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2-Aryl-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ols as a class of antitumor agents selectively active in securin$^{-/-}$ cells

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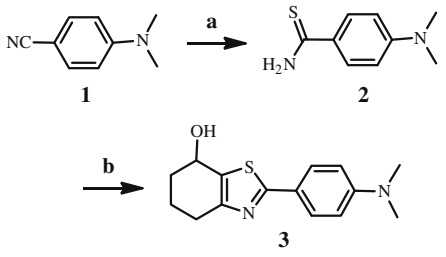
ABSTRACT

A series of 2-(4-aminophenyl)-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ols have been developed as antitumor agents that showed high selectivity against aneuploid cell lines (vs diploid cell lines). Structure–activity relationship studies showed that a hydroxymethyl group at the 2-position of the phenyl ring increased potency and selectivity. A pyrrolidinyl group at the 4-position of the phenyl ring was comparable to a dimethylamino group. The corresponding 5-aza analogs, 2-(4-aminophenyl)-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ols, retained potency and high level of selectivity against aneuploid cell growth (vs diploid cells). These 5-aza compounds exhibited higher water solubility and higher metabolic stability than the corresponding carba analogs. Compound **19** showed the highest potency against MCF-7 and MDA-MB-361 lines and was selected for further evaluation.

Chromosomal instability is a pathological process common to most solid tumors and results in chromosomal rearrangement and aneuploidy. The mitotic spindle checkpoint is the major cell-cycle regulatory mechanism controlling the transition from metaphase to anaphase and functions to maintain euploidy (diploidy). As a consequence, loss or mutation of the protein components of the mitotic spindle checkpoint, such as Mad2, Bub1, BubR1, and securin, result in chromosomal instability.[1–4] Securin regulates a proteolytic cleavage cascade that permits chromosome segregation in anaphase.[5,6] Loss of securin protein by genetic knockout results in impaired activation of the proenzyme form of separase and inhibition of this proteolytic cascade. This impairs sister chromatid separation in securin$^{-/-}$ cells, ultimately leading to the malsegregation of sister chromatids and aneuploidy.[4] Chromosomal instability allows for the amplification of oncogenes and the silencing of growth suppression genes (e.g., p53), resulting in uncontrolled cell growth.

This unregulated growth makes cancer cells more sensitive to DNA damaging agents, topoisomerase inhibitors, and microtubule polymerization inhibitors.[7] Recent evidence suggests that reliance on the unfolded protein response and dependence on protein folding chaperones makes aneuploid cells more sensitive to these inhibitors as well.[7] Since chromosomal instability is a complex cellular process and the cellular consequences are unclear, we used a cell-based screening system to identify compounds that selectively inhibited the growth of chromosomally unstable cells while sparing diploid cells. The isogenic securin$^{-/-}$ cells (clones D8 and F3) generated from the diploid HCT116 colon cancer cell line[4] demonstrate chromosomal instability with >80% of securin$^{-/-}$ cells exhibiting a loss of at least one chromosome. In this Letter, we report the discovery and the SAR of a novel series of compounds that selectively inhibit the growth of securin$^{-/-}$ cells and other aneuploid cell types, while permitting the growth of securin wild type cells and other diploid cell types.

The synthesis of 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ol (**3**) is shown in Scheme 1. Starting from 4-(dimethylamino)benzonitrile (**1**), treatment with P_2S_5 led to 4-(dimethylamino)thiobenzamide (**2**). Cyclization with 7-oxabicy-



Scheme 1. Synthesis of 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ol (**3**). Reagents and conditions: (a) P_2S_5 (0.5 equiv), MeOH, rt, 18 h, 70%; (b) 7-oxabicyclo[4.1.0]heptan-2-one (2 equiv), MeOH, 40 °C, 18 h, 45%.

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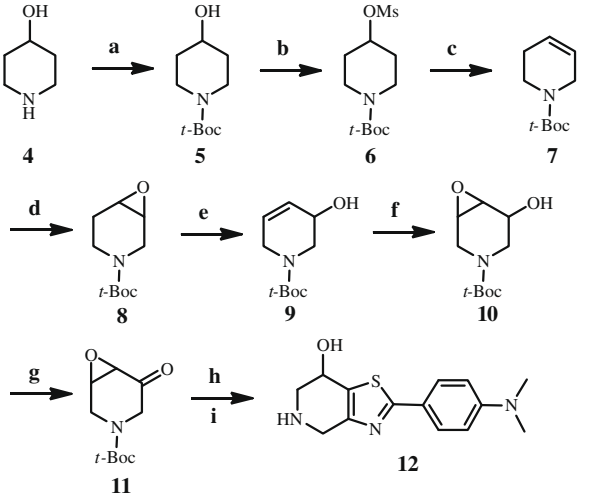
clo[4.1.0]heptan-2-one gave the desired compound **3** in racemic form. Chiral HPLC separation provided the (*R*)- and (*S*)-enantiomers of **3** (**3a** and **3b**, respectively). Analogs with R^1, R^2 substituents on the phenyl ring (Table 1) were prepared in a similar manner from the corresponding substituted benzonitriles.[8]

As shown in Scheme 2, synthesis of 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ol (**12**) started from 4-hydroxypiperidine (**4**). Protection of the amino group with *t*-Boc followed by mesylation and subsequent elimination gave 3,6-dihydro-1(2*H*)-pyridine (**7**). Epoxidation followed by ring-opening and elimination led to 3-hydroxy-3,6-dihydro-1(2*H*)-pyridine (**9**).[9] Epoxidation of **9**, followed by oxidation of the hydroxyl group in intermediate **10**, provided 5-oxo-7-oxa-3-azabicyclo[4.1.0]heptane (**11**), which was coupled with 4-(dimethylamino)thiobenzamide (**2**). Removal of the *t*-Boc group gave the desired final compound 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ol (**12**). Analogs with R^1, R^2 substituents on the phenyl ring (Table 2) were prepared in a similar manner from the corresponding substituted benzonitriles.[10]

As shown in Table 1, (*R*)-2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ol (**3a**) exhibited high selectivity in inhibiting aneuploid (vs diploid) cell growth.[11,12] Its (*S*)-enantiomer (**3b**), however, was much less potent in the aneuploid cell lines, suggesting a very specific protein binding site, with preference for the (*R*)-configuration of the hydroxyl group. Further biological studies are needed to identify this site in cells. Addition of a methyl group at the 2-position on the phenyl ring increased potency in aneuploid cell lines (**13** vs **3** and **13a** vs **3a**), while a hydroxylmethyl group at the same position further increased potency in aneuploid cell lines (**15** vs **13** and **3**; **16** vs **14**). A pyrrolidinyl group at the 4-position on the phenyl ring seemed to afford higher potency in aneuploid cell lines (**16** vs **15**).

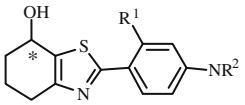
While 2-(4-aminophenyl)-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ols showed high potency (against aneuploid cell lines) and selectivity (aneuploid cell lines vs. diploid cell lines), they tended to have low water-solubility and metabolic stability (see below). The 5-aza analogs (**12**, **17**–**19**) were prepared to increase polarity in hope of improving water-solubility and possibly metabolic stability. To our delight, these 5-aza analogs were able to retain the potency (against aneuploid cell lines) and selectivity (vs diploid cell lines). Compound **19** proved to be the most potent against MCF-7 and MDA-MB-361 lines, and highly selective.[11,12] As we expected, these 5-aza compounds exhibited much higher solubility in water at pH 7.4 (20 to >100 μg/mL for **12**, **17**–**19**) than their carba analogs (2 to 15 μg/mL for **3**, **13**–**16**). More importantly, the 5-aza analogs showed much higher metabolic stability relative to their carba analogs. Oxidation at the 5- nitrogen was the major metabolic pathway for the 5-aza analogs, while dealkylation of the aniline group appeared to be the major metabolic pathway for the carba analogs. In nude mouse microsomes, the $t_{1/2}$ for **12**, **17**–**19** ranged from 13 to 26 min, while it was 3 min or less for **3**, **13**–**16**. Pharmacokinetic studies in mice showed that, when dosed orally as a free base at 50 mg/kg, compound **19** had a plasma level of 232 ng/mL at 1 h and 9 ng/mL at 4 h, and an oral bioavailability of 18% (36% when dosed as a HCl salt).



Scheme 2. Synthesis of 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ol (**12**). Reagents and conditions: (a) *t*-Boc_2O; (b) CH_3SO_2Cl; (c) DBU; (d) mCPBA; (e) TMSI; then DBU; (f) mCPBA (2 equiv), CH_2Cl_2, rt, 12 h, 94%; (g) Dess–Martin (1.2 equiv), CH_2Cl_2, rt, 12 h, 77%; (h) **2** (1.0 equiv), EtOH, 60 °C, 16 h, 45%; (i) 4 N HCl/dioxane, rt, 12 h, 79%.

In conclusion, we have developed 2-(4-aminophenyl)-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ol and their 5-aza analogs, 2-(4-aminophenyl)-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ols, that demonstrated high levels of selectivity against aneuploid cell growth (vs diploid cells). Introduction of the 5-aza moiety provided higher water solubility and higher metabolic stability, compared with the corresponding carba analogs. The aza-analog **19** showed the highest potency against MCF-7 and MDA-MB-361 cell lines. Owing to this high potency, accompanied by the very high selectivity observed against the securin$^{-/-}$ cells as well as the breast cancer cells, compound **19** was chosen for further biological mechanism of action studies and in vivo evaluations.

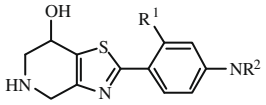
Table 1
Inhibition of cell growth by 2-(4-aminophenyl)-4,5,6,7-tetrahydro-1,3-benzothiazol-7-ols **3**, **13**–**16**



Compd	*	R^1	NR^2	IC_{50}[a] (μM)					
				HCT 116	D8	F3	MCF-10A	MCF-7	MDA-MB-361
3	Racemic	H	NMe_2	>150	1.5	3.0	>150	1.17	0.83
3a	*R*	H	NMe_2	114	0.85	1.43	>100	1.45	0.20
3b	*S*	H	NMe_2	>100	20	46	>100†	3.30†	
13	Racemic	CH_3	NMe_2	127	0.56	1.16	>100	2.15	0.16
13a	*R*	CH_3	NMe_2	>100	0.27	0.23	>100	0.73	0.083
14	Racemic	H	1-Pyrrolidinyl	>100	1.53	2.80	>100	8.70	0.20
15	Racemic	CH_2OH	NMe_2	78	0.06	0.06	>100	0.36	0.42
16	Racemic	CH_2OH	1-Pyrrolidinyl	21	0.042	0.044	17	0.056	0.032

[a] Determinations were made at 10 concentrations, in triplicate (except for † in duplicate), and repeat values agreed, on average, within 40%.

Table 2
Inhibition of cell growth by 2-[4-(dimethylamino)phenyl]-4,5,6,7-tetrahydro[1,3]thiazolo[4,5-*c*]pyridin-7-ols **12**, **17**–**19**



Compd	R^1	NR^2	IC_{50}[a] (μM)					
			HCT 116	D8	F3	MCF-10A	MCF-7	MDA-MB-361
12	H	NMe_2	121	1.33	1.63	70.2	1.60	0.17
17	H	1-Pyrrolidinyl	25	0.73	1.07	27.8	1.53	0.14
18	CH_2OH	NMe_2	>100	0.10	0.1	70	0.031	0.022
19	CH_2OH	1-Pyrrolidinyl	>100	0.07	0.06	>100	0.013	0.020

[a] Determinations were made at 10 concentrations, in triplicate, and repeat values agreed, on average, within 40%.

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8. Further synthetic details on this series can be found in Zhang, N., et al. US Patent Application 2009/02,70,363.
9. Compound **9** can also be obtained from commercial sources.
10. Further synthetic details on this series can be found in Zhang, N., et al. US Patent Application 2009/02,70,447.
11. *Cell culture*: Cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 50 μg/ml gentamicin at 37 °C in a humidified incubator, under 5% CO_2. HCT116 securin$^{+/+}$ and the securin$^{-/-}$ cell lines D8 and F3 were obtained from Prof. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). Aneuploid breast cancer cell lines MDA-MB-361 and MCF-7 and the diploid breast epithelial cell line MCF-10A were obtained from the American Type Culture Collection.
12. Cell Proliferation assays: Cells were plated in 96-well tissue culture plates. The next day, dilutions of compound (final concentrations 2 nM to 200 μM) were added and cells were cultured for 2 days. Cell survival was determined using the methods described in Rabindran, S. K.; Discafani, C. M.; Rosfjord, E. C.; Baxter, M.; Floyd, M. B.; Golas, J.; Hallett, W. A.; Johnson, B. D.; Nilakantan, R.; Overbeek, E.; Reich, M. F.; Shen, R.; Shi, X.; Tsou, H. R.; Wang, Y. F.; Wissner A. *Cancer Res.* **2004**, *64*, 3958.