The Free Internet Journal for Organic Chemistry

Paper

Archive for Organic Chemistry

Arkivoc **2017**, part iii, 225-240

Design and synthesis of *N*-benzimidazol-2-yl-*N'*-sulfonyl acetamidines

Nadezhda A. Rupakova,[a] Vasiliy A. Bakulev,[a] Uwe Knippschild,[b] Balbina García-Reyes,[b] Oleg S. Eltsov,[a] Grigoriy P. Slesarev,[a] Nikolai Beliaev,[a] Pavel A. Slepukhin,[c] Lydia Witt,[d] Christian Peifer,[d] and Tetyana V. Beryozkina*[a]

[a] *Ural Federal University, 19 Mira st.,Yekaterinburg 620 002, Russia*

[b] *Klinik für Allgemein- und Viszeralchirurgie Zentrum für Chirurgie Universitätsklinikum Ulm, 23 Albert-Einstein-Allee, 89 081 Ulm, Germany*

[c] *I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of Russian Academy of Sciences, 20 S. Kovalevskaya st., Yekaterinburg 620 990, Russia*

[d] *Institute of Pharmacy, Christian-Albrechts-University, Gutenbergstrasse 76, D-24118 Kiel, Germany*

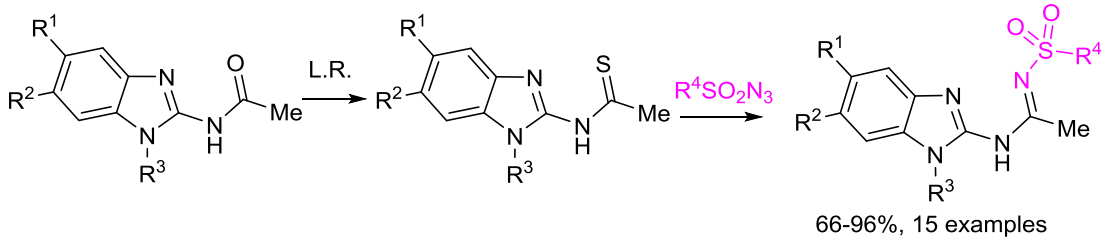
Email: tetber@mail.ru

Dedicated to Prof. Oleg A. Rakitin on the occasion of his 65th birthday

Received 06-01-2017 **Accepted** 07-21-2017 **Published on line** 07-29-2017

Abstract

N-Sulfonyl-*N'*-benzimidazol-2-yl acetamidines have been designed as CK1 inhibitors. Binding modes in the ATP pocket of CK1δ were determined by molecular modeling. The synthetic approach involves sequential acylation of 2-aminobenzimidazoles followed by reaction of amides with Lawesson's reagent and iminosulfonylation of thioamides with sulfonyl azides. The iminosulfonylation was carried out in boiling ethanol with an equivalent ratio of azides and thioamides. The synthesized compounds were tested for their ability to inhibit CK1 isoforms *in vitro* and to inhibit the growth of tumor cell lines. Among the synthesized compounds, two products showed inhibitory abilities towards CK1δ and CK1ε.



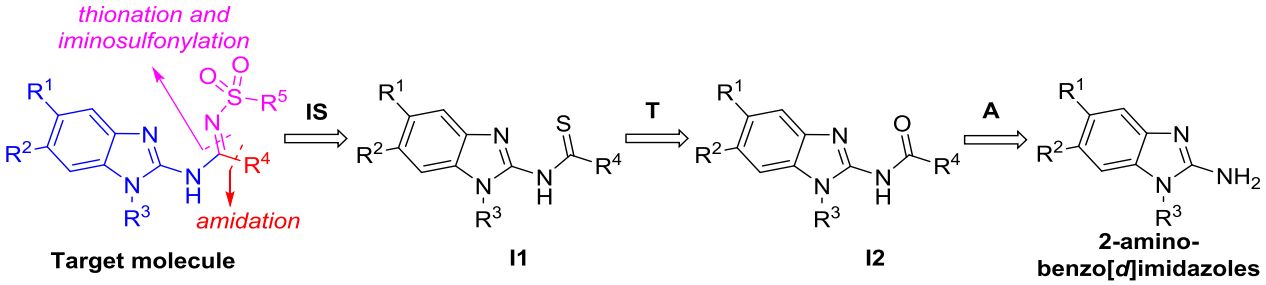
Keywords: Thioamides, *N*-sulfonyl amidines, benzimidazoles, 1,3-dipolar cycloaddition, CK1 inhibitors

Introduction

The benzimidazole nucleus is a constituent of numerous therapeutic agents, and its derivatives exhibit various types of bioactivity including anticancer, antimicrobial, antiviral, anti-inflammatory, antiparasitic, antioxidant, proton pump inhibiting, antihypertensive, etc.[1] Modification by an amino group, nitrogen atoms of imidazole ring, and CH bonds of benzene ring also results in formation of biologically active compounds.[1] Richter *et al*. have found that *N*-aminoacylated benzimidazoles inhibit casein kinase 1 (CK1) and exhibit nanomolar anticancer activity.[2] On the other hand, *N*-sulfonyl amidines have been introduced as osteoclast differentiation inhibitors, anti-resorptive agents[3], and dopamine transport inhibitors.[4] It is therefore a worthwhile challenge to develop previously unknown hybrid molecules containing both a benzimidazole and an *N*-sulfonylamidine group, with the aim of finding new biologically active compounds.

Results and Discussion

Herein, we report a novel synthetic approach to new benzimidazole derivatives bearing *N*-sulfonyl acetamidine groups, and preliminary data on their inhibition of casein kinases. The design of the structures planned for the CK1 inhibition activity testing, along with the plan to synthesize the desired compounds, are shown in Scheme 1. A series of 2-aminobenzimidazoles bearing various substituents including a dioxole ring, the use of acetic anhydride, acetyl chloride and trifluoroacetic anhydride as acylating reagents, and the use of mesyl azide and various other sulfonyl azides demonstrated the variety of the structures of the planned compounds. Acylation (**A**), thionation (**T**) and iminosulfonylation (**IS**) were steps applied for the retrosynthesis of target molecules.



Scheme 1. Retrosynthetic design of *N*-benzimidazol-2-yl-*N*′-sulfonyl acetamidines.

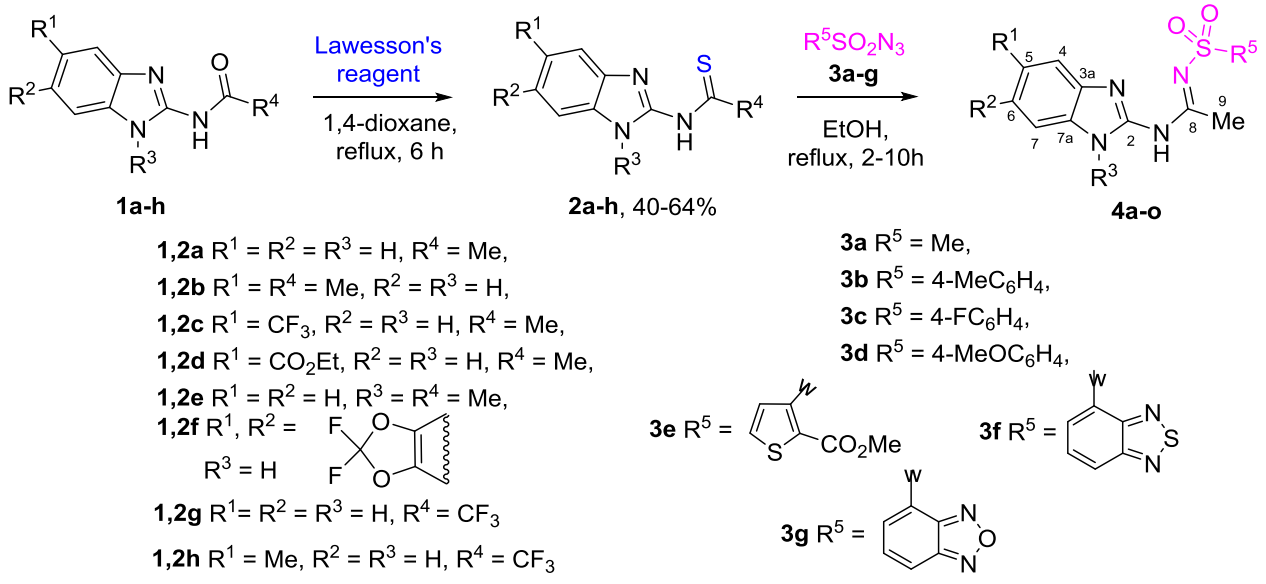
Reactions corresponding to transformation **A** are widely used in the synthesis of various acylamido derivatives of benzimidazoles.[5-8] To introduce R^4 of a small size acetic anhydride and trifluoroacetic anhydride were selected. Reactions corresponding to step **T** involve Lawesson's reagent. They have been applied for the synthesis of 2-thiocarbamoyl benzimidazoles in which R^4 is only aryl.[9] The general method for the synthesis of *N*-sulfonylamidines involves alkylation followed by treatment of alkyl thioimidates with amines[10] and then with sulfonyl chlorides (step **IS**). However, the low yield of the target compounds forced us to rule out this method from consideration. Recently a N-sulfonylamidines synthesis based on a Cu catalyzed three component reaction[11] was developed and applied to the synthesis of benzimidazol-2-yl-N-sulfonylamidines bearing R^4 aryl and long alkyl groups[12] but cannot be used for the synthesis of amidines of acetic acid (R^4 = Me) designed for testing of biological activity. We turned our attention to a one-step synthesis of sulfonylamidines involving the reaction of enamines and thiomides with sulfonyl azides.[4,13-18] This approach has successfully

been applied to the synthesis of *N*-sulfonyl amidines from cyclic thioamides[13] and active methylene thioamides,[4,14] and therefore it was selected as the method of choice for the synthesis of the target compounds.

The starting amides **1a-h** were prepared in high yields in the reaction of commercially available 2-aminobenzimidazoles with acetyl chloride (or acetic anhydride) and trifluoroacetic anhydride for the synthesis of acetamido (**1a-f**) and trifluoroacetamido (**1g,h**) benzimidazoles, respectively. The novel thioamides **2a-h** were prepared by treatment of amides **1a-h** in boiling 1,4-dioxane with Lawesson's reagent, which was found earlier to be efficient for the synthesis of various types of thioamide.[19-21]

To prepare the targeted sulfonyl amidines **4** we have studied reactions of thioamides **2a-h** with a variety of sulfonyl azides **3a-g** bearing methyl, aryl, or heteroaryl moieties in various reaction conditions. First we tried to avoid any solvent. The corresponding protocol was successfully used earlier to prepare sulfonylamidines from methylene active thioamides.[4,14] However we were not able to isolate any new compounds apart from the initial reagents and some sort of tar. The darkening of the reaction mass took place while no nitrogen evolution being a characteristic feature of azidation of thioamide[4,14] was fixed. The adding a few drops of DMF did not improve the situation. Thioamides **2c,d** did not react with sulfonyl azides **1a,b** in water, though this solvent was successfully used to prepare other types of amidine.[14,16] The reason may involve the very low solubility of thioamides **2** in water. Luckily, the reaction of (benzimidazol-2-yl)ethanethioamides **2a-h** with azides **3a-g** occurred when the mixture of thioamides **2** and sulfonyl azides **3** in equivalent ratio (for the reaction of thioamide **2f** with azide **3c** 5.0 equiv. of the azide was used) was heated in boiling ethanol for 2-10 h. We have also found that thioamides of trifluoroacetic acid **2g,h** did not react with sulfonyl azides under any conditions. (Scheme 2; Figure 1)

The structures of all new compounds were reliably confirmed by the combination of ^{1}H and ^{13}C NMR spectroscopy including 2D HMBC and HSQC experiments, as well as mass spectrometry. Thus, ^{1}H NMR spectra of amides **1d,f**, thioamides **2a-h**, and amidines **4a-o** display methyl group singlets and benzene ring protons at 2.16-2.71 and 7.04-8.10 ppm, respectively. The characteristic signals in the ^{13}C NMR spectra at 201.3–203.7 ppm of the thioamides **2a-h**, corresponding to carbons of the thioamide group, were observed. Signals of carbons of amidine groups of compounds **4a-o** appear at 163.3-167.5 ppm, *i.e*. close to signals for methylene active *N*-sulfonyl amidines.[4,14]



Scheme 2. Synthesis of (benzimidazol-2-yl)ethanethioamides **2a-h** and (benzimidazol-2-yl)acetamidines **4a-o**.

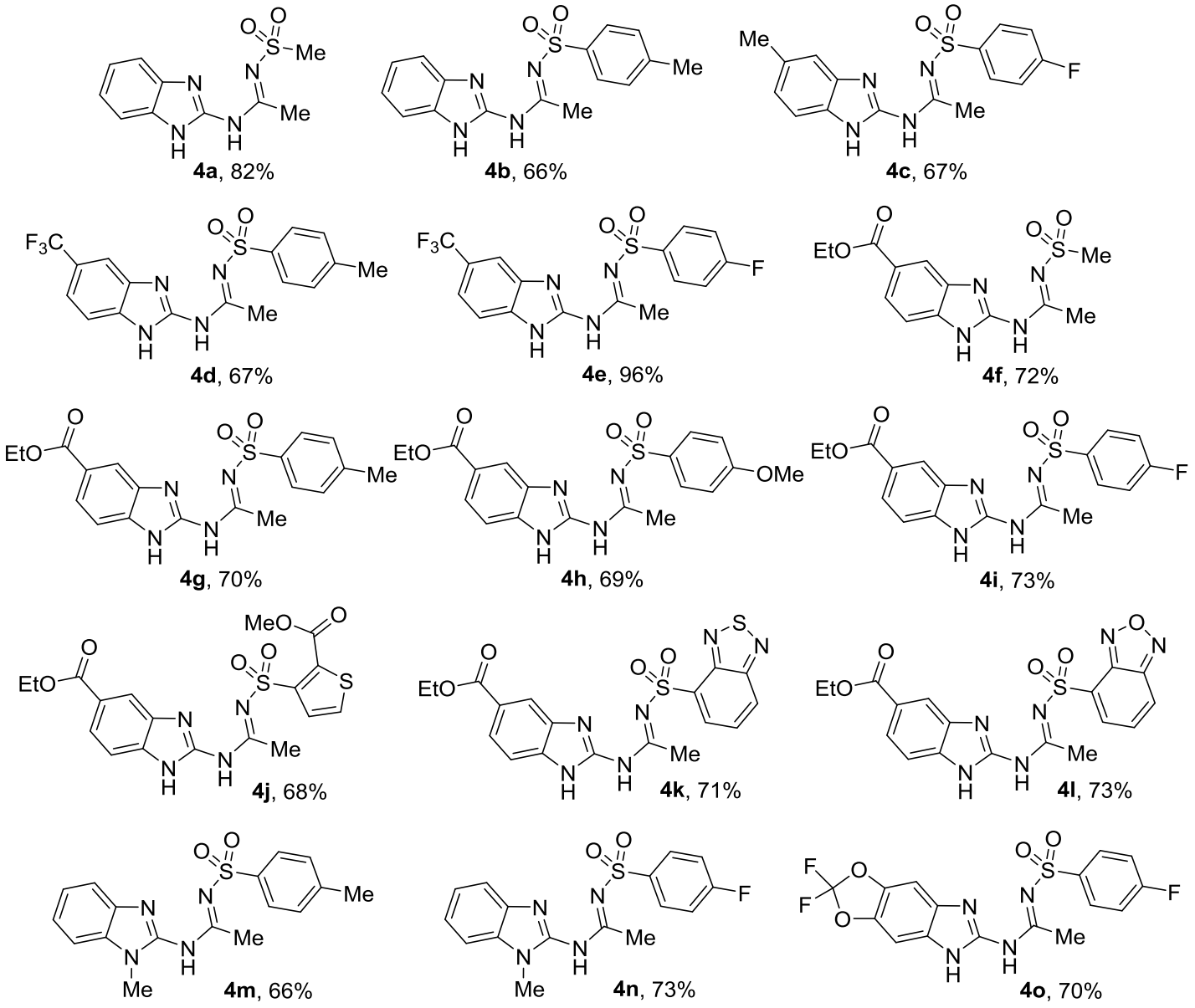


Figure 1. Structures of *N*-sulfonylacetamidines **4a-o** prepared according to Scheme 2.

Additional proof for the proposed structures of compounds **4a-o** was achieved by X-ray analysis of a single crystal of **4o** which was successfully obtained by crystallization from ethyl acetate (Figure 2). The compound crystallized in the centrosymmetric space group of the triclinic system. The molecule has a Γ-like configuration. The bonds lengths and interatomic angles in the molecules do not show any significant deviations from standard. The amidine moiety is fixed in the plane of the heterocycle in the limits 0.1 Å by intramolecular H-bonds, the C-atom of CF_2-group deviated from the least-squares plane of the heterocycle by 0.2 Å. The fluorophenyl substituent deviated from the plane of the heterocycle, the dihedral angle between the planes of the rings is 72^o and the interatomic angle N(2)–S(1)–C(8) is 102.55(15)^o. In the crystal the molecules form dimeric intermolecular H-bonds NH···N with participation of the amidine NH-group and N-atom of the imidazole, and NH···O H-bonds between imidazole and O-atom of the SO_2-group.

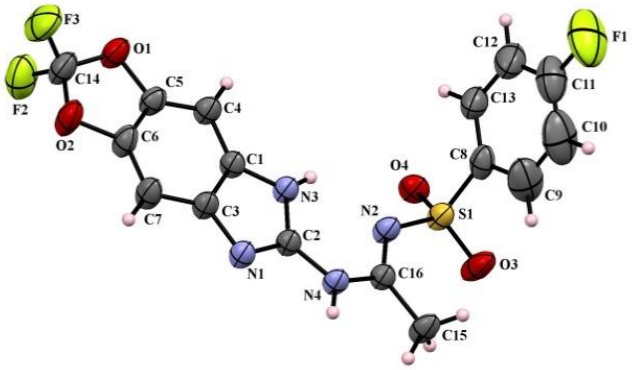
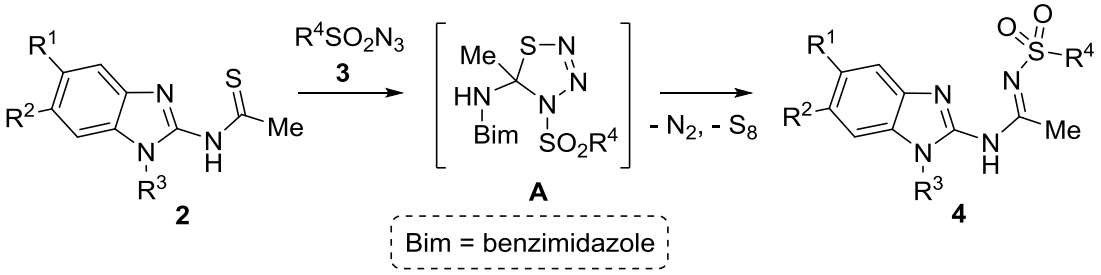


Figure 2. Compound **4o** according to XRD data in the thermal ellipsoids of the 50% probability level.

A plausible mechanism of the formation of amidines **4** is depicted in Scheme 3. We propose the formation of intermediate thiatriazolines **A** via 1,3-dipolar cycloaddition with inverse electron demand of the azido group of sulfonyl azides to the C=S bond of thioamides **2**. The fact that thioamides **2g,h** bearing electron withdrawing CF_3 group does not react with sulfonyl azides is in accord with this interpretation. The elimination of dinitrogen and sulfur from thiatriazoline **A** finalizes the formation of amidines **4**.



Scheme 3. Plausible mechanism of formation of *N*-sulfonyl amidines **4**.

Molecular Modeling

Energetically minimized ligand conformations were docked (see Experimental part) into the active site of CK1δ and CK1ε, respectively. Binding poses were determined and subsequently ranked based on their calculated binding affinities. While we did not obtain a plausible binding mode for CK1ε, the top ranked binding poses and the corresponding 2D ligand interaction diagrams for CK1δ are shown in Figure 3. Consistent with the binding mode of benzimidazole inhibitors in CK1δ determined by X-ray crystallography in our study a highly similar situation could be obtained for **2c**. Herein, the 2-amino-benzimidazole core is involved in key H-bonds towards the hinge motif Leu85. The CF_3 motif occupies the hydrophic pocket surrounded by Ile148/23, Met80, Tyr56, and Pro66. On the other site, the thioacetate moiety is oriented towards the hydrophobic region which opens to the solvent exposed area. Thus, based on the binding mode of hit fragment **2c**, a structure based optimization approach towards more potent CK1δ inhibitors will be developed.

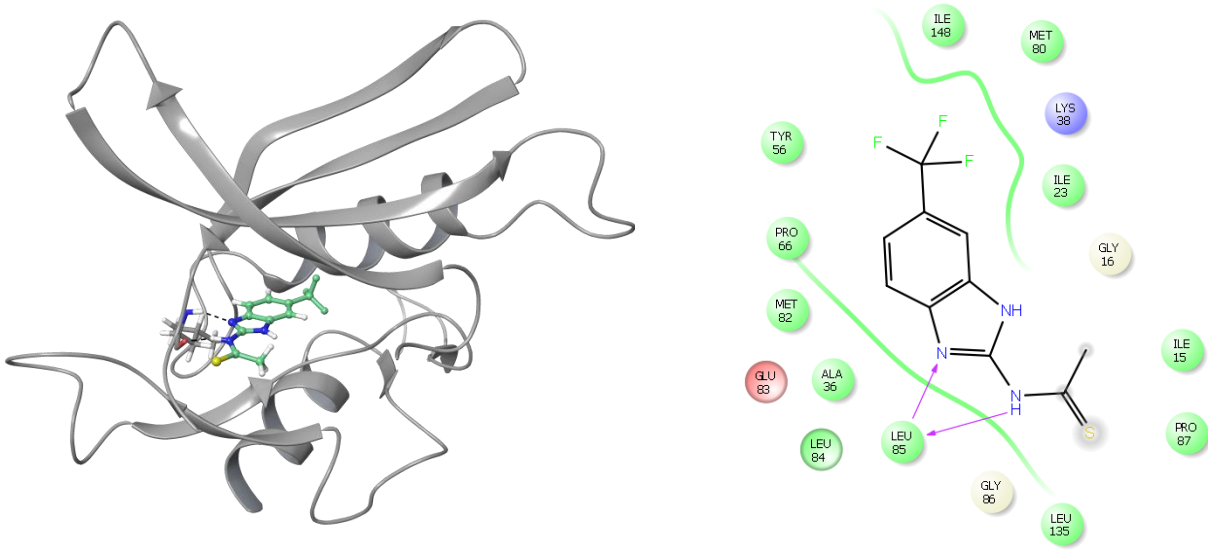


Figure 3. Modeled binding modes of **2c** in the ATP-binding pocket of CK1δ (pdb 4TWC). Key amino acid residues and ligand-active site interactions are shown (left). Right: corresponding 2D ligand interaction diagram.

Inhibitory effects of selected compounds on the kinase activity of CK1 isoforms

Selected compounds were initially screened for their activity against different CK1 isoforms against a concentration of 10 µM ATP. Under these conditions, the synthesized compounds did not show activities on CK1α and CK1γ3. Modest activities were found for **2c** and **4l** which slightly inhibit CK1δ and CK1ε respectively. Hit compound **2c** showed an IC_{50} value of approximately 7 µM for CK1δ and **4l** an IC_{50} of 4.86 µM for CK1ε (Figure 4). Although these values are significantly weaker than IC_{50} values of known highly potent CK1-specific inhibitors,[22] our results suggest that our scaffolds might be improved for further development of potent CK1 isoform kinase inhibitors.

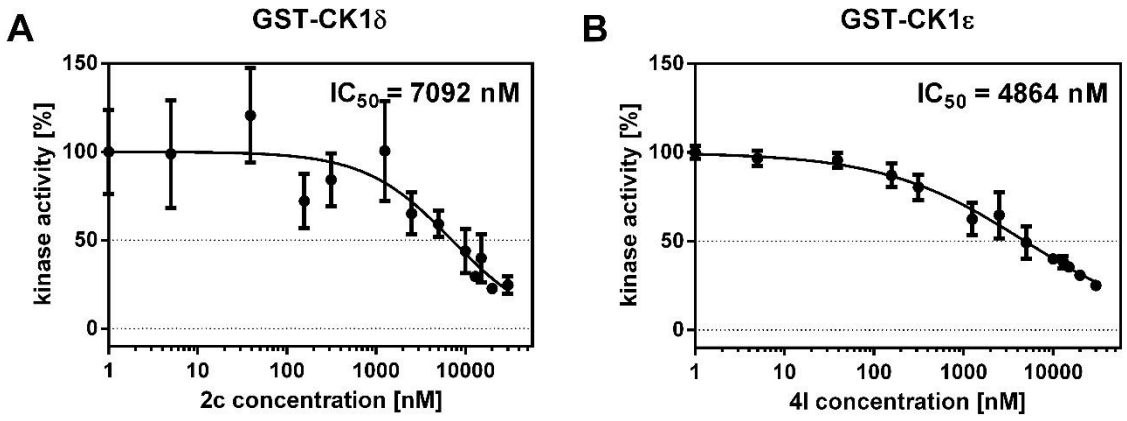


Figure 4. IC_{50} curves of relevant compounds against CK1δ and CK1ε. Curve **A**: **2c**; curve **B**: **4l**.

Conclusions

We have demonstrated a simple, efficient and convenient three-step synthesis of *N*-benzimidazol-2-yl-*N*′-sulfonyl amidines of acetic acid from commercially available reagents. Fifteen compounds were successfully obtained and tested for inhibition of casein kinases. Among the synthesized compounds, two products showed inhibitory abilities towards CK1δ and CK1ε. The search for more active compounds using the developed synthetic approach is in progress in our laboratories.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra (including 2D HMBS and HSQC) were recorded with Bruker Avance II spectrometer in DMSO-d_6 at 50 ^{o}C (400 and 100 MHz, respectively) using Me_4Si as an internal standard. Mass spectra were recorded with Shimadzu GCMS-QP2010 Ultra instrument in electron ionization (EI) mode. Electron energy - 70eV. IR spectra were obtained with Bruker Alpha (NPVO, ZnSe) IR-Fur spectrometer. Microanalyses were performed with PerkinElmer Series II CHNS/O 2400 elemental analyzer. Melting points were determined using a Stuart SMP 3 apparatus. The progress of the reactions and the purity of the compounds were monitored by TLC on TLC Silica gel 60 F245 aluminum sheets (Merck KGaA) in EtOAc-PE system.

X-Ray analysis. The single-crystal X-ray diffraction data for **4o** were collected with a "Xcalibur 3" diffractometer (Oxford Diffraction) with CCD detector applying the standard procedure (CuK_α-irradiation (λ = 1.54184 Å), graphite monochromator, ω-scans with step 1^{o} at T = 295(2) K). Empirical absorption correction was applied. The structure was solved by direct method with SHELX97 and was refined by full-matrix least squares on F^2 using SHELX97.[23]

Synthesis. The amides **1a-c,e,g** were synthesized by known procedures.[5-7,24]

Ethyl 2-acetamido-1*H*-benzimidazole-5-carboxylate (**1d**). A solution of ethyl 2-amino-1*H*-benzimidazole-5-carboxylate (1.20 g, 5.85 mmol) in excess of acetic anhydride (5 mL) was refluxed for 1 h. Water (30 mL) was added to the cooled reaction mixture and the resulting suspension was stirred for 30 min. The formed precipitate was filtered off, washed with water, dried and crystallized from ethyl acetate. Colorless powder, yield 1.02 g (70%), mp 293-295 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (t, *J* 8.0 Hz, 3H, $C\underline{H}_3CH_2O$), 2.18 (s, 3H, CH_3), 4.31 (q, *J* 8.0 Hz, 2H, $CH_3C\underline{H}_2O$), 7.50 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 7.76 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 8.10 (s, 1H, $CH_{arom.}$), 11. 58 (br. s, 1H, NH), 12. 19 (br. s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6) δ 14.1 ($\underline{C}H_3CH_2O$), 23.0 (CH_3), 60.0 ($CH_3\underline{C}H_2O$), 114.0 (C-7), 115.2 (C-4), 122.3 (C-6), 122.5 (C-5), 134.9 (C-3a), 140.4 (C-7a), 148.6 (C-2), 168.2 (C-5′), 169.7 (C-2′). MS *m/z* EI (70 eV), (I,%): 247 [M^+] (50), 205 (100), 177 (30), 160 (80), 132 (30), 105 (29). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1470, 1509, 1585, 1620, 1684, 1724, 3210, 3303. Anal. Calcd for $C_{12}H_{13}N_3O_3$ (247.25): C, 58.29; H, 5.30; N, 17.00. Found: C, 58.58; H, 5.49; N, 16.85.

***N*-(2,2-Difluoro-5*H*-[1,3]dioxolo[4,5-*e*]benzimidazol-6-yl)acetamide** (**1f**). To 2,2-difluoro-5*H*-[1,3]dioxolo[4,5-*e*]benzimidazol-6-amine hydrobromide (1.46 g, 4.96 mmol, 1.0 equiv.) in anhydrous DCM (6 mL) DIPEA (0.77 g, 0.98 mL, 5.95 mmol, 1.2 equiv.) was added. The resulting solution was stirred at room temperature for 10 min and then cooled to 10 ^{o}C. Then acetyl chloride (0.467 g, 0.42 mL, 5.95 mmol, 1.2 equiv.) was added to the solution. The resulting suspension was stirred for 5 h. The formed precipitate was filtered off. The filtrate was evaporated to dryness and water was added to the residue. An additional portion of crude acetamide **1f** was filtered off. The combined precipitates were transferred to a glass beaker and saturated aq. $NaHCO_3$ (25 mL)

was added. The resulting suspension was stirred for 20 min and the precipitate was filtered off, washed with water and dried. The crude product was crystallized from ethanol. Colorless powder, yield 0.98 g (77%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.16 (s, 3H, CH_3), 7.40 (s, 2H, $H_{arom.}$), 11.53 (br. s, 1H, NH), 12.12 (br. s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6) δ 23.0 (C-10), 94.1 (C-7), 98.3 (C-4), 128.0 (C-3a), 131.2 (t, *J* 250.0 Hz, C-8), 135.9 (C-7a), 138.3 (2C, C-5, C-6), 146.9 (C-2), 169.5 (C=O). MS EI (70 eV), *m/z* (I,%): 255 $[M]^+$ (38), 213 (100), 147 (25), 119 (40), 43 (42). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1129, 1213, 1245, 1463, 1604, 1650, 1683, 2943, 3331. Anal. Calcd for $C_{10}H_7F_2N_3O_3$ (255.18): C, 47.07; H, 2.77; N, 16.47. Found: C, 47.38; H, 2.52; N, 16.60%.

2,2,2-Trifluoro-*N*-(5-methyl-1*H*-benzimidazol-2-yl)acetamide (**1h**). Trifluoroacetic anhydride (6.0 mmol, 1.26 g, 0.77 mL, 2.0 equiv.) was added to a solution of 5-methyl-1*H*-benzimidazol-2-amine (3.0 mmol, 0.44 g, 1.0 equiv.) in anhydrous 1,4-dioxane (15 mL) at 10 °C. The reaction mixture was stirred at room temperature for 4 h. After reaction completion, the mixture was concentrated to dryness under vacuum and water (20 mL) was added to the solid residue. The resulting mixture was stirred for 20 min. The precipitate was filtered off, washed with water, ethanol, dried at 60 °C and used in the next stage without further purification. Colorless precipitate, mp 275-279 °C, yield 80% (0.58 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3), 7.08 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 7.25 (s, 1H, $CH_{arom.}$), 7.33 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 12.83 (br. s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.0 (CH_3), 111.7 (C-7), 111.8 (C-4), 117.3 (q, *J* 286.3 Hz, CF_3), 124.5 (C-6), 126.8 (C-7a), 129.0 (C-3a), 133.0 (C-5), 152.2 (C-2), 163.1 (q, *J* 33.6 Hz, C=O). MS *m/z* EI (70 eV), (I,%): 243 $[M^+]$ (52), 174 (100), 146 (20). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1131, 1195, 1573, 1587, 1620, 1636. Anal. Calcd for $C_{10}H_8F_3N_3O$ (243.19): C, 49.39; H, 3.32; N, 17.28. Found: C, 49.75; H, 3.62; N, 17.50%.

General procedure for the synthesis of (benzimidazol-2-yl)ethanethioamides 2a-f. A mixture of the corresponding amide **1** (3.20 mmol, 1.0 equiv.) and Lawesson's reagent (1.76 mmol, 0.55 equiv.) was refluxed in anhydrous 1,4-dioxane (20 mL) for 6 h. After completion, the reaction mixture was concentrated under vacuum to dryness. The crude product was purified by silica gel column chromatography (EtOAc/PE) to afford the corresponding thioamide **2**.

***N*-(1*H*-Benzimidazol-2-yl)ethanethioamide** (**2a**). Yellow powder, mp 203-205 °C, yield 51% (0.31 g), eluent: EtOAc/PE (1:2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.67 (s, 3H, CH_3), 7.20-7.22 (m, 2H, $CH_{arom.}$), 7.55-7.58 (m, 2H, $CH_{arom.}$), 12.92 (br. s, 2H, NH_2). ^{13}C (100 MHz, DMSO-d_6) δ 35.9 (CH_3), 113.9 (2C, C-4, C-7), 122.2 (2C, C-5, C-6), 133.4 (2C, C-3a, C-7a), 148.6 (C-2), 203.7 (C=S). MS *m/z* EI (70 eV), (I, %): 191 $[M]^+$ (47), 176 (20), 150 (30), 133 (100), 105 (25), 59 (45). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1258, 1360, 1441, 1527, 1583, 1630, 1690, 2829, 2930, 3331. Anal. Calcd for $C_9H_9N_3S$ (191.25): C, 56.52; H, 4.74; N, 21.97. Found: C, 56.74; H, 4.57; N, 21.83 %.

***N*-(5-Methyl-1*H*-benzimidazol-2-yl)ethanethioamide** (**2b**). Light yellow powder, mp 201-203 °C, yield 64% (0.42 g), eluent EtOAc/PE (1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.03 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 7.35 (s, 1H, $CH_{arom.}$), 7.44 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 12.96 (br. s, 2H, NH_2). ^{13}C (100 MHz, DMSO-d_6) δ 21.2 (C-8), 36.3 (C-10), 113.7 (2C, C-4, C-7), 131.0 (C-7a), 131.8 (C-5), 133.1 (C-3a), 148.9 (C-2), 203.6 (C-9). MS EI (70 eV), *m/z* (I,%): 205 $[M]^+$ (72), 190 (42), 164 (46), 146 (100), 77 (29), 59 (57). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1303, 1326, 1350, 1436, 1478, 1581, 1600, 1635. Anal. Calcd for $C_{10}H_{11}N_3S$ (205.28): C, 58.51; H, 5.40; N, 20.47. Found: C, 58.32; H, 5.68; N, 20.27%.

***N*-(5-(Trifluoromethyl)-1*H*-benzimidazol-2-yl)ethanethioamide** (**2c**). Yellow powder, mp 225-227 °C, yield 40% (0.33 g), eluent EtOAc/PE (1:7). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.73 (s, 3H, CH_3), 7.50 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 7.76 (d, *J* 8.0 Hz, 1H, $CH_{arom.}$), 7.94 (br. s, 1H, $CH_{arom.}$), 13.10 (br. s, 2H, NH_2). ^{13}C NMR(100 MHz, DMSO-d_6) δ 35.1 (CH_3), 112.1 (C-4), 115.1 (C-7), 118.5 (d, *J* 3.0 Hz, C-6), 122.2 (q, *J* 32.0 Hz, C-5), 124.8 (q, *J* 270.0 Hz, C-11), 134.7 (C-3a), 137.1 (C-7a), 149.3 (C-2), 202.7 (C=S). MS EI (70 eV), *m/z* (I,%): 259 $[M]^+$ (63), 218 (22), 59 (100). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1102, 1329, 1528, 1606, 1640, 2824, 3184. Anal. Calcd for $C_{10}H_8F_3N_3S$ (259.25): C, 46.33; H, 3.11; N, 16.21. Found: C, 46.50; H, 2.89; N, 16.05%.

Ethyl 2-(ethanethioamido)-1*H*-benzimidazole-5-carboxylate (**2d**). Yellow powder, mp 198-200 °C, yield 70% (0.59 g), eluent EtOAc/PE (1:2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.71 (s, 3H, CH$_3$), 4.34 (q, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.58-7.60 (m, 1H, H$_{arom.}$), 7.81-7.84 (m, 1H, H$_{arom.}$), 8.17-8.20 (m, 1H, H$_{arom.}$), 13.08 (br. s, 2H, NH$_2$). ^{13}C (100 MHz, DMSO-d_6) δ 14.2 (C-10), 35.4 (C-12), 60.3 (C-9), 114.5 (C-7), 116.2 (C-4), 123.3 (C-6), 123.9 (C-5), 133.2 (C-3a), 139.1 (C-7a), 149.6 (C-2), 166.1 (C-11), 203.3 (C=O). MS EI (70 eV), *m/z* (I, %): 263 [M$^+$] (100), 248 (36), 222 (46), 205 (47), 160 (68), 59 (81). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1254, 1361, 1518, 1576, 1597, 1637, 1694, 2931, 3079, 3205. Anal. Calcd for $C_{12}H_{13}N_3O_2S$ (263.31): C, 54.74; H, 4.98; N, 15.96. Found: C, 55.03; H, 5.13; N, 15.85%.

***N*-(1-Methyl-1*H*-benzimidazol-2-yl)ethanethioamide** (**2e**). Beige powder, mp 119-122 °C, yield 57% (0.38 g), eluent EtOAc/PE (1:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.62 (s, 3H, CH$_3$), 3.68 (s, 3H, CH$_3$), 7.31-7.35 (m, 2H, H$_{arom.}$), 7.58 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.70 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 13.51 (br. s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6) δ 28.7 (C-10), 38.4 (C-9), 110.0 (C-7), 113.9 (C-4), 123.1 (C-5), 123.3 (C-6), 129.4 (C-3a), 130.4 (C-7a), 150.9 (C-2), 208.8 (C-8). MS EI (70 eV), *m/z* (I,%): 205 [M]$^+$ (70), 190 (100), 172 (25), 164 (30), 132 (45), 77 (28), 59 (26). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1395, 1542, 1614, 2934. Anal. Calcd for $C_{10}H_{11}N_3S$ (205.28): C, 58.51; H, 5.40; N, 20.47. Found: C, 58.83; H, 5.58; N, 20.56%.

***N*-(2,2-Difluoro-5*H*-[1,3]dioxolo[4,5-*e*]benzimidazol-6-yl)ethanethioamide** (**2f**). Light yellow powder, mp 152-154 °C, yield 51% (0.44 g), eluent EtOAc/PE (1:3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.69 (s, 3H, CH$_3$), 7.55 (s, 2H, CH$_{arom.}$), 12.92 (br. s, 2H, NH). ^{13}C (100 MHz, DMSO-d_6) δ 34.9 (CH$_3$), 96.8 (2C, C-4, C-7), 130.9 (2C, C-3a, C-7a), 131.2 (t, *J* 253.0 Hz, C-8), 139.1 (2C, C-5, C-6), 147.5 (C-2), 201.3 (C=S). MS EI (70 eV), *m/z* (I,%): 271 [M]$^+$ (82), 230 (54), 213 (83), 147 (24), 119 (55), 59 (100). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1140, 1246, 1460, 1610, 1647, 1692, 2938, 3327. Anal. Calcd for $C_{10}H_7F_2N_3O_2S$ (271.24): C, 44.28; H, 2.60; N, 15.49. Found: C, 44.11; H, 2.85; N, 15.65%.

***N*-(1*H*-Benzimidazol-2-yl)-2,2,2-trifluoroethanethioamide** (**2g**). Bright yellow powder, mp 183-187 °C, yield 66% (0.52 g), eluent EtOAc/PE (1:8). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37-7.41 (m, 2H, H$_{arom.}$), 7.63-7.66 (m, 2H, H$_{arom.}$), 13.64 (br. s, 2H, NH). ^{13}C (100 MHz, DMSO-d_6) δ 112.7 (2C, C-4, C-7), 118.4 (q, *J* 278.0 Hz, C-9), 124.3 (2C, C-5, C-6), 128.4 (2C, C-3a, C-7a), 151.6 (C-2), 187.5 (q, *J* 34.0 Hz, C-8). MS EI (70 eV), *m/z* (I,%): 245 [M]$^+$ (49), 176 (100), 149 (28), 118 (24), 88 (16). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 973, 1131, 1267, 1430, 1553, 1614, 3022, 3351. Anal. Calcd for $C_9H_6F_3N_3S$ (245.22): C, 44.08; H, 2.47; N, 17.14. Found: C, 43.90; H, 2.60; N, 17.25 %.

2,2,2-Trifluoro-*N*-(5-methyl-*1H*-benzimidazol-2-yl)ethanethioamide (**2h**). Bright yellow powder, mp 226-239 °C, yield 57% (0.47 g), eluent EtOAc/PE (1:4→1:2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3H, CH$_3$), 7.22 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.43 (s, 1H, CH$_{arom.}$), 7.54 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 13.62 (br. s, 2H, NH). MS EI (70 eV), *m/z* (I, %): 259 [M]$^+$ (40), 190 (100), 95 (30), 77 (20). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1117, 1182, 1270, 1452, 1563, 1610, 2923, 3096. Anal. Calc for $C_{10}H_8F_3N_3S$ (259.25): C, 46.33; H, 3.11; N, 16.21. Found: C, 46.68; H, 3.39; N, 16.04%.

General procedure for the synthesis of (*E*)-*N*-(1*H*-benzimidazol-2-yl)-*N*′-sulfonylacetimidamides 4a-o. A solution of thioamide **2** (1.3 mmol, 1.0 equiv.) and sulfonyl azide **3** (1.3 mmol, 1.0 equiv.) in anhydrous EtOH (8 mL) was refluxed for 2-10 h. After completion, the reaction mixture was concentrated under vacuum to dryness, and the residue was purified by flash chromatography over silica gel (60−120) using EtOAc/PE mixtures or crystallized from EtOH.

(*E*)-*N*-(1*H*-Benzimidazol-2-yl)-*N*′-(methylsulfonyl)acetimidamide (**4a**). Reaction time 5 h. Colorless powder, mp 285-287 °C (from EtOH), yield 82% (0.27 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H, CH$_3$), 3.22 (s, 3H, SO$_2$CH$_3$), 7.15-7.17 (m, 2H, CH$_{arom.}$), 7.48-7.50 (m, 2H, CH$_{arom.}$), 11.83 (br. s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9 (C9), 42.8 (C-10), 113.9 (2C, C-4, C-9), 121.8 (2C, C-5, C-6), 134.9 (2C, C-3a, C-7a), 146.5 (C-2), 165.0 (C-8). MS EI (70 eV), *m/z* (I, %): 252 [M$^+$] (25), 132 (100), 105 (42). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1061, 1285,

1455, 1514, 1565, 1618, 2815, 3360. Anal. Calcd for $C_{10}H_{12}N_4O_2S$ (252.29): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.83; H, 4.95; N, 22.09%.

(*E*)-*N*-(1*H*-Benzimidazol-2-yl)-*N′*-tosylacetimidamide (**4b**). Reaction time 5 h. Colorless powder, mp 243-245 °C (from EtOH), yield 66% (0.28 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, 4-C$\underline{H}_3$C$_6$H$_4$), 2.41 (s, 3H, CH$_3$), 7.19-7.21 (m, 2H, CH$_{arom.}$), 7.35 (d, *J* 8.0 Hz, 2H, CH$_{arom.}$), 7.51-7.53 (m, 2H, CH$_{arom.}$), 7.86 (d, *J* 8.0 Hz, 2H, CH$_{arom.}$), 12.03 (br. s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9 (C-10), 21.4 (C-9), 113.8 (2C, C-4, C-9), 122.3 (2C, C-5, C-6), 126.3 (2C-*o*), 129.3 (2C-*m*), 133.8 (2C, C-3a, C-7a), 140.2 (C-*i*), 142.2 (C-*p*), 147.1 (C-2), 167.5 (C-8). MS EI (70 eV), *m/z* (I, %): 328 [M$^+$] (20), 223 (38), 155 (22), 132 (100), 105 (38), 91 (83). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1093, 1143, 1271, 1454, 1517, 1645, 2897, 3032, 3329. Anal. Calcd for $C_{16}H_{16}N_4O_2S$ (328.39): C, 58.52; H, 4.91; N, 17.06. Found: C, 58.32; H, 5.10; N, 17.14%.

(*E*)-*N′*-[(4-Fluorophenyl)sulfonyl]-*N*-(5-methyl-1*H*-benzimidazol-2-yl)acetimidamide (**4c**). Reaction time 2 h. Colorless powder, mp 254-257 °C (from EtOH), yield 67% (0.26 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 6H, 2CH$_3$), 7.03 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.29 (s, 1H, CH$_{arom.}$), 7.37-7.39 (m, 3H, CH$_{arom.}$), 8.00-8.04 (m, 2H, CH$_{arom.}$), 12.02 (br. s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.1 (C-8), 22.1 (C-10), 113.2 (2C, C-4, C-7), 115.9 (d, *J* 22.5 Hz, C-*m*), 123.8 (C-6), 129.2 (d, *J* 9.2 Hz, C-*o*), 130.8 (C-7a), 131.9 (C-5), 132.9 (C-3a), 139.9 (d, *J* 29.0, C-*i*), 147.7 (C-2), 163.8 (d, *J* 248.6 Hz, C-*p*), 168.3 (C-9). MS EI (70 eV), *m/z* (I, %): 346 [M$^+$] (18), 159 (18), 146 (100), 95 (50). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1025, 1089, 1148, 1236, 1281, 1345, 1472, 1542, 2886, 3037, 3324. Anal. Calcd for $C_{16}H_{15}FN_4O_2S$ (346.38): C, 55.48; H, 4.37; N, 16.18. Found: C, 55.78; H, 4.04; N, 16.07%.

(*E*)-*N′*-Tosyl-*N*-[5-(trifluoromethyl)-1*H*-benzimidazol-2-yl]acetimidamide (**4d**). Reaction time 3 h. Colorless powder, mp 213-216 °C (from EtOH), yield 67% (0.40 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, 4-CH$_3$C$_6$H$_4$), 2.47 (s, 3H, CH$_3$), 7.38 (d, *J* 8.0 Hz, 2H, CH$_{arom.}$), 7.49 (t, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.71 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.78-7.94 (m, 3H, CH$_{arom.}$), 12.22 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.2 (C-10), 20.9 (C-17), 112.1 (C-4), 115.2 (C-7), 118.6 (C-6), 122.3 (q, *J* 31.0 Hz, C-5), 125.1 (q, *J* 270.0 Hz, CF$_3$), 126.4 (C-16), 126.5 (C-12), 129.5 (2C, C-13, C-15), 135.3 (C-3a), 138.1 (C-7a), 139.4 (C-11), 142.7 (C-14), 147.7 (C-2), 166.0 (C-9). MS EI (70 eV), *m/z* (I,%): 396 [M$^+$] (16), 291 (40), 200 (20), 155 (36), 91 (100). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1032, 1092, 1110, 1150, 1275, 1333, 1526, 1632, 1650, 2920, 3017, 3300. Anal. Calcd for $C_{17}H_{15}F_3N_4O_2S$ (396.39): C, 51.51; H, 3.81; N, 14.13. Found: C, 51.80; H, 3.98; N, 14.04%.

(*E*)-*N′*-[(4-Fluorophenyl)sulfonyl]-*N*-[5-(trifluoromethyl)-1*H*-benzimidazol-2-yl]acetimidamide *(*4e). Reaction time 5 h. Colorless powder, mp 255-258 °C (fashed with EtOH), yield 96% (0.51 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.52 (s, 3H, CH$_3$), 7.40 (t, *J* 8.0 Hz, 2H, CH$_{arom.}$), 7.48 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.70 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.89 (s, 1H, CH$_{arom.}$), 8.05-8.08 (m, 2H, CH$_{arom.}$), 12.08 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.4 (C-10), 112.0 (C-4), 115.5 (C-7), 116.1 (d, *J* 22.6 Hz, C-*m*), 118.6 (C-6), 122.6 (q, *J* 31.4 Hz, C-5), 125.0 (q, *J* 269.9 Hz, C-8), 129.4 (d, *J* 9.3 Hz, C-*o*), 136.0 (C-3a), 138.4 (C-7a), 138.9 (C-11), 147.8 (C-2), 163.1 (d, *J* 49.2 Hz, C-*p*), 163.4 (C-9). MS EI (70 eV), *m/z* (I, %): 400 [M$^+$] (19), 295 (36), 200 (44), 173 (16), 159 (53), 95 (100). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1029, 1087, 1144, 1274, 1331, 1484, 1525, 1630, 1649, 2863, 3013, 3311. Anal. Calcd for $C_{16}H_{12}F_4N_4O_2S$ (400.35): C, 48.00; H, 3.02; N, 13.99. Found: C, 47.82; H, 3.25; N, 13.83%.

Ethyl (*E*)-2-[*N′*-(methylsulfonyl)acetimidamido]-1*H*-benzimidazole-5-carboxylate (**4f**). Reaction time 7 h. Colorless powder, mp 276-279 °C (for purification the crude product was refluxed in ethanol (5 mL) for 5 min and filtered off), yield 72% (0.30 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.34 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.50, (s, 3H, CH$_3$), 3.25 (s, 3H, C$\underline{H}_3$SO$_2$), 4.33 (qv, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.57 (d, *J* 8.0 Hz, 2H, H$_{arom.}$), 7.82 (d, *J* 8.0 Hz,1H, H$_{arom.}$), 8.18 (s, 1H, H$_{arom.}$), 11.94 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1 (C-7′), 20.2 (C-3′), 42.7 (C-4′), 60.2 (C-6′), 114.5 (C-7), 115.3 (C-4), 122.9 (-C6), 123.2 (C-5), 134.9 (C-3a), 140.0 (C-7a), 147.9 (C-2), 164.3 (C-2′), 166.0 (C=O). MS EI (70 eV), *m/z* (I,%): 324 (M$^+$, 58), 283 (18), 245 (16), 204 (100), 176 (24),

160 (30), 131 (31), 104 (23), 79 (37). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1132, 1232, 1254, 1286, 1521, 1556, 1698, 2960, 3243. Anal. Calc for $C_{19}H_{20}N_4O_4S$ (324.36): C, 48.14; H, 4.97; N, 17.27. Found: C, 47.90; H, 4.83; N, 17.40%.

Ethyl (*E*)-2-(*N*′-tosylacetimidamido)-1*H*-benzimidazole-5-carboxylate (**4g**). Reaction time 4 h. Colorless powder, mp 224-227 °C (from EtOH), yield 70% (0.36 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.34 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.37, (s, 3H, CH$_3$), 2.48 (s, 3H, 4-C$\underline{H}_3$C$_6$H$_4$), 4.33 (q, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.38 (d, *J* 8.0 Hz, 2H, H$_{arom}$), 7.58 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.82 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.88 (d, *J* 8.0 Hz, 2H, H$_{arom.}$), 8.18 (s, 1H, H$_{arom.}$), 12.04 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (C-7′), 20.3 (C-3′), 20.7 (C-4′), 60.2 (C-6′), 114.2 (C-7), 115.6 (C-4), 123.1 (C-5), 123.4 (C-6), 126.3 (2C-*o*), 129.2 (2C-*m*), 134.6 (C-3a), 139.4 (C-7a), 139.6 (2C-*i*), 142.3 (C-*p*), 147.8 (C-2), 165.9 (C=O), 166.2 (C-2′). MS EI (70 eV), *m/z* (I,%): 400 [M$^+$] (18), 295 (54), 204 (43), 155 (36), 91 (100). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1154, 1307, 1521, 1701, 2984, 3280. Anal. Calcd for $C_{19}H_{20}N_4O_4S$ (400.45): C, 56.99; H, 5.03; N, 13.99. Found: C, 57.29; H, 5.09; N, 13.75%.

Ethyl (*E*)-2-{*N*′-[(4-methoxyphenyl)sulfonyl]acetimidamido}-1*H*-benzimidazole-5-carboxylate (**4h**). Reaction time 10 h. Colorless powder, mp 240-244 °C (from EtOH), yield 69% (0.37 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.48, (s, 3H, CH$_3$), 3.83 (s, 3H, 4-C$\underline{H}_3$OC$_6$H$_4$), 4.33 (q, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.09 (d, *J* 8.0 Hz, 2H, H$_{arom.}$), 7.58 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.82 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.94 (d, *J* 8.0 Hz, 2H, H$_{arom.}$), 8.18 (s, 1H, H$_{arom.}$), 12.04 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ 14.0 (C-7′), 20.1 (C-3′), 55.4 (C-4′), 60.2 (C-6′), 114.0 (2C-*m*), 114.2 (C-7), 115.2 (C-4), 123.0 (C-6), 123.4 (C-5), 128.4 (2C-*o*), 134.2 (2C-*i*), 135.2 (C-3a), 139.9 (C-7a), 147.9 (C-2), 162.0 (C-*i*), 165.8 (C-2′), 166.0 (C=O). MS EI (70 eV), *m/z* (I,%): 416 [M]$^+$ (13), 311 (100), 204 (35), 171 (59), 107 (62), 77 (59). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1158, 1315, 1529, 1700, 2989, 3283. Anal. Calcd for $C_{19}H_{20}N_4O_4S$ (416.45): C, 54.80; H, 4.84; N, 13.45. Found: C, 55.14; H, 5.05; N, 13.26%.

Ethyl (*E*)-2-{*N*′-[(4-fluorophenyl)sulfonyl]acetimidamido}-1*H*-benzimidazole-5-carboxylate (**4i**). Reaction time 2 h. Colorless crystals, mp 259-262 °C (from EtOH), yield 73% (0.38 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.50, (s, 3H, CH$_3$), 4.32 (qv, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.42 (t, *J* 8.0 Hz, 2H, H$_{arom.}$), 7.59 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.83 (dd, *J* 4.0 Hz, *J* 8.0 Hz, 1H, H$_{arom.}$), 8.08-8.11 (m, 2H, H$_{arom.}$), 8.20 (s, 1H, H$_{arom.}$), 12.21 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.2 (C-7′), 20.7 (C-3′), 60.5 (C-6′), 114.5 (C-7), 115.7 (C-4), 116.1 (d, *J* 22.0 Hz, C-*m*), 123.3 (C-5), 123.5 (C-6), 129.6 (d, *J* 9.0 Hz, C-*o*), 134.5 (C-3a), 138.9 (d, *J* 3.0 Hz, C-*i*), 139.4 (C-7a), 147.9 (C-2), 164.06 (d, *J* 249.0 Hz, C-*p*), 166.1 (C=O), 166.7 (C-2′). MS EI (70 eV), *m/z* (I,%): 404 [M$^+$] (27), 299 (46), 204 (100), 159 (68), 95 (75). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1153, 1309, 1521, 1701, 2997, 3297. Anal. Calcd for $C_{18}H_{17}FN_4O_4S$ (404.42): C, 53.46; H, 4.24; N, 13.85. Found: C, 53.62; H, 3.97; N, 13.98%.

Ethyl (*E*)-2-(*N*′-{[2-(methoxycarbonyl)thien-3-yl]sulfonyl}acetimidamido)-1*H*-benzimidazole-5-carboxylate (**4j**). Reaction time 6 h. Colorless crystals, mp 234-236 °C (from EtOH), yield 68% (0.40 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.34 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.58, (s, 3H, CH$_3$), 3.87 (s, 3H, C$\underline{H}_3$OCO), 4.32 (q, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.57 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.62 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.83 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.96 (d, *J* 8.0 Hz, 2H, H$_{arom.}$), 8.13 (s, 1H, H$_{arom.}$), 12.32 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (C-7′), 20.9 (C-3′), 52.8 (C-8′), 60.3 (C-6′), 113.9 (C-7), 115.1 (C-4), 123.3 (C-6), 123.6 (C-5), 129.6 (C-12), 131.2 (C-11), 131.3 (C-13), 133.9 (C-3a), 138.6 (C-7a), 145.5 (C-10), 148.3 (C-2), 160.4 (C-9′), 165.8 (C-5′), 166.3 (C-2′). MS EI (70 eV), *m/z* (I,%): 450 [M$^+$] (11), 345 (100), 313 (82), 285 (85), 268 (47), 240 (39), 205 (49), 134 (30), 64 (35). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1142, 1248, 1310, 1519, 1538, 1706, 2915, 3233. Anal. Calcd for $C_{18}H_{18}N_4O_6S_2$ (450.48): C, 47.99; H, 4.03; N, 12.44. Found: C, 48.20; H, 4.25; N, 12.55%.

Ethyl (*E*)-2-{*N*′-[(3,1,2-benzothiadiazol-4-yl)sulfonyl]acetimidamido}-1*H*-benzimidazole-5-carboxylate (**4k**). Reaction time 5 h. Colorless crystals, mp 185-187 °C (from EtOH), yield 71% (0.41 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.34 (t, *J* 8.0 Hz, 3H, C$\underline{H}_3$CH$_2$O), 2.71, (s, 3H, CH$_3$), 4.32 (qv, *J* 8.0 Hz, 2H, CH$_3$C$\underline{H}_2$O), 7.53 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.80 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 7.88-7.92 (m, 1H, H$_{arom.}$), 8.05 (s, 1H, H$_{arom.}$), 8.36-8.39 (m, 2H, H$_{arom.}$), 12.14 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (C-7′), 21.1 (C-3′), 60.2 (C-6′), 113.9 (C-7),

115.4 (C-4), 123.2 (C-6), 123.6 (C-5), 125.9 (C-*o*), 128.6 (C-*m*), 129.4 (C-*p*), 133.5 (C-*i*), 134.4 (C-3a), 138.7 (C-7a), 148.0 (C-2), 148.9 (C-4′), 155.1 (C-8′), 165.8 (C=O), 166.5 (C-2′). MS EI (70 eV), *m/z* (I,%): 444 [M^+] (18), 339 (100), 204 (34), 176 (20), 135 (60). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1138, 1259, 1318, 1528, 1530, 1703, 2925, 3230. Anal. Calcd for $C_{18}H_{16}N_6O_4S_2$ (444.48): C, 48.64; H, 3.63; N, 18.91. Found: C, 48.97; H, 3.45; N, 18.97%.

Ethyl (*E*)-2-{*N*′-[(3,1,2-benzoxadiazol-4-yl)sulfonyl]acetimidamido}-1*H*-benzimidazole-5-carboxylate (**4l**). Reaction time 4 h. Colorless powder, mp 215-218 °C, yield 73% (0.41 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.34 (t, *J* 8.0 Hz, 3H, C<u>H</u>$_3$CH$_2$O), 2.68, (s, 3H, CH$_3$), 4.33 (qv, *J* 8.0 Hz, 2H, CH$_3$C<u>H</u>$_2$O), 7.51 (d, *J* 4.0 Hz, 1H, H$_{arom.}$), 7.76-7.80 (m, 1H, H$_{arom.}$), 7.83 (d, *J* 4.0 Hz, 1H, H$_{arom.}$), 8.06 (s, 1H, H$_{arom.}$), 8.22 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 8.34 (d, *J* 8.0 Hz, 1H, H$_{arom.}$), 12.24 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (C-7′), 21.8 (C-3′), 60.3 (C-6′), 113.6 (C-7), 115.0 (C-4), 120.7 (C-*o*), 123.4 (C-6), 123.9 (C-5), 130.4 (C-*i*), 131.5 (C-*m*), 132.3 (C-*p*), 133.6 (C-3a), 137.9 (C-7a), 144.6 (C-4′), 148.2 (C-2), 149.5 (C-8′), 165.7 (C=O), 167.4 (C-2′). MS EI (70 eV), *m/z* (I,%): 428 [M^+] (10), 323 (100), 278 (20), 206 (33), 178 (20), 64 (29). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1035, 1139, 1283, 1316, 1429, 1516, 1556, 1624, 1708, 2813, 3074, 3327. Anal. Calcd for $C_{18}H_{16}N_6O_5S$ (428.42): C, 50.46; H, 3.76; N, 19.62. Found: C, 50.30; H, 3.57; N, 19.82%.

(*E*)-*N*-(1-Methyl-1*H*-benzimidazol-2-yl)-*N*′-tosylacetimidamide (**4m**). Reaction time 4 h. Colorless powder, mp 206-210 °C (from EtOH), yield 66% (0.29 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH$_3$), 3.60 (s, 3H, N–CH$_3$), 7.29-7.35 (m, 4H, CH$_{arom.}$), 7.53 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.61 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.78 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 12.30 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9 (C-17), 28.8 (C-8), 113.6 (C-4), 123.2 (3C, C-3a, C-5, C-6), 126.3 (2C, C-*m*), 129.2 (2C, C-*o*), 130.7 (C-7a), 141.0 (C-*i*), 141.7 (C-*p*), 148.7 (C-2), 166.1 (C-9). MS EI (70 eV), *m/z* (I,%): 342 [M]$^+$ (20), 237 (38), 146 (100), 119 (43), 91 (68). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1004, 1079, 1139, 1270, 1329, 1364, 1399, 1553, 3254. Anal. Calcd for $C_{17}H_{18}N_4O_2S$ (342.42): C, 59.63; H, 5.30; N, 16.36. Found: C, 59.87; H, 5.44; N, 16.23%.

(*E*)-*N*′-((4-Fluorophenyl)sulfonyl)-*N*-(1-methyl-1*H*-benzimidazol-2-yl)acetimidamide (**4n**). Reaction time 4 h. Colorless powder, mp 209-211 °C (from EtOH), yield 73% (0.33 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH$_3$), 2.61 (s, 3H, NCH$_3$), 7.28-7.37 (m, 4H, CH$_{arom.}$), 7.54 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.60 (d, *J* 8.0 Hz, 1H, CH$_{arom.}$), 7.94-7.98 (m, 2H, CH$_{arom.}$), 12.28 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.0 (C-10), 28.8 (C-8), 110.3 (C-7), 113.3 (C-4), 115.6 (C-3a), 115.8 (d, *J* 21.0 Hz, C-*m*), 123.3 (2C, C-5, C-6), 129.2 (d, *J* 9.0 Hz, C-*o*), 130.5 (C-7a), 140.3 (d, *J* 3.0 Hz, C-*i*), 148.8 (C-2), 163.6 (d, *J* 248.0 Hz, C-*p*), 170.9 (C-9). MS EI (70 eV): *m/z* (I, %): 346 [M^+] (20), 241 (19), 146 (100), 119 (45), 95 (53). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1000, 1080, 1139 1228, 1272, 1327, 1466, 1555, 3251. Anal. Calcd for $C_{16}H_{15}FN_4O_2S$ (346.38): C, 55.48; H, 4.37; N, 16.18. Found: C, 55.63; H, 4.06; N, 16.30%.

(*E*)-*N*-(2,2-Difluoro-5*H*-[1,3]dioxolo[4,5-*e*]benzimidazol-6-yl)-*N*′-[(4-fluorophenyl)sulfonyl]acetimidamide (**4o**). Reaction time 9 h (6.6 mmol (5.0 equiv) of azide **3c** were used), light yellow crystals (column, eluent EtOAc/PE (3:1)), mp 131-134 °C, yield 70% (0.36 g). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.49, (s, 3H, CH$_3$), 7.37-7.42 (m, 2H, H$_{arom.}$), 7.52 (s, 2H, H$_{arom.}$), 8.02-8.05 (m, 2H, H$_{arom.}$), 11.92 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.0 (C-3′), 96.6 (2C, C-4, C-7), 115.9 (d, *J* 22.0 Hz, C-*m*), 129.2 (d, *J* 9.5 Hz, C-*o*), 130.9 (2C, C-3a, C-7a), 131.0 (t, *J* 250.0 Hz, C-8), 138.8 (d, *J* 3.0 Hz, C-*i*), 138.9 (2C, C-5, C-6), 145.5 (C-2), 163.9 (d, *J* 250.0 Hz, C-*p*), 165.6 (C-2′). MS EI (70 eV), *m/z* (I,%): 412 [M^+] (26), 212 (100), 159 (47), 118 (20), 95 (77). IR (ATR, ZnSe): ν_{max} (cm^{-1}) 1143, 1234, 1463, 1550, 2898, 3004, 3107, 3277. Anal. Calcd for $C_{16}H_{11}F_3N_4O_4S$ (412.34): C, 46.61; H, 2.69; N, 13.59. Found: C, 46.53; H, 2.88; N, 13.70%.

Crystal Data for 4o. Crystal is triclinic, space group *P*-1, *a* = 6.823(7), *b* = 10.989(17), *c* = 11.360(9) Å, α = 76.93(10)°, β = 80.77(8)°, γ = 87.12(10)°, *V* = 818.9(16) Å^3, *Z* = 2, μ(CuK$_\alpha$) = 2.398 mm^{-1}. On the angles 4.04 < θ < 67.75° 16712 reflections were collected, 2913 independent reflection (R_{int} = 0.0515), completeness for θ = 67.75° 98.1%. Final R-factors of the refinement: R_1 = 0.0411, wR_2 = 0.1016 [I>2σ(I)]; R_1 = 0.0553, wR_2 = 0.1048

(all data), GooF = 1.002. Maximum and minimum electron density: 0.221 and -0.373 Åe^{-3}.
Single crystal data for compound **4o** (CCDC 1552962) have been deposited in the Cambridge Crystallographic Data Center and can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Molecular modeling

Molecular modeling was performed on a DELL Precision T5500 eight core workstation. For visualization Maestro, version 10.4, 2014 (Schrödinger LLC, New York, NY, USA) was used.[25] Protein structures were prepared prior to docking by Schrödinger Protein Preparation Wizard synchronizing the following modules: Epik,[26] Impact, and Prime.[27] Water molecules beyond 5 Å from hetero atoms have been deleted. H-bond optimization was performed in a standard sampling, the Root-mean-square deviation for atomic positions cutoff of heavy atoms in subsequent protein minimization was set to 0.3 Å. By using this workflow, for CK1δ, we generated a model based on the high-resolution structure PDB 4TWC,[28] and for CK1ε based on PDB 4HNI.[29] Ligands were prepared by MacroModel[30] to generate energetically minimized structures. Ionization and tautomeric states were processed by LigPrep utilizing Hammet and Taft methodology-based Epik. Additionally, Epik state penalties were implemented. Receptor grid were generated by Glide SP settings.[29] Energetically minimized ligand conformations were docked into the active site of the protein, respectively. Binding poses were determined and subsequently ranked based on their calculated binding affinities.

Biological testing. Materials and methods

***In vitro* kinase assays.** *In vitro* kinase assays were performed with different CK1 isoforms and selected compounds at an ATP concentration of 10 µM and using DMSO controls as described previously.[32,33] Bovine GST-CK1α (FP296)[34] human GST-CK1γ (FP1054)[35] human GST-CK1δ TV1 (FP1417),[36] and recombinant human GST-CK1ε (FP455)[37] were used as sources of enzyme. Phosphorylated proteins were separated by SDS-PAGE and stained with Coomassie. α-casein served as a substrate for all kinase assay reactions. Phosphate incorporation was detected by autoradiography of dried gels. The phosphorylated protein bands were cut out and quantified by Cherenkov counting. Dose-response analyses were carried out using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla CA, USA) statistical software. *In vitro* kinase assays were performed in the presence or absence of the selected compounds. CK1α, CK1γ3, CK1δ, and CK1ε were used as sources of enzyme and α-casein as substrate. All compounds were used at a concentration of 10 µM. Kinase reactions were separated by SDS-PAGE and quantification of phosphate incorporation was performed by Cherenkov counting. Results are shown as normalized bar graphs using DMSO as a control for 100% kinase activity (dotted line). Error bars represent the standard deviation (SD) (DMSO: dimethyl sulfoxide).

Cell culture. The glioblastoma cell line DK-MG[38] was obtained from the Leibniz Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). Cells were grown in RPMI medium. The medium was supplemented with 10% fetal calf serum (FCS; Biochrom, Berlin, Germany), 100 units/ml penicillin, 100 µg/ml streptomycin (Gibco, Karlsruhe, Germany) and 2 mM glutamine. The cultures were kept at 37 ^{o}C in a humidified 5% carbon dioxide atmosphere.

Cell viability assay. Cells were seeded at a concentration of 5 x 10^4 cells/ml in 96-well cell culture plates and allowed to attach overnight at 37 ^{o}C and 5% CO_2. To investigate the effects of selected compounds on cancer cell proliferation, cells were treated with 10 µM of each compound, with untreated and DMSO-treated cells serving as a control. All media were exchanged every two days for fresh treated or control media. After an incubation period of 7 days at 37 ^{o}C, 10 µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 12 mM solution in PBS] were added, followed by further incubation for 4 h at 37 ^{o}C. Media containing MTT was then removed carefully and 100 µl of 0.04 N HCl in isopropanol were added. To dissolve

the formazan crystals, the plates were placed for 30 min on an orbital shaker. The resulting purple solution was spectrophotometrically measured at 570 nm with TECAN Spectra II Plate Reader using Magellan3 (TECAN) as software.

IC_{50} values for **2c** and **4l** were evaluated for their inhibition of CK1δ and CK1ε, respectively (Figure 4). Determination of the 50% inhibitory concentration (IC_{50}) curves of these compounds on the kinase activities was performed using serial dilutions with CK1δ and CK1ε as enzymes. Kinase reactions were separated by SDS-PAGE and the phosphate incorporation into α-casein was measured by Cherenkov counting. Obtained data were normalized towards their respective DMSO control reactions. Dose-response analyses were performed using GraphPad Prism 6, curves are shown as mean. Error bars represent the standard deviation (SD) (DMSO: dimethyl sulfoxide).

Acknowledgements

We gratefully acknowledge financial support of this work by the Russian Foundation for Basic Research (project 17-03-00641). B.G.R. was kindly supported by the German Academic Exchange Service (DAAD). We also thank Marie-Therese Bammert for her technical support.

Supplementary Material

^{1}H and ^{13}C NMR spectra (including 2D HMBC and HSQC) of all new compounds and details of biological testing.

References

1. Bansal, Y.; Silakari, O. *Bioorg. Med. Chem.* **2012**, *20*, 6208. https://doi.org/10.1016/j.bmc.2012.09.013
2. Richter, J.; Bischof, J.; Zaja, M.; Kohlhof, H.; Othersen, O.; Vitt, D.; Alscher, V.; Pospiech, I.; García-Reyes, B.; Berg, S.; Leban, J.; Knippschild, U. *J. Med. Chem.* **2014**, *57*, 7933. https://doi.org/10.1021/jm500600b
3. Lee, M. Y.; Kim, M. H.; Kim, J.; Kim, S. H.; Kim, B. T.; Jeong, I. H.; Chang, S.; Kim, S. H.; Chang, S.-Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 541. https://doi.org/10.1016/j.bmcl.2009.11.104
4. Beryozkina, T.; Bakulev, V.; Dianova, L.; Berseneva, V.; Slepukhin, P.; Leban, J.; Kalaba, P.; Aher, N. Y.; Ilic, M.; Sitte, H. H.; Lubec, G. *Synthesis* **2016**, *48*, 1046. https://doi.org/10.1055/s-0035-1561350
5. Mandel, L. R.; Porter, C. C.; Kuehl, F. A. *J. Med. Chem.* **1970**, *13*, 1043. https://doi.org/10.1021/jm00300a005
6. Garg, K.; Bansal, Y.; Bansal, G.; Goel, R. K. *Med. Chem. Res*. **2015**, *23*, 2690. https://doi.org/10.1007/s00044-013-0856-1
7. Ohemengt, K. A.; Roth, B. *J. Med. Chem*. **1991**, *34*, 1383. https://doi.org/10.1021/jm00108a022

8. Pilyugin, V. S.; Sapozhnikov, Y. E.; Davydov, A. M.; Chikisheva, G. E.; Vorob'eva, T. P.; Klimakova, E. V.; Kiseleva, G. V.; Kuznetsova, S. L.; Davletov, R. D.; Sapozhnikova, N. A.; Yumadilov, R. K. *Russ. J. Gen. Chem*. **2006**, *76*, 1653. https://doi.org/10.1134/S1070363206100264
9. Koeller, S.; Kadota, J.; Peruch, F.; Deffieux, A.; Pinaud, N.; Pianet, I.; Massip, S.; Leger, J.-M.; Desvergne, J.-P.; Bibal, B. *Chem. Eur. J.* **2010**, *16*, 4196. https://doi.org/10.1002/chem.200902912
10. Hong, D. J.; Baek, J. O.; Oh, H. S.; Lee, M. S.; Ha, T. H.; Suh, K. H. PCT Int. Appl WO 2015152550, 2015.
11. Kim, S. H.; Park, S. H.; Choi, J. H.; Chang S. *Chem. Asian J.* **2011**, *6*, 2618. https://doi.org/10.1002/asia.201100340
12. Yavari, I.; Sheikhi, A.; Nematpour, M.; Taheri, Z. *Synth. Commun*. **2015**, *45*, 1089. https://doi.org/10.1080/00397911.2014.974613
13. Aswad, M.; Chiba, J.; Tomohiro, T.; Hatanaka, Y. *Chem. Commun*. **2013**, *49*, 10242. https://doi.org/10.1039/c3cc46055j
14. Dianova, L.; Berseneva, V.; Beryozkina, T.; Efimov, I.; Kosterina, M.; Eltsov, O.; Dehaen, W.; Bakulev, V. *Eur. J. Org. Chem.* **2015**, 6917. https://doi.org/10.1002/ejoc.201500968
15. Efimov, I.; Beliaev, N.; Beryozkina, T.; Slepukhin, P.; Bakulev, V. *Tetrahedron Lett*. **2016**, *57*, 1949. https://doi.org/10.1016/j.tetlet.2016.03.058
16. Filimonov, V. O.; Dianova, L. N.; Galata, K. A.; Beryozkina, T. V.; Novikov, M. S.; Berseneva, V. S.; Eltsov, O. S.; Lebedev, A. T.; Slepukhin, P. A.; Bakulev, V. A. *J. Org. Chem.* **2017**, 82, 4056. https://doi.org/10.1021/acs.joc.6b02736
17. Efimov, I.; Bakulev, V.; Beliaev, N.; Beryozkina, T.; Knippschild, U.; Leban, J.; Zhi-Jin, F.; Eltsov, O.; Slepukhin, P.; Ezhikova, M.; Dehaen, W. *Eur. J. Org. Chem*. **2014**, 3684. https://doi.org/10.1002/ejoc.201402130
18. Bakulev, V. A.; Morzherin, Yu. Yu.; Dankova, E. F.; Kolobov, M. Yu.; Shafran, Yu. M.; Lebedev, A. T. *Bull. Soc. Chim. Belg*. **1993**, *102*, 493. https://doi.org/10.1002/bscb.19931020708
19. Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S.-O. *Bull. Soc. Chim. Belg*. **1978**, *87*, 223. https://doi.org/10.1002/bscb.19780870310
20. Jensen, O. E.; Lawesson, S. O.; Bardi, R.; Piazzesi, A. M.; Toniolo, C. *Tetrahedron* **1985**, *41*, 5595. https://doi.org/10.1016/S0040-4020(01)91361-4
21. Cava, M. P.; Levison, M. I. *Tetrahedron* **1985**, *41*, 5061. https://doi.org/10.1016/S0040-4020(01)96753-5
22. Knippschild, U.; Kruger, M.; Richter, J.; Xu, P.; Garcia-Reyes, B.; Peifer, C.; Halekotte, J.; Bakulev, V.; Bischof, J. *Front. Oncol.* **2014**, *4*, 96.
23. Sheldrick, G. M. *Acta Cryst*. **2008**, *A64*, 112. https://doi.org/10.1107/S0108767307043930
24. Vigorita, M. G.; Ficarra, R.; Ficarra, P.; Tommasini, A. *Boll. Chim. Farm.* **1981**, *120*, 278.
25. Sastry, G. M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. J. *Comput. Aided Mol. Des.* **2013**, *27*, 221. https://doi.org/10.1007/s10822-013-9644-8
26. Greenwood, J. R.; Calkins, D.; Sullivan, A. P.; Shelley, J. C. *J. Comput. Aided Mol. Des.* **2010**, *24*, 591. https://doi.org/10.1007/s10822-010-9349-1

27. Jacobson, M. P.; Pincus, D. L.; Rapp, C. S.; Day, T. J. F.; Honig, B.; Shaw, D. E.; Friesner, R. A. *Proteins* **2004**, *55*, 351. https://doi.org/10.1002/prot.10613
28. Bischof, J.; Leban, J.; Zaja, M; Grothey, A.; Radunsky, B.; Othersen, O.; Strobl, S.; Vitt, D.; Knippschild, U. *Amino Acids*, **2012**, *43*, 1577. https://doi.org/10.1007/s00726-012-1234-x
29. Long, A. M.; Zhao, H.; Huang, X. *J. Med. Chem.* **2012**, *55*, 10307. https://doi.org/10.1021/jm301336n
30. Watts, K. S.; Dalal, P.; Murphy, R. B.; Sherman, W.; Friesner, R. A.; Shelley, J. C. *J. Chem. Inf. Model.* **2010**, *50*, 534. https://doi.org/10.1021/ci100015j
31. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177. https://doi.org/10.1021/jm051256o
32. Knippschild, U.; Milne, D.; Campbell, L., Meek, D. *Oncogene* **1996**, *13*, 1387.
33. Meng, Z.; Bischof, J.; Ianes, C.; Henne-Bruns, D.; Xu, P.; Knippschild, U. *Amino Acids*, **2016**, *48*, 1185. https://doi.org/10.1007/s00726-015-2154-3
34. Milne, D. M.; Palmer, R. H.; Campbell, D. G.; Meek, D. W. *Oncogene* **1992**, *7*, 1361.
35. Sillibourne, J. E.; Milne, D. M.; Takahashi, M.; Ono, Y.; Meek, D.W. *J. Mol. Biol.* **2002**, *322*, 785. https://doi.org/10.1016/S0022-2836(02)00857-4
36. Richter, J.; Bischof, J.; Zaja, M.; Kohlhof, H.; Othersen, O.; Vitt, D.; Alscher, V.; Pospiech, I.; García-Reyes, B.; Berg, S.; Leban, J.; Knippschild, U. *J. Med. Chem.* **2014**, *57*, 7933. https://doi.org/10.1021/jm500600b
37. Knippschild, U.; Milne, D. M.; Campbell, L. E.; DeMaggio, A. J.; Christenson, E.; Hoekstra, M. F.; Meek, D.W. *Oncogene* **1997**, *15*, 1727. https://doi.org/10.1038/sj.onc.1201541
38. Kruse, C. A.; Varella-Garcia, M.; Kleinschmidt-Demasters, B. K.; Owens, G. C.; Spector, E. B.; Fakhrai, H.; Savelieva, E.; Liang, B.C. *In Vitro Cell. Dev. Biol.-Animal* **1998**, *34*, 455. https://doi.org/10.1007/s11626-998-0078-x

