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# Novel pyridinyl and pyrimidinylcarbazole sulfonamides as antiproliferative agents

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**Abstract**—A series of azaheterocyclic carbazole sulfonamides was synthesized and evaluated for antiproliferative activity. The most potent compounds *N*-(2,6-dimethoxypyridine-3-yl)-9-ethyl and 9-methylcarbazole-3-sulfonamide (**13** and **14)** gave significant cytotoxicity ($IC_{50}$ = 122 and 101 nM). Compound **13** displayed submicromolar activities against seven human tumor cell lines. The SARs of this series of sulfonamides which includes the influence of azaheterocycle rings, sulfonamide linkage, and the carbazole ring are described.


Since the discovery of E-7010 in 1992 (**1**, Fig. 1),[1] sulfonamides have emerged as an important class of anticancer agents which interact with a wide range of different cellular targets.[2] For example, sulfonamides cause disruption of microtubule assembly, show carbonic anhydrase inhibition, and target transcription factor NF-Y and matrix metalloproteinase (MMP). Some of these compounds, such as T-138067, E-7010, E7070, and HMN-214 (**1**–**4**, Fig. 1), have entered clinical trials.[3–6] Recently, more sulfonamide anticancer agents with novel molecular targets, such as methionine aminopeptidase type II inhibitors, antagonists of MDM2 oncoprotein, and inhibitors of tyrosine and Raf-kinases, have been reported.[7–9] Various novel sulfonamides with different mechanisms of action can be useful for the treatment of drug-resistant malignant tumors, which remain a major challenge in cancer chemotherapy.[10]

Recently, we reported *N*-phenyl carbazole sulfonamides which are structurally related to combretastatin A4 (CA4) (**5**, Fig. 1)[11,12] and exhibit potent antiproliferative activities in vitro and in vivo antitumor effectiveness.[13] As is well known, CA4 strongly inhibits the polymerization

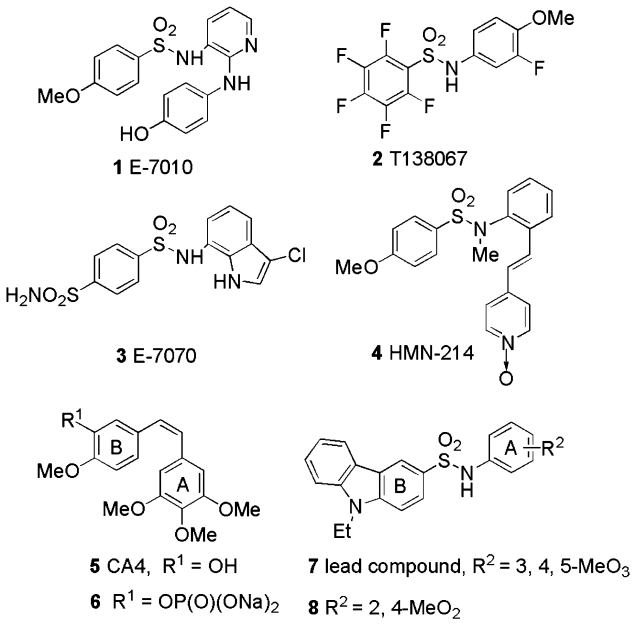


**Figure 1.** Antitumor sulfonamides in clinical trials, CA4 and carbazole sulfonamides.

*Keywords*: Antiproliferative agents; Sulfonamides.
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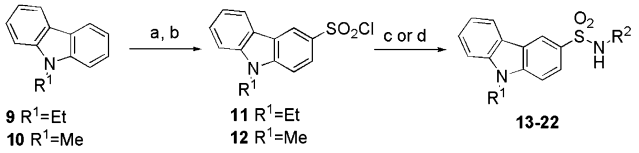
of tubulin by binding to the colchicine site and exerts irreversible vascular shutdown within solid tumors.[11,12] A water-soluble phosphate prodrug of CA4 (**6**, Fig. 1) is now in phase II clinical trials.[14] Preliminary studies with the lead carbazole sulfonamide **7** (Fig. 1) showed that it induced $G_2$/M phase arrest and apoptosis, however, it only weakly inhibited tubulin assembly. These results clearly demonstrated that the mode of action of these new sulfonamides is different from that of CA4.[13] The SARs of this series of carbazole sulfonamides also were very different from those of CA4 analogues,[15,16] both the 3,4,5-trimethoxy phenyl lead compound **7** and the 2,4-dimethoxy substituted compound **8** (Fig. 1) have similar potent activities against human tumor cell lines.

As a part of our continuing efforts to discover more potent carbazole sulfonamides and explore the SAR of this system, we have examined the replacement of 2,4-dimethoxyphenyl with pyridinyl and pyrimidinyl groups. Moreover, in the CA4 series, 4-methoxy-3-pyridinyl has been successfully used as a replacement for 4-methoxy-3-hydroxyphenyl (B ring of CA4) and it also gave improved water solubility.[17] Here, we present our results for the novel *N*-azaheterocyclic carbazole sulfonamides.

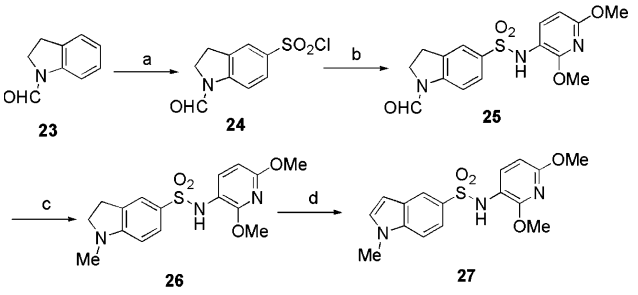
The synthesis of *N*-azaheterocyclic carbazole sulfonamides **13**–**22** used our previously reported approach[13] by the reaction of various commercially available substituted azaheteroarylamines with 9-ethyl- or 9-methyl carbazole sulfonyl chlorides[18] (Scheme 1).

The 2,6-dimethoxypyridinyl indole sulfonamide **27** was synthesized by the route illustrated in Scheme 2. 1-Formyl-5-indolinesulfonyl chloride **24** was obtained from commercially available 1-formyl-5-indoline **23** using Gupta's method.[19] Then, **24** was allowed to react with 3-amino-2,6-dimethoxypyridine using the TEA/DMF procedure to generate sulfonamide **25**. After reduction of **25**, the indoline compound **26** was aromatized to yield the indole sulfonamide **27**.[19]

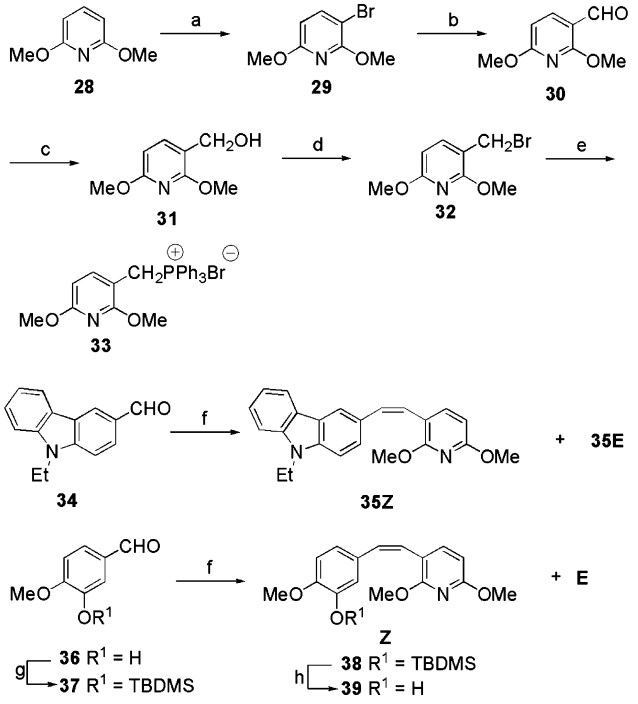
Carbazole compound **35** and the CA4 pyridine analogue **39** were prepared through classical Wittig couplings using ylide **33** and aldehydes **34** and **37** (Scheme 3). Regioselective bromination of **28** was achieved by *N*-bromosuccinimide (NBS) with pyridine in acetonitrile.[20] Metalation of 3-bromo-2,6-dimethoxypyridine **29** with *n*-butyllithium followed by the addition of DMF gave the corresponding aldehyde **30**.[21] Then, following Pettit's procedure: reduction, bromination, and reaction




**Scheme 1.** Reagents and conditions: (a) $ClSO_3H$, $CH_2Cl_2$, −5 °C, 2 h, **9**: 89%; **10**: 92%; (b) $POCl_3$, $PCl_5$, 90 °C, 2 h, **11**: 57%; **12**: 64%; (c) **13**–**19**: $R^2$–$NH_2$, $NEt_3$, DMF, rt, 3 h; (d) **20**–**22**: $R^2$–$NH_2$, Py, refulx, 2 h.




**Scheme 2.** Reagents and conditions: (a) $ClSO_3H$, $ClCH_2CH_2Cl$, −5 °C; then $SOCl_2$, 0–5 °C, 3 h, 91%; (b) 2,6-$MeO_2$Py-3–$NH_2$HCl, $NEt_3$, DMF, rt, 3 h, 85%; (c) $NaBH_4$, $CF_3CO_2H$, THF, 0–5 °C, 3 h, 91%; (d) DDQ, THF, 0-25 °C, overnight, 78%.

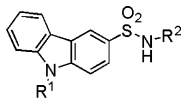



**Scheme 3.** Reagents and conditions: (a) NBS, $CH_3CN$, 0 °C, 2 h, 77%; (b) BuLi, THF, −78 °C, 1 h; then DMF, 2 h, 71%; (c) $NaBH_4$, $CH_3OH$, 0 °C, 2 h, 89%; (d) $PBr_3$, $CH_2Cl_2$, 0 °C, 12 h, 74%; (e) $PPh_3$, Toluene, reflux, 2 h, 89%; (f) **33**, NaH, $CH_2Cl_2$, 0–22 °C, 18 h, **35**: 65%; **38**: 45%, *Z*/*E* = 1.5:1; (g) *t*-$BuMe_2SiCl$, $(i\text{-}Pr)_2NEt$, DMF, 1 h, 94%; (h) TBAF, THF, 0.5 h, 90%.

with triphenylphosphine ($PPh_3$), we obtained ylide **33**.[22] Finally, carbazole compounds **35** *Z*/*E* were prepared by Wittig coupling of ylide **33** and *N*-ethyl-3-carbazolecarboxaldehyde **34** with NaH in $CH_2Cl_2$.[23] *tert*-Butyldimethylsilyl (TBDMS)-protected **38** was obtained using the same procedure employing TBDMS-protected benzaldehyde **37**.[24] After deprotection of the TBDMS group with tetra-butylammonium fluoride in THF, the desired compounds **39** *Z*/*E* were obtained.[24]

Antiproliferative activities of the new compounds against human CEM Leukemia cells were evaluated as previously reported[13] (Table 1). The new 2,6-dimethoxypyridinyl carbazole sulfonamide **13** has significant activity with an $IC_{50}$ value of 122 nM, about 2-fold less

**Table 1.** Antiproliferatative activity of new compounds in CEM leukemia cells



| Compound | $R^1$ | $R^2$ | Cytotoxicity $IC_{50}$[a] (nM) |
|---|---|---|---|
| **7** | Et | 3,4,5-$MeO_3$Ph | 56 |
| **8** | Et | 2,4-$MeO_2$Ph | 57 |
| **13** | Et | 2,6-$MeO_2$Py-3-yl | 122 |
| **14** | Me | 2,6-$MeO_2$Py-3-yl | 101 |
| **15** | Et | 6-MeOPy-3-yl | 649 |
| **16** | Et | 2-MeOPy-3-yl | 786 |
| **17** | Et | 2,6-$Me_2$Py-3-yl | >2000 |
| **18** | Et | 2,6-$Cl_2$Py-3-yl | >2000 |
| **19** | Et | 2,6-$Cl_2$Py-4-yl | >2000 |
| **20** | Et | 6-MeOPm-4-yl | >2000 |
| **21** | Et | 2,6-$MeO_2$Pm-4-yl | >2000 |
| **22** | Et | 4,6-$MeO_2$Pm-2-yl | >2000 |
| **26** | | | >2000 |
| **27** | | | 712 |
| **35***Z* | | | >2000 |
| **35***E* | | | >2000 |
| **39***Z* | | | 342 |
| **39***E* | | | >2000 |
| Podophyllotoxin | | | 7.2 |
| CA4 | | | 1.9 |

[a] Values were determined as described in Ref. 13.

than that of lead compound **7** and the close analogue **8**. The cytotoxicity of the *N*-9 methyl substituted compound **14** is slightly more potent than that of *N*-9 ethyl compound **13** in accordance with the SAR previously noted for *N*-phenyl carbazole sulfonamides.[13] The monomethoxypyridinyl compounds **15** and **16** are 5–6 times less effective than the 2,6-dimethoxypyridinyl compound **13**. The *ortho*-methoxy analogue **16** is less active than the *para*-substituted analogue **15**. However, the *para*-methoxy pyridinyl carbazole sulfonamide **15** ($IC_{50}$ value 0.65 μM) is more active than the 4-methoxy and 3,4-dimethoxy substituted phenyl carbazole sulfonamides ($IC_{50}$ value >10 and 2.4 μM).[13] These results suggest that a *meta*-pyridinyl ring is a reasonable replacement for the phenyl group and provides good antiproliferative activity. Also, a different influence on the cytotoxicity was noted for the pyridinyl system as compared to the phenyl one. The 2,6-dimethyl and dichloro substituted pyridinyl compounds **17**, **18**, and **19** were much less active than the 2,6-dimethoxypyridinyl compound **13**. These results demonstrate that dimethoxy substitution of the pyridine ring plays a pivotal role in affecting cytotoxicity. In marked contrast to the pyridine results, replacement of ring A with mono- or dimethoxy substituted pyrimidines as in **20**–**22** results in complete loss of activity. This result may be due to the reduced electron density of pyrimidine ring, we previously noted that replacement of the 3,5-dimethoxy groups in the sulfonamide series by the strong withdrawing group $CF_3$ in ring A also resulted in loss of activity.[13] Based on the above results, the 2,6-dimethoxypyridinyl substituted carbazole sulfonamides **13** and **14** merit further study.

Replacement of the carbazole by *N*-methylindole yielded compound **27** which resulted in a 7-fold decrease in cytotoxicity compared to **14**. The corresponding *N*-methylindolinyl compound **26** also showed a reduction in activity. These results indicate that the carbazole ring is important to achieve significant activity. Finally, the isomeric olefin compounds **35** were both inactive against the CEM cell line. This result shows that the sulfonamide linkage is necessary for activity in this system. We also replaced the 3,4,5-trimethoxyphenyl with a 2,6-dimethoxypyridinyl in the CA4 molecule by preparation of the analogues **39***Z*/*E*. The *Z* isomer of **39** was 180-fold less potent than CA4 and **39***E* has no activity. These data are consistent with the SARs of CA4 analogues and further demonstrate that 3,4,5-trimethoxyphenyl group in the A ring of CA4 is necessary for achieving potent antiproliferative activity.[15,16] However, **39***Z* has better activity than its close 2,3,4-trimethoxyphenyl CA4 analogue which reduced the cytotoxicity by more than five orders of magnitude in comparison with the corresponding 3,4,5-trimethoxyphenyl compound and CA4.[15]

Both the 2, 6-dimethoxypyridinyl substituted carbazole sulfonamides **13** and **14** showed good activity against human CEM cell line and a very slight increase in water solubility. Compound **13** was selected for evaluation against seven different human tumor cell lines for direct comparison with **7** and **8**. The data for **13** along with those of **7**, **8**, CA4, and podophyllotoxin are shown in Table 2. The $IC_{50}$ value of **13** are below 1 μM in the cell lines studied. The $IC_{50}$ values of **13** in the least sensitive cell line Bel-7402 hepatoma ($IC_{50}$ = 976 nM) is 5- and 10-fold less than that for **7** and **8**. Molt-3 leukemia cells showed the highest sensitivity ($IC_{50}$ = 22 nM), about the same as lead compound **7** and 2-fold more than compound **8** but 2-fold less than CA4. The sensitivities

**Table 2.** Antiproliferatative activity of **13** in human tumor cell lines

| Cell line | Human tumor | $IC_{50}$[a] (nM) | | | | |
|---|---|---|---|---|---|---|
| | | **7** | **8** | **13** | CA4 | Podophyllotoxin |
| CEM | T-cell leukemia | 56 | 57 | 122 | 1.9 | 7.2 |
| Molt-3 | T-cell leukemia | 20 | 48 | 22 | 9.3 | 14 |
| Bel-7402 | Hepatoma | 201 | 96 | 976 | 10 | 9.1 |
| MCF-7 | Breast cancer | 89 | 48 | 241 | 12 | 15 |
| DU-145 | Prostate cancer | 603 | 144 | 729 | 9.3 | 52 |
| PC-3 | Prostate cancer | 201 | 144 | 603 | 2.8 | 10 |
| DND-1 | Melanoma | 89 | 120 | 193 | 2.5 | 12 |

[a] Values were determined as described in Ref. 13.

of MCF-7 breast cancer cells, melanoma, and DU-145 and PC-3 prostate cancer cells to compound **13** were 1–3 times less than **7**.

To summarize, we have described the synthesis and SAR of novel azaheterocycle carbazole sulfonamides based upon lead compound **7**. 2,6-Dimethoxypyridinyl substituted carbazole sulfonamides **13** and **14** displayed potent antiproliferative activities, slightly less active than lead compound **7**. Compound **13** showed good activities against several human tumor cell lines. Interestingly, preliminary data suggest that **14** does not phosphorylate bcl-2 as did **7.** Further studies are underway to explain the differences in the activity of pyridinyl series. In vivo efficacy studies for both **13** and **14** are ongoing.

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