Short communication

Synthesis of Novel 5-(*N*-Boc-*N*-Benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxamides and Their Inhibition of Cathepsins B and K

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Dedicated to Professor Emeritus Miha Tišler, University of Ljubljana, on the occasion of his 90th birthday.

Abstract

Eight novel 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxamides were prepared in three steps from methyl 3-amino-1*H*-pyrazole-4-carboxylate and methyl 5-(benzyl(*tert*-butoxycarbonyl)amino)-3-oxopentanoate. The synthetic procedure comprises cyclocondensation of the above starting compounds, hydrolysis of the ester, and bis(pentafluorophenyl) carbonate (BPC)-mediated amidation. Title carboxamides were tested for inhibition of cathepsins K and B. The *N*-butylcarboxamide **5a** exhibited appreciable inhibition of cathepsin K (IC_{50} ~ 25 μM), while the strongest inhibition of cathepsin B was achieved with *N*-(2-picolyl)carboxamide **5c** (IC_{50} ~ 45 μM).

Keywords: Pyrazolo[1,5-*a*]pyrimidines, cathepsin inhibition, cyclization, synthesis

1. Introduction

Various 5–6 annulated heterocycles are important scaffolds for the preparation of compound libraries for medicinal and pharmaceutical applications.[1,2] Due to biological activity of many of its derivatives, pyrazolo[1,5-*a*] pyrimidine is an important heterocycle among 5–6-fused systems.[3,4] The importance of pyrazolo[1,5-*a*]pyrimidine is reflected in the results of a literature search[5] showing around 150,000 known pyrazolo[1,5-*a*]pyrimidine derivatives within 6,500 references and with preparation, biological study, and uses as the predominant substance roles. For 2016 alone, 74 references can be found for a term "pyrazolo[1,5-*a*]pyrimidines". Among bioactive pyrazolo[1,5-*a*]pyrimidines there are hepatitis C virus inhibitors,[6] antagonists of serotonin 5-HT6 receptors,[7] kinase inhibitors,[8–10] PET tumor imaging agents,[11] and inhibitors of amyloid *β*-peptide aggregation.[12] Sedative agents zaleplon and indiplon and the anxiolytic agent ocinaplon are approved drugs containing a pyrazolo[1,5-*a*]pyrimidine core (Figure 1).

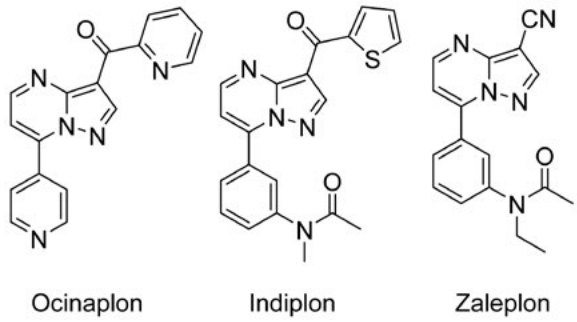
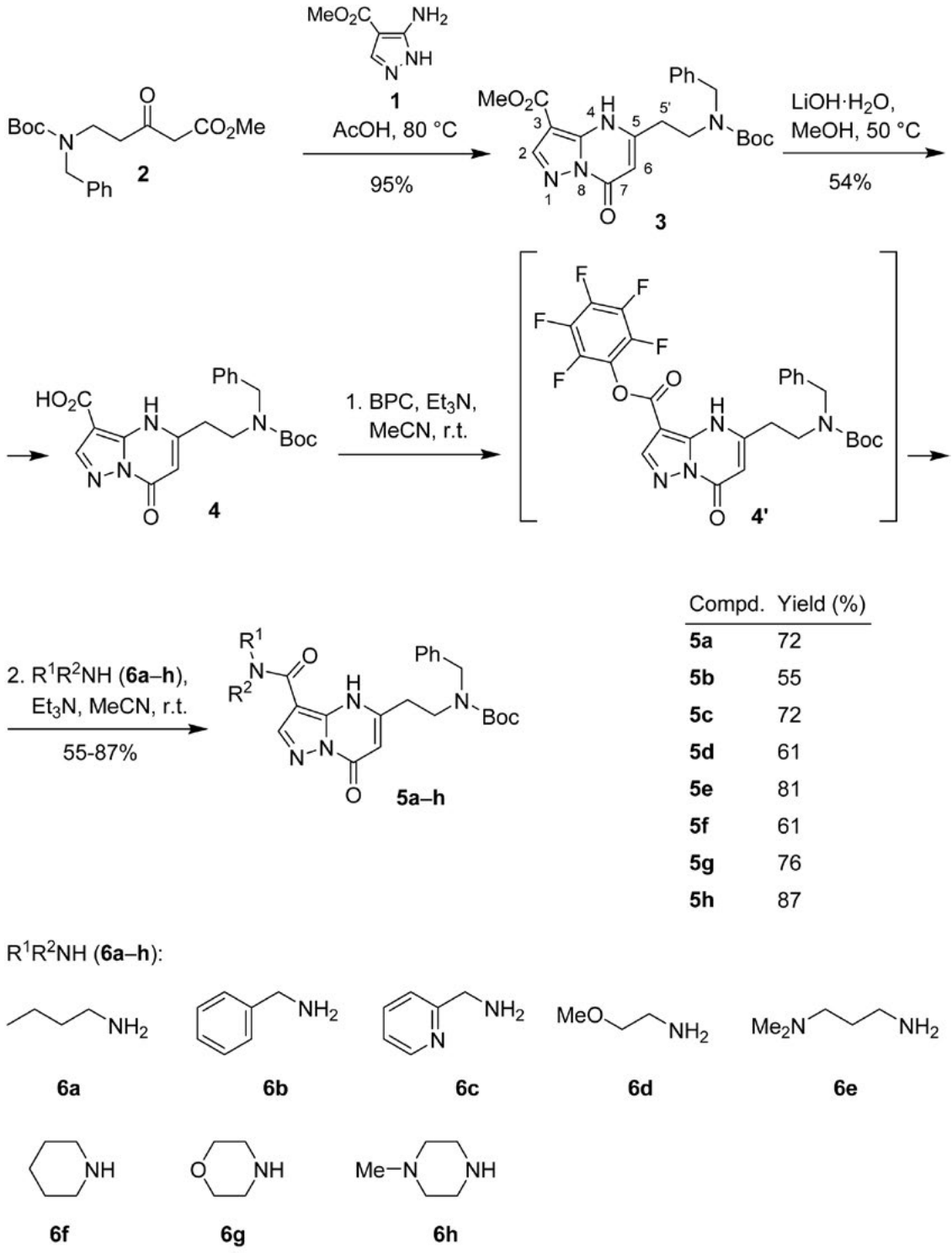


Figure 1. Approved drugs based on a pyrazolo[1,5-*a*]pyrimidine scaffold.

Cathepsin K, a cysteine protease that is selectively and abundantly expressed within osteoclasts, is believed to be crucial for the resorption of bone matrix.[13–17] The ability to degrade type I collagen allows cathepsin K to make a unique contribution to the balance between bone resorption and bone formation.[18,19] Inhibitors of cathepsin K could prevent bone resorption and may provide a promising approach for the treatment of osteoporosis, therefore inhibition of cathepsin K has been proposed as a promising strategy for the treatment of osteoporosis, cancer, and other diseases.[13–15] Several inhibitors have progressed into

clinical trials but there are, as yet, no inhibitors on the market.[20]

Pyrazolo[1,5-*a*]pyrimidines are commonly available by cyclocondensation of a 3-aminopyrazole derivative with a 1,3-dicarbonyl compound or its synthetic equivalent.[3,21] Due to this ease of access, a plethora of known pyrazolo[1,5-*a*]pyrimidine derivatives is not surprising. Nevertheless, a more detailed literature search also reveals that 5-(2-aminoethyl) substituted pyrazolo[1,5-*a*]pyrimidines are much less known – 135 examples can be found by SciFinder®, however, without any literature reference available. Furthermore, the 5-(2-aminoethyl)pyrazolo



Compd.	Yield (%)
5a	72
5b	55
5c	72
5d	61
5e	81
5f	61
5g	76
5h	87

R^1R^2NH (**6a–h**):

Scheme 1. Synthesis of title carboxamides **5a**–**h**.

[1,5-*a*]pyrimidine-3-carboxamides are, to the best of our knowledge, unknown. Recently, a substantial part of our studies were focused on the synthesis of novel pyrazolo[1,5-*a*]pyridine and pyrazolo[1,5-*c*]pyridine derivatives. In this connection, we reported (parallel) syntheses of libraries of novel 7-heteroarylpyrazolo[1,5-*a*]pyridine-3-carboxamides,[22] 7-oxopyrazolo[1,5-*a*]pyrimidine-3-carboxamides,[23] 7-(1-aminoethyl)pyrazolo[1,2–*a*]pyrimidines,[24] and tetrahydropyrazolo[1,5-*c*]pyrimidine-3-carboxamides.[25] In extension, we explored another synthetic approach based on direct cyclisation of methyl 5-amino-1*H*-pyrazole-4-carboxylate (**1**) with methyl 5-[benzyl(*tert*-butoxycarbonyl)amino]-3-oxopentanoate (**2**) to obtain a 5-(2-aminoethyl)pyrazolo[1,5-*a*]pyrimidine central building block for a late-stage derivatization at the carboxy function. Herein we report the results, the synthesis of 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxamides **5a**–**h** and their evaluation for inhibition of cathepsins B and K.

2. Results and Discussion

The starting β-keto ester, methyl 5-[benzyl(*tert*-butoxycarbonyl)amino]-3-oxopentanoate (**2**) was prepared in four steps from benzylamine (**6b**) and methyl acrylate following the literature procedures.[23,26] Subsequent cyclisation of **2** with methyl 5-amino-1*H*-pyrazole-4-carboxylate (**1**)[27] was performed in acetic acid at 80 °C for 24 h to afford methyl 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxylate (**3**) in 95% yield. Notably, heating at temperatures above 80 °C shortened the reaction times at the expense of the product yield due to partial acidolytic removal of the Boc group and concomitant formation of undesired by-products. Somewhat expectedly,[23,25] attempted hydrolysis of the ester function with aq. NaOH failed. Fortunately enough, hydrolysis of **3** into the desired carboxylic acid **4** could be performed upon prolonged treatment of the ester **3** with excess LiOH in aq. methanol to furnish the central intermediate **4** in 54% yield. For the final amidation step 1,1'-carbonyldiimidazole (CDI), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), and bis(pentafluorophenyl) carbonate (BPC) were tested as the reagents for the activation of the carboxy group of **4**. As we already experienced previously in amidation of related hetarenecarboxylic acids,[22–26] BPC proved to be the most suitable reagent, because it gave the corresponding carboxamides **5** reproducibly and in good yields. Thus, upon activation of **4** with BPC to form the intermediate pentafluorophenyl ester **4'**, further treatment with 1:1 mixtures of amines and triethylamine for 12 h furnished the target carboxamides **5a**–**h** in 55–87% yields upon chromatographic workup (Scheme 1).

The structures of novel compounds **3**, **4**, and **5a**–**h** were determined by spectroscopic methods (^{1}H NMR, ^{13}C NMR, IR, MS, HRMS). Spectral data for compounds **3**, **4**, and **5a**–**h** were in agreement with the data of closely related pyrazolo[1,5-*a*]pyrimidin-7(4*H*)-ones.[1,3,4,21–23]

Some physicochemical properties were calculated to estimate the drug-likeness of compounds **3**, **4**, and **5a**–**h**. The compounds have molecular weight (MW) between 412 and 503, number of atoms between 54 and 72, clogP between 1.3 and 3.6, number of hydrogen bond donors (HBD) ≤ 2, number of hydrogen bond acceptors (HBA) ≤ 5, and polar surface area (PSA) below 116 Å^2. These calculated physicochemical properties are compliant with Lipinski's rule of five[28–30] indicating promising drug-likeness of the synthesized compounds **3**, **4**, and **5a**–**h** (Table 1).

The biological activity of compounds **3**, **4**, and **5a**–**h** was tested against the cysteine peptidases cathepsins B and K, which are both important drug targets.[31] All compounds were initially tested for their activity at a concentration of 100 μM. As shown in Table 2, compound **5a** had the strongest inhibitory effect on cathepsin K, with an IC_{50} value of 25 ± 5 μM under the experimental conditions used in the assay and complete (100%) inhibition was observed at concentrations of 600 μM or higher. The effect of other compounds was significantly weaker and resulted in less than 50% inhibition. Cathepsin B was most strongly inhibited by compound **5c** (IC_{50} value of 45 ± 15 μM) and to a lesser extent by compounds **5a** and **5d**. Altogether these results identify three compounds, **5a**, **5c** and **5d**, as potential lead compounds for further development (Table 2).

Table 1. Calculated physicochemical properties of compounds **3**, **4**, and **5a**–**h**.

Compd.	MW (g mol^{-1})	No. of atoms	ClogP	No. of HBD	No. of HBA	PSA (Å^2)
3	426.47	57	2.62	1	4	100.5
4	412.45	54	2.41	2	4	111.5
5a	467.57	67	3.19	2	4	103.3
5b	501.59	68	3.57	2	4	103.3
5c	502.57	67	2.07	2	5	115.7
5d	469.54	65	1.81	2	5	112.6
5e	496.6	72	2.29	2	5	106.6
5f	479.58	68	2.34	1	4	94.6
5g	481.55	66	1.31	1	5	103.8
5h	494.60	70	1.87	1	5	97.8

Table 2: Effect of compounds **3**, **4** and **5a–h** on the activity of cathepsins K and B.[a]

Compound	Cathepsin K		Cathepsin B	
	RA (%)[b]	IC_{50} (μM)	RA (%)[b]	IC_{50} (μM)
control	100		100	
3	89		89	
4	84		84	
5a	29	25 ± 5	36	110 ± 30
5b[c]	–		–	
5c	94		20	45 ± 15
5d	95		23	150 ± 50
5e	69		104	
5f	60		–	
5g	74		61	
5h	112		101	

[a]) All experiments were performed in 50 mM sodium acetate buffer pH 5.5 containing 1 mM EDTA, 2.5 mM DTT and the fluorigenic substrate Z-Phe-Arg-AMC (5 μM final concentration). Final enzyme concentrations were 1 nM. IC_{50} values were determined from titration curves. [b]) Residual activity at saturation. [c]) Activity of **5b** could not be determined fluorometrically due to strong absorption of the compound at the excitation wavelength.

3. Experimental

3. 1. General Methods

Melting points were determined on a Stanford Research Systems MPA100 OptiMelt automated melting point system. The NMR spectra were obtained on a Bruker Avance III UltraShield 500 plus at 500 MHz for ^{1}H and 126 MHz for ^{13}C, using $CDCl_3$ and DMSO-d_6 (with TMS as the internal standard) as solvents. Mass spectra were recorded on an Agilent 6224 Accurate Mass TOF LC/MS spectrometer, IR spectra on a Bruker FTIR Alpha Platinum ATR spectrophotometer. Flash column chromatography (FC) was performed on silica gel (Fluka, Silica gel 60, particle size 35–70 μm).

Amines **6a–h**, bis(pentafluorophenyl) carbonate (BPC), triethylamine, and LiOH · H_2O are commercially available. Methyl 5-amino-1*H*-pyrazole-4-carboxylate (**1**)[27] and methyl 5-(benzyl(*tert*-butoxycarbonyl)amino)-3-oxopentanoate (**2**)[26] were prepared following the literature procedures.

3. 2. Synthesis of methyl 5-(*N*-Boc-*N*-benzyl- 2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxylate (3)

A mixture of **1** (1.413 g, 10 mmol), **2** (3.694 g, 10 mmol), and AcOH (20 mL) was stirred at 80 °C for 24 h. Volatile components were evaporated in vacuo and the residue was purified by FC (EtOAc). Fractions containing the product were combined and evaporated in vacuo to give **3**. Yield: 4.059 g (95%) of pale beige solid; m.p. 161–165 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (9H, s, *t*-Bu); 2.95 (2H, t, *J* = 10.0 Hz, CH_2); 3.54 (2H, t, *J* = 10.0 Hz, CH_2); 3.86 (3H, s, OMe); 4.45 (2H, s, C$\underline{H}_2$Ph); 5.72 (1H, s, 6-H); 7.29 (5H, m, Ph); 8.15 (1H, s, 2-H); 11.45 (1H, s, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 27.6, 44.8, 48.3, 51.3, 59.7, 78.7, 96.5, 99.4, 127.1, 127.4, 128.3, 138.3, 143.0, 143.3, 154.4, 155.1, 162.0, 170.3. *m*/*z* (ESI) = 427 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{22}H_{27}N_4O_5$, 427.1976; found, 427.1971. Anal. Calcd for $C_{22}H_{26}N_4O_5$: C 61.96, H 6.15, N 13.14. Found: C 61.90, H 6.29, N 13.17. IR (ATR) *ν* 3344, 2963, 1710, 1671, 1620, 1580, 1529, 1495, 1466, 1442, 1414, 1365, 1323, 1303, 1259, 1247, 1185, 1167, 1145, 1124, 1115, 1051, 1019, 963, 933, 887, 847, 791, 776, 729, 695, 683, 657, 632 cm^{-1}.

3. 3. Synthesis of 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo [1,5-*a*]pyrimidin-3-carboxylic acid (4)

A mixture of the ester **3** (3.408 g, 8 mmol), LiOH · H_2O (2.016 g, 48 mmol), and methanol (30 mL) was stirred at 50 °C for 48 h. The reaction mixture was cooled to room temperature, and acidified to pH ~ 4 by careful addition of 1 M aq. $NaHSO_4$. The precipitate was collected by filtration and washed with cold (0 °C) water (5 mL) to give **4**. Yield: 2.215 g (54%) of white solid; m.p. 166–172 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (9H, s, *t*-Bu); 2.90 (2H, t, *J* = 10.0 Hz, CH_2); 3.36 (2H, t, *J* = 10.0 Hz, CH_2); 4.45 (2H, s, C$\underline{H}_2$Ph); 5.68 (1H, s, 6-H); 7.29 (5H, m, Ph); 8.26 (1H, s, 2-H); 12.78 (1H, s, NH), CO_2H exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 27.5, 31.3, 44.9, 48.2, 78.7, 97.5, 98.7, 127.0, 127.4, 128.4, 138.3, 143.2, 144.2, 153.7, 154.8, 155.4, 163.3. *m*/*z* (ESI) = 413 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{21}H_{25}N_4O_5$, 413.1806; found, 413.1812. Anal. Calcd for $C_{21}H_{24}N_4O_5 \cdot H_2O$: C 58.60, H 6.09, N 13.02. Found: C 58.50, H 5.74, N 12.89. IR (ATR) *ν* 3648, 3368, 2977, 1682, 1635, 1575, 1495, 1464, 1446, 1404, 1366, 1345, 1302, 1281, 1252, 1218, 1200, 1160, 1131, 1073, 1047, 1015, 963, 940, 858, 841, 812, 780, 758, 725, 695, 669, 653 cm^{-1}.

3. 4. Synthesis of 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo [1,5-*a*]pyrimidin-3-carboxamides 5a–h

A mixture of carboxylic acid **4** (207 mg, 0.5 mmol), MeCN (5 mL), and Et_3N (70 μL, 0.5 mmol) was stirred at room temperature for 5 minutes. Then, BPC (197 mg, 0.5 mmol) was added and the reaction mixture was stirred at r.t. for 2 h (activation of carboxylic acid **4** via formation of the pentafluorophenyl ester **4'**). Next, amine **6** (0.5 mmol) and Et_3N (70 μL, 0.5 mmol) were added and stirring at room temperature was continued for 24 h. The reaction mixture was evaporated *in vacuo* (60 °C/2 mbar) and the crude semi-solid carboxamide **5** was purified by FC on silica gel (first EtOAc to elute the non-polar impurities, then CH_2Cl_2–MeOH, 10:1, to elute the product). Fractions containing the product were combined and evaporated in vacuo to give carboxamides **5a**–**h**.

3. 4. 1. *tert*-Butyl benzyl{2-[3-(butylcarbamoyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-5-yl]ethyl}carbamate (5a)

Prepared from **4** (207 mg, 0.5 mmol) and butylamine (**6a**) (50 μL, 0.5 mmol). Yield: 167 mg (72%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (3H, t, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); 1.27 (2H, m, CH_2); 1.34 (9H, s, *t*-Bu); 1.42 (2H, m, CH_2); 2.29 (2H, m, CH_2); 3.23 (2H, m, CH_2); 3.44 (2H, m, CH_2); 4.38 (2H, s, $C\underline{H}_2Ph$); 5.41 (1H, s, 6-H); 7.28 (5H, m, Ph); 7.90 (1H, s, 2-H); 8.50 (1H, br s, $N\underline{H}Bu$); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 13.0, 13.7, 19.7, 28.2, 31.8, 38.5, 45.8, 51.0, 80.0, 101.1, 126.0, 127.2, 127.5, 127.9, 128.4, 128.7, 138.1, 155.7, 156.0, 159.0, 164.0. *m*/*z* (ESI) = 468 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{25}H_{34}N_5O_5$, 468.2605; found, 468.2601. IR (ATR) ν 3300, 2930, 2175, 2110, 1985, 1960, 1684, 1619, 1537, 1512, 1494, 1451, 1413, 1364, 1245, 1157, 1115, 1047, 980, 885, 808, 775, 733, 697 cm^{-1}.

3. 4. 2. *tert*-Butyl benzyl{2-[3-(benzylcarbamoyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*] pyrimidin-5-yl]ethyl}carbamate (5b)

Prepared from **4** (207 mg, 0.5 mmol) and benzylamine (**6b**) (54 μL, 0.5 mmol). Yield: 137 mg (55%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (9H, br s, *t*-Bu); 2.56 (2H, br s, CH_2); 3.35 (2H, br s, CH_2); 4.29 and 4.43 (4H, 2 br s, 3:1, 2 × $C\underline{H}_2Ph$); 5.57 (1H, br s, 6-H); 6.84–7.34 (10H, m, 2×Ph); 8.10 (1H, br s, 2-H); 8.76 (1H, br s, NH); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.1, 28.3, 36.5, 42.8, 46.1, 51.3, 51.9, 81.0, 100.7, 125.1, 127.3, 127.4, 128.5, 128.6, 136.7, 138.2, 138.9, 140.6, 142.7, 156.0, 159.6, 163.9. *m*/*z* (ESI) = 502 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{28}H_{32}N_5O_4$, 502.2449; found, 502.2444. IR (ATR) ν 3278, 2975, 2114, 1618, 1535, 1494, 1451, 1413, 1364, 1244, 1207, 1156, 1115, 976, 884, 809, 774, 728, 696, 665, 630 cm^{-1}.

3. 4. 3. *tert*-Butyl benzyl(2-{7-oxo-3-[(pyridin-2-ylmethyl)carbamoyl]-4,7-dihydro-pyrazolo[1,5-*a*]pyrimidin-5-yl}ethyl) carbamate (5c)

Prepared from **4** (207 mg, 0.5 mmol) and 2-picolylamine (**6c**) (51 μL, 0.5 mmol). Yield: 190 mg (72%) of yellowish resin. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.27 and 1.32 (9H, 2 br s, 2:1, *t*-Bu); 2.67–2.77 (2H, br s, CH_2); 3.42–3.50 (2H, br s, CH_2); 4.33 and 4.37 (2H, 2 br s, 1:2, $C\underline{H}_2Ph$); 4.60 (2H, d, *J* = 5.7 Hz, $C\underline{H}_2Py$); 5.48 and 5.50 (1H, 2 br s, 2:1, 6-H); 7.20–7.30 (5H, m, Ph); 7.30–7.37 (2H, m, 2H of Ph); 7.71 (1H, td, *J* = 7.7, 1.8 Hz, 1H of Py); 8.09 (1H, br s, 2-H); 8.47 (1H, br d, *J* = 4.2 Hz, 1H of Py); 9.16 (1H, br t, *J* = 6.0 Hz, NHCO); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, DMSO-d_6): δ 27.8, 43.8, 45.6, 45.8, 49.0, 78.8, 100.2, 120.9, 122.0, 127.0, 127.2, 127.4, 128.4, 136.7, 138.1, 138.5, 140.0, 141.5, 148.8, 155.0, 157.2, 159.0, 162.9. *m*/*z* (ESI) = 503 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{27}H_{31}N_6O_4$, 503.2397; found, 503.2394. IR (ATR) ν 3679, 3607, 2926, 1730, 1624, 1537, 1497, 1393, 1368 cm^{-1}.

3. 4. 4. *tert*-Butyl benzyl(2-{3-[(2-methoxyethyl) carbamoyl]-7-oxo-4,7-dihydropyrazolo-[1,5-*a*]pyrimidin-5-yl}ethyl)carbamate (5d)

Prepared from **4** (207 mg, 0.5 mmol) and 2-methoxyethylamine (**6d**) (63 μL, 0.5 mmol). Yield: 143 mg (61%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (9H, s, *t*-Bu); 2.72–2.83 (2H, br s, CH_2); 3.40 (3H, br s, OMe); 3.51–3.59 (4H, m, 2×CH_2); 3.59–3.64 (2H, m, CH_2); 4.44 (2H, br s, $C\underline{H}_2Ph$); 5.69 (1H, s, 6-H); 7.14–7.29 (6H, m, Ph and $N\underline{H}CO$); 8.03 (1H, br s, 2-H); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.4, 39.2, 46.2, 51.8, 59.0, 71.1, 81.3, 99.2, 126.0, 127.8, 128.8, 132.2, 137.6, 139.0, 143.5, 151.0, 154.1, 155.8, 156.3, 163.1. *m*/*z* (ESI) = 470 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{24}H_{32}N_5O_5$, 470.2398; found, 470.2393. IR (ATR) ν 3313, 2978, 2916, 1685, 1624, 1585, 1532, 1513, 1479, 1453, 1414, 1365, 1244, 1156, 1122, 1051, 1012, 993, 976, 858, 819, 774, 733, 698, 660 cm^{-1}.

3. 4. 5. *tert*-Butyl benzyl[2-(3-{[3-(dimethylamino) propyl]carbamoyl}-7-oxo-4,7-dihydro-pyrazolo[1,5-*a*]pyrimidin-5-yl)ethyl] carbamate (5e)

Prepared from **4** (207 mg, 0.5 mmol) and 3-dimethylaminopropylamine (**6e**) (63 μL, 0.5 mmol). Yield: 200 mg (81%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (9H, s, *t*-Bu); 1.96–2.05 (2H, m, CH_2); 2.71 (6H, br s, NMe_2); 2.67–2.81 (2H, m, CH_2); 3.03–3.12 (2H, m, CH_2); 3.43–3.51 and 3.55–3.63 (4H, 2m, 3:1, 2 × CH_2); 4.37 (2H, br s, $C\underline{H}_2Ph$); 5.70 (1H, s, 6-H); 7.16–7.34 (5H, m, Ph); 8.14 (1H, br s, 2-H); 8.65 (1H, br s, $N\underline{H}CO$); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 25.9, 28.4, 28.5, 35.9, 43.4, 43.5, 45.7, 56.2, 79.7, 95.4,

101.1, 127.3, 127.7, 128.6, 137.3, 138.1, 139.1, 141.0, 156.0, 159.3, 165.0. *m*/*z* (ESI) = 497 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{26}H_{37}N_6O_4$, 497.2857; found, 497.2863. IR (ATR) ν 3285, 2937, 1995, 1690, 1619, 1537, 1493, 1450, 1411, 1364, 1243, 1158, 1112, 1020, 886, 806, 776, 735, 698, 665, 631 cm^{-1}.

3. 4. 6. *tert*-Butyl benzyl{2-[7-oxo-3-(piperidine-1-carbonyl)-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-5-yl]ethyl}carbamate (5f)

Prepared from **4** (207 mg, 0.5 mmol) and piperidine (**6f**) (37 μL, 0.5 mmol). Yield: 128 mg (61%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*-Bu); 1.68 (4H, br s, 2 × CH_2); 1.74 (2H, br s, CH_2); 2.76 (2H, br s, CH_2); 3.53 (2H, br s, CH_2); 3.73 (4H, br s, 2 × CH_2); 4.41 (2H, br s, C$\underline{H}_2$Ph); 5.69 (1H, s, 6-H); 7.13–7.34 (5H, m, Ph); 7.96 (1H, br s, 2-H); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 24.6, 26.2, 28.5, 33.0, 46.0, 50.9, 52.1, 80.9, 99.1, 127.4, 127.7, 128.8, 137.8, 141.1, 141.1, 145.1, 150.7, 155.6, 156.3, 162.8. *m*/*z* (ESI) = 480 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{26}H_{34}N_5O_4$, 480.2605; found, 480.2599. IR (ATR) ν 2931, 2849, 1687, 1617, 1578, 1578, 1495, 1438, 1410, 1364, 1258, 1159, 1122, 1002, 970, 875, 851, 814, 764, 731, 698, 672, 629 cm^{-1}.

3. 4. 7. *tert*-Butyl benzyl{2-[3-(morpholine-4-carbonyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-5-yl]ethyl}carbamate (5g)

Prepared from **4** (207 mg, 0.5 mmol) and morpholine (**6g**) (44 μL, 0.5 mmol). Yield: 184 mg (76%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*-Bu); 2.76 (2H, br s, CH_2); 3.50–3.59 (2H, m, CH_2); 3.79 (4H, br s, 2 × CH_2); 3.81 (4H, br s, 2 × CH_2); 4.44 (2H, br s, C$\underline{H}_2$Ph); 5.71 (1H, s, 6-H); 7.16–7.35 (5H, m, Ph); 7.97 (1H, br s, 2-H); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.5, 33.3, 45.9, 51.7, 60.6, 66.8, 81.4, 99.3, 127.8, 128.8, 133.6, 137.7, 140.9, 143.7, 145.3, 151.1, 155.7, 156.2, 163.2. *m*/*z* (ESI) = 482 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{25}H_{32}N_5O_5$, 482.2398; found, 482.2393. IR (ATR) ν 2974, 2922, 2843, 1685, 1619, 1580, 1532, 1513, 1453, 1434, 1412, 1365, 1245, 1157, 1114, 1065, 1051, 1010, 978, 935, 884, 817, 765, 733, 699, 630 cm^{-1}.

3. 4. 8. *tert*-Butyl benzyl{2-[3-(4-methylpiperazine-1-carbonyl)-7-oxo-4,7-dihydro-pyrazolo[1,5-*a*]pyrimidin-5-yl]ethyl}carbamate (5h)

Prepared from **4** (207 mg, 0.5 mmol) and 4-methylpiperazine (**6h**) (56 μL, 0.5 mmol). Yield: 215 mg (87%) of yellowish resin. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (9H, s, *t*-Bu); 2.40 (3H, br s, NC$\underline{H}_3$); 2.59 (4H, br t, *J* = 5.1 Hz, 2 × CH_2); 2.70 and 2.76 (2H, 2br s, 1:1, CH_2); 3.54 (2H, br s, CH_2); 3.85 (4H, br s, 2 × CH_2); 4.42 (2H, br s, C$\underline{H}_2$Ph); 5.70 (1H, s, 6-H); 7.16–7.31 (5H, m, Ph); 7.96 (1H, br s, 2-H); pyrimidone NH exchanged. ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.5, 33.2, 43.8, 45.8, 46.1, 52.7, 54.8, 80.9, 98.9, 127.7, 128.8, 136.6, 137.2, 137.8, 138.9, 140.6, 141.3, 155.7, 156.6, 163.3. *m*/*z* (ESI) = 495 (MH^+). HRMS–ESI (*m*/*z*): [MH^+] calcd for $C_{26}H_{35}N_6O$, 495.2714; found, 495.2707. IR (ATR) ν 2977, 2958, 1685, 1621, 1583, 1531, 1495, 1414, 1364, 1243, 1155, 976, 879, 807, 767, 731, 698, 606 cm^{-1}.

3. 5. Activity assays against cathepsins K and B

The activity of all compounds was tested against recombinant human cathepsins K and B produced in-house according to the known protocol.[32] All assays were performed in 50 mM sodium acetate buffer pH 5.5 containing 1 μM EDTA and 2.5 mM DTT. The hydrolysis of the synthetic substrate Z-Phe-Arg-AMC (5 μM final concentration) was followed fluorimetrically at an excitation wavelength of 370 nm and an emission wavelength of 455 nm. Final concentrations of the enzymes in the reaction mixtures were 1 nM. Experiments were first performed at a fixed compound concentration of 100 μM. Compounds with significant inhibitory activity were re-tested by measuring residual enzyme activity in the presence of increasing concentrations of the compounds and IC_{50} values were calculated from these titration curves.

4. Conclusions

Eight novel 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxamides **5a-h** were prepared in three synthetic steps from methyl 3-amino-1*H*-pyrazole-4-carboxylate (**1**) and methyl 5-(benzyl(*tert*-butoxycarbonyl)amino)-3-oxopentanoate (**2**). The synthetic procedure comprises cyclocondensation of the above starting compounds, hydrolysis of the ester function, and BPC-mediated amidation. This method offers a quick access to various 5-(2-aminoethyl) substituted pyrazolo[1,5-*a*]pyrimidin-3-carboxamides **5** from easily available starting materials. Testing of the intermediates **3** and **4** and title compounds **5a–h** for inhibition of cathepsins B and K revealed that most of them were weak inhibitors at 100 mM concentration. Carboxamide **5a** had the strongest inhibitory effect on cathepsin K, with an IC_{50} value of 25 ± 5 μM. Cathepsin B was most strongly inhibited by compounds **5c** and **5d** with the respective IC_{50} values of 45 ± 15 μM and 150 ± 50 μM and to a lesser extent by compound **5a** as well. Inhibitory activities of compounds **5a**, **5c**, and **5d** against cysteine peptidases cathepsins B and K identify them as potential leads for drug development. In summary, the synthetic method allows for a simple preparation of libraries of title compounds that could be useful for medicinal and pharmaceutical applications.

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Povzetek

Izhajajoč iz metil 3-amino-1*H*-pirazol-4-karboksilata in metil 5-(benzil(*terc*-butoksikarbonil)amino)-3-oksopentanoata (**2**) smo v treh sinteznih stopnjah pripravili osem novih 5-(*N*-Boc-*N*-benzil-2-aminoetil)-7-okso-4,7-dihidropirazolo[1,5-*a*]pirimidin-3-karboksamidov **5a-h**. Sintezni postopek sestavljajo ciklokondenzacija izhodnih spojin, hidroliza estra in amidiranje tako nastale karboksilne kisline z uporabo bis(pentafluorofenil) karbonata (BPC) kot aktivacijskega reagenta. Karboksamide **5a-h** smo testirali na inhibicijo katepsinov B in K. Najbolj aktiven inhibitor katepsina K (IC50 ~ 25 μM) je bil *N*-butilkarboksamid **5a**, medtem ko smo najmočnejšo inhibicijo katepsina B izmerili z *N*-(2-pikolil) karboksamidom **5c** (IC50 ~ 45 μM).