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Structure-based drug design to the discovery of new 2-aminothiazole CDK2 inhibitors

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Abstract

N-(5-Bromo-1,3-thiazol-2-yl)butanamide (compound **1**) was found active (IC_{50} = 808 nM) in a high throughput screening (HTS) for CDK2 inhibitors. By exploiting crystal structures of several complexes between CDK2 and inhibitors and applying structure-based drug design (SBDD), we rapidly discovered a very potent and selective CDK2 inhibitor 4-[(5-isopropyl-1,3-thiazol-2-yl)amino] benzenesulfonamide (compound **4**, IC_{50} = 20 nM). The syntheses, structure-based analog design, kinases inhibition data and X-ray crystallographic structures of CDK2/inhibitor complexes are reported.

Keywords: CDK2; Cyclin-dependent kinase; Ligand docking; Ligand–protein interactions; Structure-based drug design; Crystal structures

1. Introduction

Cyclin-dependent kinase 2 (CDK2) is one of the cyclin-dependent Ser/Thr kinases and plays an essential role in the cell cycle [1,2]. To date, 13 distinct CDKs and 25 different cyclin-box containing proteins have been identified from human genome sequencing [3]. Different CDK/cyclin combinations serve to regulate distinct check points in the cell division cycle. CDK2 activity is required for progression through G1 to the S phase of the cell cycle [1,2].

A large number of CDK2 crystal structures, including the apo-form and binary as well as ternary complexes, are available from the public domain. Molecules complexed with CDK2 include the co-factor ATP, various ATP-competitive inhibitors, Cyclin A and Cyclin M, p27, KAP as well as combinations thereof [4,5].

N-(5-Bromo-1,3-thiazol-2-yl)butanamide (IC_{50} CDK2/Cyclin A = 808 nM, compound **1** of Fig. 1) emerged as an active compound from a HTS screening for CDK2 inhibition. In this article, we report the syntheses, activities and crystal structures of derivatives of **1** in complex with CDK2 and we show how, following a structure-based approach, we rapidly identified more potent and selective ATP-competitive CDK2/Cyclin A/E inhibitors, i.e. **2** (IC_{50} = 192 nM) and **4** (IC_{50} = 20 nM) of Fig. 1. The potency of the thiazole class was thus improved by synthesizing, in the early phase of the drug discovery program, only few analogs.

2. Material and methods

2.1. Inhibitors docking

The docking program QXP [6] was used to dock the designed molecules in a crystal structure of CDK2/CyclinA (PDB code 1JST). QXP was employed using the docking algorithm, referred to as MCdock [7]. This algorithm allows for fully flexibility of the inhibitor and simultaneous flexibility of selected active site side-chains, which can move under the influence of the molecular mechanics force field during energy minimization. The sections designated for movement can be made fully flexible or partially flexible (using distance-based constraints). The CDK2 glycine-rich loop region (Gly11, Glu12, Gly16 and Val17) and Glu8 was treated as partially flexible, Lys33, Lys89, Tyr15 and Gln85 as completely flexible. The docking run included 1000 steps of Monte Carlo perturbation and final energy minimization. The results were

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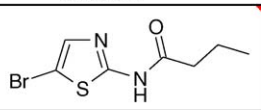
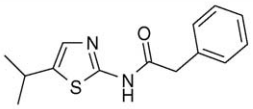
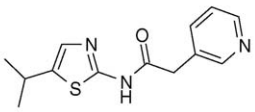
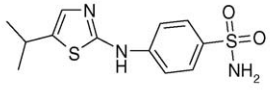
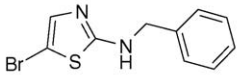
Compound	Structure	IC50 CDK2/Cyclin A (nM)
1		808
2		192
3		95
4		20
5		>1000

Fig. 1. Chemical structures of investigated thiazoles (**1–5**).

evaluated in term of total estimated binding energy, internal strain energy of the ligand, van der Waals and electrostatic interaction energies. The calculation was performed on Octane SGI Workstation.

2.2. CDK/cyclin kinase assays

The CDK2 inhibitory activity of the mentioned compounds was determined in a Scintillation Prossimity Assay (SPA, Amersham Pharmacia Biotech. Technology), as previously described [8].

2.3. Crystal structure determination

Human CDK2 for crystallization was produced and purified following a published protocol [9]. Crystals of the apo-protein were grown at 20 °C by vapour diffusion, with the hanging drop technique, mixing equal volumes of protein solution (at a protein concentration of 3 mg/ml) and reservoir solution (10% PEG3350, 50 mM ammonium acetate, 0.1 M Hepes pH 7.4).

Compounds **3** and **4** of Fig. 1 were dissolved separately in DMSO at a concentration of 50 mM. The inhibitor stock solutions were then added to the crystals' mother liquor up to a final DMSO concentration of about 15%. Crystals were soaked for up to one week and then transferred to a cryoprotectant solution (10% PEG3350, 50 mM ammonium acetate, 0.1 M Hepes pH 7.4, 25% glycerol).

Diffraction data were collected at the X-ray beamline 5.2R of the Elettra synchrotron, Trieste, Italy, using a 345 mm MAR imaging plate detector. X-ray data were processed with the DENZO/HKL [10] package and reduced with the CCP4 suite [11]. The structures were solved by difference Fourier and refined with the program REFMAC [12]. Cycles of refinement were alternated with manual rebuilding of the protein and inhibitors using the graphics program O [13]. A summary of the diffraction data and the refinement statistics are shown in Table 1.

2.4. Chemistry

Compounds **1–5** were prepared as depicted in Schemes 1–3. 2-Amino-5-isopropyl-1,3-thiazole **8b** was prepared by reacting 3-methyl-butanaldehyde with bromine, followed by thiourea [14] in 42% yield over the two steps (Scheme 1). Acylation of **8b** and of the commercially available 2-amino-5-bromo-1,3-thiazole **8a** led to compounds **1–3** [14].

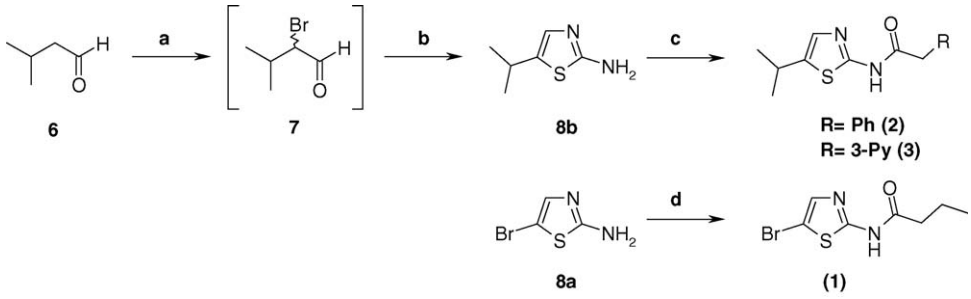
The reductive amination of **8b** (Scheme 2) with benzaldehyde, $Me_4NBH(OAc)_3$ and acetic acid in 1,2-dichloroethane provided compound **5** [15].

Compound **4** was synthesized according to Scheme 3. The 2-bromo-aldehyde 7 was reacted with 4-aminosulfonyl-phenyl thiourea, which can be easily obtained from the corresponding aniline [16].

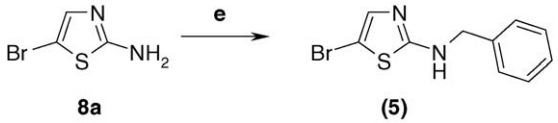
Flash chromatography was performed using silica gel (E. Merck, Grade 60, 230–400 mesh) with the eluent indicated. Melting points are uncorrected. Proton NMR spectra were recorded with Bruker (300 MHz). Less common abbreviations

Table 1
Data collection and refinement statistics for the two structures

	Compound **3**	Compound **4**
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimension (Å)	$a = 53.22$, $b = 71.61$, $c = 71.59$	$a = 53.23$, $b = 72.21$, $c = 73.27$
Resolution (Å)	1.85	2.0
No. of total reflection (unique)	87319 (23407)	68914 (19633)
Completeness (%) (last shell)	97.4 (98.4)	96.6 (90.5)
R_{merge} (%) (last shell)	5.4 (21.6)	4.2 (27.3)
I/σ (last shell)	12.2 (4.0)	19.3 (4.0)
Refinement		
Resolution range	20–1.85	20–2.0
R-factor (%) (R_{free}%)	22.6 (27.2)	21.6 (26.1)
rms bonds (Å)	0.016	0.016
rms angles (Å)	1.5	1.6
B-factor (average) (Å^2)	29.4	34.2



Scheme 1. Preparation of compounds **1**–**3**: (a) bromine, 1,4-dioxane/dichloromethane, 0 °C to room temperature, 6 h; (b) thiourea, ethanol, room temperature; (c) carboxylic acid (1.2 eq.), EDCI (1.2 eq.), dichloromethane, 0 °C to room temperature, o/n, 66% (**2**), 61% (**3**); (d) butanoyl chloride, TEA, dichlorometane, −5 °C, 2 h, 48%.



Scheme 2. Preparation of compound **5**: (e) PhCHO, acetic acid, $Me_4NBH(OAc)_3$, DCE, room temperature, 32 h, 60%.

are as follows—EDCI, *N*-ethyl-*N*-(diisopropylaminoethyl)carbodiimide; TEA, triethylamine; DCE, 1,2-dichloroethane.

2.4.1. 2-Amino-5-isopropyl-1,3-thiazole (*8b*)

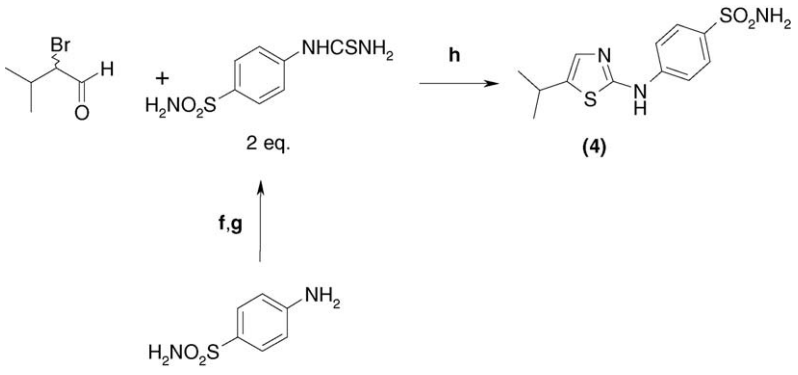
3-Methylbutanaldehyde (2 ml; 18.6 mmol) was dissolved in dichloromethane (15 ml) and treated with 2% bromine in 1,4-dioxane (47.81 ml, 18.6 mmol) drop wise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring was continued for about 2 h. Thiourea (2.83 g, 37.2 mmol) and ethanol (10 ml) were then added and the mixture was stirred at room temperature for about 6 h. The solvent was evaporated and the residue was treated with dichloromethane and extracted with 1 M hydrochloric acid. The aqueous layer was made alkaline with 30% ammonium hydroxide and extracted with dichloromethane. The combined extracts were dried (Na_2SO_4), filtered and the solvent was evaporated off under reduced pressure. The crude product was purified by column chromatography (30% EtOAc in hexane) to give **8b** as an orange oil (1.1 g, 42%): NMR (DMSO-d_6) δ ppm: 6.6 (s, 2H), 6.58 (s, 1H), 2.9 (m, 1H), 1.18 (s, 3H), 1.17 (s, 3H).

2.4.2. N-(5-Bromo-1,3-thiazol-2-yl)-butanamide (*1*)

Triethylamine (0.97 ml; 6.34 mmol) and butanoyl chloride (0.52 ml; 5.07 mmol) were added in this order to a solution of 2-amino-5-bromo-1,3-thiazole (0.76 g; 4.23 mmol) in dichloromethane (8 ml), cooled to −5 °C. The reaction mixture was stirred at −5 °C for 2 h and then warmed to room temperature. After additional 4 h, the organic layer was washed with water, saturated sodium bicarbonate, 1N hydrochloric acid and brine, dried (Na_2SO_4) and evaporated. The residue was recrystallized from cyclohexane to yield 0.45 g (48%) of the title compound as a colourless solid: mp 163–164 °C; NMR (DMSO-d_6) δ ppm: 12.20 (bs, 1H); 7.29 (m, 5H); 7.13 (s, 1H); 3.70 (s, 2H); 3.07 (m, 1H); 1.22 (d, 6H).

2.4.3. N-(5-Isopropyl-1,3-thiazol-2-yl)-phenylacetamide (*2*)

EDCI (0.53 g, 2.78 mmol) was added to a solution of phenylcetic acid (0.38 g, 2.78 mmol) in dichloromethane (5 ml) under ice cooling. After stirring for 1 h, a solution of 2-amino-5-isopropyl-1,3-thiazole (0.33 g, 2.31 mmol) and diisopropylethylamine (0.40 ml, 2.34 mmol) in dichloromethane (5 ml) was added drop wise and the mixture was kept at 0 C for 1 h, then at room temperature overnight. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The organic layer was further washed with water, 5% citric acid, water, saturated sodium bicarbonate and water. Drying over sodium sulfate and evaporation gave a solid which was triturated with isopropyl ether to give the title compound as a colourless solid (0.4 g; 66%): mp 135–137 °C;



Scheme 3. Preparation of compound **4**: (f) benzoylisothiocyanate, acetone, rt, 4 h 84%; (g) 2N NaOH, THF, reflux, 5 h, 63%; (h) 9 (2 eq.) ethanol, 20 h, 10%.

NMR (DMSO-d_6) δ ppm: 12.20 (bs, 1H, CONH); 7.29 (m, 5H); 7.13 (s, 1H); 3.70 (s, 2H); 3.07 (m, 1H); 1.22 (d, 6H); Anal. Calcd. for $C_{14}H_{16}N_2OS$: C, 64.59; H, 6.19; N, 10.76. Found: C, 64.69; H, 6.16; N, 9.96.

2.4.4. *N-(5-Isopropyl-1,3-thiazol-2-yl)-pyrid-3-yl-acetamide (**3**)*

The mp 178–180 °C (dec.); NMR (DMSO-d_6) δ ppm: 12.20 (bs, 1H); 8.45,7.7,7.35 (m, 4H); 7.17 (s, 1H); 3.78 (s, 2H); 3.14 (m, 1H); 1.22 (d, 6H); Anal. Calcd. for $C_{13}H_{15}N_3OS$: C, 59.75; H, 5.79; N, 16.08. Found: C, 60.17; H, 5.81; N, 16.27.

2.4.5. *4-Aminosulfonyl-phenyl thiourea (**9**)*

A solution of 4-aminosulfonyl-aniline (5.61 g, 32.5 mmol) in acetone (150 ml) was reacted with benzoylisothiocyanate (4.95 g, 30 mmol) at room temperature for about 4 h. The resulting solid was filtered (9.2 g, 26.85 mmol, 84%) and treated with 2N NaOH (29.4 ml, 59.1 mmol) in THF (120 ml). The mixture was refluxed for about 6 h and allowed to warm to room temperature. The solvent was evaporated off under vacuum. The residue was diluted with water (20 ml) and neutralized to pH 7 with 1N HCl. The resulting solid was filtered and dried under vacuum to afford (**9**) as a colourless solid (1.7 g, 63%): mp 212–213 °C.

2.4.6. *2-[(4-Aminosulfonyl)-phenylamino]-5-isopropyl-1,3-thiazole (**4**)*

Thiourea (**9**) (2.2 g, 9.3 mmol) and ethanol (10 ml) were added to a solution of 1,4-dioxane (5 ml) containing (**7**) (4.65 mmol); the mixture was stirred at room temperature for about 24 h. The solvents were evaporated off under vacuum and the crude material was chromatographed (dichloromethane; then 3% methanol in dichloromethane) to afford (**4**) as a colourless powder (0.09 g, 7%): mp 182–183 °C; NMR (DMSO-d_6) δ ppm: 10.4 (s, 1H); 7.65 (m, 4H); 7.15 (s, 2H); 7.0 (s, 1H); 3.05 (m, 1H); 1.22 (d, 6H); Anal.Calcd. for $C_{12}H_{15}N_3O_2S_2{\cdot}0.5H_2O$: C, 47.00; H, 5.26; N, 13.69. Found: C, 46.90; H, 4.99; N, 13.23.

2.4.7. *2-Benzylamino-5-bromo-1,3-thiazole (**5**)*

Benzaldehyde (0.5 g, 4.7 mmol) and 2-amino-5-bromo-1,3-thiazole (0.44 g, 2.48 mmol) in DCE (15 ml) were treated with sodium triacetoxyborohydride (1.5 g, 6.9 mmol) and glacial acetic acid (0.74 g, 12.4 mmol) at 0 °C. After stirring 72 h, the reaction mixture was diluted with ethyl acetate and quenched with water. The pH of the water layer was adjusted to 7 with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated to give the title compound, which was purified by crystallization from diethyl ether. Colourless solid (0.4 g, 60%): mp 108–111 °C; NMR ($CDCl_3$) δ ppm: 7.36 (m, 5H); 7.0 (s, 1H); 5.45 (bs, 1H); 4.42 (s, 2H).

3. Results and discussion

Compound **1** of Fig. 1 was a commercial compound present in our research compound collection. Its ATP-competitive binding to CDK2 was confirmed by kinetic analysis of biochemical assay data. Several crystal structures of CDK2 have been reported in literature. These include CDK2 or CDK2/Cyclin A in complex with ATP [17], isopentenyladenine [18,19], olomucine [20], roscovitine [18], staurosporine [21], des-chloro-flavopiridol [22], purvalanol B [23], indirubin [24] O^6-cyclohexylmethylguanines [25], 2-anilino-4-(thiazol-5yl)pyrimidine [26] as well as CDK2/Cyclin A with and without ATP [27,28] and pseudo-peptitic substrate bound [24]. The association of CDK2 with Cyclin A results in modification of the ATP-binding site. Thus, in the present modelling studies, the fully activated CDK2 structure in complex with Cyclin A available from the Protein Data Bank [29] was used (1JST PDB code).

Compound **1** was docked into the ATP-binding site of CDK2 with the thiazole ring occupying part of the region of the purine ring of ATP. The model was subsequently energy minimized, allowing flexibility to the protein.

The relative binding modes of compound **1** and ATP are shown in Fig. 2. The planar aromatic thiazole ring is sandwiched between two large planar hydrophobic surfaces formed by a pool of lipophilic residues: Val18, Ala31, Val64, Leu134, Ala144 (for simplicity not shown in Fig. 2). The main chain atoms from residues Glu81 to Leu83 are involved in the hydrogen-bonding network of both the ATP and the inhibitor. The nitrogen atom of the thiazole ring can accept a hydrogen bond from the amide nitrogen of Leu83 and the amide-NH is able to donate a hydrogen bond to the carbonyl oxygen of Leu83. The bromine atom extends into a hydrophobic pocket surrounded by the side-chains of Phe80, Val18, Val64 and Ala144.

Recently, crystal structures of CDK2 in complex with different aminothiazoles were also published [30–33]. In these crystal structures, the thiazole ring-binding mode is the same as that of our compounds.

The comparative analysis of the docking model of compound **1** with the crystal structure of CDK2 in complex with roscovitine [18] (Fig. 3a–c) prompted us to introduce a (hetero)aromatic benzyl moiety in the amidic side-chain and to

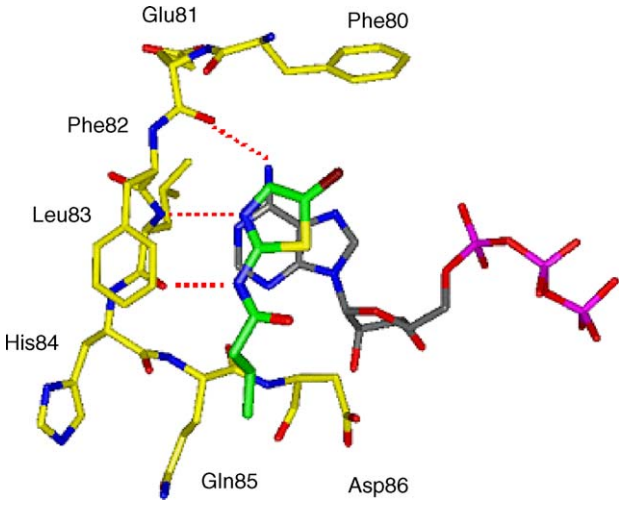


Fig. 2. Overlay of the CDK2/ATP crystal structure with that of CDK2/compound **1**. Residues from Phe80 to Asp86 are shown.

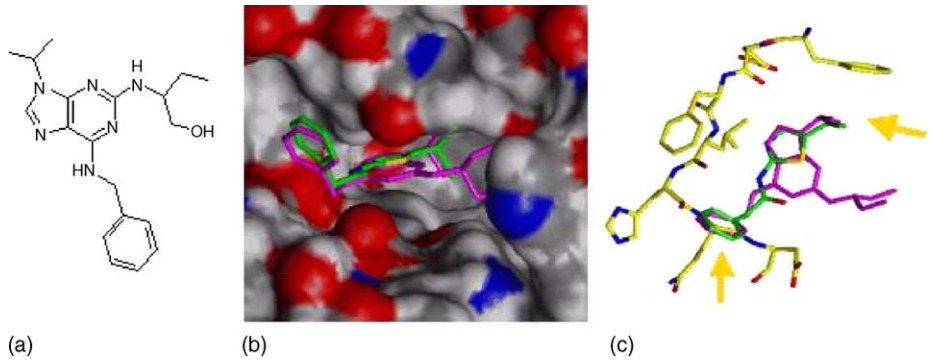


Fig. 3. (a) Chemical structure of roscovitine. (b) Surface representation of the ATP pocket of CDK2 with compound **3** and roscovitine modelled into it. (c) Superimposition of compound **3** (green) with roscovitine (magenta). The yellow arrows highlight the perfect overlap of the two aromatic moieties and the two isopropyl groups in these two structurally unrelated structures.

further elaborate the hydrophobic substitution in 5. To this end, a new 2-benzylcarbonylamino 4-isopropyl thiazole derivative was designed: compound **2** of Fig. 1. Modelling experiments supported the possibility of changing the bromine atom into a bigger hydrophobic isopropyl group, as present in the roscovitine molecule (see Fig. 3a–c). The 2-benzyl carbonyl amino moiety in position 2 of the thiazole in compound **2** perfectly mimics the interaction with the protein of the N6-benzyl group present in the purine CDK2 inhibitors, such as roscovitine (Fig. 3) and olomucine. This group was proposed to be an important determinant for specificity in the purine class of CDK inhibitors [18,19]. In fact, roscovitine and olomucine inhibit CDK1, CDK2 and CDK5 while are inactive against CDK4 and CDK6 [20]. One of the two residues contacting the N6-benzyl moiety (Phe82 of CDK2) is different between these two sub-families: phenylalanine in CDK1/CDK2/CDK5 and histidine in CDK4/CDK6 kinases (see Table 2). Compound **2** showed to be more active than the starting hit (compound **1**) with an IC_{50} = 192 nM and was selective against CDK4/Cyclin D1 (IC_{50} CDK4/Cyclin D1 > 10 μM).

Other hydrophobic 5- or 6-member rings can replace the phenyl ring of compound **2** with essentially conserved inhibitory efficacy (data not shown). In particular, the replacement of the phenyl ring with a meta-pyridine aromatic ring gave compound **3** (Fig. 1) showing an IC_{50} of 95 nM. To elucidate the details of the interaction between compound **3** and CDK2, the crystal structure of the protein–inhibitor complex was determined and refined to 1.9 Å resolution (crystallographic data are summarized in Table 1). The CDK2 protein, common with other protein kinases, comprises an amino-terminal lobe that is almost entirely β-sheet and a mostly α-helical C-terminal lobe. Compound **3** binds into the ATP-binding cleft, locating in between the two lobes, with its thiazole ring occupying part of the region of the purine ring of ATP, as described above for the binding model of compound **1**.

The pyridine ring is well ordered and makes hydrophobic interactions with residues Ile10 and Phe82. From the calculated electron density map, it is not obvious which of the two possible positions the pyridine nitrogen atom occupies. However, in one of the two orientations a water-mediated hydrogen bond can be formed with the side-chain of Lys20 (see Fig. 4). This bridging water molecule is very well located and has been observed to be often present in other in-house CDK2 crystal structures. The presence of this additional hydrogen bond leads to an

Table 2
Alignment of the CDKs human sequences

CDK1	E	I	G	E	G	T	Y	G	V	V	K	V	A	K	E	L	V	L	L
CDK2	E8	I10	G11	E12	G13	T14	Y15	G16	V17	V18	K20	V29	A31	K33	E51	L55	V64	L66	L78
CDK3	E	I	G	E	G	T	Y	G	V	V	K	L	A	K	E	L	V	L	L
CDK4	A	I	G	V	G	A	Y	G	T	V	K	F	A	K	E	L	V	L	K
CDK5	E	I	G	E	G	T	Y	G	T	V	K	I	A	K	E	L	V	L	L
CDK6	A	I	G	E	G	A	Y	G	K	V	K	F	A	K	E	L	V	L	L
CDK7	D	L	G	E	G	Q	F	A	T	V	K	I	A	K	E	L	I	L	L
CDK9	A	I	G	Q	G	T	F	G	E	V	K	K	A	K	E	L	V	L	L
CDK1	F	E	F	L	S	M	D	K	K	K	P	Q	N	L	L	A	D	F	G
CDK2	F80	E81	F82	L83	H84	Q85	D86	K88	K89	K129	P130	Q131	N132	L133	L134	A144	D145	F146	G147
CDK3	F	E	F	L	S	Q	D	K	K	K	P	Q	N	L	L	A	D	F	G
CDK4	F	E	H	V	D	Q	D	R	T	K	P	E	N	I	L	A	D	F	G
CDK5	F	E	F	C	D	Q	D	K	K	K	P	Q	N	L	L	A	D	F	G
CDK6	F	E	H	V	D	Q	D	L	T	K	P	Q	N	I	L	A	D	F	G
CDK7	F	D	F	M	E	T	D	E	V	K	P	N	N	L	L	A	D	F	G
CDK9	F	D	F	C	E	H	D	A	G	K	A	A	N	V	L	A	D	F	G

The residues lining the ATP pocket are shown.

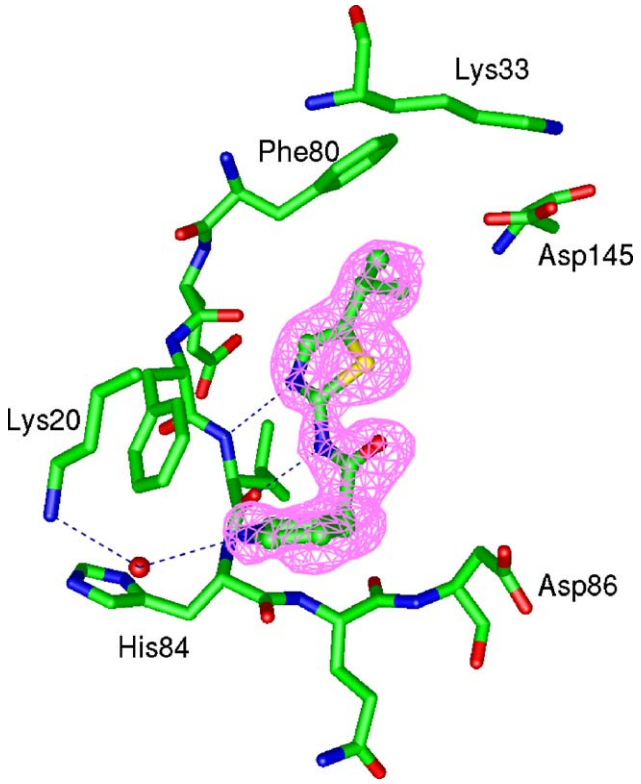


Fig. 4. ATP-binding pocket residues of the X-ray structure of CDK2 in complex with **3**. The electron density for the ligand only is shown in pink.

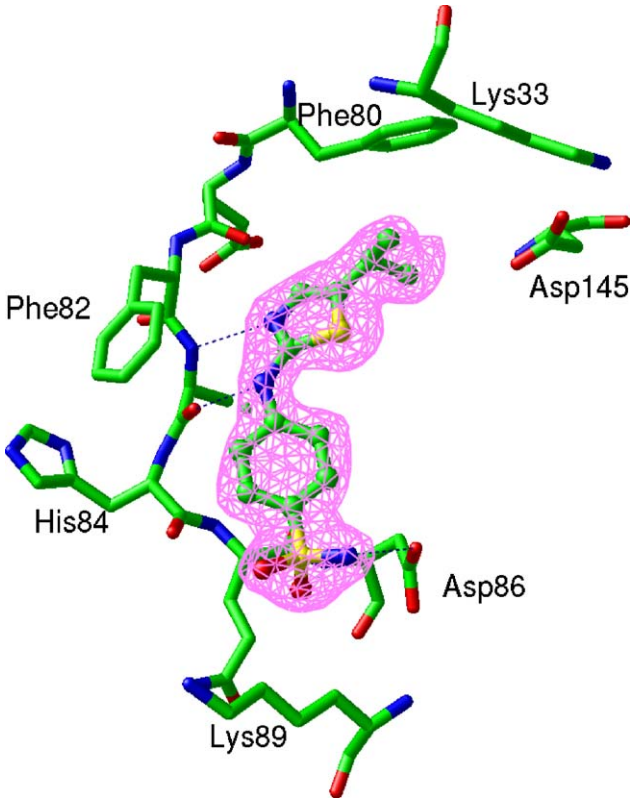


Fig. 5. ATP-binding pocket residues of the X-ray structure of CDK2 in complex with **4**. The electron density for the ligand only is shown in pink.

improvement in CDK2 inhibitory activity. Compound **3**, as compound **2**, displays selectivity against CDK4/Cyclin D1 (see Table 3).

Examination of the initial docked ligands and the subsequent crystal structure of compound **3** with CDK2 provided insight for the design of a more potent analogue.

To pick up potential additional interactions with Asp86, we envisaged substitution of the *n*-propyl amide moiety in a *para* sulfonamide aniline substituent (compound **4** of Fig. 1, IC_{50} = 20 nM). The structural modification implemented in this compound (with respect to the modification in compounds **2** and **3**) provides a more rigid compound with an associated favourable entropic contribution to binding affinity. The aniline group packs closely towards the side-chain of the hydrophobic residues Ile10. The key interactions of the sulfonamide moiety with both the main and the side-chain atoms of Asp86 were confirmed by the crystal structure of CDK2 in complex with compound **4** refined at 2.0 Å resolution (Table 1). A weak hydrogen-bond interaction with Lys89, which is solvent exposed and highly flexible (Fig. 5), is also formed.

The Lys89 hydrogen-bond interaction might be important in imparting selectivity to this inhibitor against CDK4/Cyclin D1 (see Table 3) [34]. In fact, the corresponding residue in CDK4 is a threonine (see Table 2). The combination of the two modifications, the isopropyl in position 5 and the *para* sulfonamide aniline in position 2, resulted in an increase of activity by two orders of magnitude for compound **4** with respect to the original hit, compound **1**.

It was lately confirmed and reported by others that the *para* sulfonamide aniline substituent brings about an improvement of both potency and selectivity. This effect was reported in the oxindole-based inhibitors [35,36] (see 1FVT and 1KE5 PDB codes) and in the purine-based inhibitors [37–39] (see 1H1S, 1JSV and 1OIT PDB codes).

Several other modes of expansion were not pursued based on structural inspection coupled with single molecule experimental proof. As an example, we have explored the effect of a 3-*N*-benzylamino group in the 2-amino thiazole scaffold. Compound **5** of Fig. 1 was synthesized and no CDK2 inhibition was detected (% inhibition @ 10 μM = 8%). A conformational analysis was carried out on compound **5** by rotating the two torsion angles of the benzyl group while maintaining fixed the thiazole core. Fig. 6 reports three representative conformational minima of compound **5**. Conformations A and B cannot fit into CDK2 pocket due to severe steric interactions with Ile10, Asp86; conformation C has a poor binding affinity as the benzyl group is not making any direct contact with the protein.

Table 3
In vitro CDKs activity, IC_{50} (μM)

Protein kinase	Compound **1**	Compound **3**	Compound **4**
CDK1/Cyclin B	2.215	0.463	0.173
CDK2/Cyclin A	0.808	0.095	0.020
CDK2/Cyclin E	0.692	0.112	0.041
CDK4/Cyclin D1	>10	>10	>10
CDK5/p25	2.988	0.264	0.105

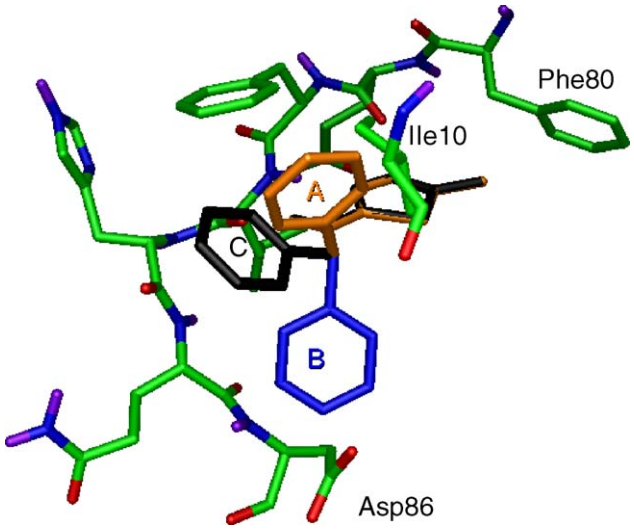


Fig. 6. Representative conformational minima of compound **5**. Conformation A (orange) and B (blue) cannot fit into CDK2 pocket due to severe steric interactions with Ile10, Asp86; conformation C (black) has a poor binding affinity as the benzyl group is not making any contact with the protein.

4. Conclusion

In summary, compound **1** was identified by HTS screening of our research compound collection. It inhibited CDK2/Cyclin A in an ATP-competitive manner with an IC_{50} of 808 nM and was selected as a promising template for expansion within a small-molecule CDK2 inhibition program.

The combination of X-ray structure determination and molecular modelling allowed us to rationally design two novel and potent CDK2 inhibitors by evaluating the optimal substitutions at two critical regions of the thiazole template.

Designed modifications at positions 2 and 5 of the thiazole core system were examined and resulted in a rapid and effective improvement of in vitro CDK2 inhibition.

5. Accession numbers

The atomic coordinates and structure factors have been deposited in the Protein Data Bank (Accession code: 2btr and 2bts).

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