Bioorganic & Medicinal Chemistry Letters 19 (2009) 3078–3080

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel carbazole and acyl-indole antimitotics

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ARTICLE INFO

Article history:
Received 16 January 2009
Revised 1 April 2009
Accepted 3 April 2009
Available online 9 April 2009

Keywords:
Tubulin
Colchicine
Carbazole
Oncology
Cancer
Antiproliferative
Antimitotic

ABSTRACT

In the course of our Heat Shock 90 program, certain carbazole compounds were identified which had an off-target antiproliferative activity. To understand the off-target activity, we studied one analog with strong activity. We discovered that it had an effect on tubulin polymerization kinetics and was competitive with colchicine. Additional analogs were made, and a number of potent compounds were identified.

Microtubule polymerization is an important cellular process and interruption of this process can lead to cell death by apoptosis. Tubulin interactive agents have been shown to have considerable value as cancer chemotherapeutics in clinical practice, with the drug paclitaxel achieving peak sales in 2000 of $1.6 billion.[1] Researchers have characterized three distinct sites toward which one can direct small molecule tubule disrupters: the taxane site; the vincamycin site; and the colchicine site. Colchicine (**1a**) is less complex and much lower in molecular weight than either vincamycin or taxane, so it is not surprising that it has captured the imagination of medicinal chemists.[2] A number of structurally distinct lead series are under study (Fig. 1). This Letter reports the discovery and optimization of a new series of colchicine-competitive antimitotics based on a screening lead (**5a**) from our compound library.

Benzamide **5a** was prepared as part of our Heat Shock Protein 90 (HSP90) program, and, upon ring substitution, we identified a number of analogs with amino-substitution *ortho* to the carboxamide which displayed HSP90 activity in our assays (e.g., **5e**, Table 1).[3] In the course of the program, to our surprise, we also identified a number of compounds (e.g., **5h**) which potently inhibited cellular proliferation against a variety of cancer cell lines in the absence of either demonstrable in vitro HSP90 activity or measurable affinity towards against any specific molecular target identifiable in purine proteome mining experiments.[4] Out of a desire to identify the off-target activity, we selected our most active analog, the urea **6a**, for detailed biological study.

Using high content cellular analysis (HCA) assays it was determined that treatment of cells with **6a** led to blockage of the G2/M progression in the cell cycle; increased phospho-Histone 3(ser10) levels; and elicited a strong apoptotic response, reminiscent of small molecule tubulin polymerization disrupting compounds such as paclixatol and vinblastine.[5] Capitalizing on this observation, **6a** was tested in tubulin polymerization assays, and was shown to disrupt polymerization with kinetics similar to vinblastine (**6a** IC_{50} = 1.64 ± 0.72 μM; vinblastine IC_{50} = 1.15 ± 0.36 μM). HCA also demonstrated that the resulting cellular phenotype of **6a**-treated cells closely matched that which was routinely observed

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doi:10.1016/j.bmcl.2009.04.010

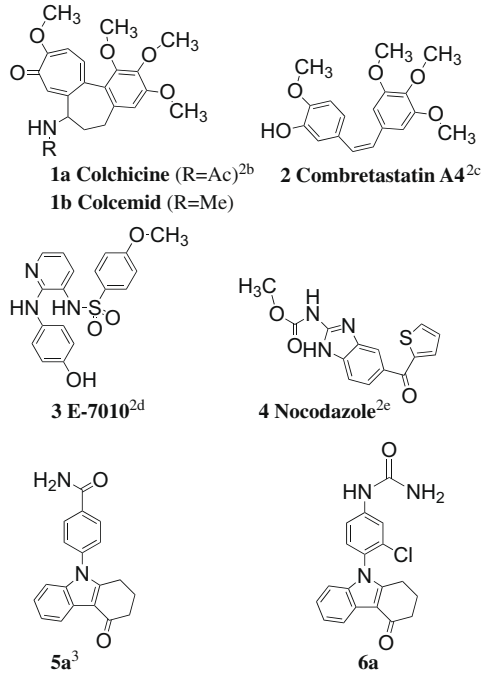
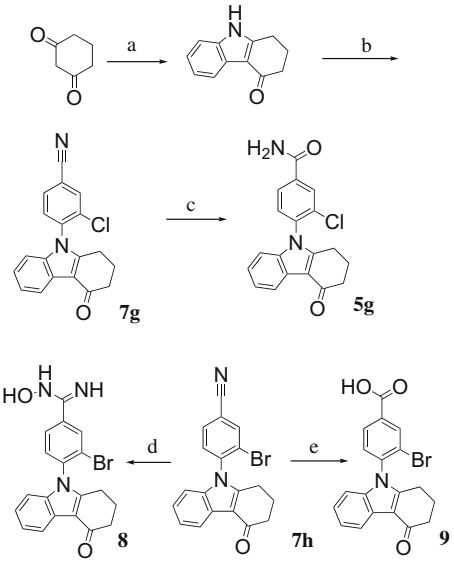
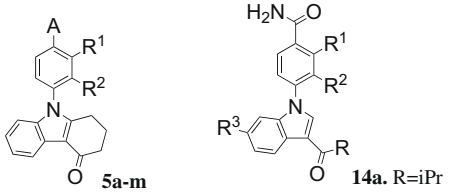


Figure 1. Colchicine competitive tubulin inhibitors.



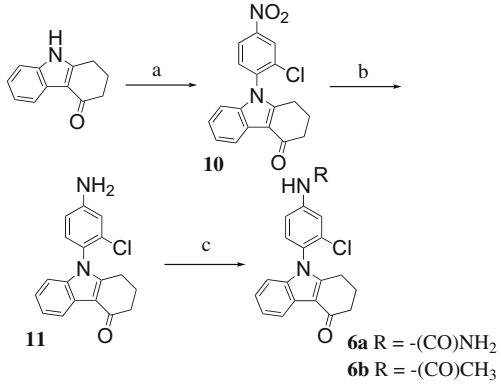
Scheme 1. Synthesis of substituted carbazoles. Reagents and conditions: (a) phenylhydrazine (0.83 equiv), TFA, 140 °C (microwave; very high absorbance), 600 s, 34%; (b) 3-chloro-4-fluorobenzonitrile (1.4 equiv), NaH (2 equiv), 0 °C, 5 min, then 50 °C for 1 h, 59%; (c) DMSO, EtOH, KOH (~5 equiv), 30% aq H_2O_2 (~5 equiv), 15 min, 41%; (d) hydroxylamine, TEA, MeOH; (e) HCl, THF, H_2O.

Table 1
Mitotic block results[6,7]



Compd	A	R^1	R^2	M-block (micromolar)
5a[a]	$-CONH_2$	–H	–H	>10
5b[a]	$-CONH_2$	–OMe	–H	>10
5c[a]	$-CONH_2$	–Br	–H	>10
5d[a]	$-CONH_2$	–NHbenzyl	–H	6.2
5e[a]	$-CONH_2$	–N-allyl	–H	0.99
5f	$-CONH_2$	–H	–F	~5
5g	$-CONH_2$	–H	–Cl	0.42
5h	$-CONH_2$	–H	–Br	0.15
5i	$-CONH_2$	–H	–OMe	0.42
5j	$-CONH_2$	–H	–Ph	>10
5k	$-CONH_2$	–H	–Me	0.96
5l	$-CONH_2$	–H	$-CF_3$	0.24
5m	$-CONH_2$	–H	$-NH_2$	~10
7l	–CN	–H	–CF3	>10
8	–CNHNHOH	–H	–Br	~2
9	–COOH	–H	–Br	>10
6a	$-NHCONH_2$	–H	–Cl	0.12
6b	$-NHCOCH_3$	–H	–Cl	0.41
14a[b]	$-CONH_2$	–H	–Cl	~5
14b[b]	$-CONH_2$	–H	–Cl	0.14
14c[b]	$-CONH_2$	–H	–OMe	0.17
14d[b]	$-CONH_2$	–OMe	–H	0.10
14e[b]	$-CONH_2$	–H	–Cl	0.21
1a	Colcemid			0.043

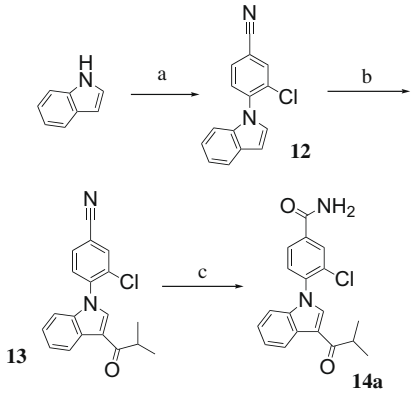
[a] These compounds reported in Ref. 3.
[b] **14a–d** R^3 = –H; **14e** R^3 = –Cl.



Scheme 2. Preparation of **6a,b**. Reagents and conditions: (a) 3-chloro-4-fluoronitrobenzene (1.33 equiv), Cs_2CO_3 (1.33 equiv), DMF, 45–65 °C, ~20 h, 72%; (b) 10% Pd/C H_2, EtOAc, ~20 h 47%; (c) AcOH, H_2O, KCNO (xs), 1 h, 63% for **6a**; H_3CN, pyridine, Ac_2O, for **6b**.

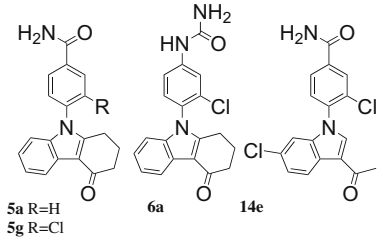
for vinblastine and colchicine treated cells. Additional testing proved that **6a** was competitive with [^{3}H]-colchicine binding to tubulin (**6a** IC_{50} = 1.61 ± 0.49 μM; colcemid (**1b**) IC_{50} = 3.58 ± 0.18 μM), but not with labeled vinblastine binding to tubulin, providing the molecular target for this compound.[5] Having established a plausible molecular target for 6a, we continued our research with the working assumption that related analogs acted by the same mechanism. We used G2/M blockage, M-block, as a convenient screening assay.[6,7]

Preparation of benzamide **5a** was straightforward (Scheme 1), requiring only three steps, and the route allowed for facile synthesis of analogs. The carbazole ring subunit was obtained using a microwave-based modification of the Fischer indole synthesis.[7] The sodium salt of the carbazole could be reacted with substituted 4-fluorobenzonitriles, such as 3-chloro-4-fluorobenzonitrile or 4-fluoro-2-methoxybenzonitrile to afford the various carbazolebenzonitriles **7a–l**, which could be isolated and hydrolyzed in basic peroxide to the desired benzamides **5a–l**.[7] Synthesis of the *ortho*-amino analogs, such as **5d**, has been previously described.[3] The hydroxyamidine **8** and the carboxylic acid **9** were prepared as shown (Scheme 1). Ana-



Scheme 3. Synthesis of acyl indole analog **14a**. Reagents and conditions: (a) 3-chloro-4-fluorobenzonitrile (1.4 equiv), NaH (1.4 equiv), DMF, 0 °C 5 min, then 45 °C for 90 min (~quant); (b) isobutyric anhydride (1.5 equiv), $Yb(OTf)_3$ (0.25 equiv), nitromethane, 50 °C, 90 min, 32%; (c) DMSO, EtOH, KOH (~5 equiv), 30% aq H_2O_2 (~5 equiv), 20 min, 65%.

Table 2
Activity[7] of selected analogs in cancer cell lines



Cancer cell line (nM)	1b	5a	5g	6a	14e
K562	16	2000	210	5.5	0.085
PC3	31	7000	140	790	2.2
MCF-7	7.8	2300	85	0.87	1.2
SW620	31	1200	94	15	6.5
HT29	25	4500	300	3.2	9.3

logs with a urea or N-acyl moieties in place of the carboxamide were accessible by starting from substituted 4-fluoro-nitrobenzenes (Scheme 2). The resulting nitrobenzene compounds were reduced to the corresponding anilines catalytically. Treatment with potassium isocyanate afforded the urea **6a**; acetic anhydride could be employed to obtain the acetamide **6b**.

Compounds incorporating simple acyl groups in the place of the carbazole cyclohexanone ring (**14a–e**) were obtained via lanthanoid salt-mediated Friedel–Crafts acylation (Scheme 3)[8] using the appropriate carboxylic acid anhydride, followed by synthetic elaboration using methods similar to what we had used with the carbazole analog.

Structure–activity trends are evident in Table 1: small lipophilic groups, like halogen, alkoxy, and alkyl, *meta*- to the carboxamide (**5f–l**) lead to analogs with potent mitotic block activity. Substitution *ortho*- to the carboxamide typically lead to inactive compounds in the carbazole series (**5b,c**), though certain small secondary amines, such as the allylamino analog, **5e**, demonstrated mitotic block activity.[6]

The carboxamide group can be replaced by other functionalities. While the nitrile (**7l**), hydroxyamide (**8**), and carboxylic acid (**9**) derivatives did not significantly block mitosis, we have already seen that the urea, **6a**, and the acetamide, **6b** are potent analogs.

We see also that a number of the acyl indole analogs **14b–e** (Scheme 3) had excellent in vitro activity, demonstrating that the rigidity of the entire carbazole ring is not necessary for potency (Table 1).

All compounds were tested in a variety of cancer cell lines.[7] Table 2 shows the antiproliferative activity of the lead compound, **5a**, and three optimized analogs in comparison to colcemid. Analogs **6a** and **14e** demonstrate superior activity to colcemid in a number of cell lines.

There are many diverse chemical structures that influence tubulin polymerization.[1] This Letter summarizes our investigations into some simple carbazoles and acyl indoles with promising biological activity which appear to be tubulin inhibitors. Further results will be presented in due course.

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