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Synthesis of novel spiropyrrolizidines as potent antimicrobial agents for human and plant pathogens

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Abstract—A series of novel dispirooxindolopyrrolizidine derivatives have been synthesized through 1,3-dipolar cycloaddition reaction of azomethine ylide generated from proline and isatin with the dipolarophile (*E*)-2-arylidine-1-keto carbazoles. The synthesized cycloadducts were evaluated for antimicrobial activities. Compounds **7d** and **7e** showed relatively good antibacterial and antifungal activities.

Functionalized pyrrolidine, pyrrolizidine and oxindole alkaloids constitute a class of compounds with significant biological activity.[1] Spiro compounds represent an important class of naturally occurring substances characterized by highly pronounced biological properties.[2] The spirooxindole ring system forms the core structure of many pharmacological agents and alkaloids. For example, spirotryprostatin A (Fig. 1), a natural product isolated from the fermentation broth of *Aspergillus funigatus*, has been identified as a novel inhibitor of microtubule assembly.[3] Spirooxindoles have been reported to behave as aldose reductase, poliovirus and rhinovirus 3C-proteinase inhibitors.[4] Moreover, the derivatives of carbazoles show well-known pharmacological activities.[5] It was found to possess antileukemic[6] and anti-HIV activities.[7]

The chemistry of azomethine ylides has gained significance in recent years as it serves as an important route for the construction of nitrogen containing five-membered heterocycles.[8] The 1,3-dipolar cycloaddition of azomethine ylides with olefinic and acetylenic dipolarophiles offers an excellent route for the construction pyrrolizidines.[9] Although there are reports available for the synthesis of substituted pyrrolizidines, there seems to

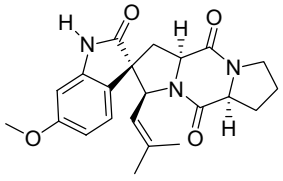

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Figure 1. Spirotryprostatin A.

be no reports to the best of our knowledge for the synthesis of a rare class of dispirooxindolopyrrolizidines having ketocarbazole skeleton. Because of their remarkable biological activities, significant efforts have been devoted to the synthesis of dispiropyrrolizidines.

An effort to synthesize biologically important compounds we have synthesized a rare class of novel spiro heterocycles wherein oxindole, pyrrolizidine and carbazole rings are fused together. The synthesis was accomplished by 1,3-dipolar cycloaddition reaction of azomethine ylide with arylidine-1-keto carbazoles as dipolarophiles. The required dipolarophiles (*E*)-2-arylidine-1-keto carbazoles were prepared by the reaction of 1-keto carbazole[10] with substituted benzaldehydes **4a**–**g** (Scheme 1).

The reaction of azomethine ylide (generated in situ by decarboxylative condensation of isatin and sarcosine) with the dipolarophiles **4a**–**g** using dioxane and metha-

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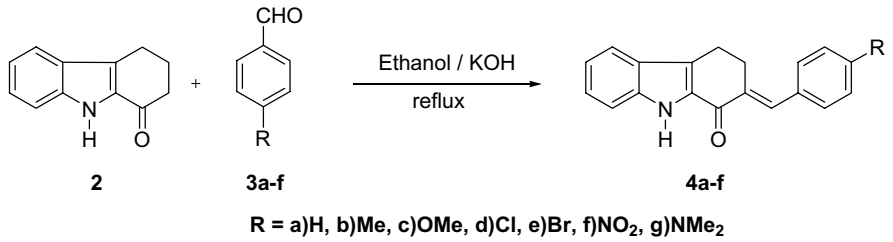


Scheme 1.

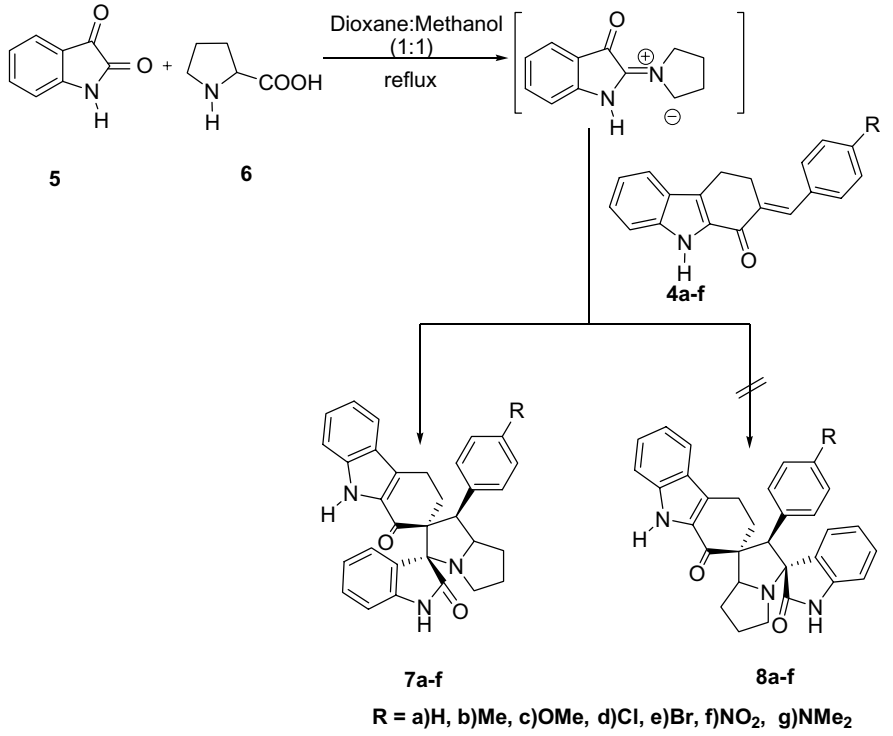
nol under reflux condition afforded dispirooxindolopyrrolizidines **7a**–**g** in good (50–68%) yields (Scheme 2).

The structure of the products was assigned on the basis of their spectral analyses.[11] In particular, the regiochemistry proposed for the product **7a** was decided on the basis of its ^{1}H NMR spectrum exhibiting a doublet at δ 4.61 for the benzylic proton. If the other isomer **8a** were formed, one would expect a singlet instead of a doublet. The ^{13}C NMR spectrum of **7b** showed two peaks at δ 64.1 and 78.2 ppm for two spirocarbons and at δ 66.2 for one methine carbon. The oxindole and keto carbazole carbonyl carbons exhibited peaks at δ 177.3 and 190.8 ppm, respectively. Identical results were obtained for compounds **7b**–**g**. The structure of **7b** was further confirmed by X-ray (Fig. 2) single crystal analysis.[12]

However, when the above reaction was carried out under solvent-free microwave irradiation (600 W) grinding with K-10 montmorillonite clay, we found that the products **7a**–**g** were obtained in better yield, 80–95% (Table 1).

In the present study, minimum inhibitory concentration[13] of seven different newly synthesized dispirooxindolopyrrolizidine compounds were evaluated against four human pathogens and two plant fungal pathogens viz. *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salamonella typhi*, by well diffusion method[14] and two plant fungal pathogens *Fusarium oxysporum*, *Macrophomena phaseolina*, by poison food technique.[15] The compounds at the concentration range 5–200 μg/ml in 0.25% DMSO was used in this study with tetracycline and carbendazim, respectively, for bacteria and fungi being used as control.

*Effect of dispirooxindolopyrrolizidines (***7a**–**g***) on the growth of human pathogens:* The MIC of the tested compounds (**7a**–**g**) against bacterial pathogen ranged from 10 to 65 μg/ml. The compound **7d** was effective in controlling all the pathogens at the lowest concentration ranging from 10 to 35 μg/ml, followed by **7c** and **7e** (15–45 and 25–45 μg/ml) as compared with control, tetracycline (10–20 μg/ml). The MIC values are relatively high for **7b**, **7f** and **7g** as compared to control (Table 2).



Scheme 2.

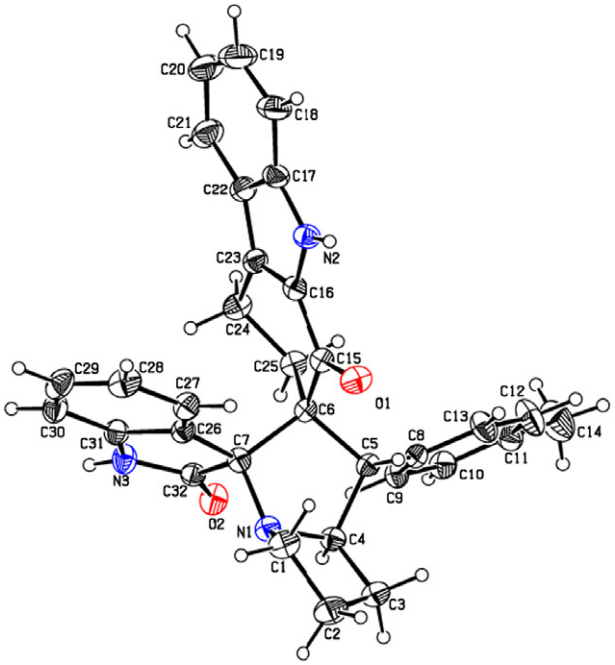


Figure 2. ORTEP diagram of **7b**.

Table 1. Synthesis of dispirooxindolopyrrolizidines (**7a**–**g**)

Entry	Compound	R	Conventional heating Yield in 6 h (%)	Microwave heating Yield in 10 min (%)
1	**7a**	H	62	95
2	**7b**	Me	50	92
3	**7c**	OMe	65	88
4	**7d**	Cl	65	94
5	**7e**	Br	68	95
6	**7f**	NO_2	60	90
7	**7g**	NMe_2	30	80

Table 2. Effect of dispirooxindolepyrrolizidines (**7a**–**g**) on the growth of human pathogens

Compound	Minimum inhibitory concentration (MIC) (μg/ml)			
	Proteus vulgaris	*Proteus mirabilis*	*Streptococcus aureus*	*Salamonella typhi*
7a	30	55	0	20
7b	40	65	45	35
7c	15	25	40	45
7d	10	35	20	30
7e	25	30	45	30
7f	35	55	25	40
7g	45	50	35	35
Tetracycline	15	20	10	15

*Effect of dispirooxindolopyrrolizidines (***7a**–**g***) on the mycelial of plant fungal pathogens:* The MIC of the tested compounds (**7a**–**g**) against plant fungal pathogens ranged from 25 to 150 μg/ml. The synthesized compound **7e** was effective in controlling both fungal pathogens namely *F. oxysporum* and *M. phaseolina* with MIC values of 50 and 25 μg/ml, respectively, followed by **7c** (75 and 25 μg/ml) and **7b** (75 and 50 μg/ml). Compounds **7a**, **7d**, **7f** and **7g** inhibit mycelial growth at higher concentration (50–150 μg/ml) compared to control, carbendazim (ranged 15 and 18 μg/ml) (Table 3).

Table 3. Effect of dispirooxindolepyrrolizidines (**7a**–**g**) on mycelial growth of plant fungal pathogens

Fungal pathogens	Minimum inhibitory concentration (MIC) compound (μg/ml)							
	7a	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	Carbendazim
Fusarium oxysporum	125	75	75	125	50	75	150	18
Macrophomena phaseolina	50	50	25	75	25	100	75	15

In conclusion, we have developed an efficient method for the synthesis of dispiropyrrolizidine derivatives by [3+2] cycloaddition methodology. Of the various conditions employed, the solvent-free solid support approach accelerated by microwave irradiation was found to be synthetically useful in achieving high yields. Though all the compounds **7a**–**g** showed antibacterial activity, the compounds **7d**, **7c** and **7e** showed pronounced activity against four human pathogens. Similarly it has been observed that **7e**, **7c** and **7b** exhibited good bioactivity against fungal pathogens.

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11. *General procedure for the synthesis of dispirooxindolopyrrolizidines:* A mixture of isatin **5** (1 mmol), proline **6** (1 mmol), and (*E*)-2-aryledene-1-keto carbazole **4a**–**g** was refluxed in dioxane and methanol (1:1). Completion of the

reaction was evidenced by TLC analysis. The solvent was removed in vacuo. The crude product was subjected to column chromatography using petroleum ether–ethyl acetate (9:1) as an eluent. The products was crystallized from hexane/ethyl acetate (1:1).

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-phenyl-pyrrolizidine spiro [2′,3″] indoline-2″-one (**7a**)*. Pale yellow solid, mp 190 °C; ν_{max} (KBr) 3281, 1702, 1643 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.48–1.51 (m, 1H); 1.80–1.82 (m, 1H); 2.50–2.78 (m, 7H); 3.00–3.02 (m, 1H); 3.17–3.20 (m, 1H); 4.61 (d, 1H, J = 11.6 Hz); 6.81–7.50 (m, 13H, ArH); 9.98 (s, 1H, *N*H); 10.16 (s, 1H, *N*H). ^{13}C (100 MHz, DMSO-d_6): δ 18.4, 20.8, 21.0, 28.3, 32.2, 53.7, 64.1, 66.2, 78.2, 110.2, 112.7, 119.5, 120.9, 121.0, 124.8, 126.4, 127.4, 128.0, 128.9, 129.0, 129.6, 129.7, 131.3, 133.9, 136.4, 138.7, 142.0, 177.3, 190.8; EI-MS *m*/*z* 473.5 (M^+). Anal. Calcd for $C_{31}H_{27}N_3O_2$: C, 78.62; H, 5.75; N, 5.76. Found: C, 78.81; H, 5.59; N, 5.81.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-methyl)phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7b**)*. Pale yellow solid, mp 195 °C; ν_{max} (KBr) 3283, 1705, 1639 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.51–1.54 (m, 1H); 1.81–1.83 (m, 1H); 2.31 (s, 3H); 2.58–2.83 (m, 7H); 3.03–3.06 (m, 1H); 3.22–3.25 (m, 1H); 4.66 (d, 1H, J = 11.6 Hz); 6.64–7.51 (m, 13H, ArH); 10.12 (s, 1H, *N*H); 10.38 (s, 1H, *N*H). ^{13}C (100 MHz, DMSO-d_6): δ 18.4, 20.9, 21.0, 24.5, 28.5, 31.63, 53.7, 64.6, 66.2, 74.7, 110.2, 119.5, 120.9, 121.0, 124.8, 124.9, 126.4, 127.5, 128.0, 128.9, 129.4, 129.6, 131.3, 133.9, 136.4, 138.7, 142.0, 177.3, 190.8; EI-MS *m*/*z* 487.5 (M^+). Anal. Calcd for $C_{32}H_{29}N_3O_2$: C, 78.82; H, 5.99; N, 8.62. Found: C, 78.75; H, 6.21; N, 8.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-methoxy) phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7c**)*. Pale yellow solid, mp 260 °C; ν_{max} (KBr) 3285, 1708, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.50–1.52 (m, 1H); 1.82–1.85 (m, 1H); 2.51–2.78 (m, 7H); 3.00–3.11 (m, 1H); 3.19 (m, 1H); 3.80 (s, 3H); 4.51 (d, 1H, J = 11.5 Hz); 6.71–7.35 (m, 12H, ArH); 9.91 (s, 1H, *N*H); 10.22 (s, 1H, *N*H). ^{13}C (100 MHz, $CDCl_3$):δ 17.8, 20.8, 21.0, 28.5, 31.6, 53.7, 58.7, 64.2, 66.2, 74.3, 111.8, 119.5, 120.6, 121.0, 124.8, 124.9, 126.4, 127.5, 128.8, 129.0, 129.6, 129.7, 130.9, 133.9, 136.4, 138.7, 142.0, 156.8, 177.3, 191.0; EI-MS *m*/*z* 503.5 (M^+). Anal. Calcd for $C_{32}H_{29}N_3O_3$: C, 76.32; H, 5.80; N, 8.34. Found: C, 76.29; H, 5.91; N, 8.4.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-chloro) phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7d**)*. Pale yellow solid, mp 216 °C; ν_{max} (KBr) 3290, 1702, 1648 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.36–1.38 (m, 1H); 1.78–1.79 (m, 1H); 2.48–2.65 (m, 7H); 3.04–3.06 (m, 1H); 3.14–3.17 (m, 1H); 4.61 (d, 1H, J = 11.6 Hz); 6.92–7.58 (m, 12H, ArH); 9.98 (s, 1H, *N*H); 10.31 (s, 1H, *N*H). ^{13}C (100 MHz, DMSO-d_6): δ 18.1, 20.8, 21.1, 28.5, 31.6, 53.7, 64.5, 68.2, 75.8, 111.2, 118.7, 120.6, 121.5, 124.6, 124.9, 126.4, 127.3, 128.1, 128.9, 129.1, 129.6, 129.7, 131.3, 135.9, 138.7, 142.1, 178.1, 192.6; EI-MS *m*/*z* 508.0 (M^+). Anal. Calcd for $C_{31}H_{26}ClN_3O_2$: C, 73.29; H, 5.16; N, 8.27. Found: C, 73.48; H, 5.29; N, 8.38.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-bromo) phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7e**)*. Pale yellow solid, mp 200 °C; ν_{max} (KBr) 3288, 1710, 1645 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.35–1.36 (m, 1H); 1.75–1.77 (m, 1H); 2.50–2.61 (m, 7H); 3.09–3.12 (m, 1H); 3.16–3.18 (m, 1H); 4.68 (d, 1H, J = 11.6 Hz); 6.96–7.61 (m, 12H, ArH); 10.2 (s, 1H, *N*H); 10.31 (s, 1H, *N*H). ^{13}C (100 MHz, $CDCl_3$): δ 18.4, 20.8, 21.1, 28.4, 31.6, 52.9, 64.5, 67.6, 79.8, 110.8, 119.5, 120.7, 121.5, 124.6, 124.9, 126.6, 127.4, 128.1, 128.3, 129.4, 129.6, 129.7, 130.7, 133.1, 138.7, 142.1, 177.3, 191.0; EI-MS *m*/*z* 552.4 (M^+). Anal. Calcd for $C_{31}H_{26}BrN_3O_2$: C, 67.40; H, 4.74; N, 7.61. Found: C, 67.72; H, 5.02; N, 7.83.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-nitro) phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7f**)*. Pale yellow solid, mp 212 °C; ν_{max} (KBr) 3293, 1706, 1651 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.57–1.60 (m, 1H); 1.85–1.87 (m, 1H); 2.56–2.78 (m, 7H); 3.11–3.13 (m, 1H); 3.30–3.31 (m, 1H); 4.97 (d, 1H, J = 11.7 Hz); 6.92–7.63 (m, 12H, ArH); 10.13 (s, 1H, *N*H); 10.61 (s, 1H, *N*H). ^{13}C (100 MHz, DMSO-d_6): δ 18.4, 20.9, 21.1, 28.4, 31.4, 53.7, 64.2, 66.2, 78.6, 110.2, 112.7, 119.6, 120.9, 121.1, 124.8,126.4, 127.4, 128.0, 128.9, 129.0, 129.4, 129.7, 131.3, 136.4, 138.7, 142.1, 178.6, 191.2; EI-MS *m*/*z* 518.5 (M^+). Anal. Calcd for $C_{31}H_{26}N_4O_4$: C, 71.80; H, 5.05; N, 10.80. Found: C, 71.68; H, 5.26; N, 10.92.

*1,2,3,4-Tetrahydro-1-ketocarbazole spiro [2,3′]-4′-(p-N,N-dimethyl) phenyl pyrrolizidine spiro [2′,3″] indoline-2″-one (**7g**)*. Pale yellow solid, mp 186–188 °C; ν_{max} (KBr) 3289, 1711, 1650 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.32–1.35 (m, 1H); 1.71–1.73 (m, 1H); 2.48–2.51 (m, 7H); 2.61 (s, 6H); 3.01–3.05 (m, 1H); 3.11–3.25 (m, 1H); 4.53 (d, 1H, J = 11.4 Hz); 6.86–7.61 (m, 12H, ArH); 9.95 (s, 1H, *N*H); 10.31 (s, 1H, *N*H). ^{13}C (100 MHz, $CDCl_3$): δ 18.40, 20.8, 21.0, 28.5, 31.6, 40.5, 53.7, 64.5, 66.2, 80.1, 110.9, 114.2, 119.6, 120.8, 121.1, 125.6, 126.8, 127.4, 127.8, 128.1, 128.9, 129.3, 130.7, 131.1, 138.4, 142.2, 143.1, 172.9, 191.5; EI-MS *m*/*z* 516.6 (M^+). Anal. Calcd for $C_{33}H_{32}N_4O_2$: C, 76.72; H, 6.24; N, 10.84. Found: C, 7.

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