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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3003–3005
Bioorganic & Medicinal Chemistry Letters

2-Cyanoaminopyrimidines as a class of antitumor agents that promote tubulin polymerization

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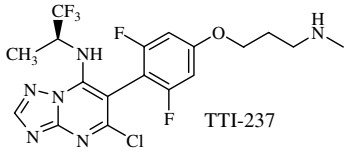
Received 31 January 2007; revised 21 March 2007; accepted 21 March 2007
Available online 25 March 2007

Abstract—A series of 4-chloro-2-cyanoamino-6-fluoroalkylamino-5-phenylpyrimidines was prepared as a result of our efforts to modify a series of [1,2,4]triazolo[1,5-*a*]pyrimidines that proved to be potent anticancer agents with a unique mechanism of tubulin inhibition. On the cyanoamino nitrogen, a methyl group is optimal for activity among alkyl groups introduced. The structure–activity relationship for the rest of the molecule resembles that of [1,2,4]triazolo[1,5-*a*]pyrimidines. A lead compound (**5**) retained in vitro potency compared with TTI-237. In the mechanism of action studies, it behaved in the same manner as TTI-237. In addition, it is also capable of overcoming multidrug resistance due to P-gp. These findings strongly suggest that this series of 2-cyanoaminopyrimidines binds at the same site and in the same binding mode as TTI-237. Further modifications of the 2-cyanoamino group are underway.

Antimitotic agents that disrupt microtubule dynamics have attracted much interest in development of anticancer therapies.[1] We recently reported a series of [1,2,4]triazolo[1,5-*a*]pyrimidines as small, synthetic anticancer agents with a unique mechanism of action in promoting tubulin polymerization.[2] This series of compounds promotes tubulin polymerization in vitro, but does not bind competitively with paclitaxel. The lead compound of this series, TTI-237, is currently in Phase I clinical trials. As a subsequent effort to modify the [1,2,4]triazolo[1,5-*a*]pyrimidine core, we developed a new series of compounds, 2-cyanoaminopyrimidines, that retained in vitro potency and the unique mechanism of action observed with [1,2,4]triazolo[1,5-*a*]pyrimidines. We now report the synthesis, SAR, and mechanism of action studies for this series of compounds (Schemes 1 and 2).



TTI-237

Starting from 5-chloro-6-(2,4,6-trifluorophenyl)-*N*-(2,2,2-trifluoroethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine **1**,[2] treatment with 1 equivalent of sodium hydride in the presence of an excess amount of iodomethane in DMSO led to an isomeric mixture of mono-methylated compounds.[3] These isomers were separated and their structures were elucidated with extensive NMR studies. One of the methylated compounds, *N*-[5-chloro-3-methyl-6-(2,4,6-trifluorophenyl)[1,2,4]triazolo[1,5-*a*]pyrimidin-7(3*H*)-ylidene]-2,2,2-trifluoroethanamine (**2**), was further treated with 1 equiv sodium hydride in DMSO. It underwent a ring-opening reaction to afford 4-chloro-6-[(2,2,2-trifluoroethyl)amino]-5-(2,4,6-trifluorophenyl)pyrimidin-2-yl(methyl)cyanamide (**3a**).[4] Compounds **3b** and **3c** were prepared analogously, using iodoethane and allyl bromide instead of iodomethane.

As in the case of [1,2,4]triazolo[1,5-*a*]pyrimidines,[2] the 4-fluoro group can be replaced in the presence of the

Keywords: 2-Cyanoaminopyrimidines; Antitumor agents; Tubulin polymerization.
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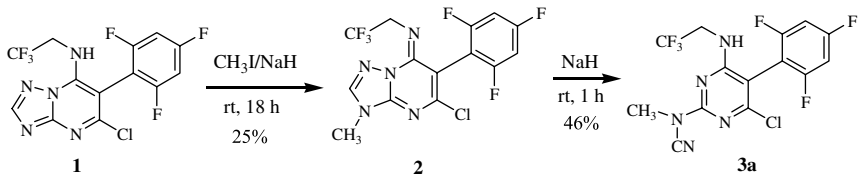
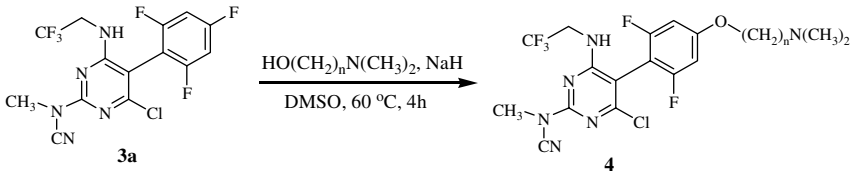
doi:10.1016/j.bmcl.2007.03.070



Scheme 1. Synthesis of 2-cyanoaminopyrimidines.



Scheme 2. Replacement of the 4-fluoro group.

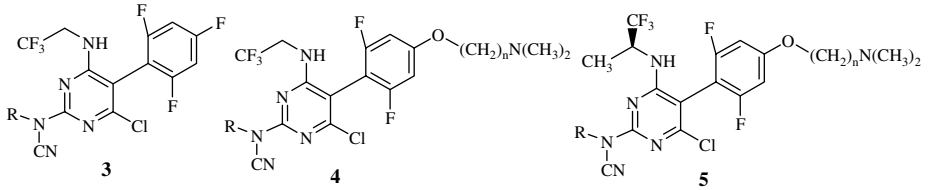
2- and 6-fluoro groups. Thus, treatment of compound **3a** with amino alcohols, in the presence of sodium hydride in DMSO, afforded compound **4** exclusively. Compound **5** was prepared analogously, using (1*S*)-2,2,2-trifluoro-1-methylethylamine[2] instead of 2,2,2-trifluoroethylamine.

These compounds were evaluated in the COLO 205 cytotoxicity assay,[2] and their IC_{50} values are shown in Table 1. Increasing the carbon chain length of the alkyl group on the cyanoamino nitrogen led to decrease in potency (**3a** vs **3b** and **3c**). The structure–activity relationship for the rest of the molecule mimics that of [1,2,4]triazolo[1,5-*a*]pyrimidines.[2] Replacing the 4-fluoro group with 3-dimethylaminopropoxy group resulted in a 4-fold increase in potency (**3a** vs **4a**). A three-methylene tether is optimal for activity. Compound **4b**, with a two-methylene tether, is much less potent. Compound **4c**, with a four-methylene tether, is less potent than **4a**, but more potent than **4b**. A possible explanation of this result is that with a flexible four-methylene tether, the dimethylamino group in **4c** can still manage to form the interaction as in **4a**. However, with a shorter two-methylene tether, the dimethylamino group in **4b** has a much harder time to form the same interaction. Replacing the achiral 2,2,2-trifluoroethylamino group with (1*S*)-2,2,2-trifluoro-1-methylethylamino group provided the best potency. The lead compound in this series (**5**) is comparable to TTI-237 in terms of cellular potency.

Compound **5** was selected to evaluate its ability to overcome resistance due to multidrug resistance (MDR) in the KB lines.[5] The results are shown in Table 2. Compound **5** is comparable to TTI-237 in the sensitive KB line and in the clinically more relevant KB 8.5 line. It showed much lower levels of recognition by P-gp compared with paclitaxel and vincristine.

Compound **5** shows a favorable profile as a pharmaceutical agent. It has good water solubility (>100 μg/mL at pH 7.4) and high microsomal stability (92% remaining after incubation in nude mouse microsomes for 30 min). Mechanistic studies[6] showed that compound **5**

Table 1. Inhibition of COLO 205 cell proliferation by compounds **3**–**5**



Compound	R	*n*	IC_{50}[a] (nM)
3a	CH_3	—	214
3b	CH_2CH_3	—	304
3c	$CH_2CH{=}CH_2$	—	477
4a	CH_3	3	52
4b	CH_3	2	532
4c	CH_3	4	229
5	CH_3	3	36
TTI-237[b]			31
Paclitaxel			3.3
Vincristine			2.6

[a] Determinations were made at 10 concentrations, in triplicate, and repeat values agreed, on average, within 40%.
[b] Tested as the HCl salt.

Table 2. Inhibition of KB, KB 8.5, and KB V1 cell proliferation by compound **5**

Compound	IC_{50}[a] (nM)			Ratio[b]	
	KB	KB 8.5	KB V1	KB 8.5	KB V1
5	24	59	82	2.5	3.5
TTI-237[c]	23	67	953	2.9	41.5
Paclitaxel	2.45	26	2013	11	822
Vincristine	2.2	58	2035	26	925

[a] Determinations were made at 10 concentrations, in duplicate, and repeat values agreed, on average, within 10%.
[b] Ratio = IC_{50} on KB 8.5 or KB V1 cells/IC_{50} on KB cells.
[c] Tested as the HCl salt.

behaved similarly to TTI-237. It promotes polymerization of either MAP-rich tubulin or pure tubulin in vitro. In tubulin binding studies,[6] it competes with [^{3}H]vinblastine, but does not competes with [^{3}H]colchicine or [^{3}H]paclitaxel. These findings, together with the similarity observed in the SAR for the [1,2,4]triazolo-[1,5-*a*]pyrimidine series and the 2-cyanoaminopyrimidine series, strongly suggest that these two series of compounds bind at the same binding site on tubulin and in the same orientation.

The 2-cyanoaminopyrimidine series shows the potential of 2-substituted pyrimidines to mimic the [1,2,4]triazolo-[1,5-*a*]pyrimidine core in the TTI-237 series. We are following this lead by introducing various aryl and heteroaryl groups to the 2-position on the pyrimidine ring.

We have developed a series of 2-cyanoaminopyrimidines[7] as a result of our efforts to modify a series of [1,2,4]triazolo[1,5-*a*]pyrimidines as anticancer agents. The structure–activity relationship for this 2-cyanoaminopyrimidine series mimics that of the [1,2,4]triazolo-[1,5-*a*]pyrimidine series. Compared with TTI-237, the lead compound (**5**) retains in vitro activity and the capability to overcome multidrug resistance due to P-gp. Mechanism of action studies showed that it behaved in the same manner as TTI-237. These findings strongly suggest that this series of 2-cyanoaminopyrimidines binds at the same site and in the same binding mode as TTI-237. Further modifications of the 2-cyanoamino group are underway.

Acknowledgment

The authors gratefully thank Dr. Joseph Ashcroft for structural elucidations of regioisomers through extensive NMR studies.

References and notes

1. Zhou, J.; Giannakakou, P. *Curr. Med. Chem. Anti-Canc. Agents* **2005**, *5*, 65.
2. Zhang, N.; Ayral-Kaloustian, S.; Nguyen, T.; Afragola, J.; Hernandez, R.; Lucas, J.; Gibbons, J.; Beyer, C. *J. Med. Chem.* **2007**, *50*, 319.
3. In addition to compound **2**, the other two isomers isolated are compounds with methylation at the *N*-4 and the 7-anilino nitrogen of compound **1**. All these three monomethylated compounds showed decreased potency in the COLO 205 assay relative to compound **1**.
4. A one-step conversion of [1,2,4]triazolo[1,5-*a*]pyrimidines to 2-cyanoaminopyrimidines with iodomethane and 1 equivalent of sodium hydride was reported in Pees, K.-J.; Pfrengle, W.; Heffernan, G. WO200196314A1; *Chem. Abstr*. 2001, *136*, 53756. We found that our stepwise conversion provided much higher yields in our hands.
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6. Detailed description of the mechanism study has been provided in the Supporting Information section in Ref. 2.
7. ^{1}H NMR spectra of selected compounds are as follows. Compound **2** ($CDCl_3$): δ 3.67 (s, 3H), 4.56 (q, J = 7 Hz, 2H), 6.70 (m, 2H), 7.86 (s, 1H). Compound **3a** ($CDCl_3$): δ 3.47 (s, 3H), 4.19 (m, 2H), 4.83 (br s, 1H), 6.88 (m, 2H). Compound **5** ($CDCl_3$): δ 1.33 (d, J = 7 Hz, 3H), 1.99 (m, 2H), 2.27, (s, 6H), 2.46 (t, J = 7 Hz, 2H), 3.45 (s, 3H), 4.06 (t, J = 7 Hz, 2H), 5.06 (m, 1H), 6.63 (m, 2H).