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Novel potent antimitotic heterocyclic ketones: Synthesis, antiproliferative activity, and structure–activity relationships

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Abstract—We report the synthesis, antiproliferative activity, and SAR of novel heterocyclic ketones derived from carbazole sulfonamides. Most of the heterocyclic ketones showed strong cytotoxicities. (*N*-1-Methylindole-5-yl)-(3,4,5-trimethoxyphenyl)-methanone **8b** gave the most potent cytotoxicity (9.2–26 nM) against seven human tumor cell lines. The mechanism of action of the heterocyclic ketones appears to involve targeting of tubulin, similar to that of CA-4 and different from the carbazole sulfonamides.

Antimitotic agents that target tubulin are effective and widely used in treating cancer.[1,2] Combretastatin A-4 (CA-4) (Chart 1) is a potent antimitotic agent, which was isolated from the Africa Willow tree *Combretum caffrum*.[3] CA-4 strongly binds to the colchicine site of tubulin and prevents tubulin polymerization.[4] CA-4 inhibits cancer cell growth at low nanomolar concentrations, including multi-drug-resistant cancer cell lines. A water soluble prodrug of CA-4 has shown promising results in phase I human cancer clinical trials.[5,6] The relatively simple structure of CA-4 as well as its selective antivascular activity have stimulated significant research efforts focused on the identification of new CA-4 analogues that exhibit more potent activities and better pharmacological profiles.[7,8] Recently, another two CA-4 analogues, CA-1P and AVE-8062 (Chart 1), have entered clinical trials.[9]

CA-4 as an antimitotic agent displays poor in vivo anticancer efficacy, partly due to its high lipophilicity and low aqueous solubility.[10] SAR studies have shown that the *Z* (cis) orientation of the olefin is essential for potent cytotoxicity.[11,12] CA-4 is prone to isomerize to the *E*-isomer (trans) during storage and administration, which dramatically reduces its antiproliferative activities.[13] By replacement of the ethene bridge of CA-4 with a carbonyl group, Phenstatin and Hydroxyphenstatin (two benzophenone analogues) (Chart 1) have been found to exhibit potent anticancer and comparable antitubulin activities with CA-4.[14,15] The sp^2-hybridized carbonyl group in Phenstatin and Hydroxyphenstatin molecules not only constrains the quasi 'cis' orientation, but often improves chemical stability and water solubility.[15b] 2′- or 3′-Aminobenzophenone analogues (Chart 1) also showed significant cytotoxicity against many human cancer cell lines.[16,17] More interestingly, 2-aroyl-5-methoxyindoles and 3-aroyl-6-methoxyindoles (Chart 1) have shown inhibition of tubulin polymerization, potential antivascular activity, and oral effectiveness in in vivo tumor models.[18,19]

We recently reported a series of carbazole sulfonamide antimitotic agents structurally related to CA-4.[20,21] The *N*-9-ethyl-3,4,5-trimethoxyphenyl-carbazolesulfonamide analogues **1**–**3** and the 2,6-dimethoxypyridin-3-yl analogue **4** (Chart 1) displayed potent antiproliferative activities against several human cancer cell lines. The lead compound **1** also showed significant anticancer activity in two in vivo models. However, the lead compound **1** does not inhibit tubulin polymerization, which

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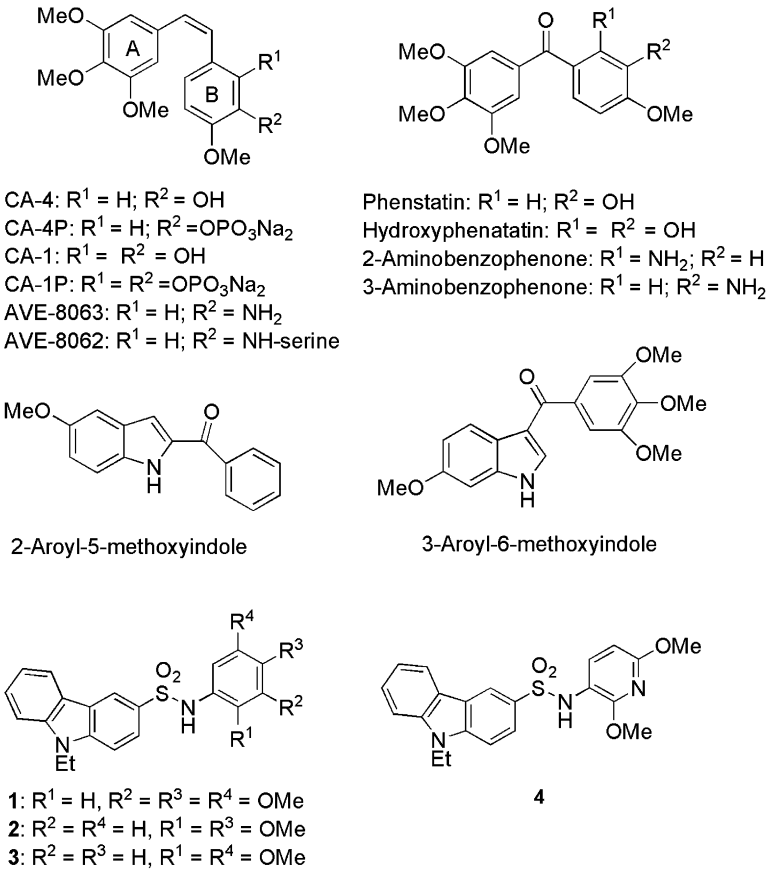
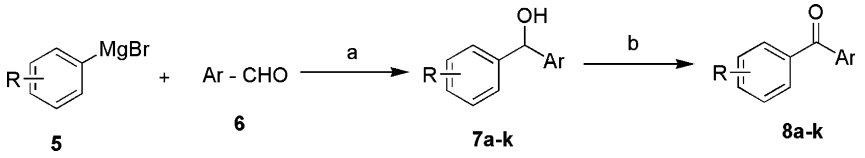


Chart 1. Antimitotic agents: CA-4, CA-4 analogues and carbazole sulfonamides.

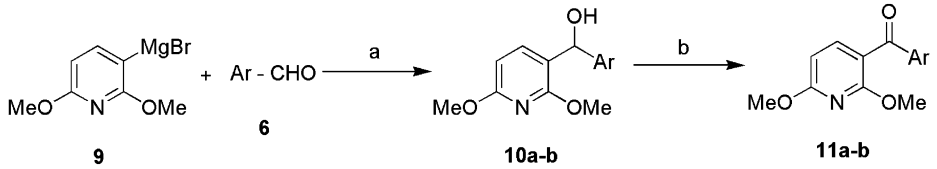
suggests the mode of action is different from CA-4.[20] For the purpose of further study of SAR of this series of carbazole sulfonamides and discovery of more effective antimitotic agents, we designed a series of aroyl carbazoles through replacing the sulfonamide linkage of carbazole sulfonamides with the carbonyl group. Also, we include aroyl indoles, benzofuran, and benzothiophene analogues to further evaluate the SAR in this series. Here, we report the synthesis and cytotoxic activities against human cancer cells of this series of novel heterocyclic ketone compounds.

The general method for the synthesis of heterocyclic ketone compounds is shown in Schemes 1 and 2.[16] The Grignard reaction of various substituted phenyl or 2,6-dimethoxypyridinyl magnesium bromide with *N*-9-ethyl-3-carbazolecarboxaldehyde, *N*-1-methyl-5, or 6, or 7-indolecarboxaldehyde, 5-benzofurancarboxaldehyde, and 5-benzothiophenecarboxaldehyde yielded the corresponding diaromatic methanols **7a**–**k**, **10a** and **10b**. We obtained the corresponding desired ketones **8a**–**k**, **11a** and **11b** by pyridinium dichromate (PDC) oxidation of the carbinols. For the synthesis of (*N*-1*H*-indole-5-yl)-(3, 4, 5-trimethoxyphenyl)-methanone **17**, it was necessary to first protect the NH group of *N*-1*H*-5-indolecarboxaldehyde **13** with TBDMS. Then, following the general procedure of Grignard reaction, oxidation, and deprotection with Bu_4NF, the ketone **17** was obtained (Scheme 3).

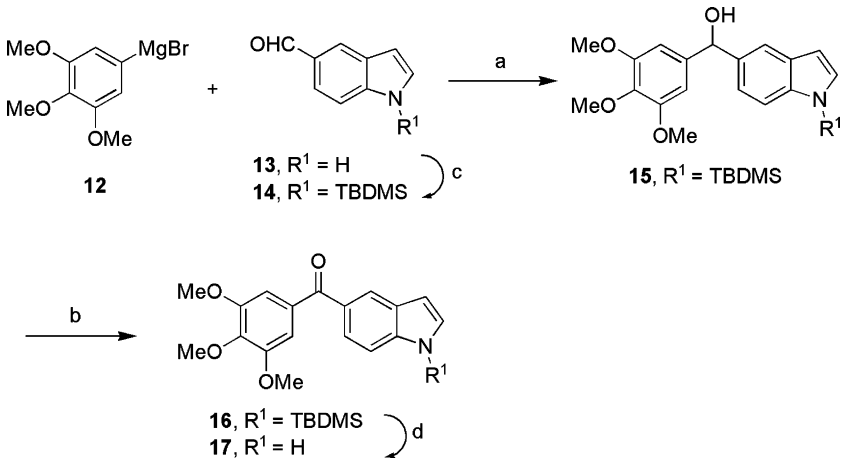
The synthesized heterocyclic ketones (Table 1) were evaluated for their cytotoxicities against the CEM leukemia cell line.[20] We first evaluated the effect of replacement of the sulfonamide group with the carbonyl group in the carbazole sulfonamide lead compound **1** on cytotoxicity. Newly synthesized ketone **8a** exhibited a slight loss of potency as compared to lead compound **1**, IC_{50} value of 90 vs 56 nM.[20] The corresponding carbinol **7a** also showed comparable activity to lead compound **1**. These results suggest that the carbonyl group is tolerated as a replacement of the sulfonamide group with retention of potent cytotoxic activity. Then, replacement of the 9-ethylcarbazole ring of ketone **8a** with *N*-methylindole ring yielded the 5-aroyl-*N*-1-methylindole analogue **8b**. It is gratifying to note that compound **8b** gave significantly increased potency (5-fold) as compared to **8a** and also a 3-fold increase of activity compared to lead compound **1**. A similar tendency has also been shown for the *N*-1-methylindole methanol **7b**, which displayed an IC_{50} value of 59 nM, almost the same value as lead compound **1**. When the position of the carbonyl group is changed to the C-6 position on the indole ring substantial potency is maintained. However, changing the keto group to the C-7 position of the indole ring (**8d**) was not tolerated and decreased cytotoxicity by 17 times as compared to **8b** was noted, suggesting that the effect of the *ortho*-steric hindrance is likely critical for cytotoxicity.



Scheme 1. Reagents and conditions: (a) THF, 0 °C; (b) PDC, CH_2Cl_2, 25 °C.



Scheme 2. Reagents and conditions: (a) THF, 0 °C; (b) PDC, CH_2Cl_2, 25 °C.



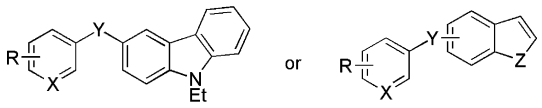
Scheme 3. Reagents and conditions: (a) THF, 0 °C; (b) PDC, CH_2Cl_2, 25 °C; (c) TBDMSCl, *t*-BuOK, THF, 0–25 °C; (d) Bu_4NF, THF.

The influence of various substitutions on the phenyl ring was also examined. 4-Methoxy, 3,5-, 3,4-,and 2,5-dimethoxy substitutions on the phenyl ring (**8e**, **8f**, **8g** and **8i**) led to significant loss of potencies more than a 50-fold reduction in activity in comparison with the 3,4,5-trimethoxyphenyl compound **8b**. However, 2,4-dimethoxy substitutions of phenyl ring (**8h**) gave an IC_{50} value of 102 nM, only a 5-fold loss of activity as compared to **8b**. The SAR of this series of compounds is different from that of the carbazole sulfonamides. For example, we reported that the 2,4- and 2,5-dimethoxy phenyl substituted carbazole sulfonamides **2** and **3** showed similar cytotoxicities to that of the 3,4,5-trimethoxyphenyl lead compound **1**.[20] The SAR of the ketone series of compounds is consistent with that of CA-4 analogues, where it is well known that the 3,4,5-trimethoxy substituents of A ring of CA-4 are indispensable for potent cytotoxicity.[11] From these observations, the mechanism of action of this series of compounds appears to be different from the carbazole sulfonamides and similar to that of CA-4 analogues.

In order to understand the effect of the nitrogen of the indole ring, we synthesized the *N*-1*H*-indole, benzofuran, and benzothiophene analogues **17**, **8j**, and **8k**. It was not surprising that these analogues resulted in more than a 10-fold decrease in potency giving IC_{50} values from 225 to 303 nM, compared to the IC_{50} value of 18 nM for **8b**. Similar results were also noted in the carbazole sulfonamide series.[20]

In the 2,6-dimethoxypyridinyl series, ketone compound **11a** led to 4.5-fold loss of potency in comparison with the corresponding carbazole sulfonamide **4**.[21] However, it is much better than the ethene compound ($IC_{50} > 2$ μM). In contrast, the (2,6-dimethoxypyridin-3-yl)-*N*-1-methylindole-ketone **11b** has decreased cytotoxicity as compared to the corresponding carbazole ketone **11a**, the IC_{50} value of 1012 versus 555 nM, indicating that it is different from 3,4,5-trimethoxyphenyl substitution. It is noteworthy that compound **11b** is less potent by 10-fold than the 2,4-dimethoxyphenyl indole compound **8h**. These results suggest that the 2,6-dimethoxypyridinyl is not suitable for ketone and ethene compounds to achieve potent cytotoxicity, perhaps due to *ortho*-steric hindrance and *meta*-nitrogen electron-withdrawing effects. The corresponding indole sulfonamide also showed much decreased activity.[21] Only 2,6-dimethoxypyridinyl carbazole sulfonamide **4** has comparable activity with the lead compound **1**, and it is likely that the mode of action of **4** is different from lead compound **1**.

Table 1. Antiproliferative activity of new compounds in CEM Leukemia cells



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Compound	Aryl type	X	R	Y	Z	Cytotoxicity IC_{50}[a] (nM)
1	I	C	3,4,5-OMe_3	SO_2NH	/	56
2	I	C	2,4-OMe_2	SO_2NH	/	57
3	I	C	2,5-OMe_2	SO_2NH	/	61
4	I	N	2,6-OMe_2	SO_2NH	/	122
7a	I	C	3,4,5-OMe_3	CHOH	/	192
8a	I	C	3,4,5-OMe_3	CO	/	90
7b	II	C	3,4,5-OMe_3	5′-CHOH	NMe	59
8b	II	C	3,4,5-OMe_3	5′-CO	NMe	18
8c	II	C	3,4,5-OMe_3	6′-CO	NMe	30
8d	II	C	3,4,5-OMe_3	7′-CO	NMe	307
8e	II	C	3,5-OMe_2	5′-CO	NMe	1693
8f	II	C	3,4-OMe_2	5′-CO	NMe	1693
8g	II	C	2,5-OMe_2	5′-CO	NMe	2370
8h	II	C	2,4-OMe_2	5′-CO	NMe	102
8i	II	C	4-OMe	5′-CO	NMe	1131
17	II	C	3,4,5-OMe_3	5′-CO	NH	225
8j	II	C	3,4,5-OMe_3	5′-CO	O	288
8k	II	C	3,4,5-OMe_3	5′-CO	S	303
11a	I	N	2,6-OMe_2	CO	/	555
11b	II	N	2,6-OMe_2	5′-CO	NMe	1012
Podophyllotoxin						7.2
CA-4						1.9

[a] Values were determined as described in Ref. 20.

Several analogues **8a**, **7b**, **8b**, **8c**, and **8h** exhibit strong antiproliferative activity against CEM leukemia cells; consequently, we further evaluated their activity against a panel of several other human cancer cell lines in vitro. Table 2 contains these results along with comparative data for lead compound **1**, CA-4, and podophyllotoxin.[20] Although carbazole ketone **8a** is slightly less potent than lead compound **1** against CEM and Molt-3 leukemia cell lines, it shows 2- to 15-fold stronger activity than **1** against the five human solid tumor cell lines. The IC_{50} values of the most potent indole ketone compound **8b** were between 9.2 and 26 nM against the panel of seven cell lines studied. Interestingly, compound **8b** is slightly more active against MCF-7 breast cancer cell line in comparison to CA-4 and podophyllotoxin. Compound **8b** is two times more potent than podophyllotoxin against DU-145 prostate cancer cell line and showed comparable activity in other five cell lines with that of podophyllotoxin. Compound **8b** also is 6–23 times more active than lead compound **1** against the five human solid tumor cell lines. The carbinol **7b** and 6-indole ketone **8c** also showed more activity than lead compound **1** in inhibiting solid tumor cell lines. The 2,4-dimethoxy substituted indole compound **8h** is more potent than lead compound **1** against Bel-7402 hepatoma and DU-145 prostate cancer cells and showed comparable activity in other cell lines to that of **1**. These results show that heterocyclic ketones are more effective against solid tumor cell lines than lead compound **1**. The sensitivity of this series of compounds is similar to that of CA-4 and Podophyllotoxin.

We have noted that the SAR of this series of heterocyclic ketones is different from that of the carbazole sulfon-

Table 2. Antiproliferative activities of **8a**, **7b**, **8b**, **8c**, **8h**, **1**, CA-4, and Podophyllotoxin in human tumor cell lines

Cell line[a]	IC_{50}[b] (nM)							
	8a	**7b**	**8b**	**8c**	**8h**	**1**	CA-4	Podophyllotoxin
CEM	90	59	18	30	102	56	1.9	7.2
Molt-3	51	38	18	18	24	20	9.3	14
Bel-7402	90	88	21	27	44	201	10	9.1
MCF-7	26	44	9.2	24	85	89	12	15
DU-145	39	103	26	75	34	603	9.3	52
PC-3	103	117	25	75	203	201	2.8	10
DND-1	21	59	15	27	118	89	2.5	12

[a] CEM; Molt-3, T-cell leukemia; Bel-7402, hepatoma; MCF-7, breast cancer; DU-145, PC-3, prostate cancer; DND-1, melanoma.
[b] Values were determined as described in Ref. 20.

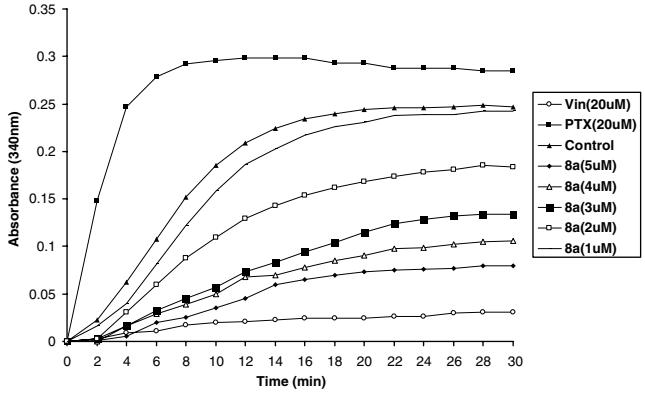

Figure 1. Effect of **8a** on tubulin assembly.[20] Free purified β-tubulin in reaction buffer was incubated with GTP and Mg^{2+} for assembly in the absence or presence of **8a** (1–5 μM), vincristine (20 μM) or paclitaxel (20 μM). Tubulin assembly was determined every 2 min by O.D. at 340 nm. Each point represents the mean of two independent experiments.

amides and similar to CA-4 analogues. The mechanism of action of this series of ketones is likely similar to CA-4 analogues by targeting on tubulin. To investigate whether the antiproliferative activities of these compounds were related to interaction with tubulin, carbazole ketone compound **8a** was evaluated for inhibition of polymerization of purified tubulin in a cell-free system. The results are shown in Figure 1. Vincristine was used as a positive control. Vincristine inhibited tubulin polymerization by 87% at 20 μM. The effect of **8a** on tubulin assembly was examined at concentrations from 1 to 5 μM. The results showed that compound **8a** inhibited tubulin polymerization in a dose-dependent manner. The IC_{50} value of inhibition of tubulin polymerization of compound **8a** was 3.4 μM; similar to that of CA-4 at 1.2 μM.[14] It is significantly lower than that of the lead compound **1** which only weakly affected tubulin polymerization, even at very high concentration.[20]

In summary, a series of novel heterocyclic ketones were synthesized by replacing the sulfonamide linkage with a carbonyl group in the lead carbazole sulfonamide **1**. Most of the heterocyclic ketones showed strong antiproliferative activities against CEM leukemia cells. Several compounds were more effective than lead compound **1** against five solid tumor cell lines. The most potent compound **8b** displayed comparable antiproliferative activities with that of CA-4 and podophyllotoxin. The SAR of the heterocyclic ketones is different from the carbazole sulfonamides and similar to that of CA-4 analogues. The carbazole ketone compound **8a** strongly inhibits tubulin assembly. Further studies of the mechanism of action of this series of novel heterocyclic ketones and evaluation of **8a** and **8b** in vivo are underway.

Acknowledgments

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