Novel pyridazin-3(2H)-one-based guanidine derivatives as potential DNA minor groove binders with anticancer activity

María Carmen Costas-Lago, a,b Noemí Vila, a,b Adeyemi Rahman, c Pedro Besada, a,b Isabel Rozas, c José Brea, d María Isabel Loza, d Elisa González-Romero, e Carmen Terán * a,b

a Universidade de Vigo, Departamento de Química Orgánica, 36310 Vigo, España
b Instituto de Investigación Sanitaria Galicia Sur, Hospital Álvaro Cunqueiro, 36213 Vigo, España
c School of Chemistry, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse street, Dublin 2, Ireland
d Drug Screening Platform/Biofarma Research Group, CIMUS Research Center. Departamento de Farmacoloxía, Farmacia e Tecnoloxía Farmacéutica. Universidade de Santiago de Compostela, 15782 Santiago de Compostela, España
e Universidade de Vigo, Departamento de Química Analítica y Alimentaria, 36310 Vigo, España

Table of Contents

Molecular modelling studies ................................................................. S1
Optimised structures of compounds 1-14 .............................................. S2
Molecular docking figures ................................................................. S4
Tabulated data of docking study .......................................................... S17
Schemes of the preparation of scaffolds and precursors needed .................... S18
Synthesis of compounds 1-14 ............................................................. S21
Biophysical studies ........................................................................... S41
Biological studies ............................................................................ S42
NMR spectra of pyridazin-3(2H)-one-based guanidine derivatives 1-14 ........... S43
References ....................................................................................... S50

Molecular modelling studies

Ligand optimization

All ligands were fully optimized at DFT level (M06-2X functional) with the 6-311+G* basis set using the Gaussian16 program [1]. Frequency calculations were performed at the same computational level to confirm that the resulting optimized structures were energetic minima. The effect of water solvation was accounted using the
SCRF-SMD approach implemented in the Gaussian16 package including dispersing, repulsing and cavitation energy terms of the solvent in the optimization. Optimised structures of all the ligands studied are shown in Figure S1.

Docking experiments

The program Autodock Vina 4.2 was used to carry out docking studies [2]. The ligands were flexibly docked into the rigid DNA minor groove model (crystal structure of a pentamidine-oligonucleotide complex, PDB: 1D64 [3]). Scores (G-scores) were measured in kcal/mol and are only indicative of the quality of the interaction with the target; they do not provide a quantitative measure of binding. Poses obtained from the docking were visualised with VMD [4].

Best poses obtained for the docking of compounds 2-14 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ complexed with the drug pentamidine, PDB: 1D64) are shown in Figures S2 to S14.
Figure S1. Optimised structures of all compounds investigated using DFT (M06-2X, 6-31+G(d,p), SMD= water). Total energies in Hartrees are also indicated.
**Figure S2.** Best pose obtained for the docking of compound 2 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG))$_2$ PDB: 1D64.
Figure S3. - Best pose obtained for the docking of compound 3 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)$_2$ PDB: 1D64).
Figure S4.- Best pose obtained for the docking of compound 4 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ PDB: 1D64).
Figure S5.- Best pose obtained for the docking of compound 5 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ PDB: 1D64).
Figure S6.- Best pose obtained for the docking of compound 6 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ PDB: 1D64).
Figure S7.- Best pose obtained for the docking of compound 7 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)$_2$ PDB: 1D64).
Figure S8.- Best pose obtained for the docking of compound 8 into the minor groove model (dodecanucleotide d(CGCGAATTCCCG)₂ PDB: 1D64).
Figure S9.- Best pose obtained for the docking of compound 9 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ PDB: 1D64).
Figure S10.- Best pose obtained for the docking of compound 10 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)$_2$ PDB: 1D64).
Figure S11.- Best pose obtained for the docking of compound 11 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)$_2$ PDB: 1D64).
Figure S12.- Best pose obtained for the docking of compound 12 into the minor groove model (dodecanucleotide d(CGCGAATTCGCG)₂ PDB: 1D64).
Figure S13.- Best pose obtained for the docking of compound 13 into the minor groove model (dodecanucleotide d(CGCGAATTCCGCG)$_2$ PDB: 1D64).
**Figure S14.** Best pose obtained for the docking of compound 14 into the minor groove model (dodecanucleotide d(CGCGAATTCTACG)₂ PDB: 1D64).
Table S1.- Distances (Å), angles (°), atoms involved and qualitative strength of the HBs observed in the docking for all compounds studied; G-scores obtained are also indicated for each ligand-oligonucleotide complex.

| Compound | H bond distance (Å) | H bond angle (°) | HBA   | HBD     | Strength | Gscore (kcal mol⁻¹) |
|----------|---------------------|------------------|-------|---------|----------|---------------------|
| 1        | 2.26                | 154.11           | DG22:04 | Gua-NH  | weak     | -8.9                |
|          | 2.38                | 170.59           | DA6:04 | Gua-NH  | weak     |                     |
|          | 2.55                | 142.9            | DT20:04| Gua-NH  | weak     |                     |
|          | 2.76                | 136.61           | DT8:04 | Gua-NH  | weak     |                     |
| 2        | 2.36                | 141.53           | DG22:04| Gua-NH  | weak     | -8                  |
|          | 2.7                 | 157.2            | DA6:04 | Gua-NH  | weak     |                     |
|          | 1.88                | 150.81           | DT7:O2 | Gua-NH  | medium   |                     |
| 3        | 2.3                 | 127.71           | DG22:04| Gua-NH  | weak     | -8.7                |
|          | 2.53                | 144.83           | DA6:04 | Gua-NH  | weak     |                     |
|          | 2.84                | 137.17           | DT20:04| Gua-NH  | weak     |                     |
|          | 2.76                | 108.66           | DT8:02 | Gua-NH  | weak     |                     |
| 4        | 2.29                | 129.5            | DT7:O2 | Gua-NH  | weak     | -7.7                |
|          | 2.45                | 130.83           | DT8:04 | Gua-NH  | weak     |                     |
| 5        | 2.29                | 136.59           | DT20:O2| Gua-NH  | weak     | -6.8                |
|          | 2.31                | 130.21           | DT20:O2| Gua-NH  | weak     |                     |
| 6        | 2.32                | 125.37           | DT20:04| Gua-NH  | weak     | -6.1                |
|          | 2.4                 | 158.58           | DT7:O2 | Gua-NH  | weak     |                     |
| 7        | 2.26                | 124.97           | DT20:O2| Gua-NH  | weak     | -6.3                |
| 8        | 2.04                | 143.2            | DG22:04| Gua-NH  | medium   | -7.3                |
|          | 2.64                | 177.08           | DA6:04 | Gua-NH  | weak     |                     |
| 9        | 2.06                | 140.24           | DG22:04| Gua-NH  | medium   | -7.1                |
|          | 2.63                | 176.68           | DA6:04 | Gua-NH  | weak     |                     |
| 10       | 2.15                | 141.92           | DT20:O2| Gua-NH  | medium   | -6.8                |
|          | 2.34                | 130.73           | DT20:O2| Gua-NH  | weak     |                     |
| 11       | 2.03                | 147.9            | DT20:O2| Gua-NH  | medium   | -6.6                |
|          | 2.28                | 129.8            | DT20:O2| Gua-NH  | weak     |                     |
| 12       | 2.69                | 161.34           | DT20:O2| Gua-NH  | weak     | -7.3                |
|          | 2.38                | 126.63           | DT7:O2 | Gua-NH  | weak     |                     |
| 13       | 2.63                | 121.51           | DT7:O2 | Gua-NH  | weak     | -7.2                |
| 14       | 2.22                | 135.77           | DT19:O4| Gua-NH  | weak     | -6.3                |
Schemes of the preparation of scaffolds and precursors needed

Scheme S1. Synthesis of pyridazinone derivatives 25-35

Scheme S2. Synthesis of dibromo derivatives 48-51

Reagents and Conditions: (a) 15, TBDPSCI, imidazole, DMF, r.t., 1.5 h (18); (b) 16 or 17 LiAlH₄, Et₂O, r.t., 45 min; TBDPSCI, imidazole, DMF, r.t., 1.5 h (19 or 20); (c) 18, O₂, hv, rose bengal, MeOH, -78 °C, 5 h; Ac₂O, pyridine, DMAP, r.t., 20 h (21); (d) 19, O₂, hv, rose bengal, MeOH, DIPEA, -78 °C, 4 h (22); (e) 20, O₂, hv, rose bengal, MeOH, DBU, -78 °C, 2 h (23 and 24); (f) 21, hydrazine monohydrate or methyl hydrazine, EtOH, reflux, 2 h (25, 14%), 3h (27); 22, hydrazine monohydrate or methyl hydrazine or benzyl hydrazine dihydrochloride and TEA, ethanol, reflux, 2 h (26, 72%), 3 h (28, 52% or 29, 66%); 23 and 24, hydrazine monohydrate or methyl hydrazine or benzyl hydrazine dihydrochloride and TEA, ethanol, reflux, 4 h (30 and 31, 56% and 5% respectively from 20), 7 h (32, 84% and 33, 73%; 34 and 35, 46% and 7% respectively from 20).
Reagents and Conditions: (a) NaH 60% mineral oil, methyl 4-bromomethylbenzoate, DMF, r.t., 24 h; (b) DIBAL-H 1M hexane, DCM, -78 °C, 3 h; (c) TBAF 1M THF, r.t., 15 min.; (d) CBr₄, Ph₃P, DCM, reflux, 3 h, 48 (65%), 49 (67%), 51 (98%) and 6 h 50 (90%).

Scheme S3. Synthesis of bromo derivatives 63-69
Scheme S4. Synthesis of bromo derivatives 74-75

Reagents and Conditions: (a) TBAF 1M THF, r.t., 15 min.; (b) CBr₄, Ph₃P, DCM, reflux, 1.5 h 63 (80%), 64 (96%), 65 (76%), 66 (82%), 67 (85%), 68 (73%), 69 (84%).

Synthesis of compounds 1-14

Reagents and Conditions: (a) NaH 60% in mineral oil, α,α'-dibromo-p-xylene, Bu₄NI, THF, r.t., 5h; (b) TBAF 1M THF, r.t., 15 min; (c) TMSBr, MeOH, reflux, 9 h; (d) CBr₄, Ph₃P, DCM, reflux, 3 h 74 (70%), 75 (94%).
**General methods**

The starting chemical materials were of commercial sources and used as provided. The solvents were purified and dried following standard protocols. Air-sensitive reactions were performed under an argon atmosphere. Melting points were measured in a Stuart Scientific SMP3 or SMP10 instrument by means of capillary tubes. $^1$H NMR, $^{13}$C NMR and $^{19}$F NMR spectra were registered on a Bruker (ARX400) spectrometer with TMS as internal reference. Chemical shifts (δ) were indicated in ppm and coupling constants in Hertz (Hz). COSY, DEPT and HSQC analysis were carried out to unequivocally assign the different signals. High resolution mass spectra were registered on a Bruker microTOF focus spectrometer using the electrospray ionization technique.

Analytical thin layer chromatography (TLC) was carried out on pre-coated aluminium sheets with silica gel (Merck 60 F254, 0.25 mm). Flash chromatography (FC) was performed on silica gel (Merck 60, 230–400 mesh). Reverse phase chromatography was performed by using reversed phase silica gel 100 C₈ (Sigma Aldrich, ≥400 mesh).

HPLC purity analysis of guanidine derivatives were carried out using a Jasco PU-980 system equipped with a UV-975 Intelligent UV/VIS detector and a manual injector (20 µL). For purity assessment, UV detection was performed at 284 nm and peak purity was confirmed by standard normalization. The stationary phase consisted of a Kinetex 5 µm C18 100Å column (250×4.6 mm), and the mobile phases used were 20% methanol buffered (aqueous formate buffer) and 40% methanol buffered eluting at 1 mL/min. Minimum requirement for purity was set at 94.0%. Synthesis of silyl protected hydroxyalkylpyridazin-3(2H)-ones 25, 27, 30-35 as well as of hydroxyalkyl (56, 57, 59-62) and bromoalkyl analogues (63, 64, 66-69) was performed as previously was described [5,6,7].

6-(**tert**-Butyldiphenylyloxy)methyl)-5-methylpyridazin-3(2H)-one (**26**). A solution of 22 [7] (800 mg, 2.09 mmol) and hydrazine monohydrate (20 µL, 4.20 mmol) in EtOH (10 mL) was stirred at reflux for 2 h. After the solvent was removed, the residue was purified by column chromatography on silica gel (50% EtOAc/hexane) to afford 26 (570 mg, 72%, white solid). Rₜ = 0.4 (50% EtOAc/hexane); m.p. = 149 – 150 °C; $^1$H NMR (CDCl₃): δ = 10.81 (s, 1H, NH), 7.69 - 7.65 (m, 4H, H-Ph), 7.50 - 7.45 (m, 2H, H-Ph), 7.44 - 7.38 (m, 4H, H-Ph), 6.74 (d, 1H, J = 1.2 Hz, H4), 4.63 (s, 2H, CH₂), 2.38 (d, 3H, J
= 1.2 Hz, CH₃), 1.07 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 162.7 (C3), 147.0 (C6), 145.4 (C5), 135.7 (CH-Ph), 132.8 (C-Ph), 130.0 (CH-Ph), 128.4 (C4), 127.9 (CH-Ph), 64.5 (CH₂), 26.9 ((CH₃)₃), 19.4 (C(CH₃)₃), 18.6 (CH₃); HRMS (ESI): m/z [M+H]⁺ calecd for C₂₂H₂₇N₂O₂Si, 379.18363; found 379.18308.

6-(tert-Butyldiphenylsilyloxyethyl)-2,5-dimethylpyrazin-3(2H)-one (28). To a solution of 22 [7] (250 mg, 0.65 mmol) in EtOH (10 mL) was added methylhydrazine (52 µL, 0.98 mmol) and the reaction mixture was refluxed under stirring for 3 h. The solvent was removed and the residue was purified by column chromatography on silica gel (20% EtOAc/hexane) to yield 28 (133 mg, 52%) as a yellowish oil. Rₛ = 0.3 (50% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 7.66 - 7.63 (m, 4H, H-Ph), 7.47 - 7.42 (m, 2H, H-Ph), 7.41 - 7.35 (m, 4H, H-Ph), 6.68 (d, 1H, J = 1.1 Hz, H₄), 4.62 (s, 2H, CH₂), 3.60 (s, 3H, CH₃N), 2.31 (d, 3H, J = 1.1 Hz, CH₃), 1.05 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 160.8 (C3), 145.7, 143.6, 135.7 (CH-Ph), 132.9 (C-Ph), 129.9 (CH-Ph), 128.0 (C4), 127.7 (CH-Ph), 64.4 (CH₂), 39.5 (CH₃N), 26.7 ((CH₃)₃), 19.3 (C(CH₃)₃), 17.9 (CH₃); HRMS (ESI): m/z [M+H]⁺ calecd for C₂₃H₂₉N₂O₂Si, 393.19928; found 393.19770.

2-Benzyl-6-(tert-butyldiphenylsililoxyethyl)-5-methylpyrazin-3(2H)-one (29). To a solution of 22 [7] (283 mg, 0.74 mmol) in EtOH (10 mL) was added benzylhydrazine dihydrochloride (216 mg, 1.11 mmol) and Et₃N (0.3 mL, 2.22 mmol) and the reaction mixture was stirred at reflux for 3 h. After the solvent was removed, CH₂Cl₂ (10 mL) and H₂O (10 mL) were added and the mixture was extracted with CH₂Cl₂ (3x10 mL). The combined organic phases were dried over Na₂SO₄, filtered and the solvent was evaporated to dryness. The residue was purified by column chromatography on silica gel (20% EtOAc/hexane) to afford 29 (146 mg, 66%) as a colourless oil. Rₛ = 0.5 (50% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 7.66 – 7.61 (m, 4H, H-Ph), 7.45 – 7.40 (m, 2H, H-Ph), 7.37 – 7.32 (m, 6H, H-Ph), 7.30 – 7.21 (m, 3H, H-Ph), 6.69 (d, 1H, J = 1.1 Hz, H₄), 5.17 (s, 2H, CH₂N), 4.62 (s, 2H, CH₂), 2.29 (d, 3H, J = 1.1 Hz, CH₃), 1.02 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 160.4 (C3), 146.0, 143.6, 136.5 (C-Ph), 135.7 (CH-Ph), 133.0 (C-Ph), 129.9 (CH-Ph), 128.7, 128.6, 128.5, 127.8 (CH-Ph), 127.8 (CH-Ph), 64.5 (CH₂), 54.6 (CH₂N), 26.8 ((CH₃)₃), 19.3 (C(CH₃)₃), 18.0 (CH₃); HRMS (ESI): m/z [M+H]⁺ calecd for C₂₉H₃₃N₂O₂Si, 469.23058; found 469.23051.
General procedure to synthesize methyl esters 36-39. A solution of compound 25-26 or 30-31 (0.25 mmol) in DMF (2 mL) was added, dropwise at 0 °C, to a suspension of NaH (0.38 mmol, 60% dispersion in mineral oil) in DMF (2 mL). After the mixture was stirred at room temperature for 1 h, methyl 4-(bromomethyl)benzoate (0.38 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, followed by quenching with MeOH. The solvent was evaporated to dryness, and the residue was purified by column chromatography on silica gel to afford the desired compound.

2-[(4-methoxycarbonyl)benzyl]-6-(tert-butyldiphenylsilyloximethyl)pyrazin-3(2H)-one (36). Compound 36 was purified by column chromatography on silica gel (40% EtOAc/hexane). Yellowish oil; yield 76%; Rf = 0.5 (50% EtOAc/hexane); 1H NMR (CDCl3): δ = 7.99 – 7.94 (m, 2H, H-Ph), 7.66 – 7.61 (m, 4H, H-Ph), 7.46 – 7.34 (m, 9H, H5, H-Ph), 6.95 (d, 1H, J = 9.5 Hz, H4), 5.25 (s, 2H, CH2N), 4.60 (s, 2H, CH2O), 3.89 (s, 3H, CH3O), 1.08 (s, 9H, (CH3)3); 13C NMR (CDCl3): δ = 166.9 (COOCH3), 160.0 (C3), 147.1 (C6), 141.3 (C-Ph), 135.6 (CH-Ph), 132.8 (C-Ph), 131.2 (C5), 130.5 (C4), 130.1 (CH-Ph), 129.9 (CH-Ph), 129.7 (C-Ph), 128.6 (CH-Ph), 128.0 (CH-Ph), 64.5 (CH2O), 54.8 (CH2N), 52.2 (CH3O), 26.9 ((CH3)3), 19.3 (C(CH3)3); HRMS (ESI): m/z [M+H]+ calcd for C30H33N2O4Si, 513.22041; found 513.21841.

2-[(4-methoxycarbonyl)benzyl]-6-(tert-butyldiphenylsilyloximethyl)-5-methylpyrazin-3(2H)-one (37). Compound 37 was purified by column chromatography on silica gel (50% EtOAc/hexane). Colourless oil; yield 96%; Rf = 0.3 (50% EtOAc/hexane); 1H NMR (CDCl3): δ = 7.98 – 7.93 (m, 2H, H-Ph), 7.65 – 7.60 (m, 4H, H-Ph), 7.47 – 7.31 (m, 8H, H-Ph), 6.71 (d, 1H, J = 1.1 Hz, H4), 5.21 (s, 2H, CH2N), 4.63 (s, 2H, CH2O), 3.89 (s, 3H, CH3O), 2.33 (d, 3H, J = 1.1 Hz, CH3), 1.04 (s, 9H, (CH3)3); 13C NMR(CDCl3): δ = 166.9 (COOCH3), 160.3 (C3), 146.3, 143.8, 141.5 (C-Ph), 135.7 (CH-Ph), 133.0 (C-Ph), 130.0 (CH-Ph), 129.9 (CH-Ph), 129.6 (C-Ph), 128.7, 128.5, 127.8 (CH-Ph), 64.5(CH2O), 54.3 (CH2N), 52.2 (CH3O) 26.9 ((CH3)3), 19.3 (C(CH3)3), 18.0 (CH3); HRMS (ESI): m/z [M+H]+ calcd for C31H35N2O4Si, 527.23606; found 527.23528.

2-[(4-methoxycarbonyl)benzyl]-5-(tert-butyldiphenylsilyloximethyl)pyridazin-3(2H)-one (38). Compound 38 was purified by column chromatography on silica gel (20% EtOAc/hexane). Colourless oil; yield 80%; Rf = 0.4 (1:2 EtOAc/hexane); 1H NMR
(CDCl$_3$): $\delta = 8.02 – 7.98$ (m, 2H, H-Ph), 7.67 – 7.60 (m, 5H, H6, H-Ph), 7.49 – 7.34 (m, 8H, H-Ph), 6.98 (dd, 1H, $J = 3.6$, 1.6 Hz, H4), 5.36 (s, 2H, CH$_2$N), 4.56 (d, 2H, $J = 1.5$ Hz, CH$_2$O), 3.90 (s, 3H, CH$_3$O), 1.09 (s, 9H, (CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$): $\delta =$ 166.9 (C$_{OOCH}$3), 160.6 (C3), 145.9 (C5), 141.4 (C-Ph), 135.6 (C6, CH-Ph), 132.4 (C-Ph), 130.3 (CH-Ph), 130.0 (CH-Ph), 128.4 (C-Ph), 128.1 (CH-Ph), 124.8 (C4), 61.9 (CH$_2$O), 54.6 (CH$_2$N), 52.2 (CH$_3$O), 26.9 ((CH$_3$)$_3$), 19.4 (C(CH$_3$)$_3$); HRMS (ESI): $m/z$ [M+H]$^+$ calcd for C$_{30}$H$_{33}$N$_2$O$_4$Si, 513.22041; found 513.22058.

2-((4-Methoxycarbonyl)benzyl)-4-(tert-butyldiphenylsililoximethyl)pyridazin-3(2H)-one (39). Compound 39 was purified by column chromatography on silica gel (10% EtOAc/hexane). Colourless oil; yield 84%; $R_f = 0.5$ (50% EtOAc/hexane); $^1$H NMR (CDCl$_3$): $\delta = 8.01 – 7.96$ (m, 2H, H-Ph), 7.86 (d, 1H, $J = 4.0$ Hz, H6), 7.66 – 7.61 (m, 4H, H-Ph), 7.53 (dt, 1H, $J = 4.0$, 1.8 Hz, H5), 7.46 – 7.33 (m, 8H, H-Ph), 5.34 (s, 2H, CH$_2$N), 4.74 (d, 2H, $J = 1.8$ Hz, CH$_2$O), 3.89 (s, 3H, CH$_3$O), 1.13 (s, 9H, (CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$): $\delta =$ 166.9 (C$_{OOCH}$3), 160.6 (C3), 145.9 (C4), 141.4 (C-Ph), 135.6 (C6), 135.6 (CH-Ph), 130.4 (CH-Ph), 132.4 (C-Ph), 130.0 (CH-Ph), 129.8 (C-Ph), 128.6 (CH-Ph), 128.1 (CH-Ph), 124.8 (C5), 61.9 (CH$_2$O), 54.6 (CH$_2$N), 52.2 (CH$_3$O), 26.9 ((CH$_3$)$_3$), 19.4 (C(CH$_3$)$_3$); HRMS (ESI): $m/z$ [M+H]$^+$ calcd for C$_{30}$H$_{33}$N$_2$O$_4$Si, 513.22041; found 513.22058.

General procedure to synthesize compounds 40-43. To a solution of compound 36-39 (0.15 mmol) in CH$_2$Cl$_2$ (5 mL) at -78 °C was added dropwise a solution of DIBAL-H 1M in hexane (0.78 mmol). The reaction mixture was stirred at this temperature for 3 h, quenched with tBuOMe (0.40 mL), H$_2$O (60 µL) and NaOH 4M (30 µL) and stirred overnight at room temperature. The resulting white precipitate was filtered off, the solvent was evaporated under reduce pressure and the residue was purified by column chromatography to afford the desired compound.

2-[(4-hydroxymethyl)benzyl]-6-(tert-butyldiphenylsililoximethyl)pyridazin-3(2H)-one (40). Compound 40 was purified by column chromatography on silica gel (60% EtOAc/hexane). Colourless oil; yield 76%; $R_f = 0.2$ (50% EtOAc/hexane); $^1$H NMR (CDCl$_3$): $\delta = 7.67 – 7.62$ (m, 4H, H-Ph), 7.48 – 7.26 (m, 11H, H5, H-Ph), 6.91 (d, 1H, $J = 9.5$ Hz, H4), 5.29 (s, 2H, CH$_2$N), 4.68 – 4.61 (m, 3H, CH$_2$OH, OH), 4.60 (s, 2H, CH$_2$Si), 1.08 (s, 9H, (CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$): $\delta =$ 160.1 (C3), 147.0 (C6), 140.8 (C-
Ph), 135.6 (C-Ph), 132.9 (C-Ph), 130.4 (C4), 130.1 (C-Ph), 129.0, 128.0, 127.2, 65.0 (CH2OH), 64.5 (CH2OSi), 55.0 (CH2N), 26.9 ((CH3)3), 19.3 (C(CH3)3); HRMS (ESI): m/z [M+H]+ calecd for C29H33N2O3Si, 485.22550; found 485.22381.

2-[(4-hydroxymethyl)benzyl]-6-(tert-butyldiphenylsililoximethyl)-5-methylpyridazin-3(2H)-one (41). Compound 41 was purified by column chromatography on silica gel (60% EtOAc/hexane). Colourless oil; yield 70%; Rf = 0.2 (50% EtOAc/hexane); 1H NMR (CDCl3): δ = 7.67 – 7.61 (m, 4H, H-Ph), 7.48 – 7.41 (m, 2H, H-Ph), 7.41 – 7.23 (m, 8H, H-Ph), 6.67 (d, 1H, J = 1.1 Hz, H4), 5.15 (s, 2H, CH2N), 4.65- 4.61 (m, 4H, CH2OH, CH2OSi), 2.39 (br s, 1H, OH), 2.30 (d, 3H, J = 1.1 Hz, CH3), 1.04 (s, 9H, (CH3)3); 13C NMR (CDCl3): δ = 160.4 (C3), 146.1, 143.6, 140.8 (C-Ph), 135.7 (CH-Ph), 135.7 (C-Ph), 133.0 (C-Ph), 130.0 (CH-Ph), 128.9 (CH-Ph), 128.6 (C4), 127.8 (CH-Ph), 127.2 (CH-Ph), 65.0 (CH2OH), 64.5 (CH2OSi), 54.5 (CH2N), 26.9 ((CH3)3), 19.4 (C(CH3)3), 18.0 (CH3); HRMS (ESI): m/z [M+H]+ calecd for C30H35N2O3Si, 499.24115; found 499.23933.

2-[(4-Hydroxymethyl)benzyl]-5-(tert-butyldiphenylsililoximethyl)pyridazin-3(2H)-one (42). Compound 42 was purified by column chromatography on silica gel (50% EtOAc/hexane). Colourless oil; yield 71%; Rf = 0.3 (50% EtOAc/hexane); 1H NMR (CDCl3): δ = 7.68 – 7.63 (m, 5H, H6, H-Ph), 7.48 – 7.43 (m, 2H, H-Ph), 7.42 – 7.36 (m, 6H, H-Ph), 7.33 – 7.29 (m, 2H, H-Ph), 6.95 (dd, 1H, J = 3.5, 1.5 Hz, H4), 5.29 (s, 2H, CH2N), 4.65 (d, 2H, J = 4.6 Hz, CH2OH), 4.55 (d, 2H, J = 1.5 Hz, CH2OSi), 2.56 (br s, 1H, OH), 1.11 (s, 9H, (CH3)3); 13C NMR (CDCl3): δ = 160.6 (C3), 145.7 (C5), 141.0 (C-Ph), 135.6 (C-Ph), 135.5 (CH-Ph), 135.5 (C6), 132.4 (C-Ph), 130.2 (CH-Ph), 129.0 (CH-Ph), 128.1 (CH-Ph), 127.2 (CH-Ph), 124.7 (C4), 64.9 (CH2OH), 61.9 (CH2OSi), 54.7 (CH2N), 26.8 ((CH3)3), 19.3 (C(CH3)3); HRMS (ESI): m/z [M+H]+ calecd for C30H35N2O3Si, 499.24115; found 499.23933.

2-[(4-Hydroxymethyl)benzyl]-4-(tert-butyldiphenylsililoximethyl)pyridazin-3(2H)-one (43). Compound 43 was purified by column chromatography on silica gel (30% EtOAc/hexane). Colourless oil; yield 56%; Rf = 0.4 (50% EtOAc/hexane); 1H NMR (CDCl3): δ = 7.84 (d, 1H, J = 4.0 Hz, H6), 7.66 – 7.60 (m, 4H, H-Ph), 7.51 (dt, 1H, J = 4.0, 1.8 Hz, H5), 7.45 – 7.28 (m, 10H, H-Ph), 5.28 (s, 2H, CH2N), 4.72 (d, 2H, J = 1.8
Hz, CH₂OSi), 4.65 (d, 2H, J = 4.4 Hz, CH₂OH), 1.65 (br s, 1H, OH), 1.12 (s, 9H, (CH₃)₃);
¹³C NMR (CDCl₃): δ = 159.4 (C3), 143.4 (C4), 140.7 (C-Ph), 136.7 (C6), 135.8 (C-Ph),
135.6 (CH-Ph), 132.9 (C-Ph), 130.1 (CH-Ph), 129.2 (CH-Ph), 128.0 (CH-Ph), 127.3 (CH-
Ph), 125.3 (C5), 65.2 (CH₂OH), 61.0 (CH₂OSi), 54.9 (CH₃N), 27.0 ((CH₃)₃), 19.5
(C(CH₃)₃); HRMS (ESI): m/z [M+H]⁺ calcd for C₂₉H₃₃N₂O₃Si, 485.22550; found
485.22451.

**General procedure to synthesize compounds 44-47.** A solution of compound 40-43
(0.79 mmol) and TBAF (0.95 mmol, 1 M in THF) in THF (15 mL) was stirred at room
temperature for 15 min. The solvent was evaporated to dryness and the residue was
purified by column chromatography on silica gel to afford the proper compound.

**6-Hydroxymethyl-2-[(4-hydroxymethyl)benzyl]pyridazin-3(2H)-one (44).**
Compound 44 was purified by column chromatography on silica gel (5% MeOH/EtOAc).
Yellowish oil; yield 87%; Rf = 0.2 (5% MeOH/EtOAc); ¹H NMR (CD₂OD): δ = 7.56 (d,
1H, J = 9.5 Hz, H5), 7.39 – 7.30 (m, 4H, H-Ph), 7.01 (d, 1H, J = 9.5 Hz, H4), 5.31 (s, 2H,
CH₂N), 4.59 (s, 2H, CH₂OH), 4.51 (s, 2H, CH₂OH-pyridazinone); ¹³C NMR (CD₂OD):
δ = 162.1 (C3), 150.0 (C6), 142.5 (C-Ph), 136.7 (C-Ph), 133.5 (C5), 130.9 (C4), 129.4
(CH-Ph), 128.1 (CH-Ph), 64.8 (CH₂OH), 63.5 (CH₂OH-pyridazinone), 56.1 (CH₃N);
HRMS (ESI): m/z [M+H]⁺ calcd for C₁₃H₁₅N₂O₃, 247.10772; found 247.10715.

**6-Hydroxymethyl-2-[(4-hydroxymethyl)benzyl]-5-methylpyridazin-3(2H)-one (45).**
Compound 45 was purified by column chromatography on silica gel (5% MeOH/EtOAc).
White solid; yield 97%; Rf = 0.3 (5% MeOH/EtOAc); m.p.: 142.3 – 142.7 °C; ¹H NMR
(CD₂OD): δ = 7.38 – 7.29 (m, 4H, H-Ph), 6.80 (d, 1H, J = 1.2 Hz, H4), 5.30 (s, 2H,
CH₂N), 4.59 (s, 2H, CH₂OH), 4.57 (s, 2H, CH₂OH-pyridazinone), 2.35 (d, 3H, J = 1.2
Hz, CH₃); ¹³C NMR (CD₂OD): δ = 162.4 (C3), 149.2, 146.3, 142.4 (C-Ph), 136.8 (C-Ph),
129.4 (CH-Ph), 128.9 (C4), 128.1 (CH-Ph), 64.8 (CH₂OH), 62.9 (CH₂OH-pyridazinone),
55.7 (CH₂N), 17.7 (CH₃); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₄H₁₇N₂O₃, 261.12337;
found 261.12260.

**5-Hydroxymethyl-2-[(4-hydroxymethyl)benzyl]pyridazin-3(2H)-one (46).**
Compound 46 was purified by column chromatography on silica gel (EtOAc). White solid; yield 96%; Rf = 0.2 (EtOAc); m.p. = 143.2 – 144.0 °C; 1H NMR (CD3OD): δ = 7.91 (d, 1H, J = 2.1 Hz, H6), 7.38 – 7.30 (m, 4H, H-Ph), 6.93 (dd, 1H, J = 3.4, 1.5 Hz, H4), 5.33 (s, 2H, CH2N), 4.59 (s, 2H, CH2OH), 4.55 (d, 2H, J = 1.5 Hz, CH2OH-pyridazinone); 13C NMR (CD3OD): δ = 162.6 (C3), 149.6 (C5), 142.5 (C-Ph), 138.2 (C6), 136.7 (C-Ph), 129.4 (CH-Ph), 128.1 (CH-Ph), 125.0 (C4), 64.8 (CH2OH), 61.0 (CH2OH-pyridazinone), 55.9 (CH2N); HRMS (ESI): m/z [M+Na]+ calcd for C13H14N2NaO3, 269.08966; found 269.08950.

4-Hydroxymethyl-2-((4-hydroxymethyl)benzyl)pyridazin-3(2H)-one (47).
Compound 47 was purified by column chromatography on silica gel (EtOAc). White solid; yield 95%; Rf = 0.4 (5% MeOH/EtOAc); m.p: 137.4 – 138.0 °C; 1H NMR (CD3OD): δ = 7.97 (d, 1H, J = 4.0 Hz, H6), 7.48 (dt, 1H, J = 4.0, 1.7 Hz, H5), 7.39 – 7.30 (m, 4H, H-Ph), 5.36 (s, 2H, CH2N), 4.60 (s, 2H, CH2OH), 4.56 (d, 2H, J = 1.7 Hz, CH2OH-pyridazinone); 13C NMR (CD3OD): δ = 161.4 (C3), 144.9 (C4), 142.5 (C-Ph), 138.7 (C6), 136.7 (C-Ph), 129.4 (CH-Ph), 128.1 (CH-Ph), 127.4 (C5), 64.8 (CH2OH), 59.5 (CH2OH-pyridazinone), 55.9 (CH2N); HRMS (ESI): m/z [M+Na]+ calcd for C13H14N2NaO3, 269.08966; found 269.08953.

General procedure to synthesize compounds 48-51. To a solution of compound 44-47 (0.10 mmol) in CH2Cl2 (5 mL) was added CBr4 (0.60 mmol) and PPh3 (0.60 mmol) and the reaction mixture was refluxed for 3 h (48, 49 and 51) or 6 h (50). After quenching with saturated aq. NaHCO3 (5 mL) the product was extracted with CH2Cl2 (3x5 mL), dried over Na2SO4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (30% EtOAc/hexane) to afford the desired compound.

6-Bromomethyl-2-[(4-bromomethyl)benzyl]pyridazin-3(2H)-one (48). White solid; yield 65%; Rf = 0.5 (EtOAc); m.p. = 92.5 – 93.0 °C; 1H NMR (CDCl3): δ = 7.41 – 7.34 (m, 4H, H-Ph), 7.32 (d, 1H, J = 9.6 Hz, H5), 6.94 (d, 1H, J = 9.6 Hz, H4), 5.26 (s, 2H, CH2N), 4.46 (s, 2H, CH2Br), 4.33 (s, 2H, CH2Br-pyridazinone); 13C NMR (CDCl3): δ = 159.4 (C3), 143.7 (C6), 137.7 (C-Ph), 136.3 (C-Ph), 132.4 (C5), 131.0 (C4), 129.5 (CH-Ph), 129.4 (CH-Ph), 55.1 (CH2N), 33.2 (CH2Br), 30.4 (CH2Br-pyridazinone); HRMS (ESI): m/z [M+H]+ calcd for C13H13Br2N2O, 370.93891; found 370.93806.
6-Bromomethyl-2-[(4-bromomethyl)benzyl]-5-methylpyridazin-3(2H)-one (49).

Colourless oil; yield 67%; \( R_f = 0.6 \) (EtOAc); \(^1\)H NMR (CDCl\(_3\)): \( \delta = 7.36 \) (m, 4H, H-Ph), 6.72 (d, 1H, \( J = 1.1 \) Hz, H4), 5.25 (s, 2H, CH\(_2\)N), 4.45 (s, 2H, CH\(_2\)Br), 4.36 (s, 2H, CH\(_2\)Br-pyridazinone), 2.30 (d, 3H, \( J = 1.1 \) Hz, CH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 160.0 \) (C3), 143.6, 142.8, 137.6 (C-Ph), 136.4 (C-Ph), 129.4 (CH-Ph), 129.3, 129.2, 54.7 (CH\(_2\)N), 33.2 (CH\(_2\)Br), 29.3 (CH\(_2\)Br-pyridazinone), 18.1 (CH\(_3\)); HRMS (ESI): \( m/z \) [M+H]\(^+\) calcd for C\(_{14}\)H\(_{15}\)Br\(_2\)N\(_2\)O, 384.95456; found 384.95333.

5-Bromomethyl-2-[(4-bromomethyl)benzyl]pyridazin-3(2H)-one (50).

White solid; yield 90%; \( R_f = 0.4 \) (50% EtOAc/hexane); m.p. = 145.5 – 146.3 \( ^\circ \)C, \(^1\)H NMR (CDCl\(_3\)): \( \delta = 7.78 \) (d, 1H, \( J = 2.2 \) Hz, H6), 7.43 - 7.39 (m, 2H, H-Ph), 7.37 - 7.34 (m, 2H, H-Ph), 6.86 (dd, 1H, \( J = 2.2, 0.6 \) Hz, H4), 5.29 (s, 2H, CH\(_2\)N), 4.46 (s, 2H, CH\(_2\)Br), 4.18 (d, 2H, \( J = 0.6 \) Hz, CH\(_2\)Br-pyridazinone); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 159.7 \) (C3), 141.9 (C5), 137.7 (C-Ph), 137.1 (C6), 136.3 (C-Ph), 129.5 (CH-Ph), 129.4 (CH-Ph), 128.0 (C4), 54.8 (CH\(_2\)N), 33.2 (CH\(_2\)Br), 26.9 (CH\(_2\)Br-pyridazinone); HRMS (ESI): \( m/z \) [M+H]\(^+\) calcd for C\(_{13}\)H\(_{13}\)Br\(_2\)N\(_2\)O, 370.93891; found 370.93891.

4-Bromomethyl-2-[(4-bromomethyl)benzyl]pyridazin-3(2H)-one (51).

White solid; yield 98%; \( R_f = 0.5 \) (50% EtOAc/hexane); m.p. = 124.3 – 124.6 \( ^\circ \)C, \(^1\)H NMR (CDCl\(_3\)): \( \delta = 7.77 \) (d, 1H, \( J = 4.1 \) Hz, H6), 7.42 (d, 2H, \( J = 8.2 \) Hz, H-Ph), 7.35 (d, 2H, \( J = 8.2 \) Hz, H-Ph), 7.32 (dt, 1H, \( J = 4.1, 0.8 \) Hz, H5), 5.33 (s, 2H, CH\(_2\)N), 4.46 (s, 2H, CH\(_2\)Br), 4.39 (d, 2H, \( J = 0.8 \) Hz, CH\(_2\)Br-pyridazinone); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 159.5 \) (C3), 139.1 (C4), 137.8 (C-Ph), 136.2 (C-Ph), 136.2 (C6), 129.8 (C5), 129.5 (2xCH-Ph), 55.4 (CH\(_2\)N), 33.2 (CH\(_2\)Br), 26.5 (CH\(_2\)Br-pyridazinone); HRMS (ESI): \( m/z \) [M+H]\(^+\) calcd for C\(_{13}\)H\(_{13}\)Br\(_2\)N\(_2\)O, 370.93891; found 370.93875.

General procedure to synthesize compounds 52-55. To a solution of compound 48-51 (0.05 mmol) in DMF (2 mL) was added 1,3-bis-(tert-butoxycarbonyl)guanidine (0.28 mmol) and K\(_2\)CO\(_3\) (0.11 mmol) and the reaction was stirred at 50 \( ^\circ \)C for 2 h. The reaction mixture was diluted with EtOAc (40 mL), washed with water (10 mL), brine (2x10 mL) and dried over anhydrous MgSO\(_4\). The solvent was evaporated to dryness and the residue was purified by column chromatography on silica gel (50% EtOAc/hexane for 52-54 and 20% EtOAc/hexane for 55) to afford the desired compound.
2-[4-((N,N′-di(tert-Butoxycarbonyl)guanidine)methyl)benzyl]-6-[(N,N′-di(tert-Butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (52). White solid; yield 60%; R_f = 0.3 (50% EtOAc/hexane); m.p. = 175.7 – 176.2 °C; ^1H NMR (CDCl_3): δ = 9.54 – 9.18 (m, 4H, 4xNH), 7.29 (d, 2H, J = 8.2 Hz, H-Ph), 7.23 – 7.15 (m, 3H, H5, H-Ph), 6.89 (d, 1H, J = 9.5 Hz, H4), 5.24 (s, 2H, CH_2N), 5.13 (s, 2H, CH_2NH), 5.06 (s, 2H, NHCH_2-pyridazinone), 1.47 (s, 18H, 2x(CH_3)_3), 1.34 (s, 9H, (CH_3)_3), 1.28 (s, 9H, (CH_3)_3); ^13C NMR (CDCl_3): δ =163.9 (CO), 163.6 (CO), 161.0 (CO), 160.5 (CO), 160.1 (C3), 155.1 (C=N), 154.6 (C=N), 144.7 (C6), 138.6 (C-Ph), 135.1 (C-Ph), 131.2 (C5), 130.4 (C4), 128.6 (CH-Ph), 127.5 (CH-Ph), 84.8 (C(CH_3)_3), 84.3 (C(CH_3)_3), 79.3 (C(CH_3)_3), 78.9 (C(CH_3)_3), 55.0 (CH_2N), 47.3 (CH_2NH), 46.6 (NHCH_2-pyridazinone), 28.5 ((CH_3)_3), 28.4 ((CH_3)_3), 27.9 ((CH_3)_3); HRMS (ESI): m/z [M+H]^+ calcd for C_{35}H_{53}N_8O_9, 729.39300; found 729.39207.

2-[4-((N,N′-di(tert-Butoxycarbonyl)guanidine)methyl)benzyl]-6-[(N,N′-di(tert-Butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (53). Colourless oil; yield 60%; R_f = 0.2 (50% EtOAc/hexane); ^1H NMR (CDCl_3): δ = 9.53 – 9.19 (m, 4H, 4xNH), 7.26 (d, 2H, J = 8.2 Hz, H-Ph), 7.14 (d, 2H, J = 8.2 Hz, H-Ph), 6.66 (d, 1H, J = 1.2 Hz, H4), 5.17 (s, 2H, CH_2N), 5.11 (s, 2H, CH_2NH), 5.03 (s, 2H, NHCH_2-pyridazinone), 2.17 (d, 3H, J = 1.2 Hz, CH_3), 1.46 (s, 9H, (CH_3)_3), 1.45 (s, 9H, (CH_3)_3), 1.32 (s, 9H, (CH_3)_3), 1.25 (s, 9H, (CH_3)_3); ^13C NMR (CDCl_3): δ = 163.9 (CO), 163.6 (CO), 160.9 (CO), 160.6 (CO), 160.2 (C3), 155.1 (C=N), 154.6 (C=N), 143.7, 141.7, 138.5 (C-Ph), 135.0 (C-Ph), 128.9 (CH-Ph), 128.4 (C4), 127.3 (CH-Ph), 84.2 (C(CH_3)_3), 79.0 (C(CH_3)_3), 78.9 (C(CH_3)_3), 54.5 (CH_2N), 47.3 (CH_2NH), 44.2 (NHCH_2-pyridazinone), 28.4 ((CH_3)_3), 28.4 ((CH_3)_3), 28.0 ((CH_3)_3), 27.8 ((CH_3)_3), 17.7 (CH_3); HRMS (ESI): m/z [M+H]^+ calcd for C_{36}H_{55}N_8O_9, 743.40865; found 743.40521.

2-[4-((N,N′-di(tert-Butoxycarbonyl)guanidine)methyl)benzyl]-5-[(N,N′-di(tert-Butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (54). White solid; yield 61%; R_f = 0.4 (50% EtOAc/hexane); m.p. = 166.9 –167.5 °C; ^1H NMR (CDCl_3): δ =9.64 - 8.94 (m, 4H, 4xNH), 7.75 (d, 2H, J = 2.2 Hz, H6), 7.31 (d, 2H, J = 8.2 Hz, H-Ph), 7.20 (d, 2H, J = 8.2 Hz, H-Ph), 6.74 - 6.71 (m, 1H, H4), 5.29 (s, 2H, CH_2N), 5.14 (s, 2H, CH_2NH), 4.99 (s, 2H, NHCH_2-pyridazinone), 1.47 (s, 9H, (CH_3)_3), 1.46 (s, 9H, (CH_3)_3), 1.41 (s,
9H, (CH₃)₃), 1.32 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.9 (CO), 163.4 (CO), 160.9 (CO), 160.3 (CO), 160.0 (C3), 155.1 (C=N), 154.2 (C=N), 143.5 (C5), 138.7 (C-Ph), 136.7 (C6), 135.1 (C-Ph), 128.5 (CH-Ph), 127.5 (CH-Ph), 126.3 (C4), 85.5 (C(CH₃)₃), 84.2 (C(CH₃)₃), 79.0 (C(CH₃)₃), 79.0 (C(CH₃)₃), 54.6 (CH₂N), 47.4 (CH₂NH), 44.5 (NHCH₂-pyridazinone), 28.4 ((CH₃)₃), 28.4 ((CH₃)₃), 28.1 ((CH₃)₃), 28.0 ((CH₃)₃); HRMS (ESI): m/z [M+H]+ calcd for C₃₅H₅₃N₈O₉, 729.39172; found 729.39300.

2-[4-((N,N’-di(tert-Butoxycarbonyl)guanidine)methyl)benzyl]-4-[(N,N’-di(tert-butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (55). Colourless oil; yield 70%; Rᵢ = 0.6 (50% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 9.55 – 9.16 (m, 4H, 4xNH), 7.71 (d, 1H, J = 4.1 Hz, H6), 7.33 (d, 2H, J = 8.2 Hz, H-Ph), 7.20 (d, 2H, J = 8.2 Hz, H-Ph), 6.85 (dt, 1H, J = 4.1, 1.3 Hz, H5), 5.31 (s, 2H, CH₂N), 5.17 – 5.11 (m, 4H, NHCH₂-pyridazinone, CH₂NH), 1.47 (s, 9H, (CH₃)₃), 1.44 (s, 9H, (CH₃)₃), 1.33 (s, 9H, (CH₃)₃), 1.29 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.9 (CO), 163.4 (CO), 160.9 (CO), 160.3 (CO), 160.0 (C3), 155.1 (C=N), 154.2 (C=N), 143.5 (C5), 138.7 (C-Ph), 136.7 (C6), 135.1 (C-Ph), 128.5 (CH-Ph), 127.5 (CH-Ph), 126.3 (C4), 85.5 (C(CH₃)₃), 84.2 (C(CH₃)₃), 79.0 (C(CH₃)₃), 79.0 (C(CH₃)₃), 54.6 (CH₂N), 47.4 (CH₂NH), 44.5 (NHCH₂-pyridazinone), 28.4 ((CH₃)₃), 28.4 ((CH₃)₃), 28.1 ((CH₃)₃), 28.0 ((CH₃)₃); HRMS (ESI): m/z [M+H]+ calcd for C₃₅H₅₃N₈O₉, 729.39172; found 729.39231.

2-Benzyl-6-(hydroximethyl)-5-methylpyridazin-3(2H)-one (58). Following a similar procedure as that used for the synthesis of 44-46, compound 58 was obtained as a white solid (145 mg, 83%) from 29 (370 mg, 0.79 mmol) and TBAF (0.95 mmol, 1M in THF) in THF (15 mL) after purification by column chromatography on silica gel (EtOAc). Rᵢ = 0.4 (EtOAc); m.p. = 102.7 – 103.2 °C; ¹H NMR (CDCl₃): δ = 7.38 - 7.35 (m, 2H, H-Ph), 7.32 – 7.24 (m, 3H, H-Ph), 6.69 (s, 1H, H4), 5.26 (s, 2H, CH₂N), 4.54 (s, 2H, CH₂O), 3.55 (br s, 1H, OH), 2.17 (s, 3H, CH₃); ¹³C NMR (CDCl₃): δ = 160.4 (C3), 145.4, 142.0, 136.2 (C-Ph), 128.9, 128.7, 128.0 (CH-Ph), 61.6 (CH₂O), 54.7 (CH₂N), 17.0 (CH₃); HRMS (ESI): m/z [M+H]+ calcd for C₁₅H₁₅N₂O₂, 231.11280; found 231.11237.

2-Benzyl-6-bromomethyl-5-methylpyridazin-3(2H)-one (65). Following a similar procedure as that used for the synthesis of 48-51, compound 65 was obtained as a white solid (117 mg, 67%) from 58 (138 mg, 0.60 mmol) CBr₄ (396 mg, 1.20 mmol) and Ph₃P
(316 mg, 1.20 mmol) in CH₂Cl₂ (10 mL) after purification by column chromatography on silica gel (40% EtOAc/hexane). R_f = 0.5 (50% EtOAc/hexane); m.p. = 99.8 – 100.2 °C; ¹H NMR (CDCl₃): δ = 7.44 - 7.35 (m, 2H, H-Ph), 7.36 - 7.22 (m, 3H, H-Ph), 6.72 (d, 1H, J = 1.1 Hz, H4), 5.27 (s, 2H, CH₂N), 4.36 (s, 2H, CH₂Br), 2.29 (d, 3H, J = 1.1 Hz, CH₃); ¹³C NMR (CDCl₃): δ = 160.0 (C3), 143.4, 142.7, 136.1 (C-Ph), 129.0 (CH-Ph), 128.7, 128.6, 128.0 (CH-Ph), 55.0 (CH₂N), 29.3 (CH₂Br), 18.0 (CH₃); HRMS (ESI): m/z [M+H]^+ calcd for C₁₃H₁₄BrN₂O, 293.02840; found 293.02851.

**General procedure to synthesize compounds 70-71.** A solution of compound 25 or 31 (0.11 mmol) in THF (3 mL) was added, dropwise at 0 °C, to a suspension of NaH (0.17 mmol, 60% dispersion in mineral oil) in THF (2 mL). After the mixture was stirred at room temperature for 1 h, α,α’-dibromo-p-xylene (0.12 mmol) and Bu₄NI (0.01 mmol) were added. The reaction mixture was stirred at room temperature for 5 h, followed by quenching with MeOH. The solvent was evaporated to dryness, and the residue was purified by column chromatography on silica gel (20% EtOAc/hexane for 70 and 10% EtOAc/hexane for 71) to afford the desired compound.

**2-((4-bromomethyl)benzyl)-6-(tert-butyldiphenylsililoximethyl)-5-methylpyridazin-3(2H)-one (70).** Colourless oil; yield 52%; R_f = 0.5 (50% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 7.69 - 7.63 (m, 4H, H-Ph), 7.49 - 7.41 (m, 2H, H-Ph), 7.41 - 7.29 (m, 8H, H-Ph), 6.72 (d, 1H, J = 1.1 Hz, H4), 5.18 (s, 2H, CH₂N), 4.65 (s, 2H, CH₂O), 4.45 (s, 2H, CH₂Br), 2.33 (d, 3H, J = 1.1 Hz, CH₃), 1.07 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 160.2 (C3), 146.0, 143.5, 137.2 (C-Ph), 136.8 (C-Ph), 135.6 (CH-Ph), 132.9 (C-Ph), 129.9 (CH-Ph), 129.2 (CH-Ph), 129.1 (CH-Ph), 128.6 (C4), 127.8 (CH₂-Ph), 64.5 (CH₂O), 54.2 (CH₂N), 33.2 (CH₂Br), 26.8 ((CH₃)₃), 19.3 (C(CH₃)₃), 17.9 (CH₃); HRMS (ESI): m/z [M+H]^+ calcd for C₃₀H₃₄BrN₂O₂Si, 561.15674; found 561.15645.

**2-((4-bromomethyl)benzyl)-4-(tert-butyldiphenylsililoximethyl)pyridazin-3(2H)-one (71).** Colourless oil; yield 50%; R_f = 0.3 (10% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 7.85 (d, 1H, J = 4.0 Hz, H6), 7.66 - 7.61 (m, 4H, H-Ph), 7.53 - 7.49 (m, 1H, H5), 7.45 - 7.31 (m, 10H, H-Ph), 5.28 (s, 2H, CH₂N), 4.73 (d, 2H, J = 1.8 Hz, CH₂O), 4.45 (s, 2H, CH₂Br), 1.12 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 159.4 (C3), 143.5 (C4), 137.6 (C-Ph), 136.8 (C-Ph), 136.6 (C6), 135.6 (CH-Ph), 132.8 (C-Ph), 130.1 (CH-Ph), 129.4 (CH-Ph), 129.4 (CH-Ph), 128.0 (CH-Ph), 125.4 (C5), 60.9 (CH₂O), 54.8 (CH₂N), 33.2
(CH₂Br), 27.0 ((CH₃)₃), 19.5 (C(CH₃)₃); HRMS (ESI): m/z [M+H]+ calcd for C₂₉H₃₂BrN₂O₂Si, 547.14109; found 547.14111.

2-((4-Fluoromethyl)benzyl)-6-hydroxymethyl-5-methylpyridazin-3(2H)-one (72).
Following a similar procedure as that used for the synthesis of 44-46, compound 72 was obtained as a white solid (141 mg, 68%) from 70 (207 mg, 0.79 mmol) and TBAF (0.95 mmol, 1 M in THF) in THF (15 mL) after purification by column chromatography on silica gel (70% EtOAc/hexane). R_f = 0.3 (EtOAc); m.p.: 118.8 – 120.0 °C; ¹H NMR (CDCl₃): δ = 7.40 – 7.34 (m, 2H, H-Ph), 7.31 – 7.22 (m, 2H, H-Ph), 6.67 (d, 1H, J = 1.2 Hz, H₄), 5.30 (d, 2H, J_HF = 47.7 Hz, CH₂F), 5.25 – 5.21 (m, 2H, CH₂N), 4.53 (s, 2H, CH₂O), 3.46 (br s, 1H, OH), 2.16 (d, 3H, J = 1.2 Hz, CH₃); ¹³C NMR (CDCl₃): δ = 160.3 (C₃), 146.2, 142.5, 136.8 (C-Ph, J_CF = 3.4 Hz), 135.9 (C-Ph, J_CF = 15.5 Hz), 128.8 (CH-Ph), 128.7 (C₄), 127.7 (CH-Ph, J_CF = 5.9 Hz), 84.2 (CH₂F, J_CF = 166.1 Hz), 61.7 (CH₂O), 54.3 (CH₂N), 17.1 (CH₃); ¹⁹F NMR (CDCl₃): δ = -207.1 (CH₂F); HRMS (ESI): m/z [M+H]+ calcd for C₁₄H₁₆FN₂O₂, 263.11903; found 263.11913.

4-hydroxymethyl-2-(4-methoxymethyl)benzylpyridazin-3(2H)-one (73). A mixture of compound 71 (33 mg, 0.06 mmol) and TMSBr (18 mg, 0.12 mmol) in MeOH (2 mL) was stirred at reflux for 9 h followed by quenching with saturated aq. NaHCO₃ (0.5 mL) and H₂O (5 mL). The mixture was extracted with EtOAc (3x5 mL) and the combined organic phases were dried over Na₂SO₄, filtered and the solvent was evaporated to dryness. The residue was purified by column chromatography on silica gel (50% EtOAc/hexane) to afford compound 73 (12.1 mg, 75%) as a white solid. R_f = 0.3 (50% EtOAc/hexane); m.p.= 84.3 – 84.9 ºC; ¹H NMR (CDCl₃): δ = 7.79 (d, 1H, J = 4.0 Hz, H₆), 7.40 (d, 2H, J = 8.1 Hz, H-Ph), 7.29 (d, 2H, J = 8.1 Hz, H-Ph), 7.19 (dt, 1H, J = 4.0, 1.3 Hz, H₅), 5.32 (s, 2H, CH₂N), 4.62 (s, 2H, CH₂OH), 4.42 (s, 2H, CH₂OCH₃), 3.36 (s, 3H, CH₃), 3.13 (br s, 1H, OH); ¹³C NMR (CDCl₃): δ = 160.5 (C₃), 142.0 (C₄), 138.2 (C-Ph), 136.7 (C₆), 135.5 (C-Ph), 129.0 (CH-Ph), 128.1 (CH-Ph), 126.4 (C₅), 74.5 (CH₂OCH₃), 61.0 (CH₂OH), 58.3 (CH₃), 55.1 (CH₂N); HRMS (ESI): m/z [M+H]+ calcd for C₁₄H₁₇N₂O₃, 261.12337; found 261.12284.

6-Bromomethyl-2-((4-fluoromethyl)benzyl)-5-methylpyridazin-3(2H)-one (74).
Following a similar procedure as that used for the synthesis of 48-51, compound 74 (23 mg, 70%) was obtained as a colourless oil from 72 (26 mg, 0.10 mmol) CBr₄ (199 mg, 0.60 mmol) and PPh₃ (158 mg, 0.60 mmol) in CH₂Cl₂ (5 mL) after purification by column chromatography on silica gel (30% EtOAc/hexane). Rₛ = 0.5 (EtOAc); ¹H NMR (CDCl₃): δ = 7.45 – 7.37 (m, 2H, H-Ph), 7.36 – 7.29 (m, 2H, H-Ph), 6.71 (d, 1H, J = 1.1 Hz, H₄), 5.33 (d, 2H, JHF = 47.7 Hz, CH₂F), 5.27 (s, 2H, CH₂N), 4.35 (s, 2H, CH₂Br), 2.29 (d, 3H, J = 1.1 Hz, CH₃); ¹³C NMR (CDCl₃): δ = 160.0 (C₃), 143.6, 142.7, 136.7 (C-Ph, JCF = 3.0 Hz), 136.0 (C-Ph), 134.6, 142.7, 136.7 (C-Ph, JCF = 16.9 Hz), 129.1, 129.0, 127.8 (CH₂N), 18.0 (CH₃); ¹⁹F NMR (CDCl₃): δ = -207.3 (CH₂F); HRMS (ESI): m/z [M+H]+ calcd for C₁₄H₁₅BrFN₂O, 325.02743; found 325.02685.

4-Bromomethyl-2-(4-methoxymethyl)benzylpyridazin-3(2H)-one (75). Following a similar procedure as that used for the synthesis of 48-51, compound 75 (30 mg, 94%) was obtained as a colourless oil from 73 (26 mg, 0.10 mmol) CBr₄ (199 mg, 0.60 mmol) and PPh₃ (158 mg, 0.60 mmol) in CH₂Cl₂ (5 mL) after purification by column chromatography on silica gel (30% EtOAc/hexane). Colourless oil; yield 94%; Rₛ = 0.5 (50% EtOAc/hexane); ¹H NMR (CDCl₃): δ = 7.76 (d, 1H, J = 4.1 Hz, H₆), 7.42 (d, 2H, J = 8.1 Hz, H-Ph), 7.32 – 7.27 (m, 3H, H₅, H-Ph), 5.34 (s, 2H, CH₂N), 4.42 (s, 2H, CH₂O), 4.39 (d, 2H, J = 0.7 Hz, CH₂Br), 3.36 (s, 3H, CH₃); ¹³C NMR (CDCl₃): δ = 159.5 (C₃), 139.0 (C₄), 138.3 (C-Ph), 136.0 (C₆), 135.4 (C-Ph), 129.7 (C₅), 129.1 (C-Ph), 128.1 (CH₂N), 74.5 (CH₂O), 58.3 (CH₃), 55.5 (CH₂N), 26.5 (CH₂Br); HRMS (ESI): m/z [M+H]+ calcd for C₁₄H₁₆BrN₂O₂, 325.03847; found 325.03847.

General procedure to synthesize compounds 76-78 and 80-83. To a solution of compound 63-69 (0.25 mmol) in DMF (1.5 mL) was added 1,3-bis(tert-butoxycarbonyl)guanidine (0.28 mmol) and K₂CO₃ (0.38 mmol) and the reaction was stirred at 50 °C for 2 h. The reaction mixture was diluted with EtOAc (40 mL), washed with water (10 mL), brine (2x10 mL) and dried over anhydrous MgSO₄. The solvent was evaporated to dryness and the residue was purified by column chromatography on silica gel (50% EtOAc/hexane for 76-78 and 80-82 and 20% EtOAc/hexane for 83) to afford the desired compound.
2-Methyl-6-[(N,N’-di(tert-butoxycarbonyl)guanidine)methylpyridazin-3(2H)-one (76). White solid; yield 57%; \( R_f = 0.4 \) (EtOAc); m.p. = 162.0 – 164.0 °C; \(^1\)H NMR (CDCl\(_3\)): \( \delta = 9.50 - 9.10 \) (m, 2H, 2xNH), 7.21 (d, 1H, \( J = 9.5 \) Hz, H5), 6.85 (d, 1H, \( J = 9.5 \) Hz, H4), 5.04 (s, 2H, CH\(_2\)), 3.69 (s, 3H, CH\(_3\)N), 1.43 (s, 9H, (CH\(_3\))\(_3\)), 1.36 (s, 9H, (CH\(_3\))\(_3\)); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 163.3 \) (CO), 160.3, 160.2, 154.4 (C=N), 144.1 (C6), 131.2 (C5), 129.5 (C4), 84.5 (C(CH\(_3\))\(_3\)), 79.1 (C(CH\(_3\))\(_3\)), 46.3 (CH\(_2\)), 40.1 (CH\(_3\)N), 28.2 ((CH\(_3\))\(_3\)), 27.8 ((CH\(_3\))\(_3\)); HRMS (ESI): \( m/z \) [M+H]\(^+\) calcd for C\(_{17}\)H\(_{28}\)N\(_5\)O\(_5\), 382.2090; found 382.2072.

2,5-di-Methyl-6-[(N,N’-di(tert-butoxycarbonyl)guanidine)methylpyridazin-3(2H)-one (77). White solid; yield 55%; \( R_f = 0.6 \) (EtOAc); m.p. = 148.0 – 150.0 °C; \(^1\)H NMR (CDCl\(_3\)): \( \delta = 9.51 - 9.23 \) (m, 2H, 2xNH), 6.66 (s, 1H, H4), 5.06 (s, 2H, CH\(_2\)), 3.66 (s, 3H, CH\(_3\)N), 2.19 (s, 3H, CH\(_3\)), 1.44 (s, 9H, (CH\(_3\))\(_3\)), 1.36 (s, 9H, (CH\(_3\))\(_3\)); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 163.5 \) (CO), 160.6, 160.4, 154.6 (C=N), 143.3, 141.7, 127.7 (C4), 84.0 (C(CH\(_3\))\(_3\)), 78.9 (C(CH\(_3\))\(_3\)), 44.1 (CH\(_2\)), 39.8 (CH\(_3\)N), 28.2 ((CH\(_3\))\(_3\)), 27.7 ((CH\(_3\))\(_3\)), 17.6 (CH3); HRMS (ESI): \( m/z \) [M+Na]\(^+\) calcd for C\(_{18}\)H\(_{29}\)N\(_5\)O\(_5\)Na, 418.2066; found 418.2065.

2-Benzyl-5-methyl-6-[(N,N’-di(tert-butoxycarbonyl)guanidine)methylpyridazin-3(2H)-one (78). Yellow oil; yield 59%; \( R_f = 0.5 \) (EtOAc); \(^1\)H NMR (CDCl\(_3\)): \( \delta = 9.57 - 9.20 \) (m, 2H, 2xNH), 7.35 - 7.30 (m, 2H, H-Ph), 7.27 - 7.18 (m, 3H, H-Ph), 6.66 (s, 1H, H4), 5.18 (s, 2H, CH\(_2\)N), 5.03 (s, 2H, CH\(_2\)NH), 2.16 (s, 3H, CH\(_3\)), 1.44 (s, 9H, (CH\(_3\))\(_3\)), 1.25 (s, 9H, (CH\(_3\))\(_3\)); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 163.3 \) (CO), 160.5 (CO), 160.0 (C3), 154.4 (C=N), 143.4, 141.4, 136.2 (C-Ph), 129.0 (CH-Ph), 128.4, 128.3, 127.7 (CH-Ph), 84.0 (C(CH\(_3\))\(_3\)), 78.9 (C(CH\(_3\))\(_3\)), 54.4 (CH\(_2\)N), 44.1 (CH\(_2\)NH), 28.2 ((CH\(_3\))\(_3\)), 27.7 ((CH\(_3\))\(_3\)), 17.6 (CH3); HRMS (ESI): \( m/z \) [M+Na]\(^+\) calcd for C\(_{24}\)H\(_{33}\)N\(_5\)O\(_5\)Na, 418.2066; found 418.2065.

2-Methyl-5-[(N,N’-di(tert-butoxycarbonyl)guanidine)methylpyridazin-3(2H)-one (80). White solid; yield 94%; \( R_f = 0.3 \) (50% EtOAc/hexane); m.p. = 143.0 – 145.0 °C; \(^1\)H NMR (CDCl\(_3\)): \( \delta = 9.47 - 9.01 \) (m, 2H, 2xNH), 7.68 (d, 1H, \( J = 2.2 \) Hz, H6), 6.67 – 6.63 (m, 1H, H4), 4.95 (s, 2H, CH\(_2\)), 3.70 (s, 3H, CH\(_3\)N), 1.41 (s, 9H, (CH\(_3\))\(_3\)), 1.38 (s, 9H, (CH\(_3\))\(_3\)); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta = 163.1 \) (CO), 160.6 (C3), 159.8 (CO), 154.0 (C=N), 143.4 (C5), 136.1 (C6), 125.4 (C4), 85.3 (C(CH\(_3\))\(_3\)), 79.3 (C(CH\(_3\))\(_3\)), 44.3 (CH\(_2\)), 39.9 (CH\(_3\)),
28.2 ((CH₃)₃), 27.9 ((CH₃)₃); HRMS (ESI): m/z [M+H]+ calc for C₁₇H₂₈N₅O₅, 382.2090; found 382.2088.

2-Methyl-4-[(N,N’-di(tert-butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (81). White solid; yield 84%; Rₛ = 0.5 (50% EtOAc/hexane); m.p. = 115.0 – 118.0 °C; 'H NMR (CDCl₃): δ = 9.54 - 9.06 (m, 2H, 2xNH), 7.67 (d, 1H, J = 4.1 Hz, H6), 6.85 (d, 1H, J = 4.1 Hz, H5), 5.13 (s, 2H, CH₂), 3.79 (s, 3H, CH₃N), 1.42 (s, 9H, (CH₃)₃), 1.36 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.6 (CO), 160.2 (CO), 160.0 (C₃), 154.5 (C=N), 140.1 (C₄), 136.6 (C₆), 124.8 (C₅), 84.6 (C(CH₃)₃), 79.1 (C(CH₃)₃), 43.2 (CH₂), 40.0 (CH₃N), 28.2 ((CH₃)₃), 27.8 ((CH₃)₃); HRMS (ESI): m/z [M+H]+ calc for C₁₇H₂₈N₅O₅, 382.2090; found 382.2100.

2-Benzyl-5-[(N,N’-di(tert-butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (82). White solid; yield 90%; Rₛ = 0.5 (50% EtOAc/hexane); m.p. = 145.0 – 148.0 °C; 'H NMR (CDCl₃): δ = 9.56 – 9.11 (m, 2H, 2xNH), 7.73 (d, 1H, J = 2.1 Hz, H₆), 7.39 - 7.34 (m, 2H, H-Ph), 7.32 - 7.21 (m, 3H, H-Ph) 6.76 – 6.73 (m, 1H, H₄), 5.29 (s, 2H, CH₂N), 4.94 (s, 2H, CH₂NH), 1.42 (s, 9H, (CH₃)₃), 1.39 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.2 (CO), 160.1 (C₃), 159.8 (CO), 154.0 (C=N), 143.3 (C₅), 136.6 (C₆), 136.2 (C-Ph), 128.6 (CH-Ph), 128.5 (CH-Ph), 127.9 (CH-Ph), 126.1 (C₄), 85.3 (C(CH₃)₃), 79.2 (C(CH₃)₃), 54.8 (CH₂N), 44.3 (CH₂NH), 28.2 ((CH₃)₃), 27.9 ((CH₃)₃); HRMS (ESI): m/z [M+H]+ calc for C₂₃H₃₂N₅O₅, 458.2402.

2-Benzyl-4-[(N,N’-di(tert-butoxycarbonyl)guanidine)methyl]pyridazin-3(2H)-one (83). White solid; yield 83%; Rₛ = 0.2 (20% EtOAc/hexane); m.p. = 128.0 – 129.0 °C; 'H NMR (CDCl₃): δ = 9.51 - 9.11 (m, 2H, 2xNH), 7.70 (d, 1H, J = 4.1 Hz, H₆), 7.42 - 7.38 (m, 2H, H-Ph), 7.32 - 7.23 (m, 3H, H-Ph), 6.84 (d, 1H, J = 4.1 Hz, H₅), 5.32 (s, 2H, CH₂N), 5.13 (s, 2H, CH₂NH), 1.50 (s, 6H, 2xCH₃), 1.42 (s, 6H, 2xCH₃), 1.27 (s, 6H, 2xCH₃); ¹³C NMR (CDCl₃): δ = 163.5 (CO), 160.2 (CO), 159.6 (C₃), 154.3 (C=N), 140.9 (C₄), 136.2 (C-Ph), 135.9 (C₆), 128.7 (CH-Ph), 128.5 (CH-Ph), 127.8 (CH-Ph), 124.8 (C₅), 84.6 (C(CH₃)₃), 79.1 (C(CH₃)₃), 55.3 (CH₂N), 43.0 (CH₂NH), 28.2 ((CH₃)₃), 28.0 ((CH₃)₃), 27.7 ((CH₃)₃); HRMS (ESI): m/z [M+Na]+ calc for C₂₃H₃₁N₅O₅Na, 480.2223; found 480.2218.
2-[4-(Fluoromethyl)benzyl]-6-[(N,N’-di(tert-butoxycarbonyl)guanidine)methyl]-5-methylpyridazin-3(2H)-one (79). Following a similar procedure as that used for the synthesis of 52-55, compound 79 (15 mg, 60%) was obtained as a white solid from 74 (16 mg, 0.05 mmol) 1,3-bis(tert-butoxycarbonyl)guanidine (73 mg, 0.28 mmol) and K₂CO₃ (15 mg, 0.11 mmol) in DMF (2 mL) after purification by column chromatography on silica gel (30% EtOAc/hexane). R_f = 0.2 (50% EtOAc/hexane); m.p. = 64.1 – 65.0 °C; ¹H NMR (CDCl₃): δ = 9.56 – 9.21 (m, 2H, 2xNH), 7.36 (d, 2H, J = 7.9 Hz, H-Ph), 7.25 (d, 2H, J = 7.9 Hz, H-Ph), 6.65 (d, 1H, J = 1.0 Hz, H4), 5.28 (d, 2H, J_HF = 47.8 Hz, CH₂F), 5.18 (s, 2H, CH₂N), 5.02 (s, 2H, CH₂NH), 2.16 (d, 3H, J = 1.0 Hz, CH₃), 1.43 (s, 9H, (CH₃)₃), 1.25 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.5 (CO), 160.6 (CO), 160.1 (C₃), 154.6 (C=N), 143.7, 141.6, 136.9 (C-Ph, J_CF = 3.5 Hz), 135.9 (C-Ph, J_CF = 17.4 Hz), 129.4 (CH-Ph), 128.4 (C₄), 127.8 (CH-Ph, J_CF = 5.9 Hz), 84.3 (CH₂F, J_CF = 166.6 Hz), 84.1 (C(CH₃)₃), 79.0 (C(CH₃)₃), 54.2 (CH₂N), 44.2 (CH₂NH), 28.3 ((CH₃)₃), 27.8 ((CH₃)₃), 17.7 (CH₃); ¹⁹F NMR (CDCl₃): δ = -207.1 (CH₂F); HRMS (ESI): m/z [M+H]^+ calcd for C₂₅H₃₅FN₅O₅, 504.26110; found 504.26110.

4-[4-(N,N’-di(tert-Butoxycarbonyl)guanidine)methyl]-2-(4-methoxymethyl)benzylpyridazin-3(2H)-one (84). Following a similar procedure as that used for the synthesis of 52-55, compound 84 (15 mg, 62%) was obtained as a white solid from 75 (16 mg, 0.05 mmol), 1,3-bis(tert-butoxycarbonyl)guanidine (73 mg, 0.28 mmol) and K₂CO₃ (15 mg, 0.11 mmol) in DMF (2 mL) after purification by column chromatography on silica gel (20% EtOAc/hexane). R_f = 0.6 (50% EtOAc/hexane); m.p. = 134.3 – 135.0 °C; ¹H NMR (CDCl₃): δ = 9.54 – 9.17 (m, 2H, 2xNH), 7.72 (d, 1H, J = 4.1 Hz, H6), 7.40 (d, 2H, J = 8.1 Hz, H-Ph), 7.27 (d, 2H, J = 8.1 Hz, H-Ph), 6.85 (d, 1H, J = 4.1 Hz, H5), 5.33 (s, 2H, CH₂N), 5.14 (s, 2H, CH₂NH), 4.42 (s, 2H, CH₂O), 3.35 (s, 3H, CH₃O), 1.43 (s, 9H, (CH₃)₃), 1.29 (s, 9H, (CH₃)₃); ¹³C NMR (CDCl₃): δ = 163.7 (CO), 160.4 (CO), 159.7 (C₃), 154.5 (C=N), 141.0 (C₄), 138.1 (C-Ph), 136.1 (C₆), 135.8 (C-Ph), 129.0 (CH-Ph), 128.0 (CH-Ph), 124.9 (C₅), 84.8 (C(CH₃)₃), 79.3 (C(CH₃)₃), 74.4 (CH₂O), 58.2 (CH₂O), 55.1 (CH₂N), 43.2 (CH₂NH), 28.4 ((CH₃)₃), 27.9 ((CH₃)₃); HRMS (ESI): m/z [M+H]^+ calcd for C₂₅H₃₆FN₅O₆, 502.26617; found 502.26617.

General procedure to synthesize guanidinium salts 1-4 and 5-14. To a solution of compound 52-55 or 76-84 (0.13 mmol) in 1,4-dioxane, a solution HCl 4M in 1,4-dioxane
(0.78 mmol HCl per Boc group) was added to reach a final concentration of 0.2 M. The reaction mixture was stirred at 55 °C for 5 h. After the solvent was removed, the residue was purified by reverse phase chromatography (H₂O) to afford the corresponding hydrochloride.

**Dihydrochloride salt of 2-(4-guanidinomethyl)benzyl-6-guanidinomethylpyridazin-3(2H)-one (1).** White solid; yield 80%; m.p. = 159.6 – 159.9 °C; ¹H NMR (CD₃OD): δ = 7.46 (d, 1H, J = 9.5 Hz, H₅), 7.44 (d, 2H, J = 8.2 Hz, H-Ph), 7.34 (d, 2H, J = 8.2 Hz, H-Ph), 7.05 (d, 1H, J = 9.5 Hz, H₄), 5.35 (s, 2H, CH₂N), 4.45 (s, 2H, NHCH₂-pyridazinone), 4.42 (s, 2H, CH₂NH); ¹³C NMR (CD₃OD): δ = 161.7 (C₃), 159.3 (C=N), 158.7 (C=N), 145.0 (C₆), 137.5 (C-Ph), 137.3 (C-Ph), 133.3 (C₅), 131.4 (C₄), 130.0 (CH-Ph), 128.6 (CH-Ph), 56.2 (CH₂N), 45.6 (CH₂NH), 44.4 (NHCH₂-pyridazinone); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₅H₂₁N₈O, 329.18328; found 329.18293; HPLC: 94.2% (tR: 2.78 min).

**Dihydrochloride salt of 2-(4-guanidinomethyl)benzyl-6-guanidinomethyl-5-methylpyridazin-3(2H)-one (2).** White solid; yield 83%; m.p. = 163.0 – 163.5 °C; ¹H NMR (CD₃OD): δ = 7.44 (d, 2H, J = 8.1 Hz, H-Ph), 7.34 (d, 2H, J = 8.1 Hz, H-Ph), 6.84 (d, 1H, J = 1.0 Hz, H₄), 5.33 (s, 2H, CH₂N), 4.47 (s, 2H, NHCH₂-pyridazinone), 4.42 (s, 2H, CH₂NH), 2.27 (d, 3H, J = 1.0 Hz, CH₃); ¹³C NMR (CD₃OD): δ = 162.1 (C₃), 159.3 (C=N), 158.7 (C=N), 145.0, 144.4, 137.4 (2xC-Ph), 130.0 (CH-Ph), 129.3 (C₄), 128.6 (CH-Ph), 55.7 (CH₂N), 45.6 (CH₂NH), 43.1 (NHCH₂-pyridazinone), 17.3 (CH₃); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₆H₂₃N₈O, 343.19893; found 343.19848; HPLC: 99.0% (tR: 2.95 min).

**Dihydrochloride salt of 2-(4-guanidinomethyl)benzyl-5-guanidinomethylpyridazin-3(2H)-one (3).** White solid; yield 85%; m.p. = 151.0 – 151.7 °C; ¹H NMR (CD₃OD): δ = 7.94 (d, 1H, J = 2.1 Hz, H₆), 7.43 (d, 2H, J = 8.2 Hz, H-Ph), 7.33 (d, 2H, J = 8.2 Hz, H-Ph), 6.84 (dt, 1H, J = 2.1, 1.1 Hz, H₄), 5.36 (s, 2H, CH₂N), 4.44 (d, 2H, J = 1.1 Hz, NHCH₂-pyridazinone), 4.42 (s, 2H, CH₂NH); ¹³C NMR (CD₃OD): δ = 162.0 (C₃), 159.0 (C=N), 158.7 (C=N), 144.8 (C₅), 138.2 (C₆), 137.5 (C-Ph), 137.4 (C-Ph), 130.0 (CH-Ph), 128.6 (CH-Ph), 125.9 (C₄), 55.9 (CH₂N), 45.6 (CH₂NH), 42.3 (NHCH₂-
Dihydrochloride salt of 2-(4-guanidinomethyl)benzyl-4-guanidinomethylpyridazin-3(2H)-one (4). White solid; yield 94%; m.p. = 160.0 – 160.7 °C; ¹H NMR (CD₃OD): δ = 7.99 (d, 1H, J = 4.1 Hz, H6), 7.44 (d, 2H, J = 8.2 Hz, H-Ph), 7.36 (dt, 1H, J = 4.1, 1.1 Hz, H5), 7.33 (d, 2H, J = 8.2 Hz, H-Ph), 5.39 (s, 2H, CH₂N), 4.41 (s, 2H, CH₂NH), 4.35 (d, 2H, J = 1.1 Hz, NHCH₂-pyridazinone); ¹³C NMR (CD₃OD): δ = 161.7 (C3), 159.3 (C=N), 158.7 (C=N), 139.4 (C4), 138.5 (C6), 137.5 (C-Ph), 137.4 (C-Ph), 130.1 (CH-Ph), 129.3 (C5), 128.6 (CH-Ph), 56.1 (CH₂N), 45.6 (CH₂NH), 41.5 (NHCH₂-pyridazinone); HRMS (ESI): m/z [M+H]+ calcd for C₁₅H₂₁N₈O, 329.18296; found 329.18328; HPLC: 99.5% (tR: 3.02 min).

Hydrochloride salt of 6-guanidinomethyl-2-methylpyridazin-3(2H)-one (5). White solid; yield 77%; m.p. = 150.0 – 150.5 °C; ¹H NMR (D₂O): δ = 7.56 (d, 1H, J = 9.5 Hz, H5), 7.12 (d, 1H, J = 9.5 Hz, H4), 4.48 (s, 2H, CH₂), 3.81 (s, 3H, CH₃); ¹³C NMR (D₂O): δ = 162.1 (C3), 157.2 (C=N), 144.7 (C6), 132.6 (C5), 129.6 (C4), 43.1 (CH₂), 40.3 (CH₃); HRMS (ESI): m/z [M+H]+ calcd for C₇H₁₂N₅O, 182.1042; found 182.1038; HPLC: 99.2% (tR: 3.2 min).

Hydrochloride salt of 6-guanidinomethyl-2,5-dimethylpyridazin-3(2H)-one (6). White solid; yield 92%; m.p. = 160.0 – 162.0 °C; ¹H NMR (D₂O): δ = 6.94 (s, 1H, H4), 4.48 (s, 2H, CH₂), 3.78 (s, 3H, CH₃N), 2.27 (s, 3H, CH₃); ¹³C NMR (D₂O): δ = 162.4 (C3), 157.3 (C=N), 144.6, 144.1, 127.5 (C4), 41.9 (CH₂), 39.9 (CH₃N), 16.5 (CH₃); HRMS (ESI): m/z [M+H]+ calcd for C₈H₁₄N₅O, 196.1198; found 196.1205; HPLC: 95% (tR: 8.4 min).

Hydrochloride salt of 2-benzyl-6-guanidinomethyl-5-methylpyridazin-3(2H)-one (7). White solid; yield 86%; m.p. = 140.0 – 140.4 °C; ¹H NMR (D₂O): δ = 7.44 - 7.36 (m, 3H, H-Ph), 7.35 - 7.30 (m, 2H, H-Ph), 6.93 (s, 1H, H4), 5.33 (s, 2H, CH₂N), 4.44 (s, 2H, CH₂NH), 2.25 (s, 3H, CH₃); ¹³C NMR (D₂O): δ = 161.9 (C3), 157.4 (C=N), 144.5, 144.4, 135.7 (C-Ph), 128.7 (CH-Ph), 128.0, 127.9, 127.8 (CH-Ph), 54.7 (CH₂N), 41.7 (CH₂NH),
16.4 (CH3); HRMS (ESI): m/z [M+H]+ calcd for C14H18N5O, 272.1511; found 272.1514; HPLC: 95.6% (tR: 22.7 min).

Hydrochloride salt of 2-(4-fluoromethyl)benzyl-6-guanidinomethyl-5-methylpyridazin-3(2H)-one (8). White solid; yield 33%; m.p. = 131.7 – 132.0 °C; 1H NMR (CD3OD): δ = 7.46 – 7.36 (m, 4H, H-Ph), 6.86 (d, 1H, J = 1.2 Hz, H4), 5.36 (s, 2H, JHF = 47.9 Hz, CH2F), 5.35 (s, 2H, CH2N), 4.46 (s, 2H, CH2NH), 2.27 (d, 3H, J = 1.2 Hz, CH3); 13C NMR (CD3OD): δ = 162.1 (C3), 159.4 (C=N), 144.9, 144.4, 138.1 (C-Ph, JCF = 2.8 Hz), 137.8 (C-Ph, JCF = 17.2 Hz), 85.1 (CH2F, JCF = 164.9 Hz), 55.7 (CH2N), 43.0 (CH2NH), 17.3 (CH3); 19F NMR (CD3OD): δ = -208.7 (CH2F); HRMS (ESI): m/z [M+H]+ calcd for C15H19FN5O, 304.15681; found 304.15664; HPLC: 94.0% (tR: 3.90 min).

Hydrochloride salt of 2-(4-chloromethyl)benzyl-6-guanidinomethyl-5-methylpyridazin-3(2H)-one (9). White solid; yield 46%; m.p. = 128.9 – 129.3 °C; 1H NMR (CD3OD): δ = 7.40 (s, 4H, H-Ph), 6.86 (d, 1H, J = 1.2 Hz, H4), 5.33 (s, 2H, CH2N), 4.64 (s, 2H, CH2Cl), 4.46 (s, 2H, CH2NH), 2.27 (d, 3H, J = 1.2 Hz, CH3); 13C NMR (CD3OD): δ = 160.7 (C3), 158.0 (C=N), 143.5, 143.0, 137.8 (C-Ph), 136.4 (C-Ph), 128.6 (CH-Ph), 128.4 (CH-Ph), 127.9 (C4), 54.2 (CH2N), 45.1 (CH2Cl), 41.6 (CH2NH), 15.9 (CH3); HRMS (ESI): m/z [M+H]+ calcd for C15H19ClN5O, 320.12726; found 320.12648; HPLC: 98.2% (tR: 6.25 min).

Hydrochloride salt of 5-guanidinomethyl-2-methylpyridazin-3(2H)-one (10). White solid; yield 92%; m.p. = 160.0 – 160.5 °C; 1H NMR (D2O): δ = 8.01 (d, 1H, J = 2.1 Hz, H6), 6.98 (d, 1H, J = 2.1 Hz, H4), 4.49 (s, 2H, CH2), 3.80 (s, 3H, CH3); 13C NMR (D2O): δ = 162.2 (C3), 157.1 (C=N), 143.6 (C5), 137.7 (C6), 124.6 (C4), 41.0 (CH2), 39.9 (CH3); HRMS (ESI): m/z [M+H]+ calcd for C7H12N5O, 182.1042; found 182.1047; HPLC: 96.1% (tR: 3.4 min).

Hydrochloride salt of 4-guanidinomethyl-2-methylpyridazin-3(2H)-one (11). White solid; yield 89%; m.p. = 170.0 – 170.6 °C; 1H NMR (D2O): δ = 8.04 (d, 1H, J = 4.3 Hz, H6), 7.47 (dt, 1H, J = 4.3, 1.2 Hz, H5), 4.39 (d, 2H, J = 1.2 Hz, CH2), 3.83 (s, 3H, CH3); 13C NMR (D2O): δ = 161.4 (C3), 157.2 (C=N), 138.3 (C6), 137.6 (C4), 128.6 (C5), 40.0
Hydrochloride salt of 2-benzyl-5-guanidinomethylpyridazin-3(2H)-one (12). White solid; yield 92%; m.p. = 139.0 – 140.0 °C; ¹H NMR (D₂O): δ = 8.03 (d, 1H, J = 2.1 Hz, H6), 7.45 - 7.36 (m, 3H, H-Ph), 7.35 – 7.30 (m, 2H, H-Ph), 7.02 – 6.98 (m, 1H, H4), 5.37 (s, 2H, CH₂N), 4.48 (d, 2H, J = 1.2 Hz, CH₂NH); ¹³C NMR (D₂O): δ = 161.7 (C3), 157.1 (C=N), 143.7 (C5), 138.2 (C6), 135.4 (C-Ph), 128.8 (CH-Ph), 128.1 (CH-Ph), 127.6 (CH-Ph), 125.3 (C4), 55.1 (CH₂N), 41.0 (CH₂NH); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₃H₁₆N₅O, 258.1355; found 258.1366; HPLC: 95.3% (tR: 24.1 min).

Hydrochloride salt of 2-benzyl-4-guanidinomethylpyridazin-3(2H)-one (13). White solid; yield 90%; m.p. = 136.0 – 137.1 °C; ¹H NMR (D₂O): δ = 8.07 (d, 1H, J = 4.2 Hz, H6), 7.49 - 7.46 (m, 1H, H5), 7.45 - 7.33 (m, 5H, H-Ph), 5.41 (s, 2H, CH₂N), 4.36 (d, 2H, J = 1.2 Hz, CH₂NH); ¹³C NMR (D₂O): δ = 161.0 (C3), 157.2 (C=N), 138.7 (C6), 138.4 (C4), 135.5 (C-Ph), 128.8 (CH-Ph), 128.5 (C5), 128.1 (CH-Ph), 127.6 (CH-Ph), 55.4 (CH₂N), 40.0 (CH₂NH); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₃H₁₆N₅O, 258.1355; found 258.1366; HPLC: 95.3% (tR: 24.1 min).

Hydrochloride salt of 4-guanidinomethyl-2-(4-methoxymethyl)benzylpyridazin-3(2H)-one (14). White solid; yield 99%; m.p. = 143.2 – 143.8 °C; ¹H NMR (CD₃OD): δ = 7.98 (d, 1H, J = 4.1 Hz, H6), 7.40 (d, 2H, J = 8.2 Hz, H-Ph), 7.34 (dt, 1H, J = 4.1, 1.2 Hz, H5), 7.31 (d, 2H, J = 8.2 Hz, H-Ph), 5.38 (s, 2H, CH₂N), 4.44 (s, 2H, CH₂O), 4.34 (d, 2H, J = 1.2 Hz, CH₂NH), 3.37 (s, 3H, CH₃); ¹³C NMR (CD₃OD): δ = 161.7 (C3), 159.3 (C=N), 139.4 (C4), 139.4 (C-Ph), 138.4 (C6), 136.9 (C-Ph), 129.6 (CH-Ph), 129.3(C5), 129.1 (CH-Ph), 75.2 (CH₂O), 58.3 (CH₃), 56.3 (CH₂N), 41.5 (CH₂NH); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₅H₂₀N₅O₂, 302.16115; found 302.16018; HPLC: 98.4% (tR: 5.28 min).

Biophysical Studies
DNA thermal denaturation assays.
Thermal melting experiments were conducted with a Varian Cary 300 Bio spectrophotometer equipped with a 6 × 6 multicell temperature-controlled block. Temperature was monitored with a thermistor inserted into a 1 mL quartz cuvette containing the same volume of buffer as in the sample cells. Absorbance changes at 295 nm for st-DNA were monitored from a range of 30 °C to 90 °C with a heating rate of 1 °C/minute and a data collection rate of five points per °C. The stock solution of st-DNA was prepared in phosphate buffer solutions contained 10 mM Na₂HPO₄/NaH₂PO₄ adjusted to pH 7. The stock solution of ligands was prepared in EtOH (1 mM). A quartz cell with a 1 cm path length was filled with a 1 mL solution of DNA (150 μM base) and compound solutions (15 μM) in phosphate buffer, adjusted to pH 7 so that a ligand to DNA base ratio of 0.1 was obtained.

Figure S15. Graph showing the DNA thermal denaturation results of st-DNA alone and after adding compound 1. X-axis represents temperature (°C) and Y axis represents absorbance (cm⁻¹).

Cytotoxicity assays
Cytotoxicity studies on NCI-H460, A2780 and MCF-7 cells were performed by using a colorimetric MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay [8,9,10]. In brief, cells were seeded in 96-well plates (15000 cells per well for NCI-H460 cell line, 4000 for A2780 cell line and 10000 for MCF-7 cell line), incubated for 24 h in the culture medium and treated at 37 °C for 48 h (NCI-H460) and 96 h (A2780...
and MCF-7) with varying doses of evaluated compounds and the reference drug, cisplatin, dissolved in DMSO. Three wells were used for each of the variants tested. Aliquots of MTT solution in phosphate buffered saline (10 µL) were added to each well and incubated for 4 h. The colour formed was quantified by a spectrophotometric plate reader (Tecan Ultra evolution) at 595 nm wavelength. In all experiments, DMSO controls were included. The percentage of inhibition of cell viability was calculated by the formula % inhibition=100-((AO*100)/AT) where AO is the absorbance observed in the treated wells and AT is the absorbance observed in the DMSO control wells.

The cytotoxic potency of compounds, measured as 50% inhibitory concentrations (IC$_{50}$), was calculated from concentration-effect curves by using GraphPad Prism software, version 2.01. Correlation coefficients ($r^2$) were higher than 0.995 for the compounds tested.
$^1$H and $^{13}$C NMR spectra of pyridazin-3(2H)-one-based guanidine derivatives 1-14 and $^{19}$F NMR spectrum of compound 8
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