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2-Substituted Paullones: CDK1/Cyclin B-Inhibiting Property and In Vitro Antiproliferative Activity

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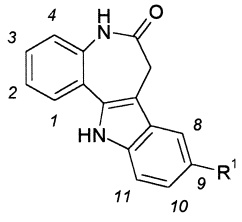
Abstract—9-Trifluoromethyl-paullones with a carbon chain in the 2-position were synthesized by palladium-catalyzed coupling reactions of a 2-iodoprecursor with terminal alkenes or alkynes, respectively. The introduction of a 2-cyanoethyl substituent led to a significant enhancement of CDK1/cyclin B inhibiting property and in vitro antiproliferative activity.

The cyclin-dependent kinases (CDKs) are a family of enzymes involved in cell cycle progression regulation.[1] In a wide variety of human tumors and tumor cell lines CDK-related mechanisms are deregulated.[2] To approach the possibility of treating neoplastic diseases with synthetic CDK-inhibiting molecules, several structural classes of CDK inhibitors have been developed.[3] The CDK inhibitor flavopiridol is currently undergoing clinical trials as an anticancer drug.[4] The paullones, a series of indolo[3,2-*d*][1]benzazepines, have recently been described as a novel class of small CDK-inhibiting molecules related to the parent structure kenpaullone **1**.[5,6] Previous structure–activity relationship investigations revealed, that the 9-trifluoromethyl-paullone **2** is equivalent to kenpaullone **1** with respect to CDK1 inhibition. However, both **1** and **2** exhibited only poor antiproliferative activity.[7] A computer model was constructed, in which kenpaullone **1** is bound to the ATP binding site of CDK2.[6] Based on this model, it was postulated that substituents in the 2-position of the paullone scaffold should be accommodated in the access channel to the ATP binding cleft. Polar terminal groups at the introduced carbon chains were envisaged to form favorable interactions with solvent molecules or amino acid side chains in the vicinity of the ATP binding site entrance. To explore this hypothesis, 9-trifluoromethyl-paullones with appropriate 2-substituents were prepared and tested for CDK1/cyclin B inhibition and in vitro antitumor activity.



1 (R^1 = Br)

2 (R^1 = CF_3)

Chemistry

The 2-iodo-9-trifluoromethyl-paullone **8** was prepared according to a method for the synthesis of similar 7,12-dihydro-indolo[3,2-*d*][1]benzazepin-6(5*H*)-ones.[8,9] Thus, 5-iodoanthranilic acid **3** was converted to the ethyl ester **4**, which was subsequently reacted with ethyl succinyl chloride to furnish the amide **5**. A Dieckmann cyclization was then accomplished by means of potassium

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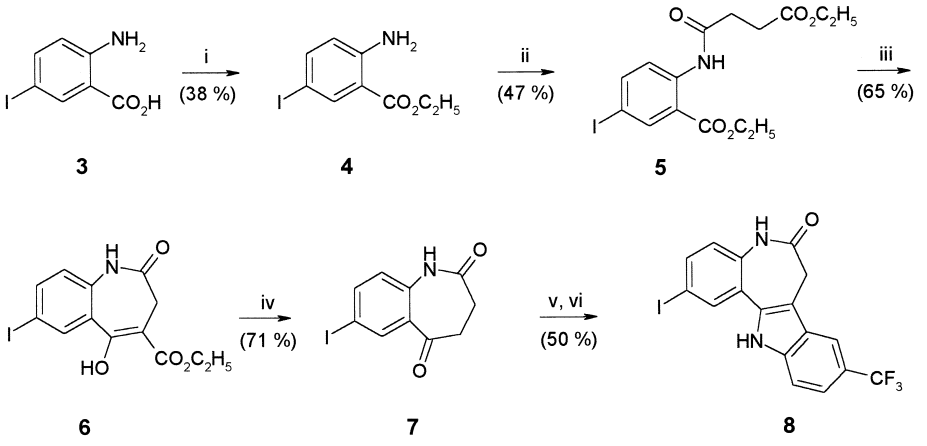
hydride, and the resulting ester **6** was dealkoxycarbonylated by heating in wet DMSO. The 7-iodo-1*H*-[1]benzazepine-2,5-(3*H*,4*H*)-dione **7** yielded the paullone **8** by a Fischer ring closure with 4-trifluoromethylphenylhydrazine (Scheme 1).

Subsequent Heck reaction of **8** with terminal alkenes under standard conditions[10] afforded the *E*-configurated 2-substituted paullones **9a–c**. The saturated analogue **11** was prepared upon refluxing the acrylonitrile derivative **9c** with magnesium turnings in methanol.[11] The reaction of terminal alkynes with **8** in the presence of cuprous iodide and a palladium catalyst in triethylamine furnished the 2-alkynyl-paullones **10a** and **10b** (Scheme 2).[12]

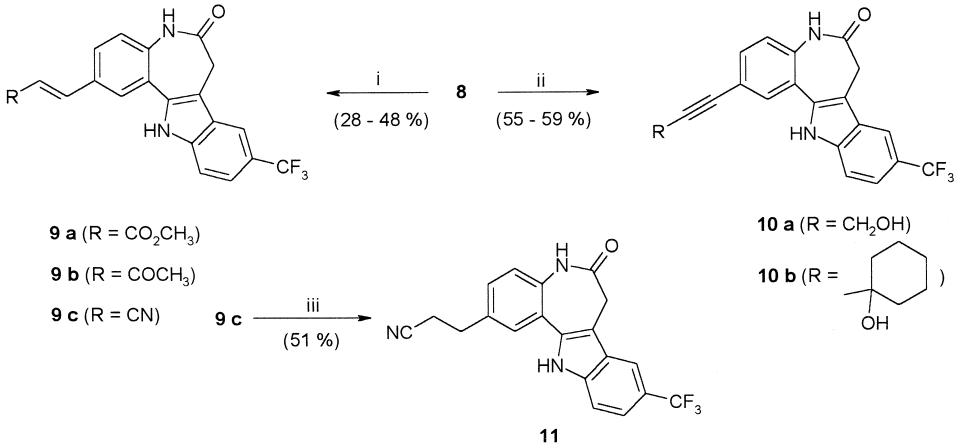
Biological Evaluation and Discussion

The paullones **8–11** were tested in a CDK1/cyclin B kinase inhibition assay described previously, using CDK1/cyclin B from starfish oocytes and histone H1 as substrate.[7] The in vitro antitumor activity was determined in the anticancer drug discovery screen of the National Cancer Institute (NCI) on 60 human tumor cell lines.[13,14] The results of this screening are processed to give an averaged parameter over all cell lines for each test compound, designated as meangraph midpoint (MGM).[15] In Table 1, the IC_{50} values for CDK1/cyclin B and the MGMs for the paullones **8–11** are reported and compared to kenpaullone **1** and its analogue **2**. Results on the colon cancer cell line HCT-116 are included, because this line proved to be especially sensitive to the paullones **1** and **2**.[7]

All newly prepared paullones turned out to be considerably active as CDK1/cyclin B inhibitors, supporting the hypothesis that larger substituents in the 2-position of paullones are tolerated. However, there were noteworthy differences in potency depending on the nature of the substituent. A loss of one order of magnitude in potency was detected for the structures **9a** and **10b** with respect to kenpaullone **1**. For **10b** the decreased potency might be accounted for by the bulky cyclohexyl group, which is kept in position by the rigid carbon chain at the paullone scaffold. Accordingly, the analogue **10a** lacking the cyclohexane ring turned out to be equipotent to **1** and **2**. To explain the low potency of



Scheme 1. Synthesis of 2-iodo-9-trifluoromethyl-paullone **8**. Reagents and conditions: (i) EtOH, gaseous HCl, Δ, 7 h; (ii) $ClCO–(CH_2)_2–CO_2C_2H_5$, toluene, pyridine, Δ, 2 h; (iii) KH, toluene, DMF, N_2, 0 °C→80 °C, 3 h; (iv) DMSO, H_2O, 150 °C, N_2, 3 h; (v) 4-trifluoromethyl-phenyl-hydrazine, AcOH, 70 °C, 1 h; (vi) H_2SO_4, AcOH, 70 °C, 1 h.



Scheme 2. Synthesis of 2-substituted paullones from **8**. Reagents and conditions: (i) R-CH=CH_2, $Pd(OAc)_2$, $P(C_6H_5)_3$, TEA, DMF, N_2, Δ, 4–14 h; (ii) R-C≡CH, $PdCl_2[P(C_6H_5)_3]_2$, CuI, TEA, N_2, 50 °C, 1–5 h; (iii) Mg, MeOH, Δ, 1 h.

Table 1. CDK1/cyclin B inhibition and in vitro antitumor activity

Compound	mp (°C)	IC_{50} CDK1/cyclin B (μM)[b]	$\log_{10} GI_{50}$[a]	
			HCT-116 (M)[c,d]	MGM (M)[d,e]
1	>330 (1,4-dioxane)	0.4	−5.7/−5.7	−4.4/−4.3
2	>330 (EtOH)	0.4	−5.4	−4.1
8	>330 (EtOH)	0.7	>−4.0	−4.07
9a	>330 (EtOH/toluene)	4.3	NA[f]	−4.02
9b	>330 (EtOH)	0.32	−5.9/−5.8[g]	−5.6/−5.3[g]
9c	>330 (EtOH)	0.27	>−4.0	−4.08
10a	>330 (EtOH)[h]	0.3	−5.5/−5.6	−4.9/−4.8
10b	>330 (EtOH)[i]	3.2	−5.6/−5.7	−5.7/−5.6
11	286 (dec.; EtOH)	0.047	−6.1/−5.7	−5.7/−5.6

[a]GI_{50} = concentration for 50% growth inhibition. The highest concentration used in the in vitro antitumor tests was 10^{-4} M.
[b]Tests were carried out in triplicate.
[c]Colon cancer cell line.
[d]Results of two test runs are separated by a slash.
[e]MGM = meangraph midpoint.
[f]NA = not available.
[g]One set of test results omitted due to high deviation.
[h]Decomposition starting at 300 °C.
[i]Decomposition starting at 275 °C.

9a with respect to **9b**, a preliminary computer model was constructed for the CDK-binding of both compounds showing that the vinyl ketone's end methyl group of **9b** was within the favorable binding region of the pocket, whereas the vinyl ester's end methyl group of **9a** protrudes into the solvent front to about 1 Å outside the binding site. Thus, the larger entropic penalty conferred by the slightly bulkier ester may account for the diminished potency of **9a**. In a similar model, the cyano nitrogen's lone pair of **9c** was favorably extended into the solvent. The remarkable increase in CDK1/cyclin B inhibitory potency of the saturated analogue **11** with regard to **9c** might either be the result of the higher flexibility of the side chain or of the lost conjugation between the cyano group and the paullone ring system. As far as 2-substituted paullones of the present study are concerned, the in vitro antitumor activity is not paralleling the CDK1 inhibitory property. Hence, the acrylonitrile-substituted derivative **9c** is nearly inactive as antiproliferative agent, although it exhibits CDK1 inhibition in submicromolar concentrations. On the other hand, **10b** shows lower CDK1 inhibition compared to **9c**, but remarkable antitumor activity. The 2-cyanoethyl derivative **11** satisfies the main rationale of this study, being a potent CDKl inhibitor as well as an interesting in vitro antitumor agent. In conclusion, the 2-substitution of the paullones appears to be a promising strategy for the development of antiproliferative agents. However, in the course of future studies it should be taken into consideration that other targets besides the CDKs might account for the antiproliferative activity of the paullones.

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