMR (75 MHz); ppm 24.12, 27.45, 30.02, 36.91, 45.52, 54.57, 62.38, 71.17, 118.37, 125.51, 126.37, 128.23, 128.91, 130.15, 130.79, 132.24, 135.47, 137.76, 179.82, 201.31. MS (EI); m/z = 767.6 (M^+). Anal. Calcd for: $C_{50}H_{46}N_4O_4$: C, 78.30; H, 6.05; N, 7.31; found; C, 78.37; H, 6.11; N, 7.27.

*(2′R,3R)-2′-(2-Hydroxybenzoyl)-1′-(4-((2′S,3S)-2′-(2-hydroxybenzoyl)-2-oxo-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizine]-1′-yl)phenyl)-1′,2′,5′,6′,7′,7a′-hexahydrospiro[indoline-3,3′-pyrrolizin]-2-one (**8c**).* Yellow solid, yield: 68%. m.p: 146°C. IR (KBr): 3052, 1739, 1619 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.67–1.78 (m, 4H), 1.78 (m, 2H), 1.86–1.92 (m, 2H), 2.22 (m, 2H), 2.53 (m, 2H), 2.95 (m, 2H), 3.82 (m, 2H), 4.49 (d, J = 11.10 Hz, 2H), 5.67 (m, 2H), 6.59–7.62 (m, 20H), 8.07 (s, 2H). 12.08 (s, 2H), ^{13}C NMR (75 MHz); ppm 26.48, 29.44, 37.48, 49.97, 54.35, 67.72, 70.97, 118.38, 120.07, 121.37, 121.84, 122.58, 123.39, 125.17, 127.15, 128.81, 130.07, 131.18, 133.83, 136.62, 136.91, 178.82, 202.11. MS (EI); m/z = 771.4 (M^+). Anal. Calcd for: $C_{48}H_{42}N_4O_6$: C, 74.79; H, 5.49; N, 7.27; found; C, 74.85; H, 5.41; N, 7.22.

*2.3. General Procedure for Synthesis of Cycloadducts, **10a–c** and **11a–c**.* To a solution of acenaphthenequinone **5**/isatin **7** (2 eq), pipecolinic acid **9** (3 eq) in dry toluene (15 mL) bischalcones **3a–c** (1eq) was added. The solution was refluxed until the completion of the reaction (6–8 h) as evidenced by TLC analysis. The solvent was removed in vacuo, and the crude product was subjected to column chromatography on silica gel (100–200 mesh) using petroleum ether/ethyl acetate (8 : 2) as eluent.

*(1′R,2′R,8a′S)-2′-Benzoyl-1′-(4-((1S,1′S,2′S,8a′R)-2′-benzoyl-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizin]-2-one (**10a**).* Yellow solid, yield: 72%. m.p: 138°C. IR (KBr): 1721, 1658 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.19–1.31 (m, 5H), 1.35–1.42 (m, 2H), 1.78 (m, 3H), 2.22 (m, 2H), 2.36 (m, 2H), 3.68 (m, 2H), 4.03 (m, 2H), 4.45 (d, J = 9.6 Hz, 2H), 4.77 (m, 2H), 6.96–8.27 (m, 26H). ^{13}C NMR (75 MHz); ppm 23.74, 25.74, 30.66, 45.86, 52.00, 61.49, 66.07, 76.11, 120.50, 121.67, 122.08, 123.43, 124.56, 127.11, 127.39, 127.67, 128.50, 128.61, 128.77, 129.37, 131.68, 131.82, 132.02, 132.64, 132.69, 133.54, 136.85, 137.64, 138.33, 142.40, 143.81, 144.79, 198.00, 210.04. MS (EI); m/z = 837.9 (M^+). Anal. Calcd for: $C_{58}H_{48}N_2O_4$: C, 83.23; H, 5.78; N, 3.35; found; C, 83.34; H, 5.67; N, 3.28.

*(1′R,2′R,8a′S)-2′-(4-Methylbenzoyl)-1′-(4-((1S,1′S,2′S,8a′R)-2′-(4-methylbenzoyl)-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizin]-2-one (**10b**).* Yellow solid, yield: 65%. m.p: 120°C. IR (KBr): 1739, 1657 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.25–1.51 (m, 5H), 1.73 (m, 3H), 1.85 (m, 2H), 1.97 (s, 6H), 2.25 (m, 2H), 2.42 (m, 2H), 3.67 (m, 2H), 4.12 (m, 2H), 4.43 (d, J = 9.3 Hz, 2H), 4.52 (m, 2H), 6.53–8.28 (m, 24H). ^{13}C NMR (75 MHz); ppm 21.68, 23.75, 25.74, 29.67, 45.88, 52.11, 61.30, 66.07, 77.50, 120.47, 122.08, 123.48, 124.50, 126.53, 127.47, 128.12, 128.66, 129.32, 132.67, 134.49, 137.66, 142.85, 143.56, 197.62, 205.61. MS (EI); m/z = 865.6 (M^+). Anal. Calcd for: $C_{60}H_{52}N_2O_4$: C, 83.30; H, 6.06; N, 3.24; found; C, 83.37; H, 6.11; N, 3.16.

*(1′R,2′R,8a′S)-2′-(2-Hydroxybenzoyl)-1′-(4-((1S,1′S,2′S,8a′R)-2′-(2-hydroxybenzoyl)-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H,2H-spiro[acenaphthylene-1,3′-indolizin]-2-one (**10c**).* Yellow solid, yield: 70%. m.p: 134°C. IR (KBr): 3021, 1729, 1662 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.25–1.47 (m, 5H), 1.70 (m, 2H), 1.79 (m, 3H), 2.20 (m, 2H), 2.34 (m, 2H), 3.65 (m, 2H), 3.99 (m, 2H), 4.43 (d, J = 9.0 Hz, 2H), 4.58 (m, 2H), 5.92–8.29 (m, 24H), 11.85 (s, 2H). ^{13}C NMR (75 MHz); ppm 23.70, 29.36, 29.70, 46.00, 51.39, 60.63, 66.42, 77.25, 117.55, 118.85, 122.08, 124.62, 127.74, 128.58, 129.76, 131.01, 131.98, 132.66, 133.44, 135.35, 145.85, 193.32, 206.12. MS (EI); m/z = 869.4 (M^+). Anal. Calcd for: $C_{58}H_{48}N_2O_6$: C, 80.16; H, 5.57; N, 3.22; found; C, 80.27; H, 5.49; N, 3.19.

*(1′R,2′R,3R,8a′S)-2′-Benzoyl-1′-(4-((1′S,2′S,3S,8a′R)-2′-benzoyl-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizin]-2-one (**11a**).* Yellow solid, yield: 65%. m.p: 198°C. IR (KBr): 1718, 1639 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.18–1.36 (m, 4H), 1.42 (m, 3H), 1.81 (m, 4H), 2.26 (m, 2H), 2.34 (m, 2H), 3.37 (m, 2H), 3.75 (m, 2H), 4.33 (d, J = 9.9 Hz, 2H), 4.57 (m, 2H), 6.75–7.86 (m, 22H), 8.42 (s, 2H). ^{13}C NMR (75 MHz); ppm 24.48, 26.38, 30.07, 45.48, 51.97, 59.37, 65.78, 71.37, 120.39, 121.44, 124.39, 125.78, 127.01, 127.67, 128.18, 128.97, 129.58, 132.78, 133.86, 137.15, 139.39, 141.15, 178.93, 197.39. MS (EI); m/z = 767.4 (M^+). Anal. Calcd for: $C_{50}H_{46}N_4O_4$: C, 78.30; H, 6.05; N, 7.31; found; C, 78.43; H, 6.01; N, 7.26.

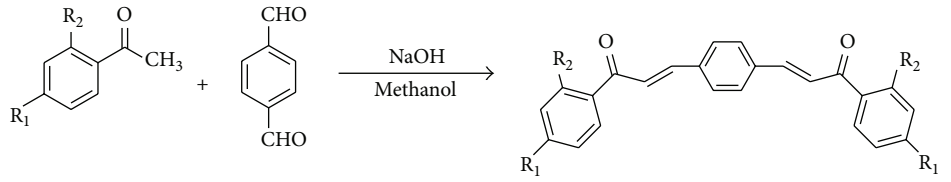
*(1′R,2′R,3R,8a′S)-2′-(4-Methylbenzoyl)-1′-(4-((1′S,2′S,3S,8a′R)-2′-(4-methylbenzoyl)-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizin]-2-one (**11b**).* Yellow solid, yield: 67%. m.p: 194°C. IR (KBr): 1721, 1646 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.18–1.38 (m, 3H), 1.51 (m, 3H), 1.88 (m, 2H), 1.92 (s, 6H), 2.09 (m, 2H), 2.32 (m, 2H), 3.22 (m, 2H), 3.61 (m, 2H), 4.09 (m, 2H), 4.40 (d, J = 9.9 Hz, 2H), 4.59 (m, 2H), 6.39–7.85 (m, 20H), 8.30 (s, 2H). ^{13}C NMR (75 MHz); ppm 22.63, 25.90, 29.47, 30.91, 44.61, 50.44, 59.69, 64.33, 71.49, 107.77, 121.74, 123.36, 125.76, 126.70, 127.11, 127.11, 127.65, 128.34, 129.91, 133.96, 137.65, 139.65, 142.11, 179.86, 196.12. MS (EI); m/z = 795.5 (M^+). Anal. Calcd for: $C_{52}H_{50}N_4O_4$: C, 78.56; H, 6.34; N, 7.05; found; C, 78.62; H, 6.28; N, 7.13.

*(1′R,2′R,3R,8a′S)-2′-(2-Hydroxybenzoyl)-1′-(4-((1′S,2′S,3S,8a′R)-2′-(2-hydroxybenzoyl)-2-oxo-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizine]-1′-yl)phenyl)-2′,5′,6′,7′,8′,8a′-hexahydro-1′H-spiro[indoline-3,3′-indolizin]-2-one (**11c**).* Yellow solid, yield: 66%. m.p: 186°C. IR (KBr): 3037, 1718, 1651 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.27–1.42 (m, 4H), 1.56 (m, 3H), 1.98 (m, 3H), 2.39 (m, 2H), 2.48 (m, 2H), 3.57 (m, 2H), 3.67 (m, 2H), 4.45 (d, J = 9.6 Hz, 2H), 4.76 (m, 2H), 6.57–7.89 (m, 20H), 8.39 (s, 2H), 11.91 (s, 2H). ^{13}C NMR (75 MHz); ppm 23.60, 25.50, 30.52, 45.70, 51.36, 60.26, 65.19, 73.04, 118.62, 119.20, 119.79, 120.19, 123.13, 126.17, 127.30, 128.49, 129.24, 133.48, 136.42, 143.55, 144.82, 180.43, 202.91. MS (EI); m/z = 799.6 (M^+). Anal. Calcd for: $C_{50}H_{46}N_4O_6$: C, 75.17; H, 5.80; N, 7.01; found; C, 75.24; H, 5.78; N, 7.08.

3. Materials and Methods for Antimicrobial Studies

We have carried out bioactivity studies for the synthesized compounds against six microorganisms, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris*, and *Proteus mirabilis* which were obtained from the Microbial Metabolite Lab Culture collection, University of Madras, Chennai.

The agar diffusion method was used for the determination of antibacterial activity of dispiropyrrolizidines against microorganism listed above. About 9 mL of nutrient agar media were poured into petri plates (9 cm in diameter) and inoculated with respective test organism. Wells are made with

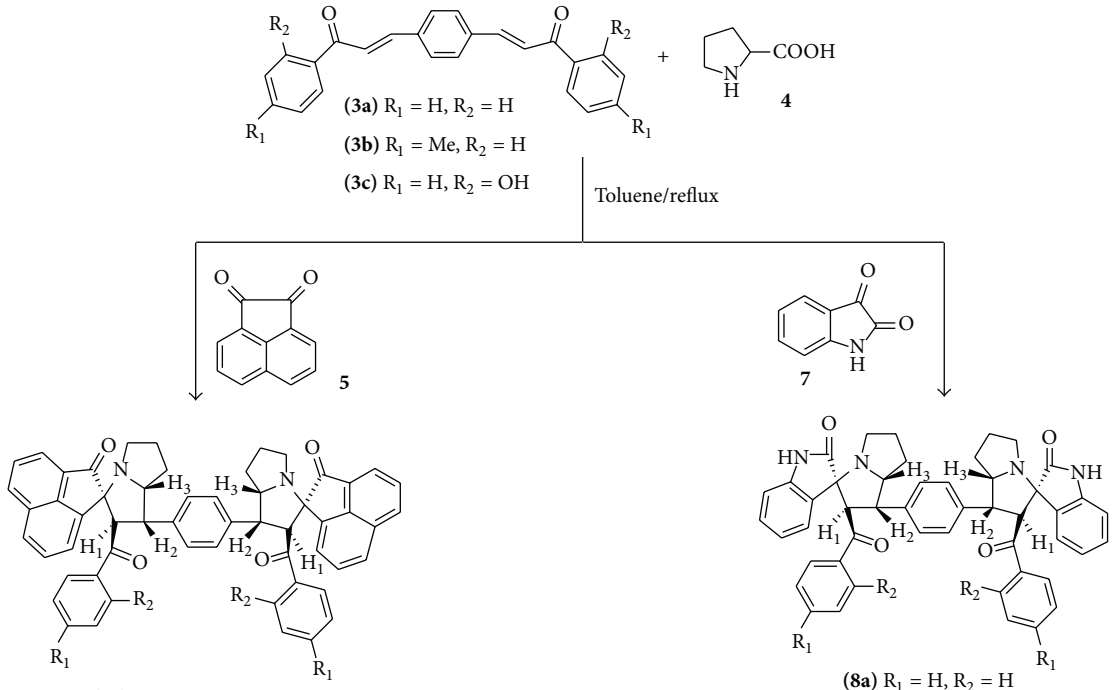


(1a) R_1 = H, R_2 = H
(1b) R_1 = Me, R_2 = H
(1c) R_1 = H, R_2 = OH

2

(3a) R_1 = H, R_2 = H
(3b) R_1 = Me, R_2 = H
(3c) R_1 = H, R_2 = OH

Scheme 1



(3a) R_1 = H, R_2 = H
(3b) R_1 = Me, R_2 = H
(3c) R_1 = H, R_2 = OH

4

5

7

(6a) R_1 = H, R_2 = H
(6b) R_1 = Me, R_2 = H
(6c) R_1 = H, R_2 = OH

(8a) R_1 = H, R_2 = H
(8b) R_1 = Me, R_2 = H
(8c) R_1 = H, R_2 = OH

Scheme 2

cork borer on the solid agar and loaded with 20–60 μg/mL of the test compound with tetracycline as control. Petri dishes were incubated at 37°C for 24 h, and the average diameter of the inhibition zone surrounding the wells was measured after specified incubation period.

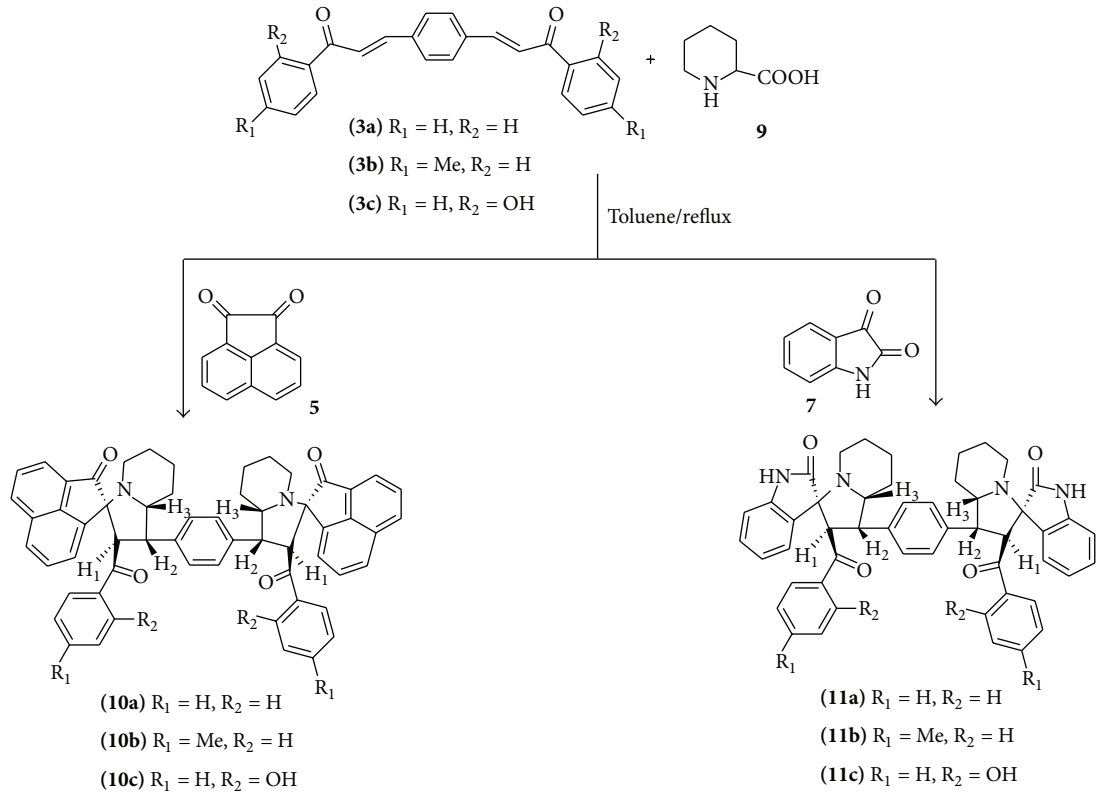
4. Results and Discussion

4.1. Chemistry. The bischalcones used as dipolarophiles for the present study were prepared according to the Claisen-Schmidt reaction, by reacting various substituted acetophenones **1a–c** with terephthalaldehyde **2** and NaOH (60%) as a base to give pale yellow products **3a–c** (80–86%) (Scheme 1).

The products **3a–c** were assigned the (*E*)-configuration based on the chemical shift value of olefinic protons in accordance with the literature data [18–22].

We have explored the reactivity of bischalcone derivatives as efficient dipolarophiles for the synthesis of a rare class of dispiroheterocycles. The azomethine ylide generated *in situ* through decarboxylative condensation reaction from L-proline **4** and acenaphthenequinone **5**/isatin **7** reacted with bischalcone derivatives **3a–c** as dipolarophiles in refluxing toluene under the Dean-Stark reaction condition to afford a series of novel dispiro pyrrolizidines **6a–c** and **8a–c** (Scheme 2).

The structures of these products were also confirmed by ^{1}H NMR, ^{13}C NMR, DEPT, COSY, NOESY, and mass spectral analysis. The ^{1}H NMR spectrum of **6b** showed a multiplet at δ 5.22 for benzylic H_2 proton. The benzoyl proton H_1 resonated as a doublet at δ 4.91 (J = 11.10 Hz), and H_3 proton appeared as a multiplet at δ 4.19–4.26. The stereochemistry of the cycloadduct **6b** was deduced on the basis of 2D NOESY experiments.



Scheme 3

The strong NOESY between H_2 and H_3 shows *cis* stereochemistry, and when there is no NOESY between H_1 and H_2 of **6b** shows *trans* stereochemistry. The ^{13}C NMR spectrum of **6b** showed a signal at 76.44 ppm for the spiro carbon. The peaks at 52.19 ppm and 63.18 ppm correspond to the benzylic –CH– carbon and *N*–CH– carbon of **10b** and were confirmed by DEPT 135 spectrum. Acenaphthenequinone carbonyl carbon appeared at 205.33 ppm, and the benzoyl carbon resonated at 196.86 ppm. Moreover, the cycloadduct **6b** exhibited a peak at m/z = 837.3 (M^+) in the mass spectrum.

With a view to explore the potential of the cycloaddition reaction of 1,3-dipole for the synthesis of spiroheterocycles, we have carried out the reaction of the azomethine ylide generated from pipecolinic acid **9** and acenaphthenequinone **5**/isatin **7** with bischalcones as dipolarophiles to give dispiro pyrrolizidines **10a–c** and **11a–c** (Scheme 3).

The IR spectrum of the cycloadduct **11c** showed peaks at 1651 and 1718 cm^{-1} which correspond to the amide and benzoyl carbonyl groups, respectively. The ^{1}H NMR spectrum of **11c** showed a multiplet at δ 4.76 for benzylic H_2 proton. The benzoyl proton H_1 resonated as a doublet at δ 4.45 (J = 9.6 Hz), and H_3 proton appeared as a multiplet at δ 3.95–4.01. The stereochemistry of the cycloadduct **11c** was deduced on the basis of 2D NOESY experiments. The strong NOESY between H_2 and H_3 shows *cis* stereochemistry, and when there is no NOESY between H_1 and H_2 of **11c** shows *trans* stereochemistry. The –NH proton of the oxindole moiety appeared as a singlet at δ 8.39, and the –OH peak appeared as a singlet at δ 11.91. The ^{13}C NMR spectrum of **11c** showed a signal at 73.04 ppm for the spiro carbon. The *N*–CH carbon of **11c** resonated at 60.26 ppm and was confirmed by DEPT 135 spectrum. The peaks at 202.91 and 180.43 ppm correspond to the benzoyl and amide carbonyl groups. Moreover, the cycloadduct **11c** exhibited a peak at m/z = 799.6 (M^+) in the mass spectrum.

5. Biology

5.1. Antimicrobial Activity of Dispiropyrrolizidines (Agar Diffusion Assay). The agar diffusion method [23] was used for the determination of antibacterial activity of dispiropyrrolizidines against microorganism listed above. The results of the antimicrobial screening of the twelve compounds have been listed in Tables 1, 2, 3, 4, 5, and 6.

It was observed that the synthetic compounds **6c**, **8b**, **8c**, **10c**, and **11c** exhibited good antimicrobial activity and inhibition zones against the pathogen *Proteus mirabilis* while **6a**, **6b**, **8a**, **10a**, **11a**, and **11b** showed moderate activity. All other derivatives did not show appreciable activity at low concentration. For *P. vulgaris* the compounds **11c**, **10c**, and **8c** showed very good antibacterial activity which is comparable to that of reference compound tetracycline, and **6a**, **6b**, **6c**, **8a**, **8b**, **11a**, and **11b** showed moderate inhibitory activity against this pathogen. The compounds **11c** and **8c** showed very good antimicrobial activity against the pathogen *Salmonella typhi* while **6c**, **10c**, **8b**, **8a**, **6a**, **11b**, and **11a** showed moderate activity. The compounds **8c**, **11c**, **10c**, and **8b**

Table 1: Effect of dispiro pyrrolizidines on the growth of human pathogen *Proteus mirabilis*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Proteus mirabilis	**6a**	—	14	19
	6b	—	13	18
	6c	—	17	21
	8a	—	10	18
	8b	—	15	23
	8c	11	19	22
	10a	—	10	15
	10b	—	—	9
	10c	—	18	22
	11a	—	11	18
	11b	—	14	19
	11c	12	18	23
	Tetracycline	16	20	24

Table 2: Effect of dispiro pyrrolizidines on the growth of human pathogen *Proteus vulgaris*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Proteus vulgaris	**6a**	—	12	18
	6b	—	13	19
	6c	—	15	21
	8a	—	14	20
	8b	—	13	21
	8c	10	18	23
	10a	—	9	13
	10b	—	—	7
	10c	—	19	23
	11a	—	10	17
	11b	9	14	19
	11c	12	19	23
	Tetracycline	18	22	25

Table 3: Effect of dispiro pyrrolizidines on the growth of human pathogen *Salmonella typhi*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Salmonella typhi	**6a**	—	11	17
	6b	—	9	11
	6c	—	15	21
	8a	—	13	18
	8b	—	12	19
	8c	11	19	25
	10a	—	9	12
	10b	—	8	12
	10c	—	16	21
	11a	—	11	18
	11b	—	14	19
	11c	12	20	26
	Tetracycline	17	21	26

Table 4: Effect of dispiro pyrrolizidines on the growth of human pathogen *Staphylococcus aureus*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Staphylococcus aureus	**6a**	—	15	19
	6b	—	9	11
	6c	—	17	21
	8a	—	10	18
	8b	—	15	22
	8c	9	17	24
	10a	—	10	15
	10b	—	—	9
	10c	—	18	23
	11a	—	11	18
	11b	—	14	19
	11c	10	19	23
	Tetracycline	18	21	24

exhibited good antibacterial activity, while **6c**, **6a**, **8a**, **11a**, and **11b** showed moderate activity against the pathogen *Staphylococcus aureus*. All other compounds showed low activity. The inhibitory activity of the compounds **10c**, **11c**, **8b**, **8c**, and **6c** against the bacteria *Bacillus subtilis* was observed to be good while **6a**, **8a**, **11a**, and **11b** showed moderate activity against this pathogen. The compounds **11c**, **10c**, **8c**, **6c**, and **8a** showed good antimicrobial activity against the pathogen *Escherichia coli* while **6a**, **8b**, **11a**, and **11b** showed moderate activity, and all other compounds showed low activity.

Some of the oxindole derived compounds and acenaphthenequinone derived compounds showed good activity against all the pathogens in low concentration, which is comparable to the reference control. It was observed that all the antibacterial activities of the presently studied compounds are dose dependent. The oxindole derived compounds **11c** and **8c** showed good activity against all the test pathogens. The activity was very much comparable to the reference drug. The acenaphthenequinone derived compounds **6c** and **10c** showed good activity against all the test pathogens. Hydroxy substituted dispiropyrrolizidines **11c**, **10c**, **8c**, and **6c** showed very good antibacterial activity against all the pathogens studied. Further clinical studies are required to validate

Table 5: Effect of dispiro pyrrolizidines on the growth of human pathogen *Bacillus subtilis*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Bacillus subtilis	**6a**	—	12	18
	6b	—	9	14
	6c	—	15	21
	8a	—	10	18
	8b	—	15	22
	8c	9	17	21
	10a	—	8	14
	10b	—	—	13
	10c	—	18	23
	11a	—	11	18
	11b	—	14	19
	11c	9	19	22
	Tetracycline	14	19	21

Table 6: Effect of dispiro pyrrolizidines on the growth of human pathogen *Escherichia coli*.

Organism	Compound	Concentration of compound (μg/mL)		
		20	40	60
		Zone of inhibition (mm)		
Escherichia coli	**6a**	—	11	18
	6b	—	7	15
	6c	—	15	21
	8a	—	15	22
	8b	—	10	18
	8c	9	18	22
	10a	—	10	15
	10b	—	—	9
	10c	—	19	23
	11a	—	14	19
	11b	—	11	17
	11c	11	19	23
	Tetracycline	16	20	23

the compounds of the present study as antimicrobial agents. The results are summarized in Tables 1, 2, 3, 4, 5, and 6.

6. Conclusion

In conclusion, we have achieved the synthesis of a variety of novel dispiro pyrrolizidines through 1,3-dipolar cycloaddition reaction and evaluated their structure by using different spectroscopic techniques. Most of the synthesized compounds showed good antimicrobial activity against all the selected human pathogens. In particular, cycloadducts, **11c**, **8c**, **10c**, and **6c**, exhibited the best antibacterial activity against all the bacterial pathogens.

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