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Microwave-Assisted [6 + 4]-Cycloaddition of Fulvenes and α-Pyrones to Azulene–Indoles: Facile Syntheses of Novel Antineoplastic Agents

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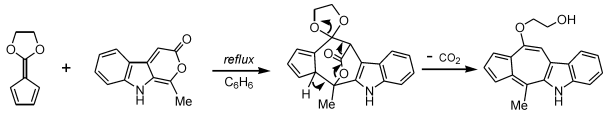
Abstract—A microwave-enhanced [6 + 4]-cycloaddition reaction between 6-aminofulvene and pyrones followed by CO_2 extrusion provides azulene–indoles which display interesting antineoplastic activity.

The widespread occurrence and interesting biological activities of indoles and azulenes in nature make them important targets for synthesis.[1] In previous studies,[2] we described a novel synthesis of azulene–indoles via a unique cycloaddition reaction of fulvene–ketene acetals and α-pyrones (Scheme 1). The formation of the azulene–indoles is envisaged to occur via a [6 + 4]-cycloaddition of fulvene with α-pyrone followed by cheleotropic extrusion of CO_2. This reaction provides a rapid construction of functionalized polycyclic azulene–indole systems with potential application to solid-phase synthesis and combinatorial libraries of these skeletons. Unfortunately, our initial results indicated that, with conventional heating, reaction times were excessive (>4 days) and product yields were low (10–25%).

Microwave-assisted organic synthesis has received much attention in recent years.[3,4] It has been demonstrated that some organic reactions proceed faster and with higher efficiency under microwave irradiation as opposed to conventional heating. The use of open focused microwave ovens[5] has added further to the evolution of this technique for accelerating thermal organic reactions. The application of microwave irradiation to the Diels–Alder reaction is also well-documented.[6] However, to the best of our knowledge, microwave-assisted higher-order cycloaddition reactions[7] (e.g., [6 + 4]-cycloaddition)[8] have not yet been explored.

In conjunction with our continuing investigations in the area of fulvene chemistry,[9] we explored the use of microwave irradiation in [6 + 4]-cycloaddition reactions between 6-dimethylaminofulvene and α-pyrones. We describe herein the application of this methodology to the synthesis of novel antineoplastic azulene–indole systems. Preliminary structure–activity relationships against a variety of human cancer cell lines are also reported.

In an initial experiment, a DMSO solution of 6-dimethylaminofulvene (**1**) and α-pyrone (**2**)[10] was irradiated by using a focused microwave oven (10% power, 130 °C, 1 h) to provide 32% of the azulene–indole **3**.[11] Experiments were carried out to test the effect of solvents and conditions on the yield of this process (Table 1). In general, DMF appears to be the best solvent. The highest yield was observed for the reaction of **1** (1 M in DMF) and **2** with 10% microwave power for 60 min at 130 °C (entry 12, 65% isolated product). Reactions performed at higher microwave power and for longer irradiation times gave lower yields of the azulene–indoles along with decomposition products (entries 3, 5, and 7). In addition, lower yields were observed when reactant concentrations were higher or lower than 1 M (entries 8, 10, and 11). It is noteworthy that reactions performed



Scheme 1. Azulene–indole forming fulvene–ketene α-pyrones [6 + 4]-cycloaddition.

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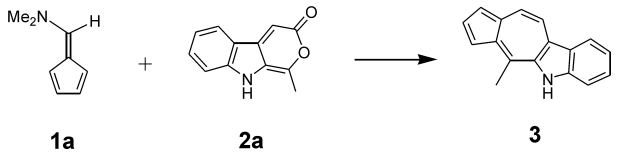
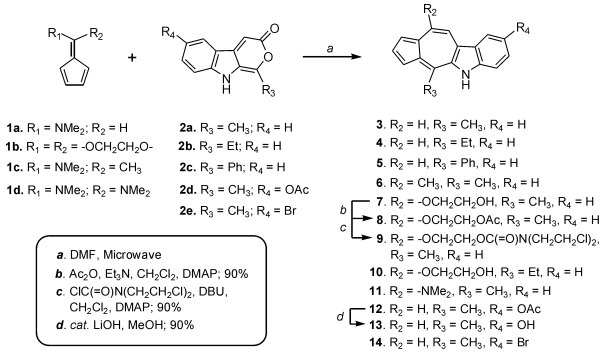
Table 1. Microwave-assisted [6 + 4]-cycloaddition of 6-aminofulvene **1a** and pyrone **2a** to azulene–indole **3**



1a **2a** **3**

Entry	Fulvene:Pyrone (molar ratio)	Solvent	Power (%)	Concn (M)	Time (min)	Temp. (°C)	Yield[a] (%)
1	1:1	DMSO	0[b]	0.5	7200	130	8
2	1:1	DMSO	10	0.5	60	130	32
3	1:1	DMSO	10	0.5	60	160	27
4	1:1	DMF	0[b]	0.5	7200	130	22
5	1:1	DMF	10	0.5	180	130	48
6	1:1	DMF	10	0.5	60	130	50
7	1:1	DMF	30	0.5	60	130	38
8	1:1	DMF	10	0.25	60	130	30
9	1:1.5	DMF	10	1	60	130	57
10	1:1.5	DMF	10	2	60	130	45
11	1:1.5	DMF	10	4	60	130	30
12	5:1	DMF	10	1	60	130	65[c]

[a]Isolated yields based on starting fulvene.
[b]Conventional oil bath heating without microwave irradiation.
[c]Isolated yield based on starting pyrone.



Scheme 2. Syntheses of various azulene–indoles.

Table 2. [6 + 4]-Cycloaddition of **1** and **2** to azulene–indole **3**

Entry	Fulvene + Pyrone	Product no.	Yield[a,b] (%) No MW	Yield[b,c] (%) with MW
1	**1a + 2a**	**3**	26	65
2	**1a + 2b**	**4**	18	46
3	**1a + 2c**	**5**	12	30
4	**1c + 2a**	**6**	23	57
5	**1b + 2a**	**7**	19	52
6	**1b + 2b**	**10**	18	48
7	**1d + 2a**	**11**	14	37
8	**1a + 2d**	**12**	26	62
9	**1a + 2e**	**14**	21	53

[a]Conventional oil bath heating without microwave irradiation for 7200 min.
[b]Isolated yields based on starting fulvene.
[c]Microwave irradiation for 60 min.

Table 3. In vitro inhibition of cancer cell lines by azulene–indoles[a,b]

Compound	NSC	Average IC_{50} (μM)[c]	Selected IC_{50} (μM)[d,e]
7	695154	2.51	EKVX (1.00), HS578T (0.93), NCI-H322M (17.0), A498 (13.8)
3	710184	2.40	U251 (1.48), UO-31 (1.62), A498 (11.7), CAK1-1 (6.6)
12	714067	2.19	NCI-H226 (1.38), IGROV1 (1.58), SR (4.57), K-562 (3.80)
14	716773	5.37	T-47D (1.70), EKVX (1.78), UACC-257 (24.0), A498 (21.9)
11	718564	3.98	HOP-92 (0.56), PC-3 (1.41), A498 (20.9), CAK1-1 (17.4)
8	710185	2.75	K-562 (0.51), HOP-92 (1.38), NCI-H322M (14.5), NCI-H226 (11.2)

[a]Results of NCI's in vitro disease-oriented tumor cell line screen assays.
[b]IC_{50}: concentration that inhibits 50% cell growth.
[c]Average IC_{50} against 60 human cell lines.
[d]Selected highest and lowest activities against various cell lines.
[e]The cancer cell lines are defined as follows: EKVX, HOP-92, NCI-H226 and NCI-H322M are non-small cell lung; HS578T and T-47D are breast; A498, UO-31 and CAK1-1 are renal; U251 is CNS, SR and K-562 are leukemia; UACC-257 is melanoma; IGROV1 is ovarian; PC-3 is prostate.

by using conventional oil bath heating require long reaction times (5 days) and give low yields of the final product along with recovered starting materials and decomposition products (entries 1 and 4). As shown in Scheme 2 and Table 2, a series of azulene–indoles were synthesized with the same reaction conditions.[12]

The azulene–indoles, prepared in this study, were evaluated according to standard NCI protocols[13] for their in vitro activity against 60 human cancer cell lines derived from 10 clinically isolated cancer subtypes (melanoma, leukemia, lung, colon, renal, ovarian, CNS, brain, breast and prostate). As shown in Table 3, these

compounds display good activity against most of the cell lines tested, with average IC_{50} values ranging between 2.2 and 5.4 μM. Also, some of the azulene–indoles show selective growth inhibitory activity. For example, compound **8** is a potent and selective growth inhibitor of the K-562 leukemia cell line ($IC_{50} = 0.51$ μM). Results from the COMPARE program of the NCI screening database seem to indicate that these azulene–indoles represent a class of antitumor agents with a novel mechanism of action.[14]

In summary, a facile and efficient microwave-assisted [6+4]-cycloaddition method for the synthesis of antineoplastic azulene–indoles has been developed. The potential biological activity of other members of this family as well as the mechanism[15] of action of these compounds are under active investigation.

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11. Focused microwave irradiation was carried out at atmospheric pressure with a Synthewave S402 Prolabo microwave reactor (300 W, monomode system, 10 mL reactors). The apparatus has a quartz reactor, visual control, PC controlled 300 W irradiation, infrared temperature measurement with continuous feedback control.
12. All new compounds were characterized by full spectroscopic data (^1H, ^{13}C, NMR, IR, MS, and HR-MS). **Typical procedure for synthesis of azulene-indole 3**: A mixture of 6-dimethylaminofulvene (**1**, 604 mg, 5 mmol), 1-methylpyrano[3,4-*b*]indol-3(9*H*)-one (**2**, 200 mg, 1 mmol) and DMF (5 mL) were placed in a 10 mL quartz vial and subjected to

programmed microwave irradiation at 30 W for 60 min. After a period of 2–3 min, the temperature reached a plateau of 130 °C where it remained throughout the reaction. After cooling, the solution was concentrated and the residue was subjected to flash column chromatography (10% EtOAc–hexane, $R_f = 0.48$ in 30% EtOAc–hexane) to give the azulene–indole **3** as a dark green solid (150 mg, 65% yield) and recovered fulvene (490 mg). Spectroscopic data for **3**: mp 157–159 °C; IR (neat): 3416, 3077, 2937, 2857, 1626, 1507, 1462, 1377, 1302, 1237, 751 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz): δ 8.24 (d, $J = 10$ Hz, 1H), 8.01 (d, $J = 8$ Hz, 1H), 7.94 (t, $J = 3.5$ Hz, 1H), 7.78 (d, $J = 10$ Hz, 1H), 7.54–7.50 (m, 3H), 7.38 (dd, $J = 8$, 8 Hz, 1H), 7.25–7.10 (m, 1H), 7.07 (dd, $J = 8.0$, 0.6 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (C_6D_6, 100 MHz): δ 139.0 (C), 138.5 (C), 137.9 (C), 135.2 (C), 134.4 (CH and C), 129.8 (CH), 128.5 (C), 127.9 (CH), 127.4 (C), 121.6 (CH), 121.1 (CH), 119.0 (CH), 114.5 (CH), 114.3 (CH), 111.7 (CH), 19.0 (CH_3); MS (*m*/*z*, relative intensity): 231 (M^+, 17), 173 (100), 158 (90), 149 (39), 130 (42); exact mass calcd for $C_{17}H_{13}N$ (M^+): 231.1049; found 231.1048.

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