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FACILE SYNTHESIS OF PYRIDAZINE-BASED α-HELIX MIMETICS

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Abstract – The synthesis of an amphiphilic, nonpeptidic scaffold that mimics the presentation of *i*, *i*+4, and *i*+7 residues of an α-helix is presented. The approach uses a pyridazine core, and minimizes the number of C-C bond forming reactions. The synthesis of this Urea-Pyridazine-Piperazine (UPP) scaffold is versatile and its synthesis makes it suitable for the preparation of small libraries of low-molecular-weight α-helix mimetics that can be targeted to specific protein/protein interactions.

INTRODUCTION

The α-helix is one of the most abundant secondary protein structures and is often a key feature for protein-protein recognition. Side chains in positions *i*, *i*+3/*i*+4, *i*+7, and *i*+11 appear on the same face of the helix are frequently crucial for the interaction.[1] Hamilton and co-workers pioneered the synthesis of non-peptidic α-helix mimetics based on terphenyl, terephthalamide, and oligopyridine scaffolds that display side chains in a manner that closely resembles those in position *i*, *i*+4, and *i*+7 of an α-helix.[2] They were shown to efficiently disrupt protein-protein interactions such as Bak/Bcl-X_L,[3] p53/HDM2,[4] calmodulin/smooth muscle myosin light-chain kinase,[5] and gp41 assembly.[6] In our general efforts towards the design of inhibitors of protein-protein interactions,[7] we were interested in developing methodology for structurally similar molecules featuring more hydrophilic components and a facile synthetic route.[8] In the present paper we describe the synthesis of a new class of low-molecular-weight α-helix mimetics featuring a pyridazine ring and hydrophobic amino-acid side chains.

The target molecules are shown in Figure 1 along with an overlay with an α-helix (Figure 1a). The scaffold mimics the position *i*, *i*+4, and *i*+7 of the α-helix. To ensure the water solubility of our inhibitors we based our scaffold on a pyridazine ring. These molecules may be thought of as synthetic counterparts

to amphiphilic α-helices; they are intended to present both a hydrophobic surface for recognition and a hydrophilic "wet edge" that is rich in hydrogen bond donors and acceptors.

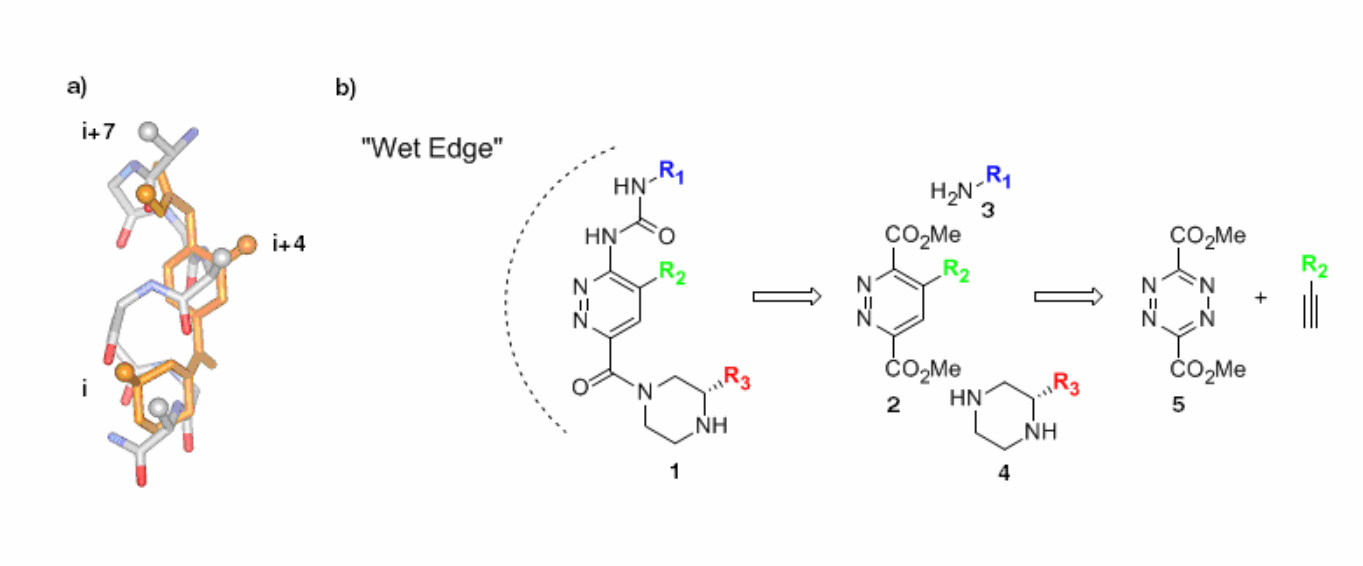


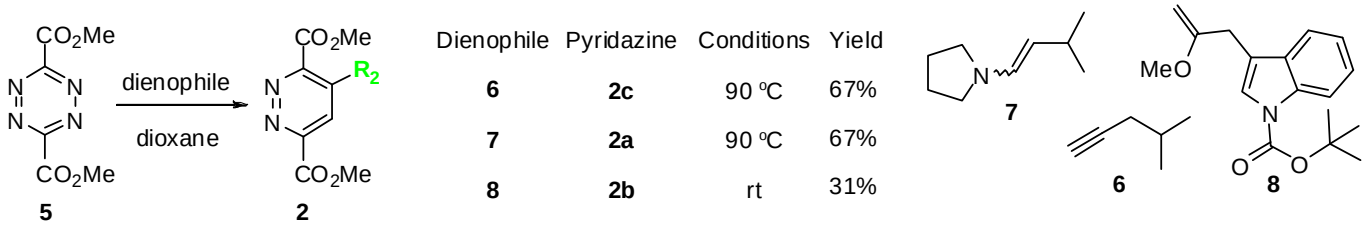
Figure 1. a) Overlay of **1** with a generic α-helix; b) General retrosynthetic approach of the UPP scaffold **1**.

RESULTS AND DISCUSSION

The general retrosynthetic approach to these molecules is laid out in Figure 1b. The major disconnections from the final urea-pyridazine-piperazine **1** (UPP) are made at the amide and urea bonds to give a pyridazine diester **2**, an amine **3**, and a piperazine **4**. This synthesis is modular, many amines are commercially available as are a variety of Boc-protected 2-substituted piperazines presenting standard hydrophobic side chains (i.e. benzyl, isobutyl, etc.). The central pyridazine ring is readily forged from the inverse electron demand Diels-Alder reaction of dimethyl-1,2,4,5-tetrazine-dicarboxylate **5** and a suitable dienophile.[9]

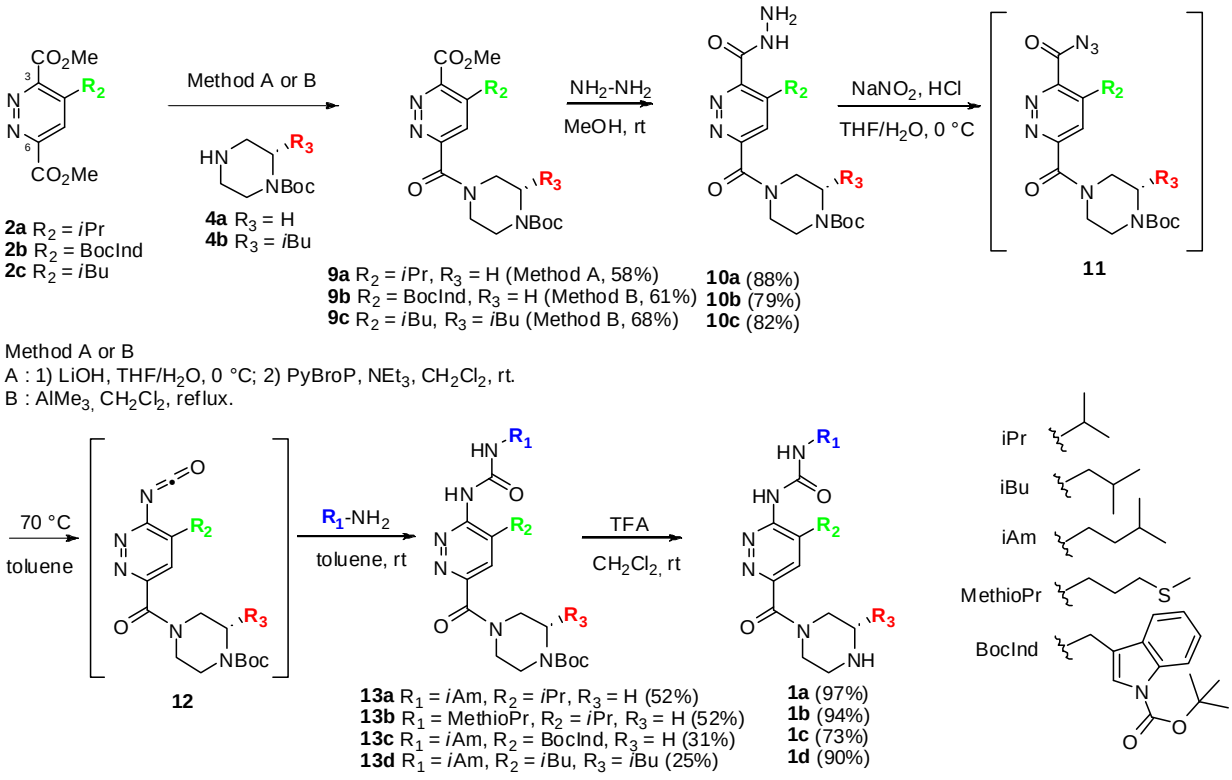
Tetrazine **5** was synthesized from ethyldiazoacetate following the procedure of Boger and co-workers[10] and reacted with a range of dienophiles to introduce the side chain R_2 and give the corresponding pyridazines (Scheme 1). Three kinds of dienophiles were employed depending on the structure of the desired R_2 group. A few suitable alkynes such as **6** are commercially available and were used. Enamines are also known to be good dienophiles for the cycloaddition with disubstituted tetrazines.[9] Thus R_2 = *i*Pr was introduced *via* the cycloaddition of the enamine **7** obtained by the condensation of isovaleraldehyde and pyrrolidine following the Mannich procedure.[11] This procedure is suitable to introduce short, low molecular weight side-chains such as *i*Pr for which the corresponding alkynes are often a gas and more difficult to handle. In the case where R_2 = CH_2-BocIndole, methyl enol ether **8** was used as the dienophile. The enol ether was obtained by methylenation of the corresponding methyl ester with Tebbe's reagent[12] and directly subjected to reaction with tetrazine **5** without any further purification. This procedure allowed us to expand the scope of the possible side chains to the wide range of methyl esters

commercially available, especially those derived from natural amino-acids.



Scheme 1. Inverse electron demand Diels-Alder reaction of tetrazine **5** with various dienophiles.

Installation of the piperazine group was performed by using either a peptide coupling (Method A) or direct coupling of the methyl ester function (Method B) (Scheme 2). A few examples were first carried out with non substituted N-Boc protected piperazines (R_3 = H). The corresponding final compounds present only two side chains but provide clear NMR spectra for characterization. The synthesis was then applied to substituted piperazines yielding compounds presenting three side chains for which the NMR spectra are more complex due to the conformational isomers of the piperazine around the tertiary amide bond. The 6-position methyl ester of the pyridazine **2a** was selectively saponified using LiOH in a THF/water mixture at 0 °C. The piperazine **4a** (R_3 = H) was coupled under standard peptide coupling conditions with PyBroP (Method A) in good yields to give pyridazine-piperazine **9a**. In the case of pyridazines **9b,c** the coupling with piperazines **4a** or **4b** (R_3 = *i*Bu) was performed directly using Weinreb amidation[13] in the presence of $AlMe_3$ (Method B) to give only the desired regioisomer.



Scheme 2. Synthesis of the UPP scaffold **1**.

To install the urea function in the 3-position, the remaining methyl ester was first converted to the acyl hydrazide **10a-c** under mild conditions using an excess of hydrazine in methanol. The acylhydrazide function was then diazotized to the acyl azide **11** using the Curtius method (sodium nitrite in acidic conditions).[14] Acyl azides were not purified and directly transformed to the corresponding isocyanates **12** by heating at 70 °C. Ureas **13a-d** were obtained by trapping these isocyanates with various amines. Final removal of the *N*-Boc group was accomplished using TFA, to give the desired UPP **1a-d** which present three side chains to mimic the *i*, *i*+4, *i*+7 positions of an α-helix.

CONCLUSIONS

In conclusion, the synthesis of the desired α-helix mimetics has been performed in few steps. While specific derivatives are prepared and disclosed here, the methodology reported is applicable for a broader, more general decoration of the scaffold to provide a diversity of compounds. A library of over thirty compounds that bear common hydrophobic amino-acid side chains was synthesized with this strategy and it is currently being evaluated for the inhibition of various protein-protein interactions.

EXPERIMENTAL

Solvents and reagents were of reagent-grade, purchased from commercial suppliers, and used without further purification unless otherwise stated. Substituted NBoc-protected piperazines were purchased from Anaspec. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} coated plates (Merck). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 250 MHz, 300 MHz, or 600 MHz spectrometers. Chemical shifts are expressed in ppm (δ), referenced to the protio impurity of the solvent as internal standard for ^{1}H and ^{13}C nuclei. High resolution mass spectra were recorded on an Agilent ESI-TOF mass spectrometer by Scripps Center for Mass Spectrometry.

Dimethyl 4-isopropylpyridazine-3,6-dicarboxylate (2a): To a stirred solution of isovaleraldehyde (1 mL, 9.2 mmol) and K_2CO_3 (1.5 g, 11.1 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 1 h pyrrolidine (780 μL, 9.3 mmol) at 0 °C. The mixture was vigorously stirred under nitrogen for 18 h. The mixture was then filtered and the solvent removed in vacuo. The crude enamine was then used directly without any further purification and added to a solution of tetrazine dimethylester **5** (1 g, 5.05 mmol) in dioxane (50 mL) at 0 °C. The mixture was stirred for 4 h at rt, heated at 90 °C for 16 h, and then concentrated in vacuo. The crude residue was purified on silica (CH_2Cl_2/AcOEt 1/0 to 9/1) to afford compound **2a** (800 mg, 3.36 mmol, 67%) as a pale yellow solid; ^{1}H NMR: (250 MHz, $CDCl_3$) δ 8.15 (s, 1H), 4.05 (s, 3H), 4.02 (s, 3H), 3.38 (hept, *J* = 6.9 Hz, 1H), 1.27 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR: (62.5 MHz, $CDCl_3$) δ 165.2, 164.2, 154.2, 151.8, 148.2, 125.5, 53.4, 53.2, 28.8, 22.5; HRMS: (ESI-TOF)

$C_{11}H_{14}N_2O_4H^+$ expected: 239.1026. found: 239.1024.

Dimethyl 4-((1-(*tert*-butoxycarbonyl)-*1H*-indol-3-yl)methyl)pyridazine-3,6-dicarboxylate (2b): To a solution of methyl (1-*tert*-butyloxycarbonyl-indol-3-yl)acetate[15] (3 g, 10 mmol) in THF (30 mL) cooled at -40 ºC was added Tebbe's reagent (22 mL, 1.1 eq, 0.5 M in toluene) after 30 min temperature was raised to ambient over a period of 2 h. The mixture was then cooled to -10 ºC and the reaction was quenched by the dropwise addition of NaOH (2.4 mL, 2 M solution). Reaction mixture was then allowed to warm to rt The dark green solution was then diluted with excess ether and filtered through a plug of celite. Solvent was removed under reduced pressure and the crude residue was directly diluted in dioxane (40 mL) and added to a solution of tetrazine **5** (2 g, 10 mmol) in dioxane (10 mL). After 18 h at rt, the volatiles were removed and the crude residue was purified on silica gel (CH_2Cl_2/AcOEt 1/0 to 15/1) to afford pyridazine **2b** (1.3 g, 3 mmol, 31%) as a solid; ^{1}H NMR: (600 MHz, $CDCl_3$) δ 8.14 (bs, 1H), 8.02 (s, 1H), 7.46 (s, 1H), 7.35 (m, 1H), 7.30 (m, 1H), 7.21 (m, 1H), 4.40 (s, 2H), 4.07 (s, 3H), 4.03 (s, 3H), 1.69 (s, 9H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 165.1, 164.0, 153.7, 151.8, 141.3, 129.3, 128.6, 124.9, 124.8, 122.8, 118.1, 116.7, 115.5, 115.1, 84.0, 53.4, 28.1, 27.1; HRMS: (ESI-TOF) $C_{22}H_{23}N_3O_6H^+$ expected: 426.1660. found: 426.1670.

Dimethyl 4-isobutylpyridazine-3,6-dicarboxylate (2c): To a solution of tetrazine **5** (500 mg, 2.52 mmol) in anhydrous 1,4-dioxane (12.5 mL) was added 4-methylpentyne (234 mg, 2.85 mmol), the reaction vessel was then sealed and heated to 90 °C for 18 h. The volatiles were removed under reduced pressure, and the crude product was purified by silica gel chromatography (CH_2Cl_2/AcOEt 95/5) to give **2c** (423 mg, 67% yield) as a yellow solid. ^{1}H NMR: (600 MHz, $CDCl_3$) δ 8.07 (s, 1H), 4.10 (s, 3H), 4.07 (s, 3H), 2.83 (d, J = 7.3 Hz, 2H), 1.97 (m, 1H), 0.95 (d, J = 6.6 Hz, 6H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 165.4, 164.5, 154.8, 151.6, 142.6, 129.9, 53.7, 53.5, 40.8, 29.7, 22.5; MS: (ESI-TOF) $C_{12}H_{16}N_2O_4H^+$ expected: 253.1183, found 253.1187.

Procedures for the coupling of pyridazine with piperazines:

Method A:

Methyl 6-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-4-isopropylpyridazine-3-carboxylate (9a): To a stirring solution of pyridazine **2a** (536 mg, 2.25 mmol) in THF (10 mL) at 0 °C was added dropwise a cold solution of LiOH (monohydrate, 99 mg, 2.36 mmol) in water (5 mL). The reaction was stirred at 0 °C until disappearance of the starting material on TLC. The pH was then made acidic (pH 1-2) with careful addition of a 3% HCl solution. The solution was extracted with AcOEt (3x30 mL), the fractions were combined, dried with Na_2SO_4, filtered and the solvent was removed in vacuo. The desired monosaponified pyridazine was obtained as a pale yellow solid. The carboxylic acid was then directly involved in the next step without any further purification. To a solution of the acid (312 mg, 1.39 mmol)

in CH_2Cl_2 (7 mL) was added NEt_3 (387 μL, 2.78 mmol), *N*-Boc-piperazine (285 mg, 1.53 mmol) and PyBroP (714 mg, 1.53 mmol). After 18 h under nitrogen, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with a solution of HCl (0.1 M, 10 mL) and then a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic layer was then dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (Hexane/AcOEt 1/0 to 6/4) and the desired piperazine adduct **9a** (516 mg, 1.31 mmol, 58%) was obtained as a pale yellow foam; ^{1}H NMR: (250 MHz, $CDCl_3$) Mixture of conformers δ 7.88 (s, 1H), 4.03 (s, 3H), 3.79 (m, 2H), 3.70 (m, 2H), 3.59-3.40 (m, 5H), 1.44 (s, 9H), 1.28 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR: (62.5 MHz, $CDCl_3$) δ 165.1, 164.5, 156.8, 154.4, 152.8, 149.0, 125.8, 80.3, 53.2, 47.3, 43.6, 43.3 (broad), 42.7, 28.8, 28.2, 22.5; HRMS: (ESI-TOF) $C_{19}H_{28}N_4O_5H^+$ expected: 393.2132. found: 393.2126.

Method B:

To a solution of piperazine (1 equivalent) in anhydrous CH_2Cl_2 (5 mL) was added slowly $AlMe_3$ (1 equivalent, 2.0 M solution in hexanes). The mixture was allowed to stir at rt for approximately 10 min. To the aluminum-amide solution was added slowly a solution of pyridazine (1.05 equivalent) in anhydrous CH_2Cl_2 (2.5 mL). The schlenk flask was sealed, and the yellow solution heated to 41 °C. After 24 h, the now orange solution was cooled to room temperature, and quenched with slow addition of water (3 mL) with vigorous stirring. The aqueous layer was extracted with CH_2Cl_2 (3x5 mL). The organic fractions were collected, dried over $MgSO_4$, and evaporated to dryness under reduced pressure. The crude residue was purified by flash chromatography on silica gel.

***tert*-Butyl 3-((6-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-3-(methoxycarbonyl)pyridazin-4-yl)methyl)-*1H*-indole-1-carboxylate (9b);** Starting from **2b** (398 mg, 0.93 mmol), column Hexane/AcOEt 7/3, white solid, yield: 331 mg, (61%);^{1}H-NMR (600 MHz, $CDCl_3$) Mixture of conformers δ 8.15 (bs, 1H), 7.67 (bs, 1H), 7.48 (bs, 1H), 7.33 (dd, *J* = 7.2, 8.4 Hz, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.19 (dd, *J* = 7.2, 8.4 Hz, 1H), 4.41 (s, 2H), 4.08 (s, 3H), 3.74 (bs, 2H), 3.63 (bs, 2H), 3.53 (bs, 2H), 3.48 (bs, 2H), 1.68 (s, 9H), 1.46 (s, 9H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 165.0, 164.3, 156.9, 154.5, 152.2, 149.5, 142.2, 129.4, 128.8, 125.1, 124.9, 122.8, 118.6, 115.6, 115.0, 84.1, 80.4, 53.4, 47.4, 42.7, 28.4, 28.2, 27.2; HRMS: (ESI-TOF) $C_{30}H_{37}N_5O_7H^+$ expected: 580.2766, found: 580.2759.

(*S*)-Methyl 6-(4-(*tert*-butoxycarbonyl)-3-isobutylpiperazine-1-carbonyl)-4-isobutylpyridazine-3-carboxylate (9c); Starting from **2c** (160 mg, 0.63 mmol), column Hexane/AcOEt 1/1, white foam, yield: 200 mg, (68%); ^{1}H NMR: (600 MHz, $CDCl_3$) Mixture of conformers δ 7.76 (s, 1H), 4.64 (m, 0.4H), 4.58 (m, 0.6H), 4.36 (m, 0.4H), 4.22 (m, 0.5H), 4.15 (m, 0.5H), 4.08 (m, 0.6H), 4.05 (s, 1.4H), 4.04 (s, 1.6H), 3.95 (m, 0.4H), 3.45 (m, 0.4H), 3.24 (m, 0.4H), 3.20-3.07 (m, 1.8H), 3.04 (m, 0.6H), 2.96 (m, 0.4H), 2.88-2.74 (m, 2H), 1.94 (m, 1H), 1.62-1.52 (m, 1H), 1.47 (s, 4.4H), 1.46 (s, 4.6H), 1.44 (m, 1H), 1.23 (m,

1H), 0.96-0.91 (m, 9.5H), 0.82 (m, 2.5H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 165.0, 164.9, 156.5, 154.4, 153.1, 152.9, 143.1, 142.8, 129.9, 129.8, 80.2, 53.2, 50.2, 47.6, 45.8, 42.7, 40.6, 37.9, 29.3, 28.3, 24.6, 24.4, 22.8, 22.6, 22.4, 22.3, 22.2; HRMS: (ESI-TOF) $C_{24}H_{38}N_4O_5H^+$ expected: 463.2915. found: 463.2915.

General procedure for the hydrazynolysis of the methyl ester pyridazines:

To a solution of pyridazine methyl ester (1 equivalent) in MeOH (30 mL) was added hydrazine monohydrate (10 equivalents). The reaction was stirred for 20 h at rt under an atmosphere of nitrogen. The volatiles were evaporated under reduced pressure, and the product was purified on silica gel.

***tert*-Butyl 4-(6-(hydrazinecarbonyl)-5-isopropylpyridazine-3-carbonyl)piperazine-1-carboxylate (10a)** : Starting from **9a** (148 mg, 0.37 mmol), column CH_2Cl_2/MeOH 97/3, white foam, yield : 130 mg, (88%); ^{1}H NMR: (600 MHz, $CDCl_3$) Mixture of conformers δ 8.95 (m, 1H), 7.94 (s, 1H), 4.23 (hept, *J* = 6.8 Hz, 1H), 4.16 (m, 2H), 3.85 (m, 2H), 3.70 (m, 2H), 3.61 (s, 2H), 3.56 (m, 2H), 1.49 (s, 9H), 1.33 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 164.7, 164.4, 157.2, 154.4, 151.1, 151.0, 126.5, 80.4, 47.3, 43.6 (broad), 42.6, 28.3, 27.9, 22.6; HRMS: (ESI-TOF) $C_{18}H_{28}N_6O_4H^+$ expected: 393.2245. found: 393.2239.

***tert*-Butyl 3-((6-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-3-(hydrazinecarbonyl)pyridazin-4-yl)methyl)-*1H*-indole-1-carboxylate (10b)**; Starting from **9b** (164 mg, 0.28 mmol), column CH_2Cl_2/MeOH 97/3, pale yellow foam, yield: 130 mg, (79%); ^{1}H NMR (250 MHz, $CDCl_3$) Mixture of conformers δ: 9.13 (bs, 1H), 8.15 (bs, 1H), 7.61 (s, 1H), 7.50 (s, 1H), 7.33 (m, 2H), 7.18 (dd, *J* = 7.0, 7.8 Hz, 1H), 4.69 (s, 2H), 3.72 (m, 2H), 3.52 (m, 4H), 3.44 (m, 2H), 1.67 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.4, 164.2, 157.2, 154.4, 150.7, 143.2, 129.7, 129.5, 125.1, 124.9, 124.8, 122.7, 118.8, 115.6, 115.5, 84.0, 80.4, 47.2, 42.5, 28.3, 28.2, 27.0; HRMS: (ESI-TOF) $C_{29}H_{37}N_7O_6H^+$ expected: 580.2878, found: 580.2871.

(*S*)-*tert*-Butyl 4-(6-(hydrazinecarbonyl)-5-isobutylpyridazine-3-carbonyl)-2-isobutylpiperazine-1-carboxylate (10c); Starting from **9c** (219 mg, 0.47 mmol), column CH_2Cl_2/AcOEt 1/1, white foam, yield: 180 mg, (82%); ^{1}H NMR (600 MHz, $CDCl_3$) Mixture of conformers δ 9.03 (d, *J* = 9.6 Hz, 1H), 7.73 (s, 0.6H), 7.71 (s, 0.4H), 4.65 (d, *J* = 12.5 Hz, 0.4H), 4.59 (d, *J* = 13.0 Hz, 0.6H), 4.16-4.07 (m, 3H), 3.94 (d, *J* = 13.2 Hz, 0.6H), 3.46 (dd, *J* = 13.6, 3.7 Hz, 0.4H), 3.20-2.94 (m, 4H), 2.00 (m, 1H), 1.60 (m, 1H), 1.53 (m, 1H), 1.47 (s, 5H), 1.46 (s, 4H), 1.20 (m, 1H), 0.96-0.92 (m, 9H), 0.83 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 165.3, 165.0, 156.8, 156.7, 151.5, 144.4, 144.3, 131.0, 130.7, 80.4, 53.4, 50.1, 47.5, 45.7, 42.6, 40.8, 38.0, 29.5, 28.3, 24.7, 24.6, 22.8, 22.7, 22.6, 22.3; HRMS: (ESI-TOF) $C_{23}H_{38}N_6O_4H^+$ expected: 463.3027, found 463.3026.

General procedure for the synthesis of the ureas :

To a solution of sodium nitrite (2 equivalents) in H_2O (0.7 mL) was added at 0 °C a solution of HCl (1 M, 4 equivalents) and a solution of acetic acid (3 equivalents). After 5 min under vigorous stirring, a solution of acyl hydrazide (1 equivalent) in THF (6 mL) was added dropwise. The mixture was stirred for another 20 min at 0 °C. Saturated aqueous Na_2CO_3 solution was then added until pH is basic, and the organic phase is extracted with Et_2O (3x10 mL). The organic fractions were collected, dried over Na_2SO_4 and concentrated under reduced pressure to give the desired acyl azide as yellow oil. The azide was then dissolved in anhydrous toluene (1 mL) and stirred for 1 h at 70 ^oC to give the corresponding isocyanate. The reaction mixture was cooled to rt and an amine (1.1 equivalent) was added. The resulting mixture was stirred over night at rt, concentrated in vacuo and the residue purified by preparative TLC.

***tert*-Butyl 4-(6-(3-isopentylureido)-5-isopropylpyridazine-3-carbonyl)piperazine-1-carboxylate (13a);** Starting from **10a** (55 mg, 0.14 mmol), Prep. TLC CH_2Cl_2/MeOH 97/3, white solid, yield: 34 mg, (52%); ^{1}H NMR: (250 MHz, $CDCl_3$) Mixture of conformers δ 9.69 (m, 1H), 8.04 (s, 1H), 7.66 (s, 1H), 3.76 (m, 4H), 3.52 (m, 4H), 3.39 (m, 2H), 3.14 (hept, *J* = 6.7 Hz, 1H), 1.67 (m, 1H), 1.53 (m, 2H), 1.46 (m, 9H), 1.29 (d, *J* = 6.7 Hz, 6H), 0.93 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR: (62.5 MHz, $CDCl_3$) δ 165.2, 154.6, 154.5, 154.2, 151.0, 136.8, 125.6, 80.3, 47.3, 42.6, 38.6, 28.3, 26.6, 25.9, 22.4, 21.2; HRMS: (ESI-TOF) $C_{23}H_{38}N_6O_4H^+$ expected: 463.3027. found: 463.3035.

***tert*-Butyl 4-(5-isopropyl-6-(3-(3-(methylthio)propyl)ureido)pyridazine-3-carbonyl)piperazine-1-carboxylate (13b);** Starting from **10a** (55 mg, 0.14 mmol), Prep. TLC CH_2Cl_2/MeOH 97/3, white solid, yield: 35 mg, (52%); ^{1}H NMR: (250 MHz, $CDCl_3$) Mixture of conformers δ 9.80 (m, 1H), 7.90 (s, 1H), 7.68 (s, 1H), 3.78 (m, 4H), 3.62-3.42 (m, 6H), 3.09 (hept, *J* = 6.7 Hz, 1H), 2.57 (m, 2H), 2.10 (s, 3H), 1.93 (m, 2H), 1.46 (s, 9H), 1.30 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR: (62.5 MHz, $CDCl_3$) δ 165.1, 154.7, 154.5, 154.1, 151.2, 136.7, 125.8, 80.3, 47.3, 43.4 (broad), 42.6, 39.1, 31.5, 29.1, 28.3, 26.6, 21.2, 15.5; HRMS: (ESI-TOF) $C_{22}H_{36}N_6O_4SH^+$ expected: 481.2591. found: 481.2590.

***tert*-Butyl 3-((6-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-3-(3-isopentylureido)pyridazin-4-yl)methyl)-*1H*-indole-1-carboxylate (13c);** Starting from **10b** (47 mg, 0.08 mmol), Prep. TLC CH_2Cl_2/MeOH 97/3, white solid, yield: 16 mg, (31%); ^{1}H NMR: (250 MHz, $CDCl_3$) Mixture of conformers δ 9.63 (m, 1H), 8.15 (m, 1H), 8.04 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.39-7.15 (m, 3H), 4.06 (s, 2H), 3.70 (m, 4H), 3.47 (m, 4H), 3.37 (m, 2H), 1.68 (s, 9H), 1.61 (m, 1H), 1.51 (m, 2H), 1.46 (s, 9H), 0.87 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR: (62.5 MHz, $CDCl_3$) δ 164.9, 154.8, 154.4, 151.0, 149.3, 135.6, 129.2, 128.9, 125.0, 122.8, 118.5, 115.6, 113.1, 84.2, 80.3, 47.3, 43.5 (broad), 42.5, 38.6, 28.3, 28.1, 25.9, 25.6, 22.4; HRMS: (ESI-TOF) $C_{34}H_{47}N_7O_6H^+$ expected: 650.3660. found: 650.3654.

(*S*)-*tert*-Butyl 2-isobutyl-4-(5-isobutyl-6-(3-isopentylureido)pyridazine-3-carbonyl)piperazine-1-carboxylate (13d); Starting from **10c** (86 mg, 0.18 mmol), Prep. TLC Hexane/AcOEt 1/2, white solid,

yield: 25 mg, (25%); ^{1}H NMR: (600 MHz, $CDCl_3$) Mixture of conformers δ 9.58 (m, 1H), 7.59 (s, 1H), 7.17 (d, *J* = 19.6 Hz, 1H), 4.62 (d, *J* = 12.5 Hz, 0.5H), 4.57 (d, *J* = 13.2 Hz, 0.5H), 3.98-4.35 (m, 3H), 2.91-3.48 (m, 5H), 2.48 (m, 2H), 2.03 (m, 1H), 1.69 (m, 1H), 1.61 (m, 0.6H), 1.54 (m, 2.4H), 1.48 (s, 4.5H), 1.47 (s, 4.5H), 1.30-1.42 (m, 2H), 0.93-1.00 (m, 15H), 0.87 (t, *J* = 5.8 Hz, 3H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 165.4, 154.9, 154.4, 154.2, 150.9, 150.8, 138.3, 129.8, 129.6, 80.3, 50.1, 47.5, 45.6, 42.8, 38.8, 38.7, 38.1, 28.4, 26.8, 26.0, 25.9, 24.8, 24.7, 22.9, 22.8, 22.6, 22.5, 22.4; HRMS: (ESI-TOF) $C_{28}H_{48}N_6O_4H^+$ expected: 533.3810. found: 533.3814.

General procedure for the Boc deprotection:

To a solution of the urea in CH_2Cl_2 (0.75 mL) at 0 °C was added TFA (0.25 mL). The mixture was stirred for 3 h at rt and then concentrated in vacuo. The crude residue was purified on a short plug of silica if necessary to afford the desired amines as trifluoroacetic acid salts. In the case of compound **1c** *i*Pr_3SiH was added for the deprotection of the indole.

1-Isopentyl-3-(4-isopropyl-6-(piperazine-1-carbonyl)pyridazin-3-yl)urea 2,2,2-trifluoroacetate (1a); Starting from **13a** (34 mg, 0.07 mmol), Column CH_2Cl_2/MeOH 9/1, white solid, yield : 34 mg, (97%); ^{1}H NMR: (600 MHz, $CDCl_3/CD_3OD$ 95/5) Mixture of conformers δ 7.69 (s, 1H), 4.06 (m, 4H), 3.75 (m, 4H), 3.35 (m, 2H), 3.04 (hept, *J* = 6.7 Hz, 1H), 1.63 (hept, *J* = 6.7 Hz, 1H), 1.48 (m, 2H), 1.25 (d, *J* = 6.7 Hz, 6H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR: (150 MHz, $CDCl_3/CD_3OD$ 95/5) δ 164.9, 154.6, 154.1, 150.1, 137.6, 125.9, 44.2, 43.5, 42.9, 39.4, 38.4, 38.3, 26.5, 25.7, 22.1, 20.8; HRMS: (ESI-TOF) $C_{18}H_{30}N_6O_2H^+$ expected: 363.2503. found: 363.2448.

1-(4-Isopropyl-6-(piperazine-1-carbonyl)pyridazin-3-yl)-3-(3-(methylthio)propyl)urea 2,2,2-trifluoroacetate (1b); Starting from **13b** (35 mg, 0.07 mmol), Column CH_2Cl_2/MeOH 9/1, white solid, yield : 34 mg, (94%); ^{1}H NMR: (600 MHz, $CDCl_3/CD_3OD$ 95/5) Mixture of conformers δ 7.71 (s, 1H), 4.07 (m, 4H), 3.60-3.40 (m, 6H), 3.04 (hept, *J* = 6.7 Hz, 1H), 2.54 (m, 2H), 2.07 (s, 3H), 1.90 (m, 2H), 1.27 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR: (150 MHz, $CDCl_3/CD_3OD$ 95/5) δ 164.8, 154.8, 154.1, 150.2, 137.5, 126.0, 44.2, 43.5, 39.5, 38.9, 31.3, 28.8, 26.6, 20.8, 15.2; HRMS: (ESI-TOF) $C_{17}H_{28}N_6O_2SH^+$ expected: 381.2067. found: 381.2052.

1-(4-((*1H*-Indol-3-yl)methyl)-6-(piperazine-1-carbonyl)pyridazin-3-yl)-3-isopentylurea (1c); Starting from **13c** (16 mg, 0.02 mmol), Column saturated solution of NH_3 in CH_2Cl_2, white solid, yield : 8 mg, (73%); ^{1}H NMR: (600 MHz, $CDCl_3$) Mixture of conformers δ 9.53 (m, 1H), 8.58 (s, 1H), 7.59 (s, 1H), 7.44 (s, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.24 (m, 1H), 7.13-7.09 (m, 2H), 4.09 (s, 2H), 3.79 (m, 2H), 3.72 (m, 2H), 3.40 (m, 2H), 2.99 (m, 2H), 2.91 (m, 2H), 1.67 (hept, *J* = 6.7 Hz, 1H), 1.53 (m, 2H), 0.93 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR: (150 MHz, $CDCl_3$) δ 164.9, 154.7, 151.4, 136.6, 129.3,

129.0, 126.5, 123.4, 122.8, 120.0, 118.3, 111.7, 108.0, 48.7, 46.4, 45.7, 43.6, 38.7, 38.6, 26.8, 25.9, 22.4; HRMS: (ESI-TOF) $C_{24}H_{31}N_7O_2H^+$ expected: 450.2612. found: 450.2610.

(*S*)-1-(4-Isobutyl-6-(3-isobutylpiperazine-1-carbonyl)pyridazin-3-yl)-3-isopentylurea (1d); Starting from **13d** (25 mg, 0.04 mmol), washed with saturated $NaHCO_3$ solution, white solid, yield : 18 mg, (90%); ^{1}H NMR (600 MHz, $CDCl_3$) Mixture of conformers δ 9.61 (m, 1H), 7.56 (s, 1H), 7.29 (d, *J* = 12.9 Hz, 1H), 4.61 (d, *J* = 12.3 Hz, 1H), 4.20 (m, 1H), 3.41 (m, 2H), 2.86-3.32 (m, 4H), 2.62 (m, 0.6H), 2.48 (d, *J* = 7.0 Hz, 2H), 2.30 (m, 0.4H), 2.04 (m, 1H), 1.77 (m, 0.4H), 1.68 (m, 1H), 1.62 (m, 0.6H), 1.53 (m, 2H), 1.35 (m, 1H), 0.88-0.99 (m, 18H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 164.8, 164.7, 154.8, 154.1, 150.9, 136.8, 129.7, 129.6, 54.0, 53.2, 47.8, 45.0, 42.8, 38.8, 38.7, 31.9, 30.3, 29.7, 29.4, 26.7, 26.0, 25.9, 24.3, 24.2, 22.7, 22.5, 22.4; HRMS: (ESI-TOF) $C_{23}H_{40}N_6O_2H^+$ expected 433.3285. found 433.3277.

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