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Exploration of the effect of sterically demanding 3′-amido substitution of 3′-deoxyadenosines towards inhibition of cyclin-dependent kinase 1

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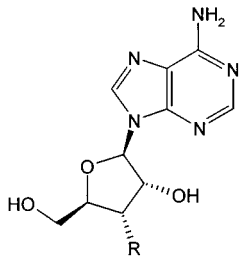
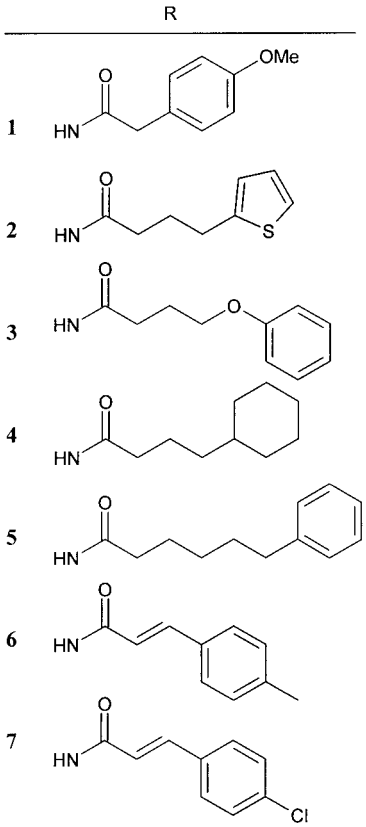
With focus on exploring the structural demands of adenosine triphosphate pockets of different target enzymes, the 3′-amido-3′-deoxyadenosines **1**–**7** were synthesized. The inhibitory activity of these compounds on a selected cyclin-dependent kinase as model target in anticancer research was evaluated *in vitro*. A starfish oocyte enzyme based assay revealed a decreased inhibitory activity in comparison to adenosine. Consequently, the introduction of spacefilling lipophilic 3′-amido substituents alters the enzyme inhibition in an unfavorable manner.

1. Introduction

The disclosure of cyclin-dependent kinases (CDK's) as targets for drug discovery opened up new pathways for attempted selective interference with the cell cycle at different stages and therefore represents a highly attractive approach in medicinal chemistry [1]. Since it is known that the interaction of small organic molecules with the ATP-binding pocket of the CDK-family is a promising way to inhibit these enzymes [2], it is an interesting topic to explore the addressable space inside this target pocket. To date, structural information derived from X-ray crystallography of CDK's is scarce and confined to CDK 2 mainly. Most potent inhibitors currently available consist of derivatives of the adenine moiety of ATP (e.g., olomoucine). Adenosine itself exhibits rather weak competitive inhibition on CDK 1 ($IC_{50} = 55\ \mu M$) [3]. Because the active site of CDK probably extends significantly beyond the volume encapsulated by olomoucine and to further establish a recognition pattern, we considered it straightforward to use 3′-amido-3′-deoxyadenosines **1**–**7** as analogs of the natural ligand ATP for the exploration of a putative binding pocket in the region of the 3′-hydroxyl group of adenosine. Generally, amido substitution of deoxyadenosines leads to compounds with retained hydrogen bond donor capabilities on the substitution site in contrast to O-acylated adenosine derivatives. The aim of our approach was to determine whether an amide derivatization in the 3′-region is favorable with respect to inhibitory potency or not. Due to the fact that only limited amounts of material are required to gain the desired biological information, we used a polymer supported microsynthesis recently reported by our group [4], in order to prepare small samples of **1**–**3** for the enzyme assay in mg quantities only. Compounds **4**–**7** have been synthesized earlier and were originally part of our program for the discovery of anti-parasite drugs and are currently under evaluation as ligands of ATP-pockets of other enzymes, too.



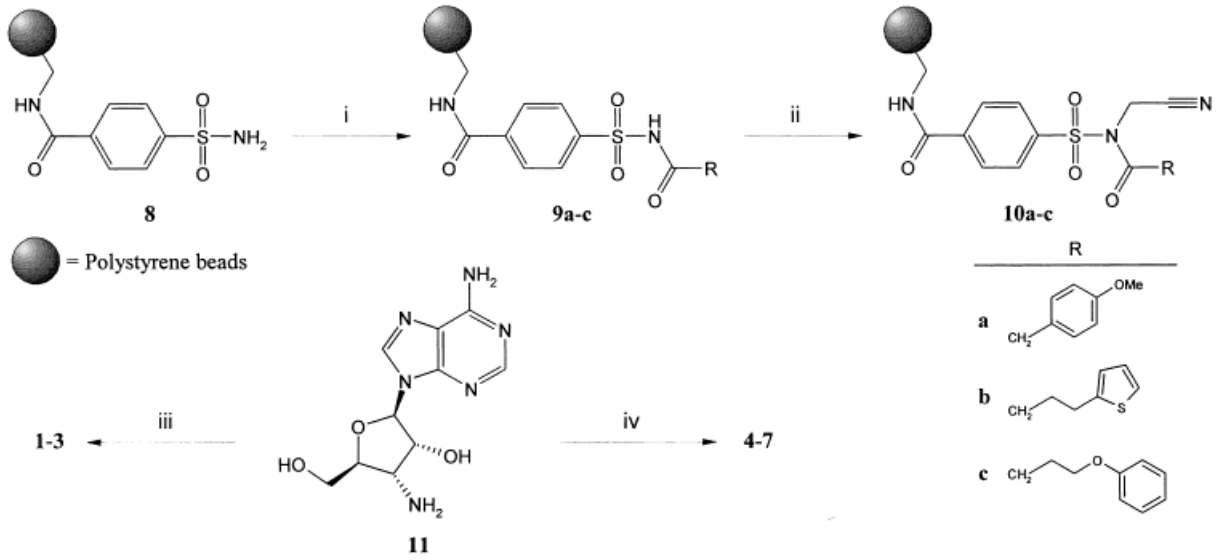
1-7



2. Investigations, results and discussion

The synthesis of 3′-amino-3′-deoxyadenosine (**11**) was performed in ten steps starting from D-xylose [5, and references cited therein]. The synthesis of compounds **1**–**3** was achieved by coupling the selected acids to the Kenner safety-catch linker **8** improved by Ellman et al. [6]. In this way different resin aliquots **9a**–**c** were prepared from commercially available acids by in situ anhydride formation using *N*,*N*-diisopropylcarbodiimide, Hüning's base and 4-(dimethylamino)pyridine as catalyst. The reaction could be followed by FTIR-spectroscopy. Advantageously,

Scheme



i: RCOOH, *N*,*N*-diisopropylcarbodiimide, Hünig's base, DMAP, CH_2Cl_2; ii: $BrCH_2CN$, Hünig's base, *N*-methylpyrrolidone; iii: **10a–c**, 50 °C; iv: appropriate carboxylic acid, DCC, *N*-hydroxysuccinimide, DMF.

acylated resins like **9a–c** are very stable and, when carefully selected and prepared in large number, can be used to create multipurpose molecular diversity. Acylation of the sulfonamide linker leads to a decrease of the intensity of the sulfonamide absorption band at 3340 cm^{-1} while a new carbonyl stretch is formed at 1718 cm^{-1}. Reaction completeness was assured by carefully weighing out the resulting washed and dried resins after termination of the coupling step. Activation for nucleophilic cleavage was accomplished by substituting the acylsulfonamide moiety with an electron withdrawing alkyl side-chain as described by Ellman et al. [6]. The resulting highly activated polymer bound acids **10a–c** could be transformed to compounds **1–3** by stirring with amine **11** in dimethylformamide (Scheme). The work-up consisted of removal of the polymer beads by filtration and subsequent removal of the solvent. Short column chromatography ("filtration") over strongly basic anion-exchange resin (Dowex® 1×2, OH^--form) and evaporation yielded analytically pure **1–3** in satisfactory yields. Compounds **4–7** were synthesized according to a procedure published in a preliminary paper [5].

Compounds **1–7** were investigated in the starfish oocyte CDK 1-asay developed by Meijer [7]. Text results are given in the Table. The outcome reveals, that a 3′-amido substitution of adenosine with sterically demanding substituents seems not favorable probably in terms of fitting into the targeted enzyme pocket. Therefore, one can conclude that bulky substituents are not well tolerated in this area.

3. Experimental

3.1. Apparatus

Melting points were determined on an Electrothermal 9100 instrument. Elemental analyses of polymers and final products were performed in the analytical department of the Institut für Pharmazie, Universität Hamburg and at the Universität Konstanz, respectively. All the results were in an acceptable range. IR spectra were recorded using KBr pellets on a Perkin Elmer 1660 FTIR spectrometer. NMR spectra were recorded on a Bruker WH 500 spectrometer, using tetramethylsilane as internal standard. HPLC analyses for purity determination were carried out on a Merck 125-4 LiChrospher® 100 RP-18 (5 µm) column using a Merck 6200 pump (flow rate 0.8 mL, MeOH/H_2O 40 : 60) and L-3000 diode array detector at 200–360 nm (260 nm for quantification by 100% method; integration from 2.0–30.0 min). TLC analyses were carried out on fluorescent Sil G/UV_{254} silica gel plates, using CH_2Cl_2/MeOH 90 : 10 as eluent. Spots were visualized under 254 nm UV illumination.

3.2. Synthesis

3.2.1. General procedure A for the synthesis of the 3′-amido-3′-deoxyadenosines **1–3**

To a flask containing 1.0 g of dry 4-sulfamylbenzoylaminomethyl polystyrene with an initial loading level of 1.0 mmol/g as determined by elemental analysis (prepared from very high load aminomethylated polystyrene, purchased from Novabiochem®, Switzerland) was added 20 ml of dichloromethane. The resin was allowed to swell at room temperature for 2 h. In another flask, 4 mmol of the appropriate acid was dissolved in 20 ml of dichloromethane and preactivated by adding 700 µl (4.5 mmol) *N*,*N*-diisopropylcarbodiimide. The mixture was kept at room temperature for 10 h and decanted from the precipitated *N*,*N*-diisopropylurea. After addition of 540 µl Hünig's base (5.5 mmol) and 11 mg (0.09 mmol) 4-(dimethylamino)pyridine as catalyst the coupling mixture was added to the swollen resin. The resulting slurry was agitated at room temperature for 24 h. The resin beads were filtered and washed exhaustively with dimethylformamide, dichloromethane and methanol. After drying the increase in weight and elemental composition were determined. An amount of resin corresponding to 0.30 mmol acid was alkylated as described by Ellman et al. [6] using bromoacetonitrile in *N*-methylpyrrolidone and subsequently agitated at 50–60 °C with a solution of 30 mg (0.11 mmol) **11** in dimethylformamide. The reaction was monitored by HPLC. After the starting material **11** had disappeared (2–12 h), the suspension was filtered, the resin was washed with dimethylformamide and the combined dimethylformamide solutions were evaporated. The obtained residue was dissolved in MeOH/H_2O (1 : 1) and eluted over Dowex® 1×2 (OH^-) resin with the same mixture. The product containing fractions were evaporated to furnish analytically pure **1–3**.

3.2.1.1. 3′-Deoxy-3′-(2-(4-methoxyphenyl)acetylamino)adenosine (**1**)

Compound **1** was prepared following the general procedure A furnishing 69% off-white product, m.p.: 226–226 °C dec.; IR 3324 cm^{-1} (NH), 1654 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 3.37–3.52 (m, $COCH_2$ and H-5B′), 3.61–3.74 (m, CH_3O and H-5A′), 3.99 (q, J = 3.1 and 6.4 Hz, H-4′), 4.39–4.51 (m, H-2′ and H-3′); 5.2 (br s, OH-5′), 5.97 (d, J = 3.0 Hz, H-1′), 6.18 (br s, OH-2′),6.85 (d, J = 8.6 Hz), 7.20 (d, arom. H), 7.33 (br s, NH_2), 8.13 (s, H-2), 8.25 (d, J = 7.7 Hz, 3′-NH), 8.39 (s, H-8); HPLC 96.8%; HRMS (LSIMS, thioglycerol) *m*/*z* 415.1692 [$MH^+C_{19}H_{22}N_6O_5$)414.1730].
$C_{19}H_{22}N_6O_5$ (414.4)

3.2.1.2. 3′-Deoxy-3′-(4-(2-thienyl)butanoylamino)adenosine (**2**)

Compound **2** was prepared following the general procedure A furnishing 77% off-white product, m.p.: 200–201 °C dec.; IR 3324 cm^{-1} (NH),

1647 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 1.84 (t, 2 H), 2.23 (t, 2 H), 2.79 [t, 2 H, $CO(CH_2)_3$], 3.52 (d, J = 11 Hz, H-5B′), 3.70 (d, H-5A′), 4.0 (br s, H-4′), 4.48 (br s, H-2′ and H-3′); 5.27 (br s, OH-5′), 5.97 (s, H-1′), 6.04 (br s, OH-2′), 6.85 (s), 6.93 (d, J = 4 Hz), 7.31 (d, thienyl H), 7.34 (br s, NH_2), 8.02 (br s, 3′-NH), 8.15 (s, H-2), 8.41 (s, H-8); HPLC 99.7%; HRMS (LSIMS, thioglycerol) *m/z* 419.1519 [$MH^+(C_{18}H_{22}N_6O_4S)$ 419.1501].
$C_{18}H_{22}N_6O_4S$ (418.5)

3.2.1.3. 3′-Deoxy-3′-(4-phenoxybutanoylamino)adenosine (**3**)

Compound **3** was prepared following general procedure A furnishing 81% off-white product, m.p.: 237–239 °C dec.; IR 3327 cm^{-1} (NH), 1653 cm^{-1}(C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 1.94 (t, 2 H), 2.34 [t, 2 H, $Ph(CH_2)_2$], 3.51 (d, H-5B′), 3.69 (d, J ≈ 11 Hz, H-5A′), 3.96 (t, OCH_2), 4.0 (br s, H-4′), 4.48 (br s, H-2′ and H-3′); 5.25 (br s, OH-5′), 5.97 (s, H-1′), 6.22 (br s, OH-2′), 6.85–6.95 (m, 3 H), 7.27 (t, J = 6.8 Hz, 2 H, arom H), 7.33 (br s, NH_2), 8.13 (s, H-2), 8.18 (br s, 3′-NH), 8.40 (H-8); HPLC 100%; HRMS (LSIMS, thioglycerol) m/z 429.1888 [$MH^+(C_{20}H_{24}N_6O_5)$429.1886].
$C_{20}H_{24}N_6O_5$ (428.5)

3.2.2. General procedure B for the synthesis of 3′-amido-3′deoxyadenosines **4**–**7**

To a solution of 3′-amino-3′-deoxyadenosine (**11**) (133 mg, 0.5 mmol) and the appropriate carboxylic acid (0.66 mmol) in 10 ml of dimethylformamide at −20 °C were added *N*-hydroxysuccinimide (0.76 mg, 0.66 mmol) and *N*,*N*-dicyclohexylcarbodiimide (136 mg, 0.66 mmol). The mixture was stirred overnight at RT, H_2O (2 ml) was added, and the solvents evaporated. The residual oil was applied to a column of Dowex® 1 × 2 (OH^--form; H_2O/MeOH 2 : 1, then 1 : 2). Evaporation of the product containing fractions gave compounds **4**–**7** as crystalline material from ethanol.

3.2.2.1. 3′-(3-Cyclohexylbutanoylamino)-3′-deoxyadenosine (**4**)

Compound **4** was prepared following the general procedure B. Yield 42% colorless crystals, m.p.: 213 °C dec.; IR 3324 cm^{-1} (NH), 1654 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 0.83 (q, 2 H), 1.05–1.25 (m, 6 H), 1.45–1.7 (m, 7 H, $C_6H_{11}-CH_2-CH_2$), 2.15 (m, CH_2CO), 3.52 (app dq, H-5B′), 3.70 (ddd, J = 2.2, 4.8, −12.2 Hz, H-5A′), 3.98 (q, H-4′), 4.45 (m, H-2′ and H-3′), 5.22 (app t, J = 5.4 Hz, 5′-OH), 5.95 (d, J = 2.3 Hz, H-1′), 5.98 (br s, 2′-OH), 7.33 (br s, NH_2), 7.90 (d, J = 7.4 Hz, 3′.NH), 8.16 (s, H-2), 8.42 (s, H-8); HRMS (LSIMS, thioglycerol) m/z 419.2407 [$MH^+(C_{20}H_{30}N_6O_4)$ 419.2394].
$C_{20}H_{30}N_6O_4$ (418.5)

3.2.2.2. 3′-Deoxy-3′-(6-phenylhexanoylamino)adenosine (**5**)

Compound **5** was prepared following the general procedure B. Yield 51% colorless crystals, m.p.: 204 °C dec.; IR 3324 cm^{-1} (NH), 1654 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 1.27 (t, 2 H), 2.17 (m, 2 H), 2.54 (t, 2 H, aliphatic H), 3.51 (m, H-5B′), 3.69 (m, H-5A′), 4.00 (s, H-4′), 4.48 (br s, H-2′ and H-3′), 5.25 (br s, 5′-OH), 5.96 (s, H-1′), 6.00 (br s, 2′-OH), 7.10–7.28 (m, arom H), 7.93 (d, J = 6.7 Hz, 3′-NH), 8.17 (s, H-2), 8.41 (s, H-8): HRMS (LSIMS, thioglycerol) m/z 441.2299 [$MH^+(C_{22}H_{28}N_6O_4)$ 441.2250].
$C_{22}H_{28}N_6O_4$ (440.5)

3.2.2.3. 3′-Deoxy-3′-(4-(4-methylphenyl)-trans-prop-3-enoylamino)-adenosine (**6**)

Compound **6** was prepared following the general procedure B. Yield 44% colorless crystals, m.p.: 234–236 °C dec.; IR 3324 cm^{-1} (NH), 1654 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 2.32 (CH_3), 3.57 (m, H-5B′), 3.74 (m, H-5A′), 4.04 (q, H-4′), 4.53 (m), 4.61 (m, H-2′ and H-3′), 5.25 (app t, J = 4.9 Hz, 5′-OH); 5.99 (d, H-1′), 6.12 (br s, 2′-OH), 6.79 (d, J = 15.6 Hz), 7.41 (d, CH=CH), 7.24 (d, J = 7.7 Hz), 7.47 (d, arom H), 7.34 (br s, NH_2), 8.16 (s, H-2), 8.25 (d, J = 8.2 Hz, 3′-NH), 8.44 (s, H-8); HRMS (LSIMS, thioglycerol) m/z 411.1790 [$MH^+(C_{20}H_{22}N_6O_4)$ 411.1781].
$C_{20}H_{22}N_6O_4$ (410.4)

3.2.2.4. 3′-Deoxy-3′-(4-(4-chlorophenyl)-trans-prop-3-enoylamino)-adenosine (**7**)

Compound **7** was prepared following the general procedure B. Yield 39% colorless crystals, M.p.: 235–237 °C dec.; IR 3324 cm^{-1} (NH), 1654 cm^{-1} (C=O); ^1H NMR (500 MHz, [D_6]-DMSO) δ (ppm): 3.57 (m, H-5B′), 3.74 (m, H-5A′), 4.05 (br s, H-4′), 4.54 (br s), 4.62 (quintet, H-2′ and H-3′), 5.26 (br s, 5′-OH), 6.00 (s, H-1′), 6.22 (br s, 2′-OH), 6.86 (d, J = 15.7 Hz), 7.44 (d, CH=CH), 7.34 (s, NH_2), 7.50 (d, J = 7.7), 7.61 (d, arom H), 8.16 (s, H-2), 8.37 (d, J = 7.9 Hz, 3′-NH), 8.43 (s, H-8); HRMS (LSIMS, thioglycerol) m/z 453.1052 [$MNa^+(C_{19}H_{22}N_6O_5)$ 453.1054].
$C_{19}H_{19}ClN_6O_4$ (430.9)

3.3. Biological evaluation

$p34^{cdc2}$/Histone H1 kinase assay. $P34^{cdc2}$/cyclin B was purified from M phase oocytes of the starfish *Marthasterias glycialis* by affinity chromatography on p^{9CKhsq}-Sepharose beads, from which it was eluted by free p^{9CKhs1}. The cdc2 (CDK 1) assay mixture (final volume: 30 µl) contained 0.5–1 µl purified enzyme, 5 µl of histone H1 (5 mg/ml), 5 µl of [γ-^{32}P]ATP (15 µM, 3000 Ci/mmol, 1 mCi/ml) and 3 µl of the inhibitor (0.1–1000 µM), all in reaction buffer C (60 mM of β-glycerolphosphate, 15 mM of p-nitrophenyl phosphate, 25 mM of 3-(*N*-morpholino)propanesulfonic acid, pH 7.2, 5 mM of EGTA, 15 mM of $MgCl_2$, 1 mM of dithiothreitol, 1 mM of sodium vanadate, 1 mM of phenyl phosphate, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 10 µg/ml soybean trypsin inhibitor, 100 µM of benzamidine). For determination of maximum phosphate incorporation, buffer C was used instead of inhibitor. Nonspecific binding was determined in the absence of histone H1 in the reaction mixture and substracted from each volume. The assays were started by addition of radioactive ATP, and after 10 min incubation at 30 °C 25 µl aliquots of the supernatant were spotted onto 2.5 × 3.0 pieces of Whatman P81 phosphocellulose paper. After 20 s, the filters were washed five times (for at least 5 min each time) in 0.1% phosphoric acid. The wet filters were transferred into 1 ml of ACS scintillation cocktail (Amersham), and after mixing, ^{32}P radioactivity was determined using a Packard Tr-Carb counter. Control analyses were also performed with appropriate dilutions of DMSO because the inhibitors were dissolved in DMSO as 100 mM stock solutions. However, the final DMSO concentration in the reaction mixture never exceeded 1%. The kinase activity is expressed as pmol of phosphate groups incorporated in histone H1 during a 10 min incubation or in % of the maximal kinase activity. Dose-response curves were drawn for every compound tested and used for calculating IC_{50}'s. All assays were carried out in triplicate.

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