

Benzodipyrazoles: a new class of potent CDK2 inhibitors

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Received 21 October 2004; revised 10 January 2005; accepted 12 January 2005

Abstract—The synthesis and the preliminary expansion of this new class of CDK2 inhibitors are presented. The synthesis was accomplished using a solution-phase protocol amenable to rapid parallel expansion and suitable to be scaled-up in view of possible lead development. Following a medicinal chemistry program aimed at improving cell permeability and selectivity, a series of compounds with nanomolar activity in the biochemical assay and able to efficiently inhibit tumor cell proliferation has been obtained. © 2005 Elsevier Ltd. All rights reserved.

1. Introduction

Recent discoveries in diverse fields of biomedical research have elucidated the molecular basis of cell cycle control and have shown how alterations to this homeostatic mechanism play an important role in cancer development.^{1,2} Cyclin-dependent kinase 2 (CDK2) in complex with cyclins E and/or A is a key cell cycle regulator and continues to be an attractive target for the discovery of new anti-tumor agents.³ In particular, inhibitors of CDK2/cyclin A/E have already progressed into clinical trials with encouraging early results.^{4,5} Benzodipyrazoles (BDPs) as CDK2 inhibitors originated from high throughput screening of our internal chemical collection. The hit compound of this class (**1**, Fig. 1), a tetrahydro-benzodipyrazole (TH-BDP) derivative bearing a sulfamidophenyl moiety was found to possess a remarkable activity on the CDK2/cyclinA complex (IC₅₀ = 7 nM) and to be an ATP competitive inhibitor.

The crystal structure of CDK2/cyclin A in complex with the compound **1** was determined (Fig. 2). The pyrazole ring A, the carboxamido and the sulfamido groups are

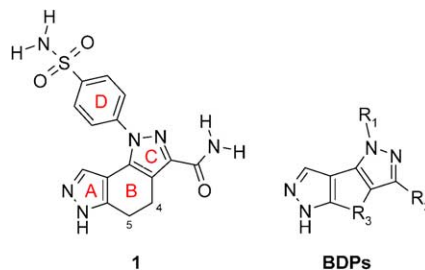


Figure 1. The structure of **1** and the general formula of BDPs.

the key features for binding of the ligand to the adenine, phosphate and ribose regions of the ATP pocket, respectively.

In spite of the notable potency in the biochemical assay, the anti-proliferative activity of this compound, measured as inhibition of the A2780 ovarian tumor cell line, was disappointing (IC₅₀ > 20 μM). This is likely due to the presence of both the sulfamoyl and carbamoyl moieties which, although important for binding, may prevent this compound from penetrating cells efficiently. A medicinal chemistry program was therefore started with the aim to improve cell permeability, while maintaining activity on CDK2/cyclin A/E in the nanomolar range.

Keywords: CDK2; Cyclins; Kinase selectivity; Tumor cell proliferation inhibition.

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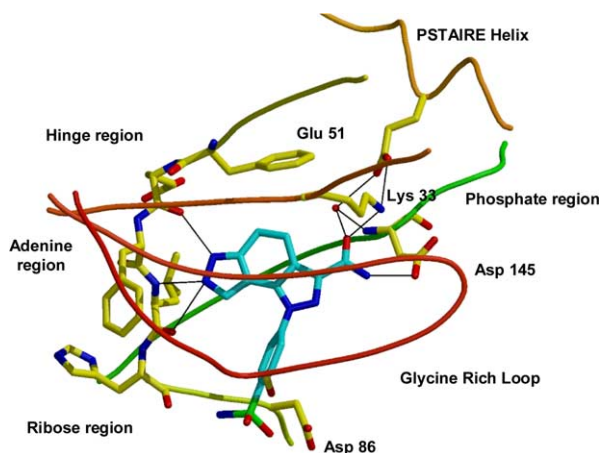


Figure 2. The crystal structure of CDK2/cyclin A in complex with compound **1**.

2. Chemistry

The synthetic methodology used to produce this class of compounds (**Scheme 1**) started from the commercially available cyclohexanedione that was condensed with *N,N*-dimethylformamide dimethyl acetal to obtain the adduct **2**.⁶

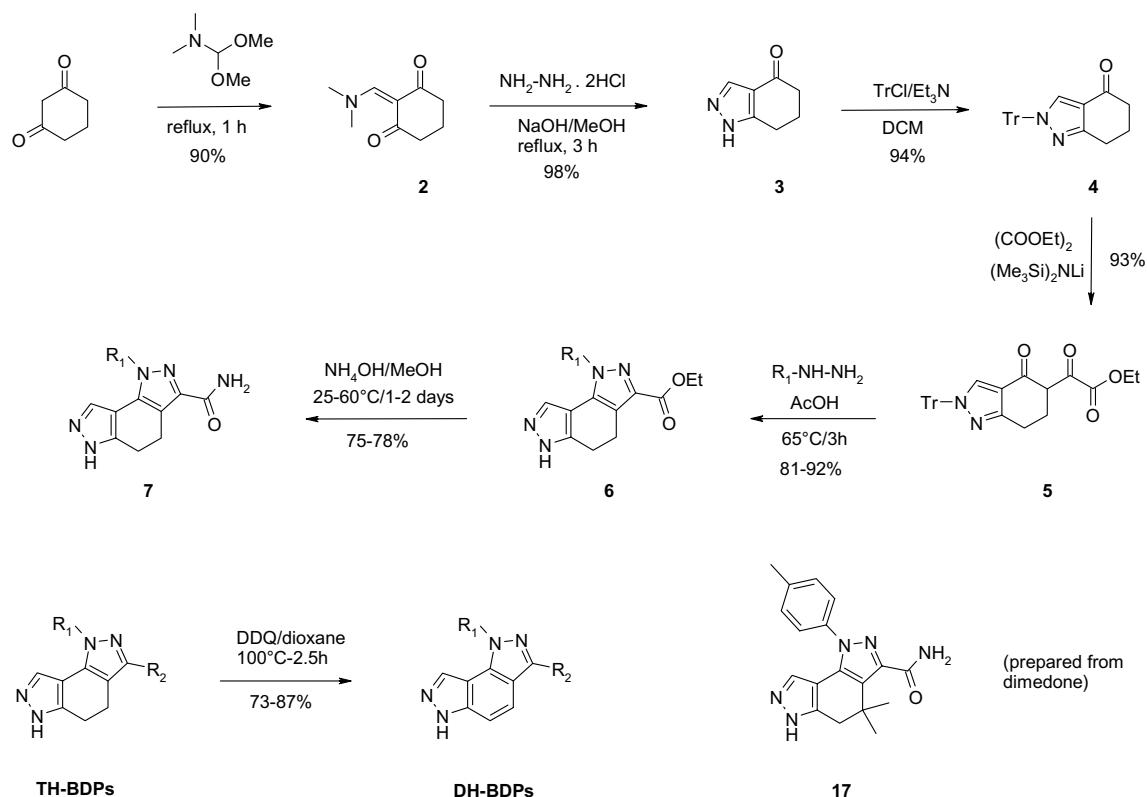
In the second step, the adduct **2** was reacted with hydrazine dihydrochloride to obtain the cyclic intermediate **3**, that was then protected with trityl chloride to give the intermediate **4**. The use of trityl as the protecting group

for ring A was particularly profitable, allowing us to increase the overall yield and to reduce reaction times.

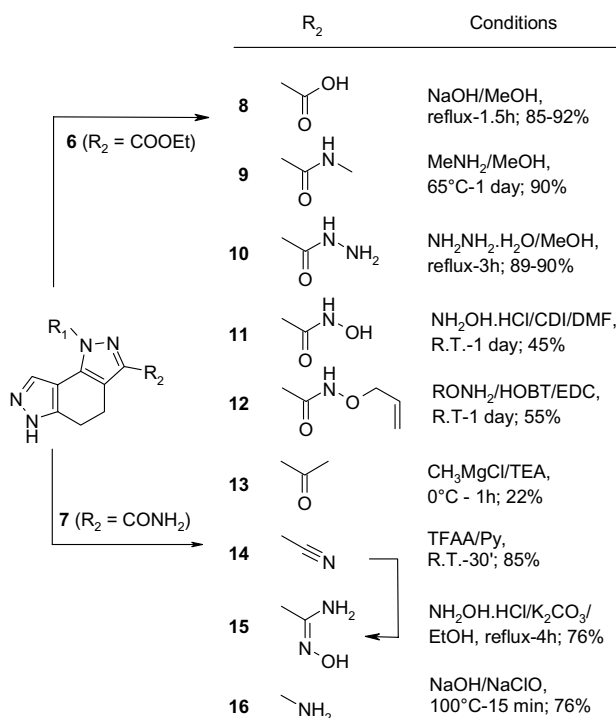
Interestingly, only one regioisomer was formed under tritylation and the protecting group was directly removed during the closure of the second pyrazole ring C. After condensation with diethyl oxalate, the diketester **5** was reacted with a suitably substituted hydrazine to form the BDP **6**. The trityl-protecting group is normally lost during the cyclization reaction if a salified form of the hydrazine is used (i.e., hydrochloride). Optionally, diluted hydrochloric acid can be added to complete the deprotection, once the cyclization has occurred. In the final step, the ester was then converted to the TH-BDP amide **7** by treatment with ammonium hydroxide in methanol. All the intermediates were crystalline and could be obtained in very good yields without chromatographic purification. The ethoxycarbonyl and aminocarbonyl groups of compounds **6** and **7** could be further manipulated to obtain compounds **8–16** as shown in **Scheme 2**.

The aromatization of the central ring B of TH-BDPs to obtain the corresponding dihydrobenzo-dipyrzole (DH-BDP) counterparts could be conveniently accomplished by the use of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (**Scheme 1**).

Finally, the preparation of the 4,4-gem-dimethyl derivative **17** (**Table 3**) was accomplished, according to the general synthetic scheme, using dimedone as a starting material.



Scheme 1. General scheme for the synthesis of TH-BDPs and DH-BDPs.

Scheme 2. Synthetic manipulations at R₂.

3. Results and discussion

Low lipophilicity and the presence of several NH bonds may contribute to the poor cell permeability of the parent compound **1**. In order to improve these features, some modifications were designed, trying to retain those functionalities directly involved in the binding to the protein as much as possible.

As a first approach, we focused our attention on R₁ that, according to X-ray data, points towards the ribose region of the ATP pocket and offered wider room for modifications. As shown in Table 1, the progressive substitution at sulfonamido (compd **7a–e**) or its replacement with other groups, while reducing the activity on CDK2, led to an increase of the anti-proliferative activity, both in TH-BDP and DH-BDP series.

Particularly, phenyl **7h** was better than benzyl **7w**, while compound **7u**, bearing a 2-pyridyl moiety was better than the phenyl derivative. Some aliphatic substituents were also explored: among them trifluoroethyl group **7y** appeared to be the most promising.

Thus, looking at the potency against CDK2/cyclin A combined with the anti-proliferative activity, the sulfonamido could be conveniently replaced by other groups. Generally, more lipophilic substituents led to compounds that were less potent on CDK2/cyclin A (due to the loss of H-bond interactions involving the

Table 1. Variations at R₁ (refer to the general formula in Fig. 1)

Compd	R ₂ = -CONH ₂ R ₁	CDK2/cyclin A IC ₅₀ (μM)		A2780 cells IC ₅₀ (μM)	
		R ₃			
		-CH ₂ -CH ₂ -	-CH=CH-	-CH ₂ -CH ₂ -	-CH=CH-
7a	-Ph-4-SO ₂ NH ₂	0.004	0.0003	>20	8.74
7b	-Ph-4-SO ₂ NHMe	0.04	0.002	>20	>20
7c	-Ph-4-SO ₂ NMe ₂	0.54	0.010	13.92	1.89
7d	-Ph-4-SO ₂ NHBu	0.21	0.002	9.76	1.25
7e	-Ph-4-SO ₂ N(CH ₂ -CH ₂) ₂ N-Me	>10	0.150		>20
7f	-Ph-4-SO ₂ Me	0.03	0.001	>20	8.81
7g	-H	0.46	0.078	18.19	3.54
7h	-Ph	0.15	0.014	5.27	3.86
7i	-Ph-4-Me	0.14	0.015	8.05	0.88
7j	-Ph-4-OMe	0.14	0.009	6.05	1.46
7k	-Ph-4-Cl	0.16	0.008	>20	2.73
7l	-Ph-4-F	0.27	0.036	8.50	1.65
7m	-Ph-4-CF ₃	0.08	0.013	>20	1.77
7n	-Ph-4-OCF ₃	0.51	0.037	>20	2.53
7o	-Ph-4-CN	0.19	0.005	>20	1.94
7p	-Ph-4-N(CH ₂ -CH ₂) ₂ O	0.56	0.018	8.75	4.07
7q	-Ph-4-(2-imidazolo)	0.25	0.075	>20	>20
7r	-Ph-3-Me	0.16	0.009	1.88	0.40
7s	-Ph-3-Cl	0.36	0.029	4.50	2.10
7t	-Ph-3-F	0.42	0.028	4.10	1.50
7u	-2-pyridyl	0.26	0.008	4.06	0.65
7v	-3-pyridyl	1.60	0.062		4.48
7w	-Bn	2.20	0.140		2.98
7x	-Me	0.29	0.018	13.45	0.58
7y	-CH ₂ -CF ₃	0.02	0.002	2.67	0.26
7z	-CH ₂ -CH ₂ -OH	1.15	0.028		0.92

Values are means of at least three experiments.

Only compd with IC₅₀ (CDK2/cyclin A) <1 μM were tested on A2780 cell line.

sulfonamido group), but more effective on cells, probably as a result of their improved permeability.

Some variations were also performed at R₂, in an attempt to conveniently replace the carboxamido group (Table 2). Primary carboxamides (**7j**, **d**, **i**) proved to be the most active derivatives with a reduction of the activity upon N-alkylation (**9d**). Esters (**6j**, **d**, **i**) were considerably less active on CDK2 than the corresponding amides, while acids (**8j**, **d**, **i**) and the ketone **16i** retained some activity, according to the binding mode of the parent compound. Among other functionalities explored, the hydroxamic acid **11j** was the most promising one, showing activity comparable to the parent amide and, at the same time, conferring higher solubility to the scaffold.

A considerable improvement on activity and selectivity was achieved through modifications of the central ring B (R₃). DH-BDPs (R₃ = –CH=CH–) were always more active than their TH-BDP counterparts (R₃ = –CH₂–CH₂–) both in terms of potency on CDK2/cyclin A complex and cytostatic activity on A2780 cell line (Tables 1 and 2). On the other hand, the higher activity of DH-BDPs corresponded to a marked reduction of their selectivity evaluated on a panel of different kinases (data not shown). It seems, therefore, that although a higher potency against CDK2/cyclin A could be obtained by

the aromatization of the central ring B, the flatter scaffold is less able to discriminate amongst the various shapes of kinase ATP pockets.

In this view, the 4,4-gem-dimethyl derivative subclass, a representative of which is shown in Table 3, has been recently designed to improve selectivity for CDK2 versus GSK3β kinase.

This has been achieved exploiting a key difference between the two kinases. Figure 3 shows the 4,4-gem-dimethyl derivative **17** docked in CDK2 and GSK3β crystal structure solved in house.⁷ The larger size of GSK3-Cys 199 compared with CDK2-Ala 144 and the nonplanar nature of the GSK3-Leu 132 as compared to CDK2-Phe 80 would be responsible for the selectivity due to gem-dimethyl clashing. As shown in Table 3, compound **17** was able to inhibit the CDK2/cyclin A complex at concentrations comparable to those of the corresponding DH-BDP **7i** without affecting GSK3β kinase.

4. Conclusions

Starting from compound **1**, a sulfonamido derivative endowed with good potency as a CDK2/cyclin A inhibitor, but devoid of anti-proliferative activity on cells, a series

Table 2. Variations at R₂ (refer to the general formula in Fig. 1)

Compd	R ₁	R ₂	CDK2/cyclin A IC ₅₀ (μM)				A2780 cells IC ₅₀ (μM)	
			R ₃					
			–CH ₂ –CH ₂ –	–CH=CH–			–CH ₂ –CH ₂ –	–CH=CH–
6j	–Ph–4–OMe	–COOEt	8.10	7.50				
7j		–CONH ₂	0.14	0.009		6.05		1.46
8j		–COOH	2.10	0.21				>20
10j		–CONHNH ₂	2.10	1.30				
11j		–CONHOH	0.69	0.029		7.50		2.82
12j		–CONHOCH ₂ CH=CH ₂	4.50					
14j		–CN		1.75				
15j	–Ph–4–SO ₂ NHBu	–C=N(OH)NH ₂		0.31				4.60
16j		–NH ₂		>10				
6d		–COOEt	10					
7d		–CONH ₂	0.21	0.002		9.76		1.25
8d		–COOH	1.20					
9d		–CONHMe	0.60	0.16		10.20		2.90
12d		–CONHOCH ₂ CH=CH ₂	2.85	0.20				8.70
6i	–Ph–4–Me	–COOEt	8.50					
7i		–CONH ₂	0.14	0.015		8.05		0.88
16i		–COMe	0.90			>20		

Values are means of at least three experiments.

Only compd with IC₅₀ (CDK2/cyclin A) <1 μM were tested on A2780 cell line.

Table 3. 4,4-gem-dimethyl series (refer to the general formula in Fig. 1)

Compd	R ₁	R ₂	R ₃	CDK2/cyclin A IC ₅₀ (μM)	GSK3β IC ₅₀ (μM)	A2780 cells IC ₅₀ (μM)
7i	–Ph–4–Me	–CONH ₂	–CH ₂ –CH ₂ –	0.140	0.460	8.05
7i			–CH=CH–	0.015	0.023	0.88
17			–CH ₂ –C(CH ₃) ₂ –	0.087	>10	1.65

Values are means of at least three experiments.

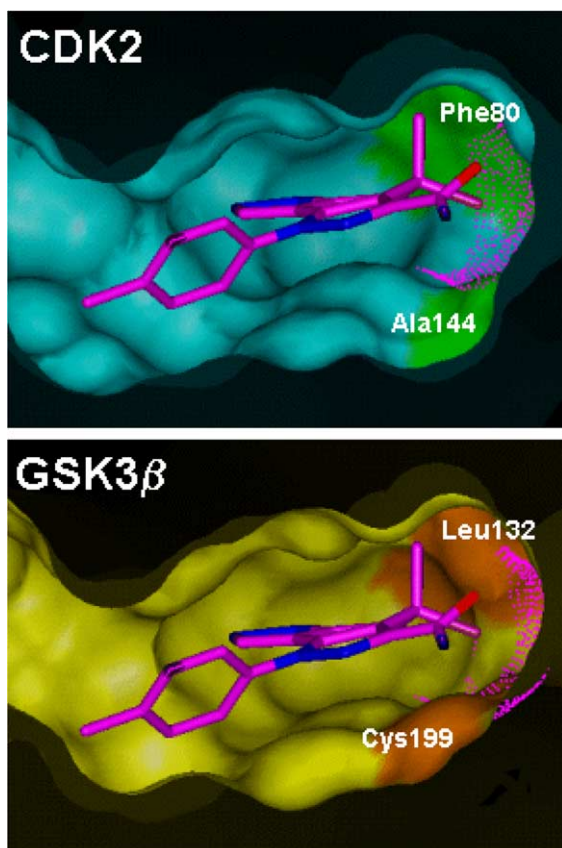


Figure 3. Compound 17 docked in CDK2 and GSK3 crystal structure.

of new derivatives was synthesized through chemical modifications designed to give the compounds both a

more reasonable lipophilicity and an improved cell permeability.

The new derivatives, still maintaining potency on CDK2/cyclin A in the nanomolar range, are able to block tumor cell growth at low micromolar concentrations. In addition, a subset of highly selective benzodipyrzoles was designed exploiting the structural diversity existing among kinase ATP pockets. Ongoing tests to assess the selectivity of this class on a wider panel of kinases and preliminary in vivo efficacy studies on tumor models will tell more about the real potential of this new class of CDK2 inhibitors as anti-cancer agents.

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