Supporting Information

Discovery and Optimization of Triazolopyrimidinone Derivatives as Selective NLRP3 Inflammasome Inhibitors

David Harrison[a,*], Mark G. Bock[b], John R. Doedens[c], Christopher A. Gabel[c], M. Katharine Holloway[e], Arwel Lewis[d], Jane Scanlon[a], Andrew Sharpe[d], Iain D. Simpson[d], Pamela Smolak[c], Grant Wishart[d], Alan P. Watt[a]

[a] *NodThera Ltd., Suite 8, The Mansion, Chesterford Research Park, Little Chesterford, Saffron Walden, Essex CB10 1XL, United Kingdom*

[b] *NodThera Inc., 430 Bedford Street, Lexington, MA 02420, USA*

[c] *NodThera Inc., 454 N 34th Street, Seattle, WA 98103, USA*

[d] *Charles River Laboratories, Chesterford Research Park, Little Chesterford, Saffron Walden, Essex CB10 1XL, United Kingdom*

[e] *Gfree Bio LLC, 10601 Ranch Rd 2222, Austin, TX 78730, USA*

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Safety Statement

No unexpected or unusually high safety hazards were encountered during the handling or in the obtaining of experimental data from the compounds disclosed herein.

Appropriate safety procedures were followed in accordance with the rules of the country and place of business where the research took place.

Assay protocols

PBMC IL-1β assay:

PBMCs were isolated from buffy coats by density gradient centrifugation on Histopaque-1077 (Sigma, cat no. 10771). Isolated cells were seeded into the wells (280,000 cells/well) of a 96-well plate and incubated for 3 h with lipopolysaccharide (LPS, 1 µg/mL diluted 1000x from a 1 mg/mL stock solution). Following medium exchange, the compounds were added (a single compound per well) and the cells were incubated for 30 min. Next, the cells were stimulated with ATP (5 mM) for 1 h and the cell culture media from the wells were collected for further analysis.

The release of IL1-β into the media was determined by a quantitative detection of IL-1β in the media using an IL-1β enzyme-linked immunosorbent assay (ELISA) Ready-SET-Go!, eBioscience cat. No. 88-7261-88. Briefly, in a first step, high affinity binding plates (Corning, Costar 9018 or NUNC Maxisorp Cat No. 44-2404) were coated overnight at 4° C with specific capture antibody included in the kit (anti-human IL-1β ref. 14-7018-68). Subsequently, plates were blocked with blocking buffer for 1 h at room temperature (rt) and after washing with a buffer (PBS with 0.05 % Tween-20) incubated with protein standard and culture media. After 2 h of incubation at rt, plates were washed and incubated with biotinylated detection antibody included in the kit (anti-human IL-1β Biotin ref. 33-7110-68) for 1 h at rt. Plates were washed and incubated with HRP-streptavidin for 30 min at rt and washed again. The signal was developed after addition of 3,39,5,59-tetramethylbenzidine-peroxidase (TMB) until color appeared and the reaction was stopped by 2 M H_2SO_4. A microplate spectrophotometer (BioTek) was used to detect signals with 450 nM. The detection range of IL-1β ELISA was 2-150 ng/mL.

Whole blood IL-1β assay:

Whole blood was drawn from single healthy donors and treated with sodium heparin anticoagulant. Blood was diluted with cell culture media at a ratio of 90 µl whole blood per 50 µL media, seeded in a 96-well plate and incubated for 3 h with lipopolysaccharide (LPS, 1 µg/mL final concentration diluted 1000x from a 1 mg/mL stock solution). The compounds were added (a single compound concentration per well) and the blood was incubated for 30 min. Next, the blood was stimulated with ATP (5 mM final concentration diluted 20x from a 100 mM stock solution) for 1 h and the cell culture media from the wells were collected for further analysis.

The release of IL-1β into the media was determined by quantitative detection of IL-1β in the media using HTRF®, CisBio cat. No. 62HIL1BPEH. Briefly, cell culture supernatant were diluted as appropriate to bring IL-1β levels within the HTRF® detection range (36-6500 pg/mL) and subsequently dispensed into the assay plate containing antibodies labelled with the HTRF® donor and acceptor. A microplate spectrophotometer (BMG) was used to detect signals at 655 nm and 620 nm.

PBMC IL-6 / TNFα assays:

Human PBMCs were isolated and enriched using a density gradient technique from freshly isolated heparinized human whole blood. Isolated PBMCs were suspended in RPMI-1640 medium containing 1 % FBS, 1 % penicillin/streptomycin and 20 mM HEPES, pH 7.3. An appropriate number of PBMCs (2.8 x 10^5 cells/well) were transferred to each well of 96-well plates. Varying concentrations of test agent were introduced into designated wells and the plates were incubated at 37° C in a 5 % CO_2 incubator to allow equilibration. LPS subsequently was introduced to achieve a final concentration of 100 ng/ml, the plates were returned to the 37° C incubator for an additional 4 h, after which they were centrifuged and

resulting supernatants harvested. IL-6 and TNFα content within the supernatants was quantitated using ELISA kits selective for each cytokine.

ASC-speck assay:

THP1-ASC-GFP cells (Invivogen) were cultured in RPMI-1640 medium containing 10 % fetal bovine serum (FBS) with or without 1 µg/mL LPS for 2 h at 37° C in a 5 % CO_2 incubator. At the end of the 2 h incubation, the cells were transferred into medium containing 1 % FBS and dispensed into a 96 well plate. Diluted compounds or equivalent dilutions of vehicle (DMSO) were added, and the cells were incubated for 30 min at 37° C. At the end of the 30 min incubation, the caspase inhibitor zVAD-FMK was added to the cells to yield a final concentration of 20 µM. Nigericin was then added to a final concentration of 5 µM, and the cells were incubated for 1 h at 37° C.

The cells were then fixed with a final concentration of 1 % paraformaldehyde and stained with Hoechst 33342 to fluorescently label DNA. Images of the cells were acquired using an ImageXpress Micro Confocal High Content Imaging System (Molecular Devices) and analyzed using CellProfiler software.

IC_{50} values were determined by nonlinear regression in GraphPad Prism software using four-parameter (variable slope) fits to plots of log(inhibitor concentration) versus fraction speck-positive cells.

PAMPA assay:

Phosphate buffered saline (PBS) was prepared by dissolving KH_2PO_4 (2.6 g) and $K_2HPO_4.3H_2O$ (18.5 g) in 1000 mL of ultra pure water. The pH was adjusted to 7.40 ± 0.05, using either 1 M sodium hydroxide or 1M hydrochloric acid.

A working solution was prepared by dissolving the sample in DMSO to 0.20 mM concentration. A donor solution was prepared by diluting the working solution (20 µL) with PBS (380 µL). 150 µL of 10.0 µM donor solutions to each well of the donor plate, whose PVDF membrane was precoated with 5 µL of 1 % lecithin/dodecane mixture. Duplicates were prepared. 300 µL of PBS was added to each well of the PTFE acceptor plate. The donor plate and acceptor plate were combined together and incubated for 4 h at room temperature with shaking at 300 rpm. Preparation of T_0 sample: 20 µL donor solution was transferred to new well followed by the addition of 230 µL PBS (DF: 12.5), 130 µL ACN (containing internal standard) as T_0 sample. Preparation of acceptor sample: The plate was removed from incubator. 250 µL solution was transferred from each acceptor well and mixed with 130 µL ACN (containing internal standard) as acceptor sample. Preparation of donor sample: 20 µL solution was transferred from each donor well and mixed with 230 µL PBS (DF: 12.5), 130 µL ACN (containing internal standard) as donor sample. Acceptor samples and donor samples were all analysed by LC-MS/MS. The equation used to determine permeability rates (Pe) was as follows.

$$P_e = C \times (-\ln(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}})) \times 10^7, where C = (\frac{V_D \times V_A}{(V_D + V_A) \times Area \times time})$$

$[drug]_{equilibrium}=([drug]_{donor} \times V_D + [drug]_{acceptor} \times V_A)/(V_D + V_A)$

V_D = 0.15 mL; V_A = 0.30 mL; Area = 0.28 cm^2; time = 14400 s.

[drug]acceptor = (Aa/Ai×DF)acceptor; [drug]donor= (Aa/Ai*DF)donor;

Aa/Ai: Peak area ratio of analyte and internal standard; DF: Dilution factor.

Thermodynamic solubility assay:

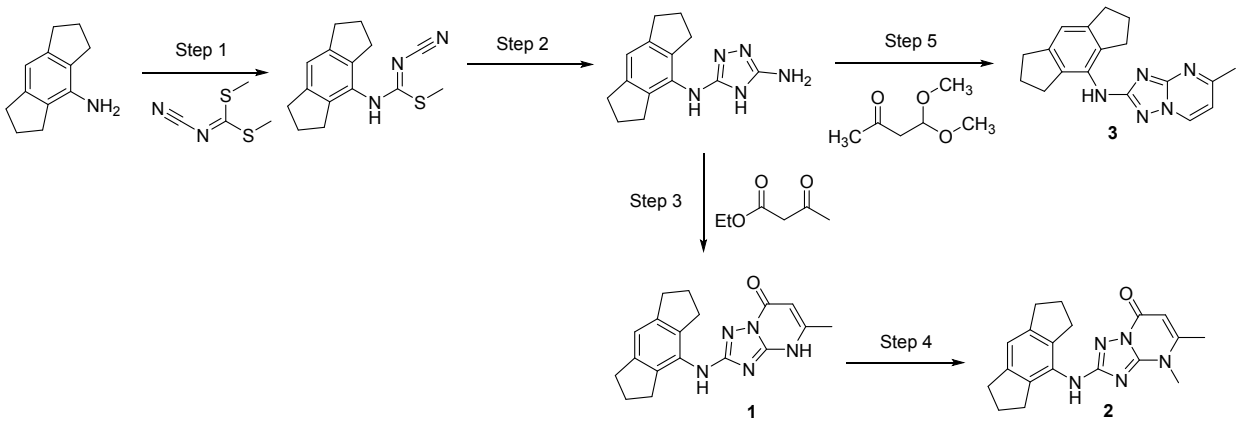
An appropriate amount of test compound and control compounds (amiodarone hydrochloride, carbamazepine, dexamethasone) were weighed into lower chambers of Whatman miniuniprep vials. Phosphate buffer (50 mM, pH 7.4, 450 µL) was added. The samples were vortexed for at least 2 minutes. The vials were shaken on a shaker for 24 hours at room temperature at a speed of 800 rpm, then centrifuged for 20 minutes at 4000 rpm. The miniuniprep vials were compressed to prepare filtrates for injection into the UPLC system (column: BEH C18 1.7 µm 50 mm; mobile phase: 0.1% TFA and 5 mM NH_4OAc in water/ACN (v:v, 95:5)); . The concentration was calculated using a standard curve.

Docking studies

The NLRP3 NACHT domain|**NP3-146** co-crystal structure (PDB ID 7ALV) was prepared for docking using the MOE QuickPrep protocol with the binding site defined by the bound ligand. Starting 3D geometries / tautomers of **NDT-30805 (50)** were generated from SMILES using the AMBER10:EHT force field with R solvation = 8. Induced fit docking was performed with enhanced pose sampling, *i.e.* 60 placement poses; 20 refinement poses. Poses were scored using a GBVI/WSA free energy metric.

Synthesis and spectral data for compounds 1 – 51

1-3:



Step 1

To a solution of 1,2,3,5,6,7-hexahydro-s-indacen-4-amine (1.0 g, 5.77 mmol) in THF (30 mL) at room temperature was added sodium hydride (60 % dispersion in oil, 369 mg, 9.23 mmol). The reaction mixture was stirred for 20 minutes. Dimethyl N-cyanodithioiminocarbonate (1.27 g, 8.66 mmol) was added and the mixture was heated at 60° C for 42 hours. The reaction was cooled to room temperature and air was bubbled through the solution for 15 minutes then quenched with saturated ammonium chloride solution (10 mL), poured into water (100 mL) and extracted with dichloromethane (3 x 100 mL). The combined organic layers were dried (hydrophobic frit) and concentrated. The residue was purified by column chromatography on silica gel, eluting with 0-100 % ethyl acetate in isohexane to give methyl (Z)-N'-cyano-N-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamimidothioate as an orange solid.

Step 2

To a suspension of methyl (Z)-N'-cyano-N-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamimidothioate (653 mg, 2.41 mmol) in ethanol (10 mL) was added hydrazine (0.23 mL, 7.22 mmol) and the reaction mixture was heated at reflux overnight. The reaction mixture was cooled to room temperature and water (15 mL) added. The resulting precipitate was filtered off, washed with water (25 mL) and dried *in vacuo* to give N^3-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (351 mg, 24% over two steps) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 7.14 (s, 1H), 6.77 (s, 1H), 5.66 (s, 2H), 2.82 - 2.74 (m, 4H), 2.70 - 2.64 (m, 4H), 1.98 - 1.89 (m, 4H).

Step 3

N^3-(1,2,3,5,6,7-Hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (10 mg, 0.039 mmol) was treated with ethyl acetoacetate (0.007 mL, 0.047 mmol) in acetic acid (0.025 mL). The reaction mixture was heated at 120° C for 1.5 hours and then cooled to room temperature. N^3-(1,2,3,5,6,7-Hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (44 mg, 0.173 mmol) was treated with ethyl acetoacetate (0.026 mL, 0.207 mmol) in acetic acid (0.088 mL). The reaction mixture was heated at 120° C for 1.5 hours. The mixture was cooled to room temperature and more ethyl acetoacetate (0.026 mL, 0.207 mmol) and acetic acid (0.088 mL) were added. The reaction mixture was heated at 120°C for 30 minutes and then cooled to room temperature. The two mixtures were combined and concentrated *in vacuo*. The residue was purified by reverse phase preparative HPLC to give **1** (14.5 mg, 21%) as a cream solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (s, 1H), 6.92 (s, 1H), 5.61 (s, 1H), 2.89 - 2.81 (m, 4H), 2.79 - 2.71 (m, 4H), 2.25 - 2.23 (m, 3H), 2.04 - 1.93 (m, 4H). Molecular formula = $C_{18}H_{19}N_5O$, MW = 321.4. MS (ESI): m/z 322 (M+1). HPLC purity: 98.0%.

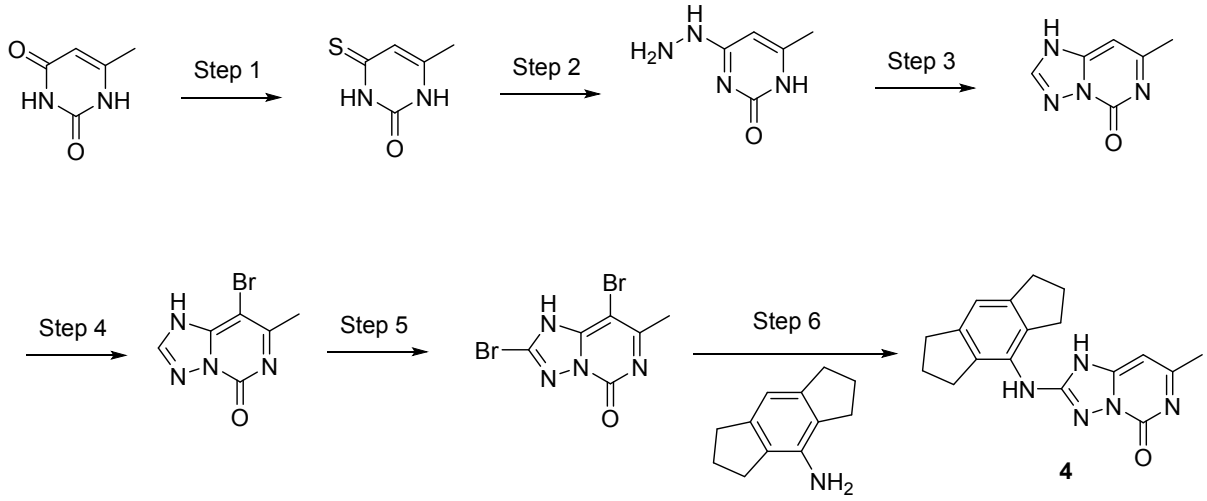
Step 4

To a solution of **1** (70 mg, 0.218 mmol) in THF (2 mL) cooled to 0° C were added dimethylsulfide (33 mg, 0.262 mmol) and 20 % NaOH (0.05 mL, 0.218 mmol). The RM was stirred at RT for 4 h, filtered, washed with diethyl ether and dried under vacuum to give **2** (15 mg) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 6.91 (s, 1H), 5.81 (s, 1H), 3.76 (s, 3H), 2.85 – 2.79 (m, 4H), 2.76 – 2.66 (m, 4H), 2.35 (s, 3H), 2.00 – 1.91 (m, 4H). Molecular formula = $C_{19}H_{21}N_5O$, MW = 335.4. MS (ESI): m/z 336.2 (M+1). HPLC purity: 99.6%.

Step 5

To a solution of N^3-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (60 mg, 0.235 mmol) in AcOH (1 mL) was added dimethoxybutanone (62 mg, 0.471 mmol). The RM was heated at reflux for 6 h. The RM was concentrated *in vacuo*. The residue was diluted with EtOAc, washed sequentially with sat. $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated under vacuum. The crude was purified by chiral prep-HPLC to give **3** as an off-white solid (20 mg, plus 20 mg of other isomer). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.40 (s, 1H), 7.01 - 6.98 (m, 1H), 6.91 (s, 1H), 2.85-2.80 (m, 4H), 2.76 – 2.70 (m, 4H), 2.60 (s, 3H), 1.99 – 1.92 (m, 4H). Molecular formula = $C_{18}H_{19}N_5$, MW = 305.4. MS (ESI): m/z 306.3 (M+1). HPLC purity: 97.7%.

4:



Step 1

To a solution of 6-methylpyrimidine-2,4(1H,3H)-dione (10 g, 79.3 mmol) in dioxane (200 mL) at RT was added Lawson's reagent (32.1 g, 79.3 mmol). The mixture was stirred at 100° C for 3 h. The reaction mixture was filtered and the solid washed with MeOH (100 mL) and dried under vacuum to give 4-methyl-6-sulfanylidene-1,3-dihydropyrimidin-2-one as a white solid.

Step 2

To a solution of 4-methyl-6-sulfanylidene-1,3-dihydropyrimidin-2-one (5.0 g, 35.2 mmol) in EtOH (50 mL) at RT was added hydrazine hydrate (1.71 mL, 35.2 mmol). The mixture was stirred at 80° C for 12 h. The reaction mixture was filtered, and the solid washed with MeOH (100 mL) and dried *in vacuo* to give 4-hydrazinyl-6-methyl-1,2-dihydropyrimidin-2-one as a white solid. Y = 61 %.

Step 3

A mixture of 4-hydrazinyl-6-methyl-1,2-dihydropyrimidin-2-one (3.0 g, 21.4 mmol) and formic

acid (30 mL) was stirred at 110° C for 3 h. The reaction mixture was filtered, then the solid was washed sequentially with H_2O (30 mL) and MeOH (30 mL) and dried *in vacuo* to give 7-methyl-1H,5H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one as a white solid. Y = 47 %.

Step 4

To a solution of 7-methyl-1H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one (1.0 g, 6.66 mmol) in MeCN (50 mL) was added NBS (1.24 g, 6.99 mmol). The mixture was stirred at RT for 12 h. The solution was filtered and the solid dried to give 8-bromo-7-methyl-1H,5H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 8.38 (s, 1H), 2.38 (s, 3H).

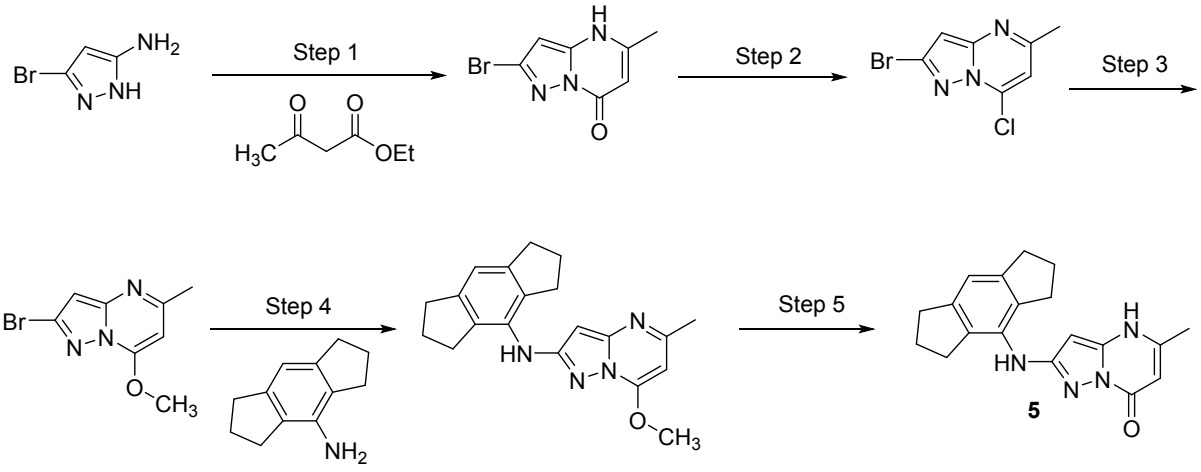
Step 5

To a solution of 8-bromo-7-methyl-1H,5H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one (860 mg, 3.75 mmol, 1 *eq*) in THF (12 mL) was added NBS (2.00 g, 11.3 mmol). The mixture was stirred at 80° C for 1 h. The solution was concentrated under reduced pressure and purified by prep-TLC (SiO_2, ethyl acetate, R_f = 0.61) to give 2,8-dibromo-7-methyl-1H,5H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1 H), 2.56 (s, 3 H).

Step 6

To a solution of 2,8-dibromo-7-methyl-1H,5H-[1,2,4]triazolo[1,5-c]pyrimidin-5-one (250 mg, 812 µmol) in *tert*-amyl alcohol (4 mL) was added 1,2,3,5,6,7-hexahydro-s-indacen-4-amine (703 mg, 4.06 mmol), sodium *tert*-butoxide (234 mg, 2.44 mmol) and Xantphos Pd G3 (234 mg, 244 µmol). The mixture was stirred at 100° C for 12 h under N_2. The solution was concentrated under reduced pressure and the resulting residue purified by prep-HPLC (column: Phenomenex Luna C_{18} 80x40 mm x 3 µm; mobile phase: [water (0.04 % HCl) - ACN]; B: 35 – 55 %, 7 min) and lyophilized to give **4** as a white solid. Y = 2.4 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.47 (s, 1H), 6.90 (s, 1H), 6.32 (s, 1H), 2.82 (t, J = 7 Hz, 4H), 2.69 (t, J = 7 Hz, 4H), 2.21 (s, 3H), 2.00 - 1.95 (m, 4H). ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 6.90 (s, 1H), 6.29 (s, 1H), 2.80 (t, J = 7 Hz, 4H), 2.66 (t, J = 7 Hz, 4H), 2.20 (s, 3H), 1.93 (m, 4H). Molecular formula = $C_{18}H_{19}N_5O$, MW = 321.4. MS (ESI): m/z 322.2 (M+1). HPLC purity: 92.0%.

5:



Step 1

A solution of 3-bromo-1H-pyrazol-5-amine (2.0 g, 12.3 mmol) in AcOH (20 mL) was treated with ethyl acetoacetate (3.21 g, 24.7 mmol) and heated at 110° C for 16 h. The RM was allowed to cool to RT, diluted with diethyl ether and filtered. The solid was washed with diethyl

ether to give 2-bromo-5-methyl-4H,7H-pyrazolo[1,5-a]pyrimidin-7-one (2.4 g) as a white solid. Y = 79 %.

Step 2

A mixture of 2-bromo-5-methyl-4H,7H-pyrazolo[1,5-a]pyrimidin-7-one (2.5 g, 11.0 mmol) and $POCl_3$ (50 mL) was cooled to 0° C and treated with DIPEA (4.04 mL, 21.9 mmol). The RM was heated to reflux for 16 h. The RM was evaporated to dryness, diluted with DCM, washed sequentially with sat. sodium bicarbonate solution and brine, dried over sodium sulfate and evaporated to dryness. The crude was purified by silica column chromatography (0 – 50 % EtOAc in hexane) to give 2-bromo-7-chloro-5-methylpyrazolo[1,5-a]pyrimidine (2.2 g) as an off-white solid.

Step 3

A solution of 2-bromo-7-chloro-5-methylpyrazolo[1,5-a]pyrimidine (2.2 g, 8.94 mmol) in dry THF (50 mL) was cooled to 0° C and treated with sodium methoxide (580 mg, 10.7 mmol). The RM was stirred at RT for 4 h, then concentrated under vacuum. The crude was diluted with EtOAc, washed with water, dried over sodium sulfate and evaporated to dryness. sodium bicarbonate solution and brine, dried over sodium sulfate and evaporated to dryness. The crude was purified by silica column chromatography (0 – 50 % EtOAc in hexane) to give 2-bromo-7-methoxy-5-methylpyrazolo[1,5-a]pyrimidine (1.6 g) as an off-white solid.

Step 4

A mixture of 2-bromo-7-methoxy-5-methylpyrazolo[1,5-a]pyrimidine (200 mg, 0.83 mmol), 1,2,3,5,6,7-hexahydro-s-indacen-4-amine (114 mg, 0.66 mmol) and potassium phosphate (525 mg, 2.48 mmol) was degassed with argon. X-phos (59 mg, 0.12 mmol) and $Pd_2(dba)_3.CHCl_3$ (60 mg, 0.066 mmol) were added and the RM heated at 120° C for 48 h.

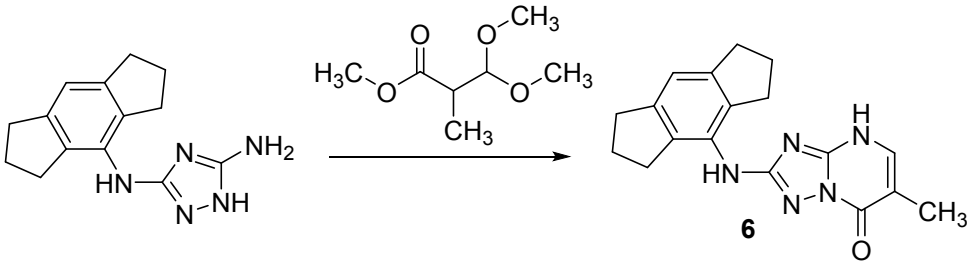
This reaction was repeated a further two times.

The combined reaction mixtures were concentrated under vacuum and purified by silica column chromatography (EtOAc / hexane). The crude was further purified by prep HPLC to give
N-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-7-methoxy-5-methylpyrazolo[1,5-a]pyrimidin-2-amine (44 mg) as an off-white solid.

Step 5

A solution of N-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-7-methoxy-5-methylpyrazolo[1,5-a]pyrimidin-2-amine (40 mg, 0.12 mmol) in DCM (2 mL) was cooled to 0° C and treated dropwise with 1.2 M BBr_3 in DCM (0.50 mL, 0.60 mmol). The RM was stirred at RT for 16 h. The RM was quenched with ice cold water and neutralised with $NaHCO_3$. The RM was extracted with 10 % MeOH in DCM, dried (sodium sulfate) and evaporated to dryness. The crude was purified by silica column chromatography (0 – 5 % MeOH in DCM) to give **5** (15 mg) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 7.80 (s, 1H), 6.85 (s, 1H), 5.39 (s, 1H), 5.25 (s, 1H), 2.85 – 2.76 (m, 4H), 2.70 – 2.63 (m, 4H)2.18 (s, 3H), 1.98 – 1.89 (m, 4H). Molecular formula = $C_{19}H_{20}N_4O$, MW = 320.4. MS (ESI): m/z 321.2 (M+1). HPLC purity: 97.5%.

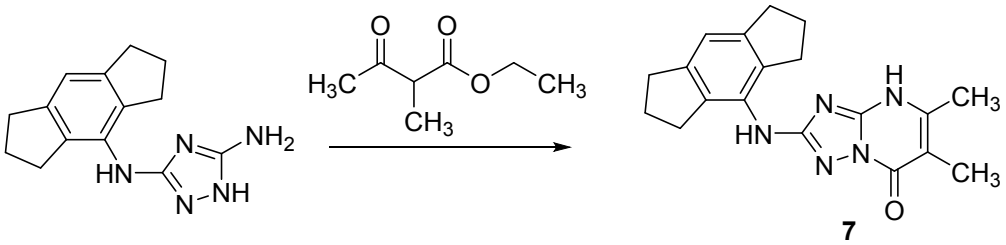
6:



6

To a solution of N^3-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (50 mg, 0.196 mmol)(*for synthesis refer to compound* **1**) in AcOH (3 mL) was added methyl 3,3-dimethoxy-2-methylpropanoate (64 mg, 0.392 mmol). The RM was heated at reflux for 48 h. The RM was concentrated *in vacuo*. The residue was diluted with EtOAc, washed sequentially with sat. $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated under vacuum. The crude was triturated with diethyl ether, filtered, washed with diethyl ether and dried to give **6** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 - 12.50 (br. s, 1H), 8.41 (s, 1H), 7.65 (s, 1H), 6.89 (s, 1H), 2.82 - 2.75 (m, 4H), 2.74 – 2.65 (m, 4H), 1.99 – 1.90 (m, 7H). Molecular formula = $C_{18}H_{19}N_5O$, MW = 321.4. MS (ESI): m/z 322.1 (M+1). HPLC purity: 100%.

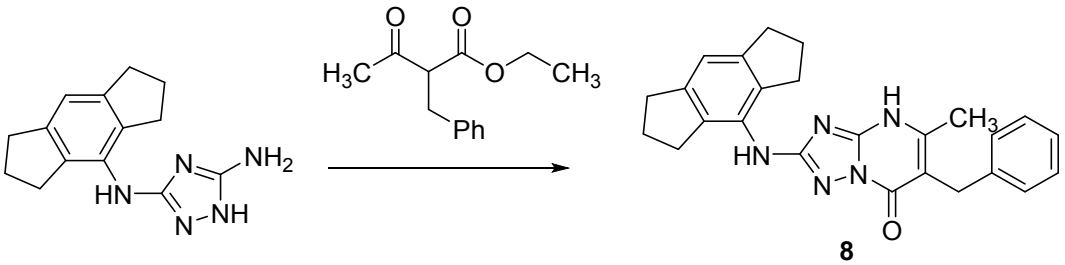
7:



7

Synthesis analogous to compound **6**, using ethyl 2-methylacetoacetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H), 8.39 (s, 1H), 6.89 (s, 1H), 2.82 - 2.76 (m, 4H), 2.73 – 2.65 (m, 4H), 2.23 (s, 3H), 1.99 – 1.86 (m, 7H). Molecular formula = $C_{19}H_{21}N_5O$, MW = 335.4. MS (ESI): m/z 336.8 (M+1). HPLC purity: 97.2%.

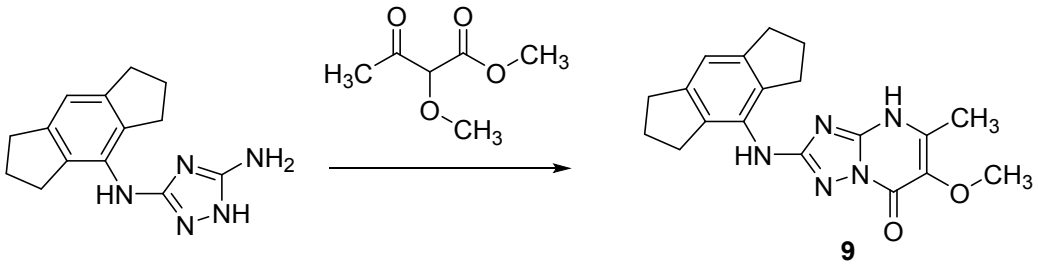
8:



8

Synthesis analogous to compound **6**, using ethyl 2-benzylacetoacetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 – 12.45 (br. s, 1H), 8.19 (s, 1H), 7.29 – 7.10 (m, 5H), 6.89 (s, 1H), 3.78 (s, 2H), 2.85 - 2.77 (m, 4H), 2.75 – 2.62 (m, 4H), 2.21 (s, 3H), 1.99 – 1.87 (m, 4H). Molecular formula = $C_{25}H_{25}N_5O$, MW = 411.5. MS (ESI): m/z 410.3 $(M-H)^-$. HPLC purity: 99.4%.

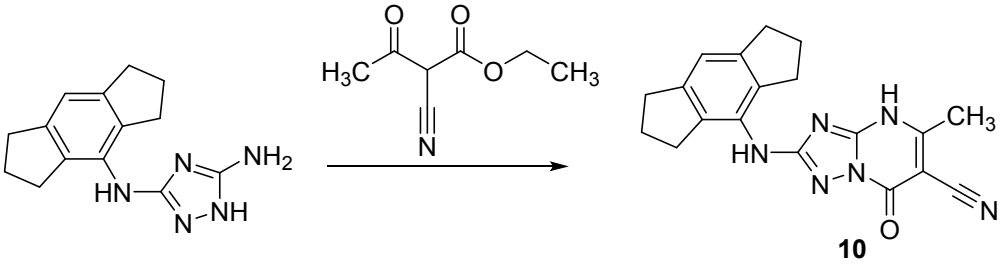
9:



9

Synthesis analogous to compound **6**, using methyl 2-methoxyacetoacetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 8.40 (s, 1H), 6.90 (s, 1H), 3.68 (s, 3H), 2.84 - 2.77 (m, 4H), 2.75 – 2.64 (m, 4H), 2.22 (s, 3H), 1.99 – 1.89 (m, 7H). Molecular formula = $C_{19}H_{21}N_5O_2$, MW = 351.4. MS (ESI): m/z 352.3 (M+1). HPLC purity: 96.2%.

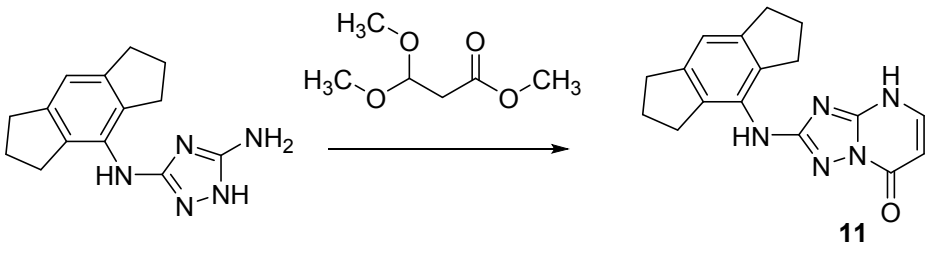
10:



10

Synthesis analogous to compound **6**, using ethyl 2-cyanoacetoacetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (s, 1H), 8.79 (s, 1H), 6.91 (s, 1H), 2.84 - 2.77 (m, 4H), 2.75 – 2.68 (m, 4H), 2.56 (s, 3H), 1.99 – 1.89 (m, 4H). Molecular formula = $C_{19}H_{18}N_6O$, MW = 346.4. MS (ESI): m/z 347.3 (M+1). HPLC purity: 93.2%.

11:

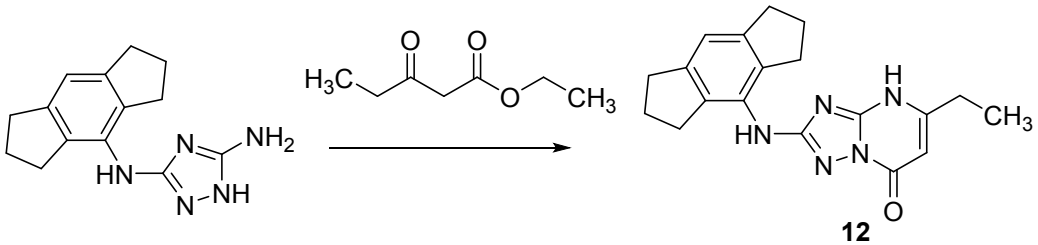


11

Synthesis analogous to compound **6**, using methyl 3,3-dimethoxypropanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (d, *J* = 4 Hz, 1H), 8.47 (s, 1H), 7.80 - 7.70 (m, 1H), 6.91 (s, 1H), 5.79 (d, *J* = 8 Hz, 1H), 2.82 (t, *J* = 7 Hz, 4H), 2.71 (t, *J* = 7 Hz, 4H), 2.10 - 1.90 (m, 4H).

^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 7.72 (d, *J* = 8 Hz, 1H), 6.91 (s, 1H), 5.80 (d, *J* = 8 Hz, 1 H), 2.80 (t, *J* = 7 Hz, 4 H), 2.71 (t, *J* = 7 Hz, 4H), 2.10 - 1.90 (m, 4H). Molecular formula = $C_{17}H_{17}N_5O$, MW = 307.4. MS (ESI): m/z 308.1 (M+1). HPLC purity: 98.9%.

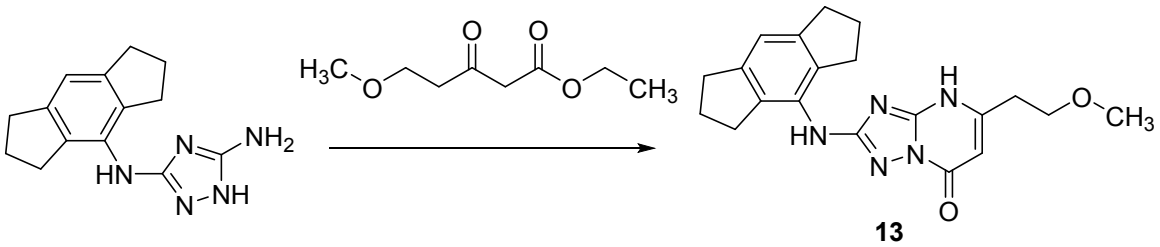
12:



12

Synthesis analogous to compound **6**, using ethyl propionylacetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 8.31 (s, 1H), 6.91 (s, 1H), 5.63 (s, 1H), 2.83 (t, *J* = 7 Hz, 4H), 2.73 (t, *J* = 7 Hz, 4H), 2.01 - 1.93 (m, 4H), 1.20 (t, *J* = 7 Hz, 3H). (Note: CH_2CH_3 peak not observed: assumed to be obscured by DMSO peak). Molecular formula = $C_{19}H_{21}N_5O$, MW = 335.4. MS (ESI): m/z 336 (M+1). HPLC purity: 98.3%.

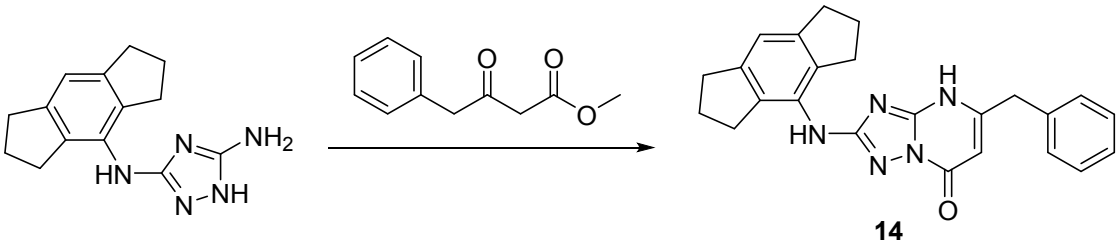
13:



13

Synthesis analogous to compound **6**, using ethyl 5-methoxy-3-oxopentanoate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.63 (s, 1H), 5.78 (s, 1H), 3.70 (t, *J* = 5 Hz, 2H), 3.40 (s, 3H), 2.88 (t, *J* = 8 Hz, 4H), 2.84 - 2.76 (m, 6H), 2.10 - 2.01 (m, 4H). Molecular formula = $C_{20}H_{23}N_5O_2$, MW = 365.4. MS (ESI): m/z 366 (M+1). HPLC purity: 95.6%.

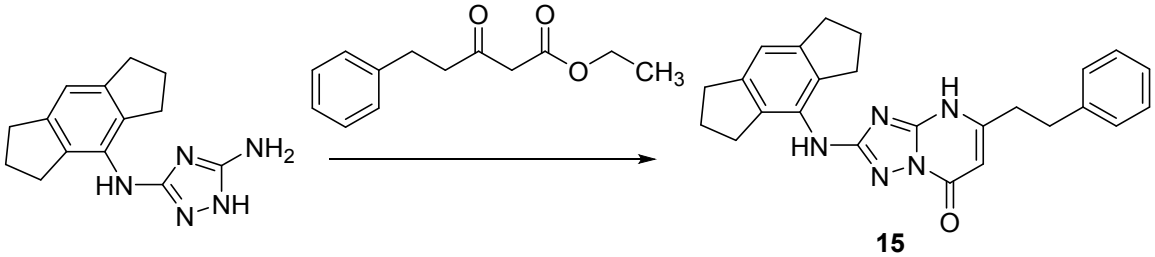
14:



14

Synthesis analogous to compound **6**, using methyl 3-oxo-4-phenylbutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 – 12.80 (br. s, 1H), 8.02 – 7.90 (br. s, 1H), 7.35 – 7.29 (m, 4H), 7.27 – 7.19 (m, 1H), 6.84 (s, 1H), 5.45 (s, 1H), 3.75 (s, 2H), 2.82 - 2.76 (m, 4H), 2.75 – 2.66 (m, 4H), 1.99 – 1.85 (m, 7H). Molecular formula = $C_{24}H_{23}N_5O$, MW = 397.4. MS (ESI): m/z 396.2 $(M-H)^-$. HPLC purity: 99.0%.

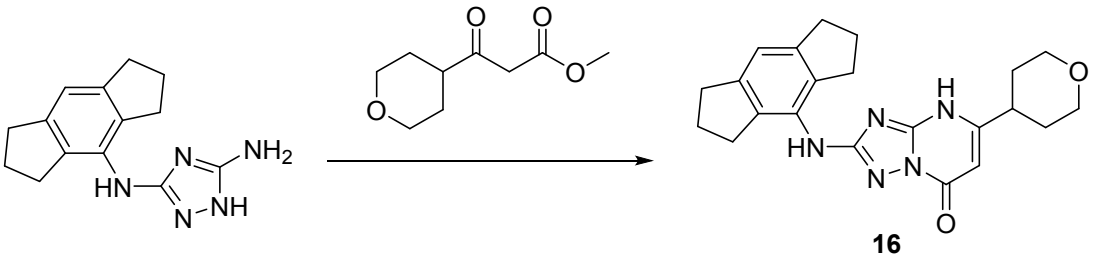
15:



15

Synthesis analogous to compound **6**, using ethyl 3-oxo-5-phenylpentanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 – 12.75 (br. s, 1H), 8.39 – 8.30 (br. s, 1H), 7.34 – 7.16 (m, 5H), 6.89 (s, 1H), 5.63 (s, 1H), 2.98 – 2.89 (m, 2H), 2.82 - 2.77 (m, 6H), 2.76 – 2.66 (m, 4H), 1.99 – 1.90 (m, 4H). Molecular formula = $C_{24}H_{23}N_5O$, MW = 397.4. MS (ESI): m/z 412.3 $(M+H)^+$. HPLC purity: 99.3%.

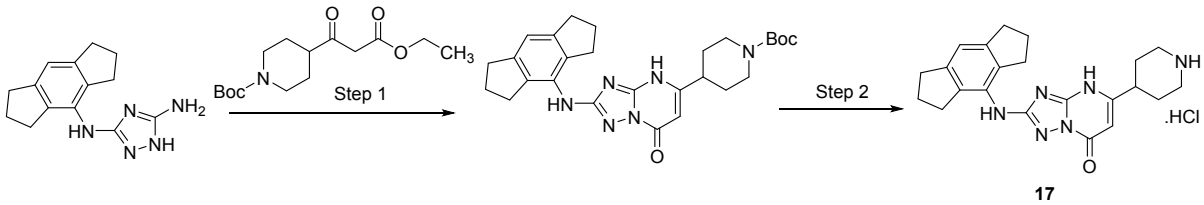
16:



16

Synthesis analogous to compound **6**, using methyl 3-(oxan-4-yl)-3-oxopropanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 – 12.52 (br. s, 1H), 8.42 (s, 1H), 6.90 (s, 1H), 5.69 (s, 1H), 3.99 – 3.90 (m, 2H), 3.40 – 3.34 (m, 2H), 2.85 – 2.78 (m, 4H), 2.77 - 2.65 (m, 5H), 1.99 – 1.90 (m, 4H), 1.78 – 1.62 (m, 4H). Molecular formula = $C_{22}H_{25}N_5O_2$, MW = 391.5. MS (ESI): m/z 392.3 $(M+H)^+$. HPLC purity: 94.1%.

17:



17

Step 1

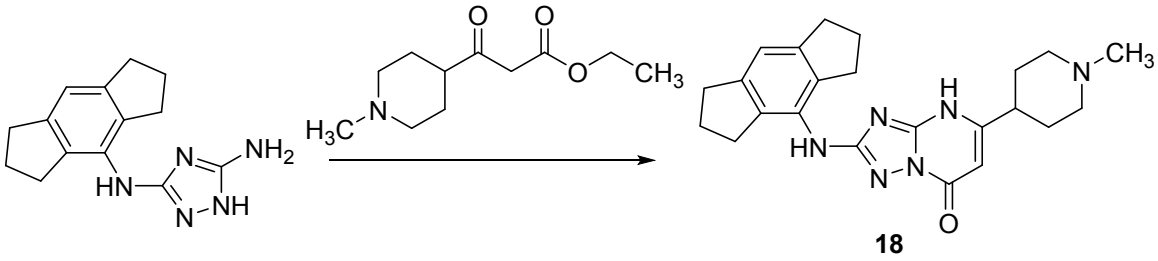
A mixture of_N^3-(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)-4H-1,2,4-triazole-3,5-diamine (100 mg, 0.392 mmol)(*for synthesis refer to compound* **1**), tert-butyl 4-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate (353 mg, 1.18 mmol) and sodium ethoxide (133 mg, 1.96 mmol) in EtOH (2 mL) was heated in a sealed tube under microwave irradiation at 120° C for 6 min. The RM was concentrated *in vacuo*. The residue was diluted with water and extracted with 10 % MeOH / DCM. The organic phase was brine, dried (Na_2SO_4) and evaporated under vacuum. The crude was purified by flash column chromatography to give tert-butyl 4-{2-[(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)amino]-7-oxo-4H,7H-[1,2,4]triazolo[1,5-a]pyrimidin-5-yl}piperidine-1-carboxylate as an off-white solid.

Step 2

Tert-butyl
4-{2-[(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)amino]-7-oxo-4H,7H-[1,2,4]triazolo[1,5-a]pyrimidin-5-yl}piperidine-1-carboxylate (20 mg, 0.041 mmol) was dissolved in DCM (5 ml) and cooled to 0° C. 4 M HCl in dioxane (2 ml) was added and the RM allowed to warm to RT and stirred for 4 h. The reaction was evaporated and the solid triturated with diethyl ether to give **17** as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 – 12.76 (br. s, 1H), 9.00 – 8.90 (br. s, 1H), 8.69 – 8.54 (br. s, 1H), 8.48 (s, 1H), 6.91 (s, 1H), 5.65 (s, 1H), 3.61 – 3.30 (m, 3H), 3.01 – 2.88 (m, 2H), 2.87 – 2.76 (m, 5H), 2.75 - 2.62 (m, 5H), 2.12 – 2.01 (m, 2H), 2.00 – 1.90 (m, 4H), 1.83 – 1.73 (m, 2H). Molecular formula = $C_{22}H_{26}N_6O$, MW = 390.5. MS (ESI): m/z 391.3 $(M+H)^+$. HPLC purity: 92.3%.

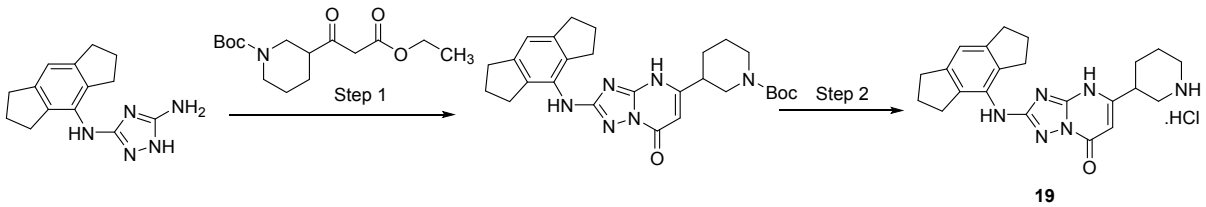
18:



18

Synthesis analogous to compound **6**, using ethyl 3-(1-methylpiperidin-4-yl)-3-oxopropanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 – 11.00 (br. s, 1H), 8.12 (s, 1H), 6.90 (s, 1H), 5.54 (s, 1H), 3.11 – 3.01 (m, 2H), 2.83 – 2.76 (m, 4H), 2.48 - 2.20 (m, 6H), 2.10 (s, 3H), 1.99 – 1.70 (m, 8H). Molecular formula = $C_{23}H_{28}N_6O$, MW = 404.5. MS (ESI): m/z 405.3 $(M+H)^+$. HPLC purity: 99.0%.

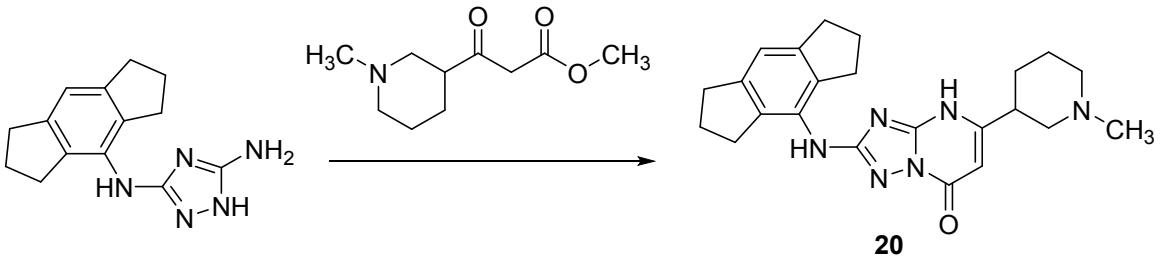
19:



19

Synthesis in two steps analogous to compound **17**, using tert-butyl 3-(3-ethoxy-3-oxopropanoyl)piperidine-1-carboxylate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 – 12.81 (br. s, 1H), 9.11 – 9.03 (s, 1H), 8.90 – 8.77 (br. s, 1H), 8.49 (s, 1H), 6.92 (s, 1H), 5.75 (s, 1H), 3.61 – 3.30 (m, 3H), 3.20 – 2.90 (m, 2H), 2.83 – 2.72 (m, 4H), 2.71 - 2.63 (m, 4H), 2.03 – 1.88 (m, 6H), 1.76 – 1.62 (m, 2H). Molecular formula = $C_{22}H_{26}N_6O$, MW = 390.5. MS (ESI): m/z 391.3 $(M+H)^+$. HPLC purity: 99.0%.

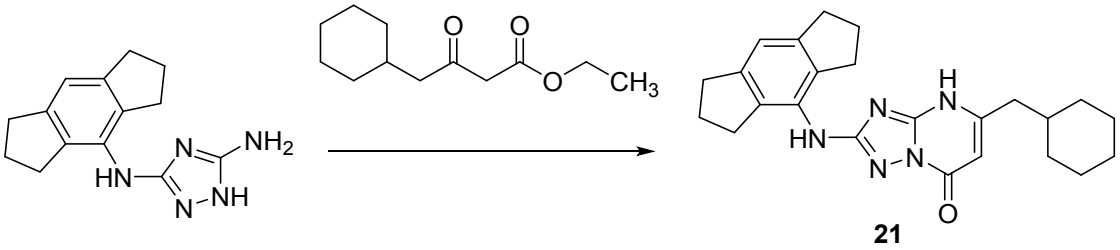
20:



20

Synthesis analogous to compound **6**, using methyl 3-(1-methylpiperidin-3-yl)-3-oxopropanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 – 10.70 (br. s, 1H), 8.07 (s, 1H), 6.89 (s, 1H), 5.54 (s, 1H), 3.15 – 3.03 (m, 2H), 2.83 – 2.65 (m, 4H), 2.48 - 2.38 (m, 3H), 1.99 – 1.70 (m, 6H), 1.68 – 1.42 (m, 2H). Molecular formula = $C_{23}H_{28}N_6O$, MW = 404.5. MS (ESI): m/z 405.3 $(M+H)^+$. HPLC purity: 99.2%.

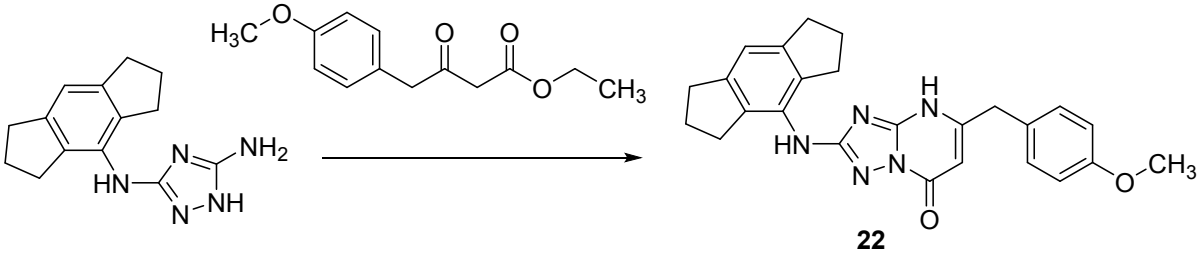
21:



21

Synthesis analogous to compound **6**, using ethyl 4-cyclohexyl-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 – 12.20 (br. s, 1H), 8.35 (s, 1H), 6.89 (s, 1H), 5.60 (s, 1H), 2.85 - 2.76 (m, 4H), 2.75 – 2.66 (m, 4H), 2.38 – 2.33 (m, 2H), 1.99 – 1.89 (m, 4H), 1.70 – 1.55 (m, 6H), 1.22 – 1.09 (m, 4H), 1.00 – 1.88 (m, 2H). Molecular formula = $C_{24}H_{29}N_5O$, MW = 403.5. MS (ESI): m/z 404.4 $(MH)^+$. HPLC purity: 99.0%.

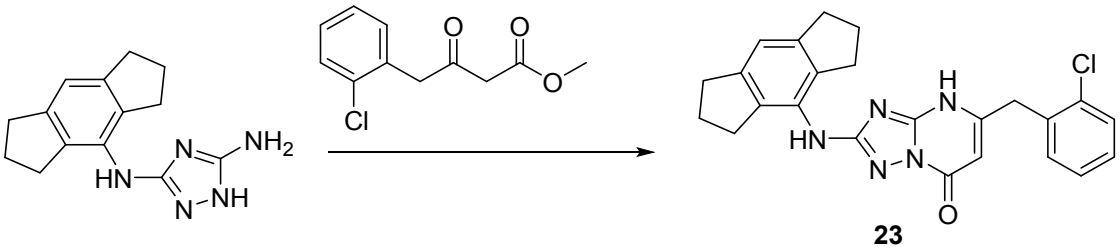
22:



22

Synthesis analogous to compound **6**, using methyl 3-oxo-4-phenylbutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 – 12.75 (br. s, 1H), 8.42 (s, 1H), 7.30 – 7.24 (m, 2H), 6.93 – 6.82 (m, 3H), 5.59 (s, 1H), 3.80 (s, 2H), 3.72 (s, 3H), 2.86 - 2.76 (m, 4H), 2.75 – 2.64 (m, 4H), 1.99 – 1.86 (m, 4H). Molecular formula = $C_{25}H_{25}N_5O_2$, MW = 427.5. MS (ESI): m/z 428.4 $(MH)^+$. HPLC purity: 92.7%.

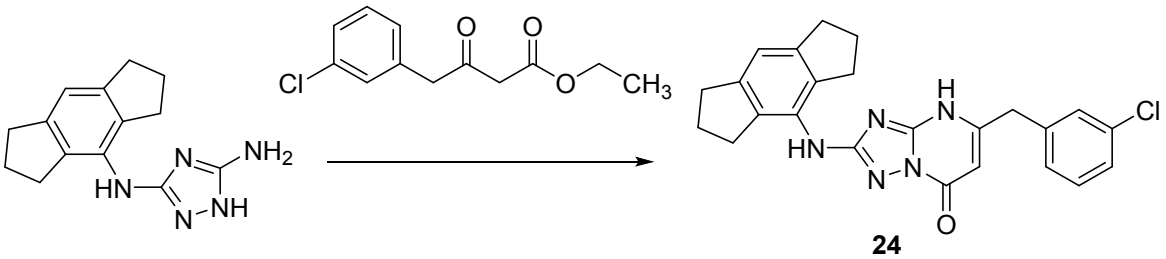
23:



23

Synthesis analogous to compound **6**, using methyl 4-(2-chlorophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.22 – 12.70 (br. s, 1H), 8.03 (s, 1H), 7.49 – 7.42 (m, 1H), 7.40 – 7.35 (m, 1H), 7.34 – 7.27 (m, 2H), 6.85 (s, 1H), 5.24 (s, 1H), 3.91 (s, 2H), 2.84 - 2.76 (m, 4H), 2.75 – 2.67 (m, 4H), 1.97 – 1.86 (m, 4H). Molecular formula = $C_{24}H_{22}ClN_5O$, MW = 431.9. MS (ESI): m/z 432.3 $(MH)^+$. HPLC purity: 97.4%.

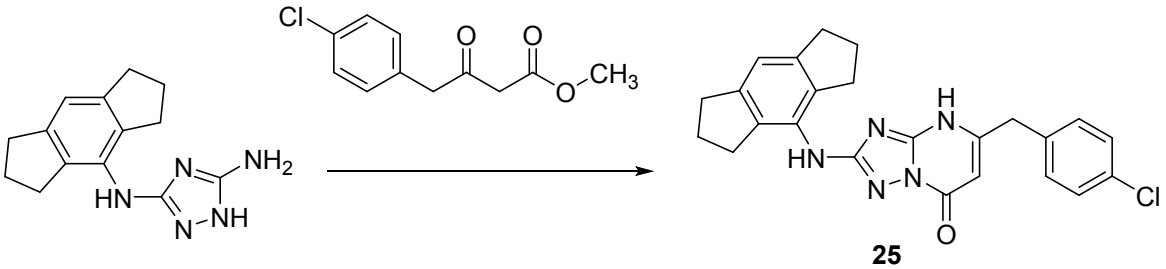
24:



24

Synthesis analogous to compound **6**, using ethyl 4-(3-chlorophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 – 12.70 (br. s, 1H), 8.29 (s, 1H), 7.45 (s, 1H), 7.40 – 7.28 (m, 3H), 6.89 (s, 1H), 5.69 (s, 1H), 3.85 (s, 2H), 2.82 - 2.76 (m, 4H), 2.75 – 2.64 (m, 4H), 1.97 – 1.88 (m, 4H). Molecular formula = $C_{24}H_{22}ClN_5O$, MW = 431.9. MS (ESI): m/z 432.3 $(MH)^+$. HPLC purity: 95.4%.

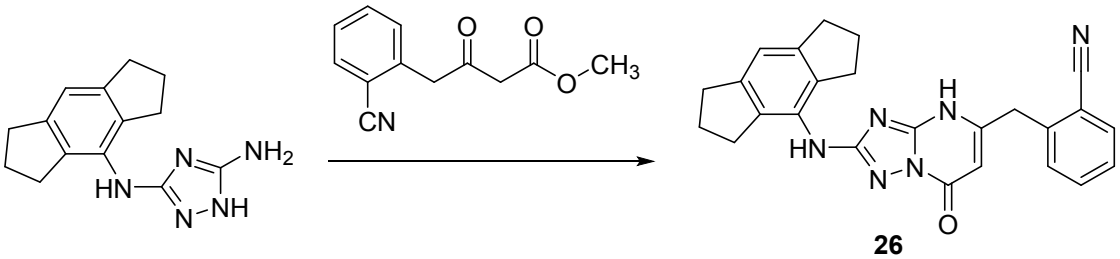
25:



25

Synthesis analogous to compound **6**, using methyl 4-(4-chlorophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 – 12.85 (br. s, 1H), 8.41 – 8.32 (br. s, 1H), 7.40 – 7.32 (m, 4H), 6.89 (s, 1H), 5.65 (s, 1H), 3.85 (s, 2H), 2.81 - 2.76 (m, 4H), 2.75 – 2.63 (m, 4H), 1.97 – 1.87 (m, 4H). Molecular formula = $C_{24}H_{22}ClN_5O$, MW = 431.9. MS (ESI): m/z 432.2 $(MH)^+$. HPLC purity: 95.7%.

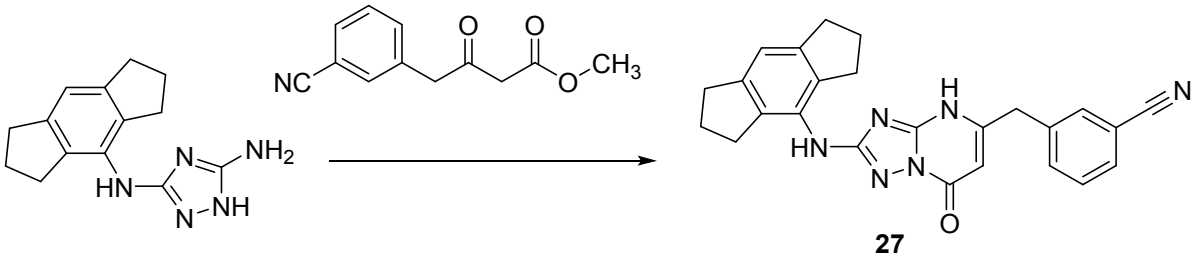
26:



26

Synthesis analogous to compound **6**, using methyl 4-(2-cyanophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 – 12.95 (br. s, 1H), 8.49 – 8.42 (br. s, 1H), 7.89 – 7.86 (m, 1H), 7.72 – 7.66 (m, 1H), 7.53 – 7.48 (m, 2H), 6.90 (s, 1H), 5.45 (s, 1H), 4.12 (s, 2H), 2.87 - 2.76 (m, 4H), 2.75 – 2.67 (m, 4H), 1.97 – 1.89 (m, 4H). Molecular formula = $C_{25}H_{22}N_6O$, MW = 422.5. MS (ESI): m/z 423.3 $(MH)^+$. HPLC purity: 95.0%.

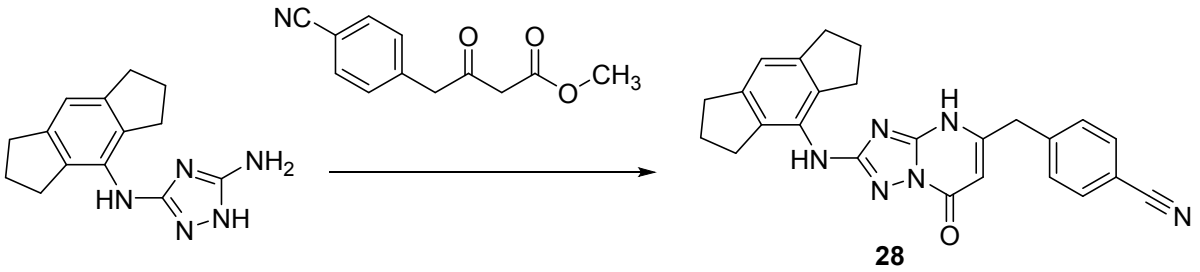
27:



27

Synthesis analogous to compound **6**, using methyl 4-(3-cyanophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 – 12.75 (br. s, 1H), 8.30 – 8.20 (br. s, 1H), 7.83 – 7.80 (m, 1H), 7.75 – 7.64 (m, 1H), 7.57 – 7.50 (m, 2H), 6.89 (s, 1H), 5.64 (s, 1H), 3.89 (s, 2H), 2.84 - 2.76 (m, 4H), 2.75 – 2.64 (m, 4H), 1.99 – 1.89 (m, 4H). Molecular formula = $C_{25}H_{22}N_6O$, MW = 422.5. MS (ESI): m/z 423.3 $(MH)^+$. HPLC purity: 99.0%.

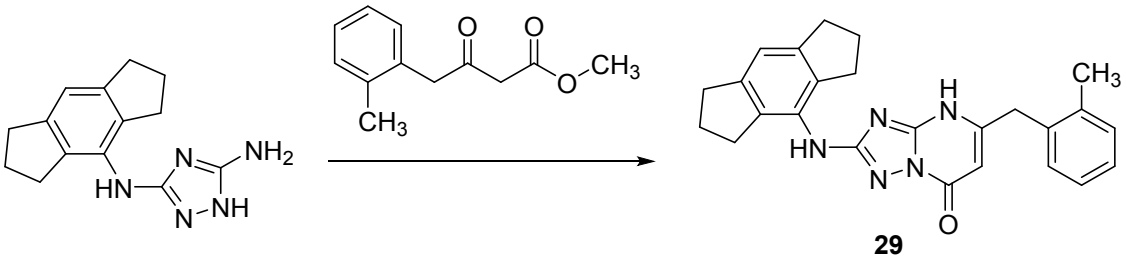
28:



28

Synthesis analogous to compound **6**, using methyl 4-(4-cyanophenyl)-3-oxobutanoate. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.21 (s, 1H), 7.80 (d, 2H), 7.52 (d, 2H), 6.88 (s, 1H), 5.67 (s, 1H), 3.98 (s, 2H), 2.84 - 2.76 (m, 4H), 2.75 – 2.64 (m, 4H), 1.97 – 1.88 (m, 4H). Molecular formula = $C_{25}H_{22}N_6O$, MW = 422.5. MS (ESI): m/z 423.3 $(MH)^+$. HPLC purity: 96.1%.

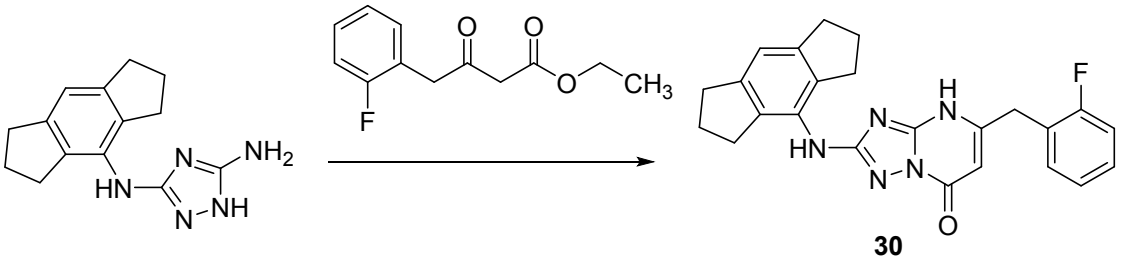
29:



29

Synthesis analogous to compound **6**, using methyl 4-(2-benzyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.03 – 12.87 (br. s, 1H), 8.38 – 8.32 (br. s, 1H), 7.24 – 7.15 (m, 4H), 6.88 (s, 1H), 5.23 (s, 1H), 3.85 (s, 2H), 2.86 - 2.75 (m, 4H), 2.74 – 2.65 (m, 4H), 1.98 – 1.88 (m, 4H). Molecular formula = $C_{25}H_{25}N_5O$, MW = 411.5. MS (ESI): m/z 412.3 $(MH)^+$. HPLC purity: 96.5%.

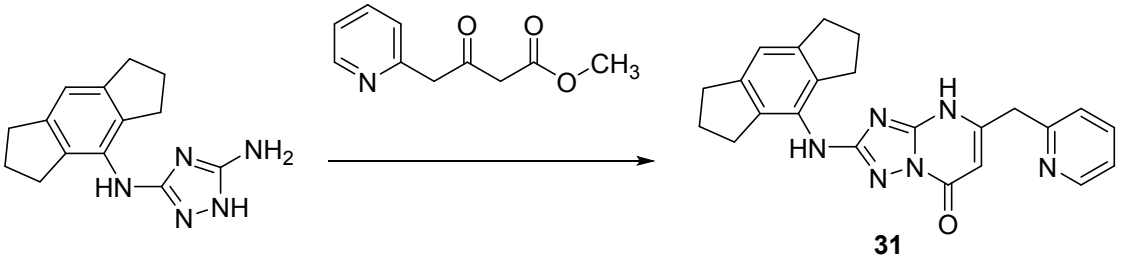
30:



30

Synthesis analogous to compound **6**, using ethyl 4-(2-fluorophenyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 – 12.90 (br. s, 1H), 8.49 – 8.40 (br. s, 1H), 7.40 – 7.32 (m, 2H), 7.27 – 7.16 (m, 2H), 6.90 (s, 1H), 5.45 (s, 1H), 3.93 (s, 2H), 2.86 - 2.76 (m, 4H), 2.75 – 2.66 (m, 4H), 2.00 – 1.88 (m, 4H). Molecular formula = $C_{24}H_{22}FN_5O$, MW = 415.5. MS (ESI): m/z 416.3 $(MH)^+$. HPLC purity: 96.7%.

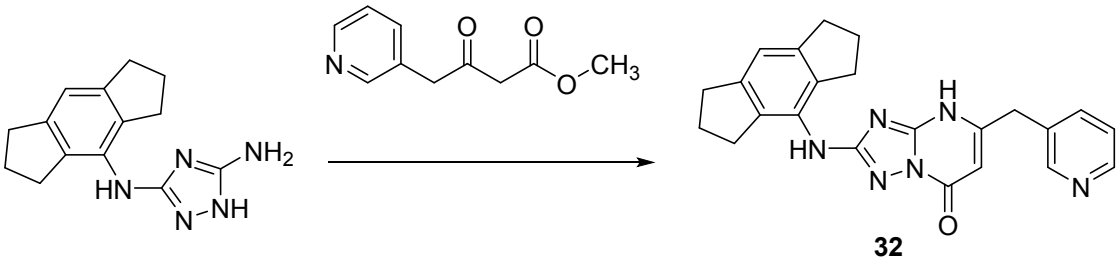
31 (NDT-30347):



31

Synthesis analogous to compound **6**, using methyl 4-(2-pyridyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (d, J = 4 Hz, 1H), 8.47 (s, 1H), 8.05 (t, J = 8 Hz, 1H), 7.63 (d, J = 8 Hz, 1H), 7.54 (t, J = 8 Hz, 1H), 6.91 (s, 1H), 5.72 (s, 1H), 4.19 (s, 2H), 2.81 (t, J = 7 Hz, 4H), 2.71 (t, J = 7 Hz, 4H), 1.99 - 1.91 (m, 4H). One exchangeable not seen. Molecular formula = $C_{23}H_{22}N_6O$, MW = 398.5. MS (ESI): m/z 399.0 $(MH)^+$. HPLC purity: 95.7%.

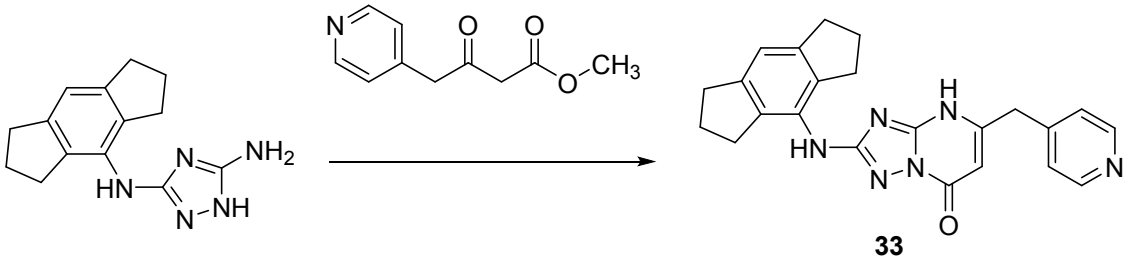
32:



32

Synthesis analogous to compound **6**, using methyl 4-(3-pyridyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 – 8.50 (br. s, 1H), 8.46 – 8.38 (br. s, 1H), 8.00 – 7.88 (br. s, 1H), 7.73 – 7.65 (m, 1H), 7.35 – 7.26 (m, 1H), 6.84 (s, 1H), 5.50 (s, 1H), 3.76 (s, 2H), 2.85 – 2.76 (m, 4H), 2.75 – 2.62 (m, 4H), 1.99 - 1.86 (m, 4H). One exchangeable not seen. Molecular formula = $C_{23}H_{22}N_6O$, MW = 398.5. MS (ESI): m/z 399.3 $(MH)^+$. HPLC purity: 97.0%.

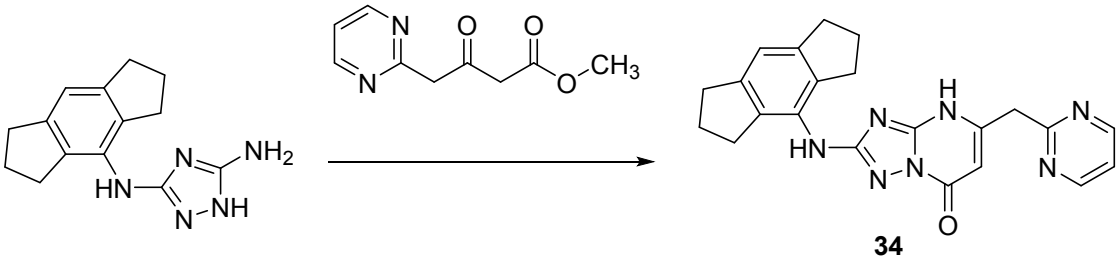
33:



33

Synthesis analogous to compound **6**, using methyl 4-(4-pyridyl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, 2H), 7.51 (s, 1H), 7.28 (d, 2H), 6.81 (s, 1H), 5.34 (s, 1H), 3.66 (s, 2H), 2.82 – 2.75 (m, 4H), 2.74 – 2.65 (m, 4H), 1.96 - 1.86 (m, 4H). One exchangeable not seen. Molecular formula = $C_{23}H_{22}N_6O$, MW = 398.5. MS (ESI): m/z 399.4 $(MH)^+$. HPLC purity: 96.3%.

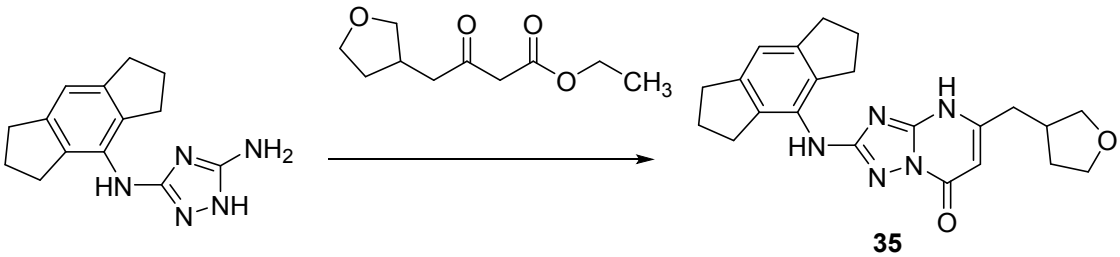
34:



34

Synthesis analogous to compound **6**, using methyl 3-oxo-4-pyrimidin-2-yl-butanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 5 Hz, 2H), 8.46 - 8.44 (br. s, 1H), 7.43 (t, J = 5 Hz, 1H), 6.91 (s, 1H), 5.76 (s, 1H), 4.25 (s, 2H), 2.82 (t, J = 7 Hz, 4H), 2.71 (t, J = 7 Hz, 4H), 1.99 - 1.92 (m, 4H). Molecular formula = $C_{22}H_{21}N_7O$, MW = 399.5. MS (ESI): m/z 400.1 $(MH)^+$. HPLC purity: 94.6%.

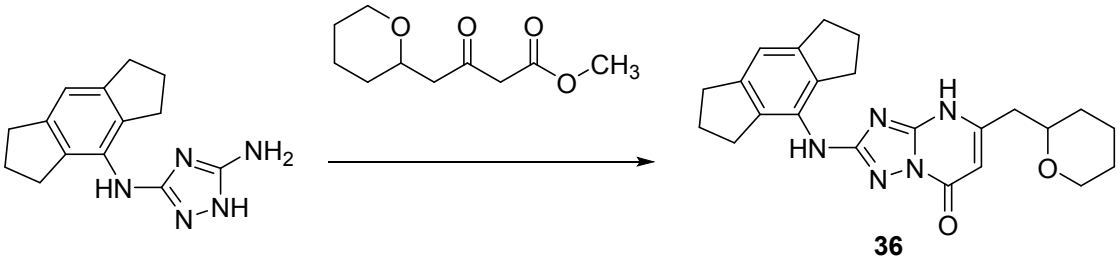
35:



35

Synthesis analogous to compound **6**, using ethyl 3-oxo-4-(oxolan-3-yl)butanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 – 12.65 (br. s, 1H), 8.41 (s, 1H), 6.90 (s, 1H), 5.71 (s, 1H), 3.79 – 3.72 (m, 2H), 3.68 – 3.59 (m, 1H), 3.38 – 3.36 (m, 1H), 2.86 – 2.77 (m, 4H), 2.76 – 2.64 (m, 4H), 2.60 – 2.54 (m, 2H), 2.01 - 1.90 (m, 5H), 1.60 – 1.50 (m, 1H). Molecular formula = $C_{22}H_{25}N_5O_2$, MW = 391.5. MS (ESI): m/z 392.3 $(MH)^+$. HPLC purity: 94.2%.

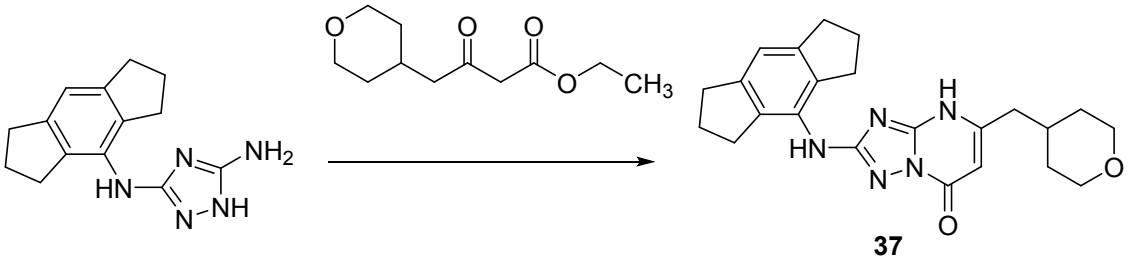
36 (NDT-30408):



36

Synthesis analogous to compound **6**, using methyl 4-(oxan-2-yl)-3-oxobutanoate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (s, 1H), 6.75 (br. s, 1H), 5.81 (s, 1H), 4.10 - 4.00 (m, 1H), 3.69 - 3.67 (m, 1H), 3.44 - 3.41 (m, 1H), 2.89 (t, *J* = 7 Hz, 4H), 2.82 (t, *J* = 7 Hz, 4H), 2.72 (d, *J* = 6 Hz, 2H), 2.10 - 2.03 (m, 4H), 1.92 - 1.83 (m, 1H), 1.68 - 1.61 (m, 1H), 1.55 - 1.53 (m, 3H), 1.43 - 1.32 (m, 1H). Molecular formula = $C_{23}H_{27}N_5O_2$, MW = 405.5. MS (ESI): m/z 406.2 $(MH)^+$. HPLC purity: 99.6%.

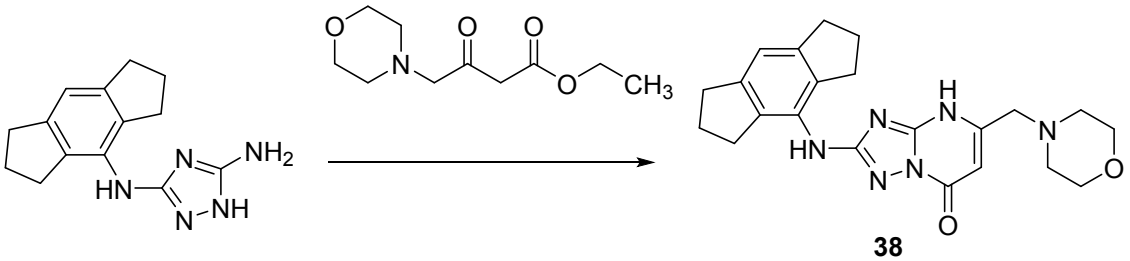
37:



37

Synthesis analogous to compound **6**, using ethyl 4-(oxan-4-yl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 8.41 (s, 1H), 6.92 (s, 1H), 5.68 (s, 1H), 3.84 (dd, *J* = 3, 12 Hz, 2H), 3.30 - 3.24 (m, 2H), 2.83 (t, *J* = 7 Hz, 4H), 2.73 (t, *J* = 7 Hz, 4H), 2.45 (d, *J* = 7 Hz, 2H), 2.02 - 1.87 (m, 5H), 1.54 (d, *J* = 13 Hz, 2H), 1.30 - 1.18 (m, 2H). Molecular formula = $C_{23}H_{27}N_5O_2$, MW = 405.5. MS (ESI): m/z 406.2 $(MH)^+$. HPLC purity: 99.8%.

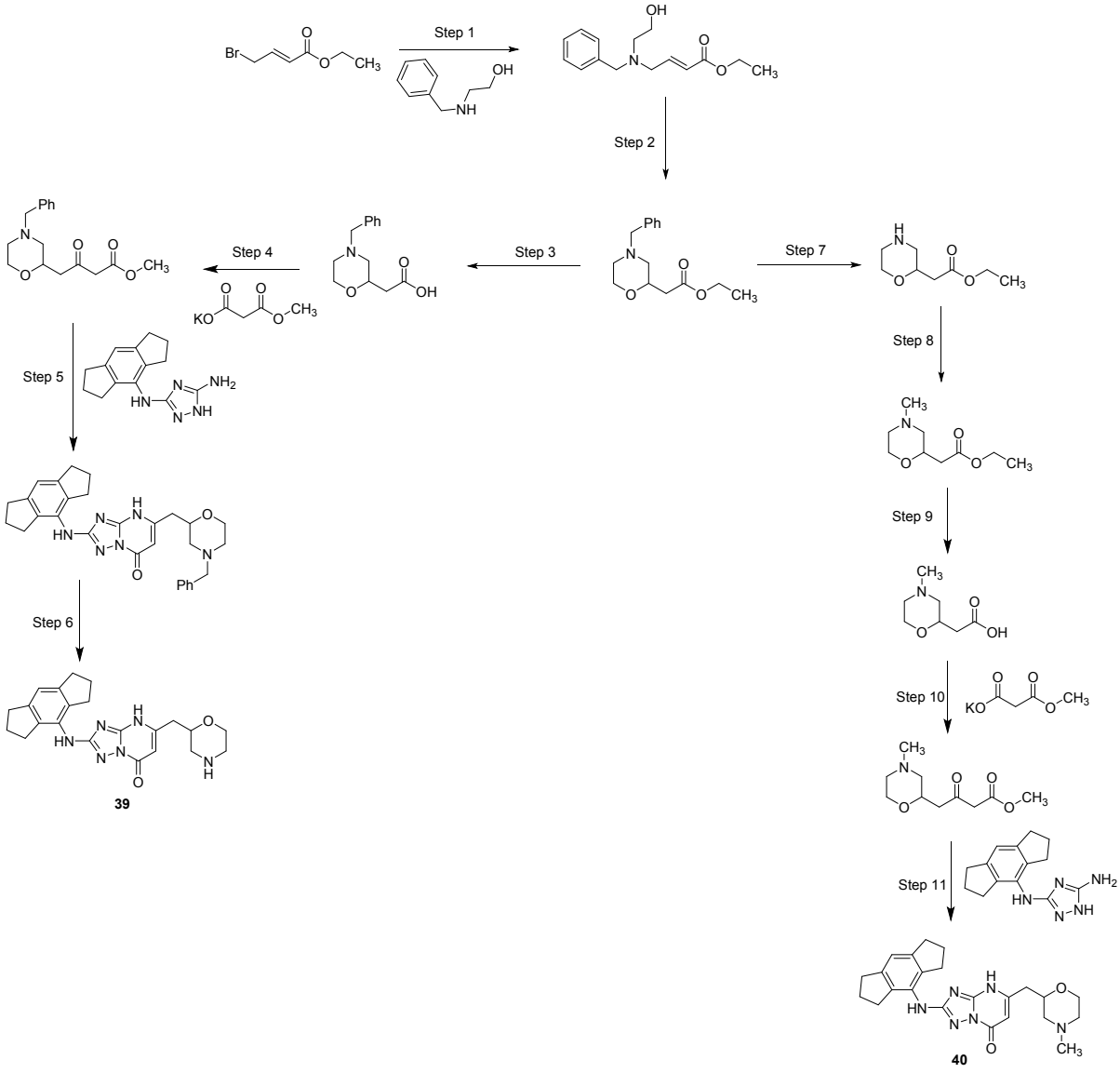
38:



38

Synthesis analogous to compound **6**, using ethyl 4-(morpholin-4-yl)-3-oxobutanoate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 6.91 (s, 1H), 5.81 (s, 1H), 3.75 - 3.60 (m, 4H), 3.50 (s, 2H), 2.84 - 2.79 (m, 4H), 2.71 - 2.67 (m, 4H), 2.50 - 2.45 (m, 4H), 1.97 - 1.93 (m, 4H). ^{1}H NMR (400 MHz, MeOH-d_4) δ 6.93 (s, 1H), 5.95 (s, 1H), 3.85 - 3.70 (m, 4H), 3.60 (s, 2H), 2.88 - 2.84 (m, 4H), 2.82 - 2.77 (m, 4H), 2.68 - 2.66 (m, 4H), 2.07 - 1.99 (m, 4H). Molecular formula = $C_{22}H_{26}N_6O_2$, MW = 406.5. MS (ESI): m/z 407.2 $(MH)^+$. HPLC purity: 97.4%.

39 and 40:



Step 1

To a solution of 2-(benzylamino)ethanol (5.0 g, 33.1 mmol) in H_2O (25 mL) was added TEA (4.60 mL, 33.1 mmol). The mixture was stirred at 100° C for 30 min. To the mixture was added ethyl (E)-4-bromobut-2-enoate (4.79 mL, 34.7 mmol). The reaction mixture was stirred at 100° C for 10 h. The resulting mixture was diluted with H_2O (40 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic phase was washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum to give ethyl (E)-4-[benzyl(2-hydroxyethyl)amino]but-2-enoate as a colourless oil. Y = 69 %. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.38 - 7.20 (m, 5H), 7.05 - 6.95 (m, 1H), 6.10 - 6.00 (m, 1H), 4.16 (t, *J* = 7 Hz, 2H), 3.69 - 3.59 (m, 4H), 3.31 - 3.29 (m, 2H), 2.63 (t, *J* = 6 Hz, 2H), 1.28 (t, *J* = 7 Hz, 3H).

Step 2

To a solution of ethyl (E)-4-[benzyl(2-hydroxyethyl)amino]but-2-enoate (5.0 g, 19.0 mmol) in toluene (50 mL) was added DBU (2.86 mL, 19.0 mmol). The mixture was stirred at 100° C for 2 h. The mixture was concentrated under reduce pressure. The residue was diluted with H_2O (20 mL) and the resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give ethyl 2-(4-benzylmorpholin-2-yl)acetate as a colourless oil. Y = 80 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 - 7.22 (m, 5H), 4.17 - 4.10 (t, *J* = 7 Hz, 2H), 4.03 - 3.92 (m, 1H), 3.88 - 3.79 (m, 1H), 3.75 - 3.65 (m, 1H), 3.49 (s, 2H), 2.78 - 2.75 (m, 1H), 2.65 - 2.62 (m, 1H), 2.54 - 2.46 (m, 1H), 2.39 - 2.31 (m, 1H), 2.25 - 2.10 (m, 1H), 1.97 - 1.87 (m, 1H), 1.23 (t, *J* = 7 Hz, 3H).

Step 3

A mixture of ethyl 2-(4-benzylmorpholin-2-yl)acetate (1.0 g, 3.80 mmol) in 6 M HCl (5 mL) was stirred at 25° C for 1 h. The mixture was concentrated under reduce pressure to give 2-(4-benzylmorpholin-2-yl)acetic acid hydrochloride as a white solid.

Step 4

To a solution of (3-methoxy-3-oxo-propanoyl)oxypotassium (996 mg, 6.38 mmol) in THF (10 mL) was added TEA (1.48 mL, 10.6 mmol) and $MgCl_2$ (243 mg, 2.55 mmol) at 25 °C. The reaction mixture was stirred at 25 °C for 2.5 h. A separate solution of 2-(4-benzylmorpholin-2-yl)acetic acid hydrochloride (500 mg, 2.13 mmol) in THF (10 mL) was treated with CDI (414 mg, 2.55 mmol) and stirred at 25 °C for 2.5 h. This was then treated with the ester/$MgCl_2$/TEA solution, and the resulting mixture was stirred at 50° C for 12 h. The mixture was concentrated under reduce pressure to give a residue. The residue was diluted with H_2O (10 mL), the resulting mixture was extracted with EtOAc (3 x 5 mL), the organic phase was dried with Na_2SO_4, filtrated and the filtrate concentrated to give methyl 4-(4-benzylmorpholin-2-yl)-3-oxo-butanoate as a yellow oil.

Step 5

Synthesis analogous to compound **6**, using methyl 4-(4-benzylmorpholin-2-yl)-3-oxo-butanoate. Product isolated as HCl salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (br. s, 1H), 10.53 (br. s, 1H), 8.47 (s, 1H), 7.65 - 7.38 (m, 5H), 6.91 (s, 1H), 5.74 (s, 1H), 4.34 (s, 2H), 4.12 - 3.93 (m, 2H), 3.83 - 3.64 (m, 1H), 3.26 - 3.16 (m, 2H), 3.10 - 2.97 (m, 1H), 2.94 - 2.86 (m, 1H), 2.82 (t, *J* = 7 Hz, 4H), 2.78 - 2.74 (m, 1H), 2.75 - 2.65 (m, 5H), 1.99 - 1.92 (m, 4H). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.51 (s, 5H), 6.94 (s, 1H), 5.85 (s, 1H), 4.36 (s, 2H), 4.17 - 4.09 (m, 1H), 4.05 - 3.96 (m, 1H), 3.85 - 3.70 (m, 1H), 3.53 - 3.45 (m, 1H), 3.39 - 3.35 (m, 1H), 3.21 - 3.09 (m, 1H), 3.09 - 3.00 (m, 1H), 2.06 - 1.99 (m, 10H), 2.03 (m, 4H).

Step 6

To a solution of 5-[(4-benzylmorpholin-2-yl)methyl]-2-(1,2,3,5,6,7-hexahydro-s-indacen-4-ylamino)-3H-[1,2,4]triazolo[1,5-a]pyrimidin-7-one hydrochloride (200 mg, 0.40 mmol) in EtOH (5 mL) was added 10 % Pd/C (200 mg, 50% in water) and ammonium formate (127 mg, 2.01

mmol) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (50 psi) at 25° C for 2 h. The reaction mixture was filtered through a pad of Celite, then the filtrate concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150x40 mm x 10 µm; mobile phase: [water (10mM NH_4HCO_3) - ACN]; B: 20 – 40 %, 8 min) and lyophilized to give **39** as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 6.90 (s, 1H), 5.75 (s, 1H), 4.06 - 3.93 (m, 2H), 3.75 - 3.65 (m, 1H), 3.23 - 3.20 (m, 1H), 3.11 - 2.95 (m, 2H), 2.90 - 2.74 (m, 10H), 2.69 - 2.59 (m, 1H), 2.07 - 1.99 (m, 4H). Molecular formula = $C_{22}H_{26}N_6O_2$, MW = 406.5. MS (ESI): m/z 407.1 $(MH)^+$. HPLC purity: 99.4%.

Step 7

To a solution of ethyl 2-(4-benzylmorpholin-2-yl)acetate (1.0 g, 3.80 mmol) in EtOH (20 mL) was added 10 % Pd/C (1.0 g, 50 % in water) and ammonium formate (1.20 g, 19.0 mmol). The resulting suspension was degassed and purged with H_2 several times. The reaction mixture was stirred at 25° C for 2 h under H_2 (15 psi). The resulting mixture was filtered through a pad of Celite and the filtrate concentrated under reduced pressure to give ethyl 2-morpholin-2-ylacetate as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (t, *J* = 7 Hz, 2H), 3.94 - 3.80 (m, 2H), 3.65 - 3.55 (m, 1H), 3.00 - 2.74 (m, 3H), 2.60 - 2.50 (m, 1H), 2.54 - 2.44 (m, 1H), 2.40 - 2.32 (m, 1H), 1.26 (t, *J* = 7 Hz, 3H).

Step 8

To a solution of ethyl 2-morpholin-2-ylacetate (800 mg, 4.62 mmol) in MeOH (10 mL) was added $NaBH_3CN$ (435 mg, 6.93 mmol) and 37 % formaldehyde (aq.) (1.72 mL, 23.1 mmol). The mixture was stirred at 25° C for 2 h, then concentrated under reduced pressure. The residue was diluted with H_2O (20 mL), and the resulting mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give ethyl 2-(4-methylmorpholin-2-yl)acetate as a white solid.

Step 9

To a solution of ethyl 2-(4-methylmorpholin-2-yl)acetate (0.5 g, 2.67 mmol) in MeOH (5 mL) and H_2O (5 mL) was added NaOH (107 mg, 2.67 mmol). The mixture was stirred at 25° C for 0.5 h. The mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 250x50 mm x 10 µm; mobile phase: [water (0.05 % HCl) - ACN]; B: 1 – 10 %, 10 min) and lyophilized to give 2-(4-methylmorpholin -2-yl)acetic acid hydrochloride as a white solid.

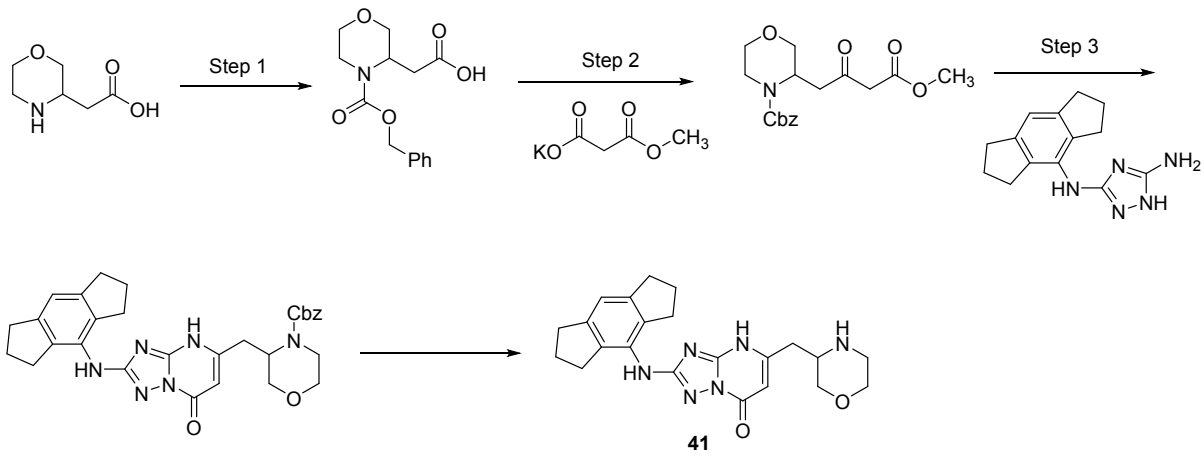
Step 10

Synthesis analogous to Step 4, using 2-(4-methylmorpholin-2-yl)acetic acid hydrochloride.

Step 11

Synthesis analogous to compound **6**, using methyl 4-(4-methylmorpholin-2-yl)-3-oxo-butanoate to give **40** as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 6.92 (s, 1H), 5.81 (s, 1H), 3.97 - 3.81 (m, 2H), 3.70 - 3.57 (m, 1H), 3.03 - 2.94 (m, 1H), 2.90 - 2.70 (m, 11H), , 2.43 (s, 3H), 2.41 - 2.33 (m, 1H), 2.23 - 2.10 (m, 1H), 2.07 - 2.00 (m, 4H). Molecular formula = $C_{23}H_{28}N_6O_2$, MW = 420.5. MS (ESI): m/z 421.2 $(MH)^+$. HPLC purity: 99.4%.

41:



Step 1

To a solution of 2-morpholin-3-ylacetic acid hydrochloride (500 mg, 2.75 mmol) in THF (3 mL) and H_2O (1 mL) was added Na_2CO_3 (875 mg, 8.26 mmol). The mixture was cooled to 0° C and treated with a solution of benzyl carbonochloridate (783 µL,5.51 mmol) in THF (1 mL). The mixture was stirred at 25° C for 12 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 x 2 mL). The combined organic layers were washed with brine (2 x 2 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, 50 % EtOAc in petroleum ether, R_f = 0.45) to give 2-(4-benzyloxycarbonylmorpholin-3-yl)acetic acid as a yellow oil. Y = 13 %.

Step 2

To a solution of (3-methoxy-3-oxo-propanoyl)oxypotassium (122 mg, 781 µmol) in ACN (3 mL) was added TEA (181 µL, 1.3 mmol) and $MgCl_2$ (149 mg, 1.56 mmol) at 25 °C. The reaction mixture was stirred at 25° C for 2.5 h. Separately, a solution of 2-(4-benzyloxycarbonylmorpholin-3-yl)acetic acid (218 mg, 781 µmol) in ACN (3 mL) was treated with CDI (152 mg, 937 µmol) at 0 °C. This was stirred at 25° C for 2.5 h, then treated with the ester/$MgCl_2$/TEA mixture. The resulting reaction was stirred at 25° C for 12 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give benzyl 3-(4-methoxy-2,4-dioxo-butyl)morpholine-4-carboxylate as a yellow solid. Y = 38 %.

Step 3

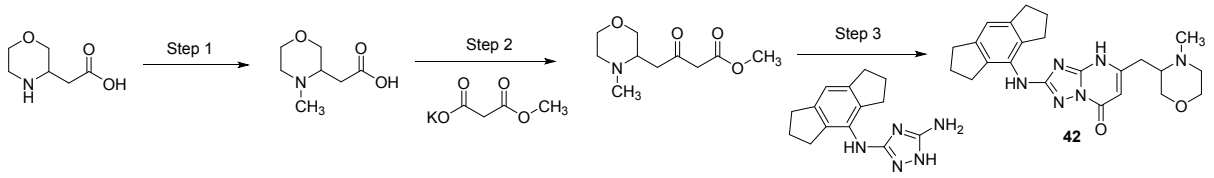
Synthesis analogous to compound **6**, using benzyl 3-(4-methoxy-2,4-dioxo-butyl)morpholine-4-carboxylate to give benzyl 3-[[2-(1,2,3,5,6,7-hexahydro-s-indacen-4-ylamino)-7-oxo-3H-[1,2,4]triazolo[1,5-a]pyrimidin-5-yl]methyl]morpholine-4-carboxylate as a yellow solid.

Step 4

To a solution of benzyl 3-[[2-(1,2,3,5,6,7-hexahydro-s-indacen-4-ylamino)-7-oxo-3H-[1,2,4]triazolo[1,5-a]pyrimidin-5-yl]methyl]morpholine-4-carboxylate (20 mg, 37.0 µmol) in MeOH (4 mL) was added 10 % Pd/C (20 mg, 50% in water) and 12 M HCl (120 µL). The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (15 psi) at 25° C for 0.5 h. The reaction mixture was filtered through a pad of Celite and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Luna Omega 5 µm Polar C18 100A; mobile phase: [water (0.04 % HCl) - ACN]; B: 22 – 38 %, 7 min) and lyophilized to give **41** as a

white solid, isolated as the hydrochloride salt. Y = 20 %. ^{1}H NMR (400 MHz, MeOH-d_4) δ 6.91 (s, 1H), 5.64 (s, 1H), 4.00 - 3.86 (m, 2H), 3.70 - 3.56 (m, 2H), 3.50 - 3.44 (m, 1H), 3.28 - 3.20 (m, 1H), 3.12 - 3.04 (m, 1H), 2.95 - 2.85 (m, 4H), 2.84 - 2.78 (m, 4H), 2.77 - 2.67 (m, 1H), 2.66 - 2.59 (m, 1H), 2.10 - 2.00 (m, 4H). Molecular formula = $C_{22}H_{26}N_6O_2$, MW = 406.5. MS (ESI): m/z 407.2 $(MH)^+$. HPLC purity: 99.5%.

42:



Step 1

To a solution of 2-(morpholin-3-yl)acetic acid hydrochloride (200 mg, 1.10 mmol) in EtOH (10 mL) at 25 °C under N_2 was added 37 % formaldehyde (164 µL, 2.20 mmol) and 20 % $Pd(OH)_2$ (40 mg, 50% in water). The suspension was degassed and purged with H_2 3 times. The mixture was stirred under H_2 (15 Psi) at 25 °C for 12 h. The reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure to give 2-(4-methylmorpholin-3-yl)acetic acid as a yellow solid.

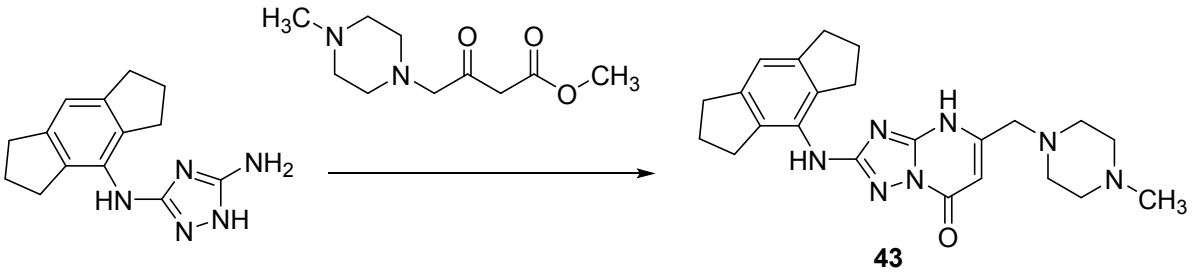
Step 2

To a solution of (3-methoxy-3-oxo-propanoyl)oxypotassium (147 mg, 942 µmol, 3 eq) in THF (3 mL) was added Et_3N (227 µL, 1.63 mmol) and $MgCl_2$ (89.7 mg, 942 µmol) at 25 °C. The reaction mixture was stirred at 25 °C for 2.5 h. Separately, a solution of 2-(4-methylmorpholin-3-yl)acetic acid (50 mg, 314 µmol) in THF (1 mL) was treated with CDI (61.1 mg, 377 µmol) at 25 °C. The reaction mixture was stirred at 25 °C for 2.5 h. This was then treated with the ester/$MgCl_2$/Et_3N reaction mixture and the resulting mixture stirred at 25 °C for 12 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give methyl 4-(4-methyl morpholin-3-yl)-3-oxobutanoate as a white solid.

Step 3

Synthesis analogous to compound **6**, using methyl 4-(4-methyl morpholin-3-yl)-3-oxobutanoate to give **42** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (br. s, 1H), 6.89 (s, 1H), 5.68 (s, 1H), 3.67 - 3.61 (m, 1H), 3.58 - 3.54 (m, 1H), 3.51 - 3.49 (m, 1H), 3.27 - 3.18 (m, 1H), 2.90 - 2.85 (m, 1H), 2.81 (t, J = 7 Hz, 4H), 2.71 (t, J = 7 Hz, 4H), 2.67 - 2.64 (m, 1H), 2.56 - 2.53 (m, 1H), 2.45 - 2.36 (m, 1H), 2.29 (s, 3H), 2.26 - 2.20 (m, 1H), 1.95 (m, 4H). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ 7.94 (s, 1H), 7.81 (s, 1H), 6.91 (s, 1H), 5.63 (s, 1H), 3.67 - 3.62 (m, 1H), 3.60 - 3.56 (m, 1H), 3.55 - 3.49 (m, 1H), 3.26 - 3.20 (m, 1H), 2.85 - 2.81 (m, 5H), 2.73 (t, J = 7 Hz, 4H), 2.66 - 2.60 (m, 1H), 2.56 - 2.51 (m, 1H), 2.42 - 2.39 (m, 1H), 2.33 - 2.21 (m, 4H), 2.00 - 1.94 (m, 4H). Molecular formula = $C_{23}H_{28}N_6O_2$, MW = 420.5. MS (ESI): m/z 421.2 $(MH)^+$. HPLC purity: 94.6%.

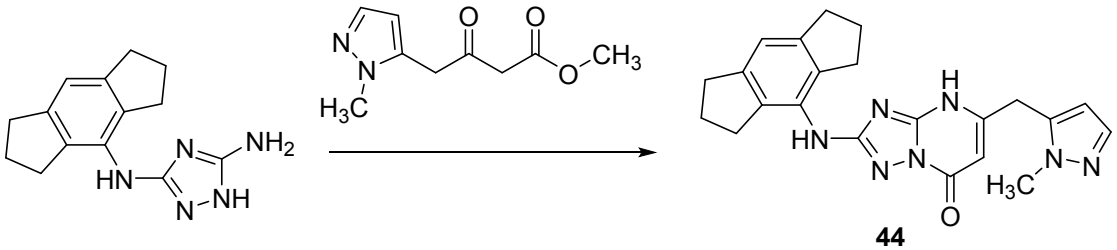
43:



43

Synthesis analogous to compound **6**, using methyl 4-(4-methylpiperazin-1-yl)-3-oxo-butanoate to give **43** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (br. s, 1H), 8.54 (s, 1H), 6.92 (s, 1H), 6.00 (s, 1H), 3.80 (s, 2H), 3.47 - 3.44 (m, 2H), 3.22 - 3.17 (m, 4H), 2.89 - 2.87 (m, 2H), 2.85 - 2.82 (m, 4H), 2.80 (s, 3H), 2.75 - 2.69 (m, 4H), 1.97 - 1.93 (m, 4H). Molecular formula = $C_{23}H_{29}N_7O$, MW = 419.5. MS (ESI): m/z 420.3 $(MH)^+$. HPLC purity: 96.6%.

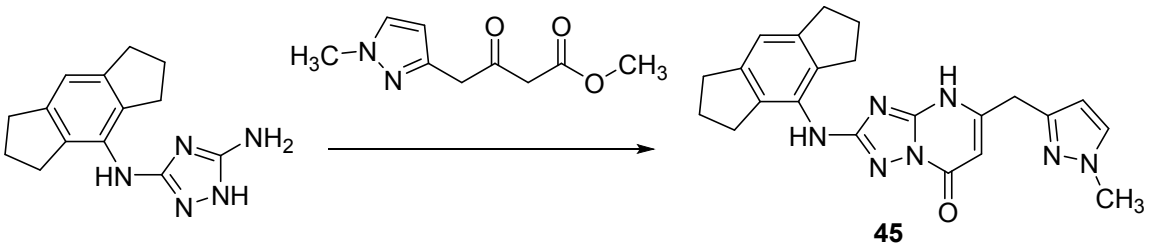
44:



44

Synthesis analogous to compound **6**, using methyl 4-(2-methylpyrazol-3-yl)-3-oxo-butanoate to give **44** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.33 (d, J = 2, 1H), 6.88 (s, 1H), 6.13 (s, 1H), 5.42 (s, 1H), 3.92 (s, 2H), 3.75 (s, 3H), 2.81 (t, J = 7 Hz, 4H), 2.71 (t, J = 7 Hz, 4H), 1.98 - 1.91 (m, 4H). Molecular formula = $C_{22}H_{23}N_7O$, MW = 401.5. MS (ESI): m/z 402.1 $(MH)^+$. HPLC purity: 100%.

45:

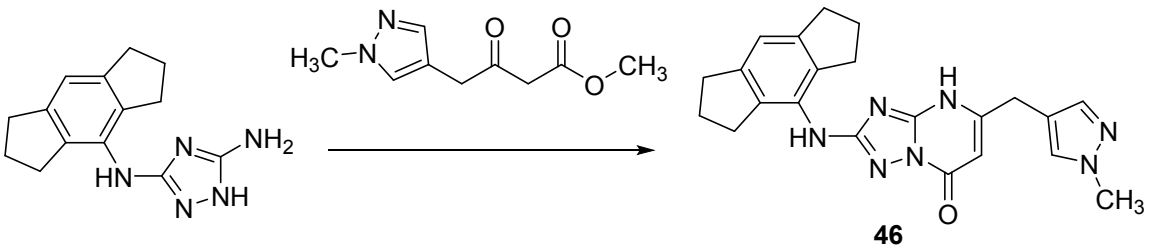


45

Synthesis analogous to compound **6**, using methyl 4-(1-methylpyrazol-3-yl)-3-oxo-butanoate to give **45** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.73 (s, 1H), 7.53 (d, J = 2 Hz, 1H), 6.83 (s, 1H), 6.03 (d, J = 2 Hz, 1H), 5.31 (s, 1H), 3.77 (s, 3H), 3.62 (s, 2H),

2.80 (t, *J* = 7 Hz, 4H), 2.70 (t, *J* = 7 Hz, 4H), 1.96 - 1.82 (m, 4H). Molecular formula = $C_{22}H_{23}N_7O$, MW = 401.5. MS (ESI): m/z 402.1 $(MH)^+$. HPLC purity: 100%.

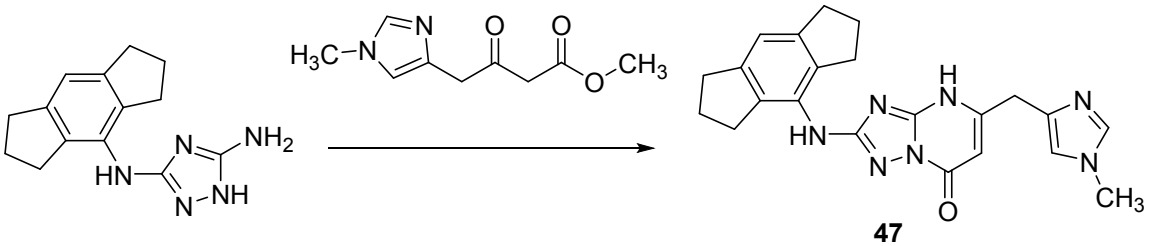
46:



46

Synthesis analogous to compound **6**, using methyl 4-(1-methylpyrazol-4-yl) -3-oxo-butanoate to give **46** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.57 (s, 1H), 7.34 (s, 1H), 6.88 (s, 1H), 5.54 (s, 1H), 3.78 (s, 3H), 3.63 (s, 2H), 2.81 (t, *J* = 7 Hz, 4H), 2.70 (t, *J* = 7 Hz, 4H), 1.98 - 1.91 (m, 4H). Molecular formula = $C_{22}H_{23}N_7O$, MW = 401.5. MS (ESI): m/z 402.2 $(MH)^+$. HPLC purity: 99.8%.

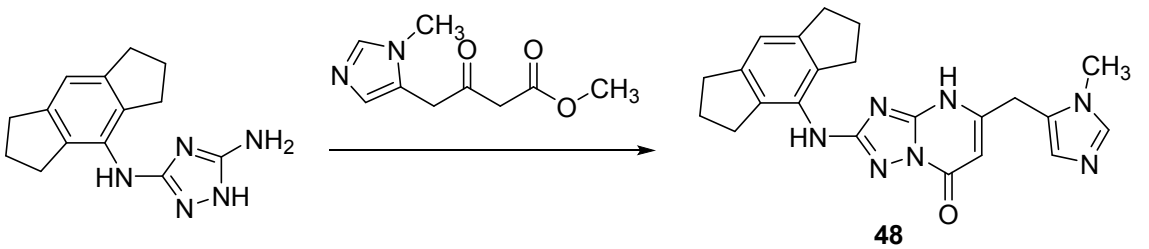
47:



47

Synthesis analogous to compound **6**, using methyl 4-(1-methylimidazol-4-yl)-3-oxo-butanoate to give **47** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.49 (s, 1H), 6.93 (s, 1H), 6.87 (s, 1H), 5.48 (s, 1H), 3.65 (s, 2H), 3.59 (s, 3H), 2.81 (t, *J* = 7 Hz, 4H), 2.71 (t, *J* = 7 Hz, 4H), 1.98 - 1.91 (m, 4H). Molecular formula = $C_{22}H_{23}N_7O$, MW = 401.5. MS (ESI): m/z 402.2 $(MH)^+$. HPLC purity: 97.1%.

48:

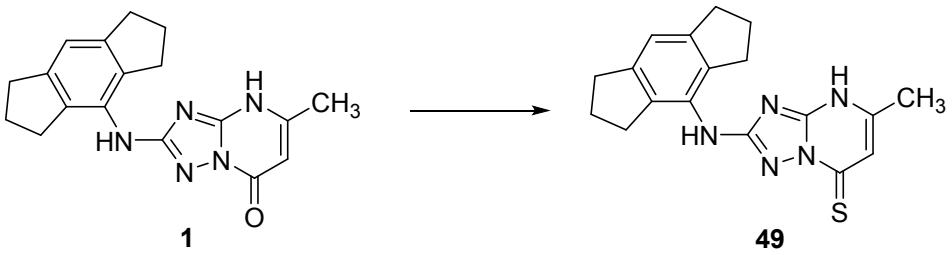


48

Synthesis analogous to compound **6**, using methyl 4-(3-methylimidazol-4-yl)-3-oxo-butanoate to give **48** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.49 (s, 1H), 7.60 (s, 1H), 6.91 (s, 1H), 5.67 (s, 1H), 4.08 (s, 2H), 3.78 (s, 3H), 2.82 (t, *J* = 7 Hz, 4H),

2.71 (t, *J* = 7 Hz, 4H), 1.99 - 1.92 (m, 4H). ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) *δ* 8.95 (s, 1H), 7.54 (s, 1H), 6.91 (s, 1H), 5.66 (s, 1H), 4.05 (s, 2H), 3.75 (s, 3H), 2.80 (t, *J* = 7 Hz, 4H), 2.68 (t, *J* = 7 Hz, 4H), 1.97 - 1.92 (m, 4H). Molecular formula = $C_{22}H_{23}N_7O$, MW = 401.5. MS (ESI): m/z 402.2 $(MH)^+$. HPLC purity: 98.4%.

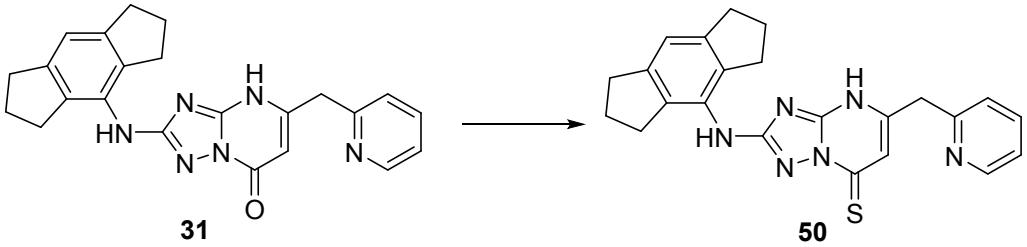
49:



1 **49**

To a solution of **1** (0.1 g, 311 µmol) in 1,4-dioxane (20 mL) at 25 °C under N_2 atmosphere was added 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Lawesson's reagent, 315 mg, 778 µmol). The resulting mixture was stirred at 70° C for 48 h under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in DMF (0.5 mL) and then treated with H_2O (0.5 mL). The resulting suspension was filtered, and the filter cake washed with H_2O (3 x 5 mL) and lyophilized to give **49** as a yellow solid. Y = 23 %. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 13.48 (br. s, 1H), 8.69 (s, 1H), 6.91 (s, 1H), 6.60 (s, 1H), 2.80 (t, *J* = 7 Hz, 4H), 2.73 (t, *J* = 7 Hz, 4H), 2.24 (s, 3H), 1.97 - 1.94 (m, 4H). Molecular formula = $C_{18}H_{19}N_5S$, MW = 337.5. MS (ESI): m/z 338.1 $(MH)^+$. HPLC purity: 98.2%.

50 (NDT-30805):

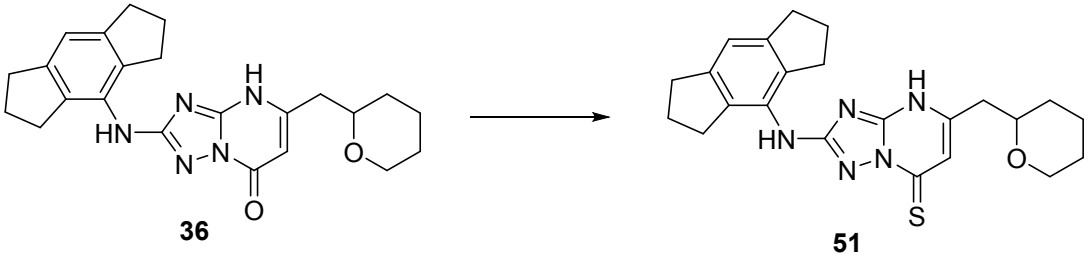


31 **50**

To a solution of 2-(1,2,3,5,6,7-hexahydro-s-indacen-4-ylamino)-5-(2-pyridylmethyl)-4H-[1,2,4]triazolo[1,5-a]pyrimidin-7-one (215 mg, 0.54 mmol) in dioxane (2 mL) was added P_2S_5 (600 mg, 2.70 mmol). The reaction mixture was bubbled with N_2 for 5 min and then stirred at 70° C for 12 h. Two identical batches were conducted in parallel and combined for work up. The mixture was treated with saturated aqueous Na_2CO_3 (5 mL) and the resulting mixture stirred at 25 °C for 3 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was washed with MeOH (10 mL) and purified by prep-HPLC (column: Phenomenex Luna C18 80x40 mm x 3 µm; mobile phase: [water (0.04 % HCl) - ACN]; B: 20 – 45 %, 7 min) and lyophilized to give **50** (13.5 % yield) as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.69 (s, 1H), 8.13 (s, 1H), 7.80 - 7.55 (m, 2H), 6.95 (s, 1H), 6.73 (s,1H), 4.24 (s, 2H), 2.82 (t, *J* = 7 Hz, 4H), 2.73 (t, *J* = 7 Hz, 4H), 2.03 - 1.89 (m, 4H). ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ 8.71 (d, *J* = 5 Hz, 1H), 8.19 (t, *J* = 8 Hz, 1H), 7.74 (d, *J* = 8 Hz, 1H), 7.70 - 7.61 (m, 1H), 6.95 (s, 1H), 6.75 (s, 1H), 4.25 (d, *J* = 8 Hz, 1H), 2.81

(t, *J* = 7 Hz, 4H), 2.71 (t, *J* = 7 Hz, 4H), 2.04 - 1.86 (m, 4H). Molecular formula = $C_{23}H_{22}N_6S$, MW = 414.5. MS (ESI): m/z 415.1 $(MH)^+$. HPLC purity: 100%.

51 (NDT-30744):



36 **51**

To a solution of **36** (100 mg, 247 µmol) in dioxane (2 mL) was added 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4dithiadiphosphetane (Lawesson's reagent, 499 mg, 1.23 mmol). The mixture was stirred at 50° C for 72 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was washed with EtOH to **51** as a yellow solid. Y = 16 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.55 (s, 1H), 8.88 (s, 1H), 6.93 (s, 1H), 6.65 (s, 1H), 3.87 - 3.80 (m, 1H), 3.68 - 3.54 (m, 1H), 3.29 - 20 (m, 1H), 2.82 (t, *J* = 7 Hz, 4H), 2.73 (t, *J* = 7 Hz, 4H), 2.69 - 2.56 (m, 2H), 1.98 - 1.94 (m, 4H), 1.81 - 1.73 (m, 1H), 1.66 - 1.56 (m, 1H), 1.50 - 1.37 (m, 3H), 1.30 - 1.17 (m, 1H). Molecular formula = $C_{23}H_{27}N_5OS$, MW = 421.6. MS (ESI): m/z 422.2 $(MH)^+$. HPLC purity: 93.3%.