Antiproliferative Activity of (−)-Isopulegol-based 1,3-Oxazine, 1,3-Thiazine and 2,4-Diaminopyrimidine Derivatives

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1. Experimental Section

1.2. General methods: $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer (500 and 125 MHz, respectively, $\delta=0$ ppm (TMS)). Chemical shifts ($\delta$) are expressed in [ppm] relative to TMS as internal reference. $J$ values are given in [Hz]. HRMS flow injection analysis was performed with a Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer coupled to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK). Optical rotations were determined with a Perkin–Elmer 341 polarimeter. Melting points were determined on a Kofler apparatus and they are uncorrected. Chromatographic separations were carried out on Merck Kieselgel 60 (230–400 mesh ASTM). Reactions were monitored with Merck Kieselgel 60 F$254$-precoated TLC plates (0.25 mm thickness). Commercially available reagents were used as obtained from suppliers (Molar Chemicals Ltd., Halásztelek, Hungary; Merck Ltd., Budapest, Hungary and VWR International Ltd., Debrecen, Hungary), while solvents were dried according to standard procedures.

1.3. Starting materials: (−)-Isopulegol (1) is commercially available from Merck Co with $ee = 95\%$, ([α]$^D_{20} = -22.0$, neat) and its enantiomer (+)-1 ($ee = 90\%$, [α]$^D_{20} = +22.0$, neat). (+)-Neoisopulegol (2) ([α]$^D_{20} = +28.7$, c = 17.2, CHCl$_3$) and its enantiomer (−)-2 ([α]$^D_{20} = -22.2$, c = 2.0, CHCl$_3$) were synthesized from (−)-1 and its isomer (+)-1 following a reported procedure, respectively.$^{[1]}$ (−)-Isopulegol- based aminodiols 2a–b and 6,$^{[2]}$ (+)-neoisopulegol-based aminodiols 10 and 12$^{[3]}$ together with aminotriol 4$^{[2]}$ as well as aminoalcohols 14$^{[4]}$ were prepared according to literature procedures. All spectroscopic data were similar to those described therein.

1.4. Docking Study: Aurora A kinase crystal structure was obtained from PDB (protein data bank). ChemBioDraw Ultra 11.0 was used to design the compound for the docking study. Docking study and In-Silico ADMET prediction were performed by Accelrys discovery studio 2.5 software.

1.4.1. Choosing the template crystal structure: Aiming to choose the most valid crystal structure to be used in our study, we first downloaded the available Aurora A crystal structure from PDB.

1.4.2. Preparation of the crystal structure of Aurora A: It is well known that the extracted crystal structure from PDB does not have hydrogen atoms, so firstly, hydrogen atoms must be added by applying several force fields (CHARMm). Adding hydrogen atoms lead to steric hindrance and subsequently to high energy and unstable molecule, which should be minimised. Minimisation of the crystal structure was performed by using adopted basis minimisation aiming at finding the most stable and less energy structure and reducing H-H interactions without affecting the basic protein skeleton atoms. Then, the active site was determined and the sphere surrounded.$^{[5]}$

1.4.3. Docking study (CDocker): By using CDocker method, we can generate all the possible conformations of the compound in the protein active site. Then the results can be assessed by both the CDocker energy and the number of interactions between the ligand and active site. This method requires preparing the crystal structure (as mentioned before) and preparing the designed compound by using Accelrys Discovery Studio protocol and applying
force field.[6] Before starting this study, it is important to make sure that the used method is valid by comparing the conformation of the reference compound with its conformations generated by the docking method, where RMSD (Root Mean Square Deviation) should not exceed 2A°.

1.5. **Experimental section and compound characterisations**

(3S,3aR,6R,7aR)-6-Methyl-3-(((S)-1-phenylethyl)amino)methyl)octahydrobenzofuran-3-ol (8a) and (3R,3aR,6R,7aR)-6-Methyl-3-(((S)-1-phenylethyl)amino)methyl)octahydrobenzofuran-3-ol (8b)

m-CPBA (70% purity, 5.87 g, 23.8 mmol) was added at 0°C to a solution of 7 (11.9 mmol) in CH₂Cl₂ (50 mL) and Na₂HPO₄·12H₂O (6.35 g, 35.7 mmol) in water (130 mL), and the mixture was stirred at room temperature. When the reaction was complete, as indicated by TLC (2 h), the mixture was separated and the aqueous phase was extracted with CH₂Cl₂ (100 mL). The organic layer was washed with a 5% KOH solution (3 × 50 mL), then dried (Na₂SO₄) and evaporated to provide a 4:1 mixture of epoxides as a pale yellow oil, which was added to the solution of (S)-methylbenzylamine (0.80 mL, 6.20 mmol) in MeCN (30 mL) and LiClO₄ (0.31 g, 2.94 mmol). The mixture was kept at reflux temperature for 6 h. When the reaction was completed (indicated by TLC), the mixture was evaporated to dryness, and the residue was dissolved in water (15 mL) and then extracted with CH₂Cl₂ (3 × 50 mL). The combined organic phase was dried (Na₂SO₄), filtered, and concentrated. The crude product was purified by column chromatography on silica gel with an appropriate solvent mixture (CHCl₃ : MeOH = 19:1) to provide 8a and 8b.

8a: Yellow crystals (33%); m.p. 54–55°C; [α]°D = −51.0 (c 0.1275, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.33-7.22 (m, 5H), 3.80-3.69 (m, 3H), 3.57-3.52 (m, 1H), 3.08 (bs, 1H), 2.60 (q, J = 12.0 Hz, 2H), 2.11-2.08 (m, 1H), 1.76-1.73 (m, 1H), 1.68-1.63 (m, 1H), 1.52-1.35 (m, 3H), 1.36 (d, J = 6.5 Hz, 3H), 1.06-1.00 (m, 1H), 0.96 (d, J = 6.6 Hz, 3H), 0.94-0.86 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 144.9 (C₆), 128.6 (CH), 127.2 (CH), 126.5 (CH), 81.4 (CH), 79.7 (CH), 78.3 (C₆), 58.7 (CH), 54.8 (CH₂), 53.3 (CH), 40.1 (CH₂), 34.3 (CH₂), 30.9 (CH), 24.4 (CH), 22.8 (CH₂), 22.0 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₁₈H₂₈N₂O₂ 290.2042, found 290.2116.

8b: White crystals (8%); m.p. 68–69°C; [α]°D = −46.0 (c 0.21, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.23-7.34 (m, 5H), 3.88-3.86 (m, 1H), 3.75-3.71 (q, J = 6.6 Hz, 1H), 3.64 (d, J = 9.5 Hz, 1H), 3.14 (td, J = 4.0, 11.1 Hz, 1H), 2.56 (q, J = 12.3 Hz, 2H), 2.05-2.02 (m, 1H), 1.82-1.77 (m, 1H), 1.70-1.68 (m, 1H), 1.59-1.54 (m, 1H), 1.43-1.31 (m, 1H), 1.36 (d, J = 6.6 Hz, 3H), 1.10 (q, J = 11.4 Hz, 1H), 0.99-0.86 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 145.1 (C₆), 128.6 (CH), 127.1 (CH), 126.2 (CH), 81.9 (CH), 78.4 (CH₂), 78.2 (CH₂), 58.8 (CH), 55.1 (CH), 51.9 (CH₂), 40.3 (CH₂), 34.6 (CH₂), 31.0 (CH), 30.9 (CH), 23.4 (CH₂), 21.8 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₁₈H₂₈N₂O₂ 290.2042, found 290.2116.

Aminoalcohols 8a–b (14.0 mmol) in MeOH (100 mL) were added to a suspension of palladium-on-carbon (5% Pd, 0.22 g) in MeOH (50 mL), and the mixture was stirred under an
H₂ atmosphere (1 atm) at room temperature. After completion of the reaction (as monitored by TLC, 24 h), the mixture was filtered through a Celite pad, and the solution was evaporated to dryness. The crude product was recrystallised in Et₂O, resulting in primary aminoalcohols 8c–d as white crystals.

8c: White crystals (90%); m.p. 190–192 °C; [α]₂⁰ D = −3.0 (c 0.26, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 7.88 (brs, 2H), 5.28 (brs, 1H), 3.98 (d, J = 9.8 Hz, 1H), 3.60 (d, J = 9.9 Hz, 1H), 3.48-3.43 (m, 1H), 2.95-2.85 (m, 2H), 2.00-1.97 (m, 1H), 1.69-1.66 (m, 2H), 1.44-1.37 (m, 1H), 1.29-1.21 (m, 1H), 1.14-1.09 (m, 1H), 0.97-0.85 (m, 5H); ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) = 80.8 (CH), 78.6 (CH₂), 77.1 (C₆), 52.6 (CH), 46.3 (CH₂), 40.3 (CH₂), 34.3 (CH₂), 30.9 (CH), 22.4 (CH₂), 22.4 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₁₀H₂₀N₂O₂ 186.1416, found 186.1491.

8d: White crystals (87%); m.p. 193–196 °C; [α]₂⁰ D = −12.0 (c 0.14, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 7.97 (brs, 2H), 5.62 (brs, 1H), 3.83 (d, J = 9.6 Hz, 1H), 3.61 (d, J = 9.6 Hz, 1H), 3.23-3.20 (m, 1H), 2.92 (d, J = 13.2 Hz, 1H), 2.81 (d, J = 12.7 Hz, 1H), 1.98-1.94 (m, 1H), 1.83-1.80 (m, 1H), 1.71-1.68 (m, 1H), 1.55-1.49 (m, 1H), 1.44-1.36 (m, 1H), 1.11-1.03 (m, 1H), 1.01 (q, J = 11.2 Hz, 1H), 0.93 (d, J = 6.6 Hz, 3H), 0.88-0.81 (m, 1H); ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) = 81.2 (CH), 77.2 (C₆), 76.7 (CH₂), 56.1 (CH), 44.1 (CH₂), 40.5 (CH₂), 34.7 (CH₂), 30.8 (CH), 22.5 (CH₂), 22.2 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₁₀H₂₀N₂O₂ 186.1416, found 186.1487.

1.5.1. General procedure for the preparation of thioureas (15a–d)

Aminodiols 2a, 6, 10 and 12 (0.53 mmol) and the appropriate phenylisothiocyanate (0.79 mmol) were dissolved in toluene (40 mL), and the mixture was stirred at room temperature for 1 h, except that in the case of 6 when a treatment at reflux temperature for 3 h was carried out. The resulting mixtures were then evaporated then the residue was purified by column chromatography on silica gel (eluted with CHCl₃ : MeOH = 19:1).

1-((S)-2-Hydroxy-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15a): Prepared from 2a. White crystals (95%); m.p. 162–163 °C; [α]₂⁰ D = −33.0 (c 0.28, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 9.79 (s, 1H), 7.51 (d, J = 7.7 Hz, 2H), 7.45 (brs, 1H), 7.30 (t, J = 5.7 Hz, 2H), 7.10-7.06 (m, 1H), 5.74 (s, 1H), 5.62 (s, 1H), 3.65-3.63 (m, 2H), 3.30 (d, J = 3.9 Hz, 1H), 1.83 (d, J = 12.0 Hz, 1H), 1.68 (d, J = 11.2 Hz, 1H), 1.58 (d, J = 12.1 Hz, 1H), 1.40-1.35 (m, 1H), 1.12 (s, 3H), 0.96-0.81 (m, 6H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 128.9 (CH), 124.4 (CH), 123.0 (CH), 71.5 (CH), 53.1 (CH₂), 48.9 (CH), 45.3 (CH₂), 34.6 (CH₂), 31.1 (CH), 26.2 (CH₂), 22.4 (CH₃), 21.8 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₁₇H₂₇N₂O₂S 323.1715, found 323.1788.

1-((R)-3-Hydroxy-2-((1S,2R,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15b): Prepared from 6. White crystals (58%); m.p. 97–98 °C; [α]₂⁰ D = −22.0 (c 0.28, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.77 (brs, 1H), 7.41 (t, J = 7.7 Hz, 2H), 7.28 (t, J = 7.1 Hz, 1H), 7.22 (d, J = 7.1 Hz, 2H), 6.98-6.92 (m, 1H), 4.08-3.99 (m, 1H), 3.72-3.63 (m, 2H), 3.59-3.47 (m, 2H), 3.01 (s, 1H), 2.05-2.04 (m, 1H), 1.93-1.91 (m, 1H), 1.69 (brs, 1H), 1.65-1.61 (m, 2H), 1.46-1.37 (m, 1H), 1.32-1.27 (m, 1H), 1.11-1.04 (m, 1H), 1.00-0.93 (m, 1H), 0.90 (d, J = 6.56 Hz, 3H), 0.88-0.80 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 180.4 (C₆), 130.0 (CH), 127.4 (CH), 125.5(CH), 71.0 (CH), 63.9 (CH₂), 46.4 (CH₂), 45.8 (CH), 44.3 (CH₂),
42.4 (CH), 34.4 (CH\(_2\)), 31.5 (CH), 27.8 (CH\(_2\)), 22.0 (CH\(_3\)); HRMS (ESI): \(m/z\) [M+H]\(^+\) calculated for \(\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_2\text{S}\) 323.1715, found 323.1784.

**I-((S)-2-Hydroxy-2-(((1R,2S,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15c):** Prepared from 10. Yellow oil (61%); \([\alpha]_D^{20} = -5.0\) (c 0.295, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 7.69 (brs, 1H), 7.42 (t, \(J = 7.5\) Hz, 2H), 7.30-7.25 (m, 2H), 6.70 (brs, 1H), 4.39 (s, 1H), 3.79 (dd, \(J = 4.2, 13.8\) Hz, 1H), 3.72-3.52 (m, 1H), 2.05 (brs, 1H), 1.79-1.61 (m, 5H), 1.28 (s, 3H), 1.25-1.24 (m, 1H), 1.15-1.09 (t, \(J = 13.0\) Hz, 1H); 0.95-0.86 (m, 1H), 0.86-0.88 (d, \(J = 6.1\) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 130.1 (CH), 127.3 (CH), 125.2 (CH), 68.1 (CH), 52.9 (CH\(_2\)), 47.2 (CH), 42.8 (CH\(_2\)), 34.6 (CH\(_2\)), 25.7 (CH\(_3\)), 24.9 (CH), 22.0 (CH\(_3\)), 20.4 (CH\(_2\)); HRMS (ESI): \(m/z\) [M+H]\(^+\) calculated for \(\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_2\text{S}\) 323.1715, found 323.1788.

**I-((S)-3-Hydroxy-2-(((1S,2S,5R)-3-phenylthiourea (15d):** Prepared from 12. White crystals (94%); m.p. 50–52 °C; \([\alpha]_D^{20} = +25.0\) (c 0.265, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 7.81 (brs, 1H), 7.40 (t, \(J = 7.8\) Hz, 2H), 7.30-7.27 (t, \(J = 7.4\) Hz, 1H), 7.21 (d, \(J = 7.7\) Hz, 2H), 4.00 (s, 1H), 3.95-3.92 (m, 1H), 3.67-3.58 (m, 3H), 2.34 (brs, 2H), 1.82-1.68 (m, 4H), 1.52-1.37 (m, 3H), 1.15-1.10 (t, \(J = 12.4\) Hz, 1H), 0.94-0.84 (m, 1H), 0.85 (d, \(J = 6.2\) Hz, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 136.2 (Cq), 130.0 (CH), 127.4 (CH), 125.6 (CH), 67.4 (CH), 61.7 (CH\(_2\)), 45.0 (C\(_1\)), 42.9 (CH), 42.6 (CH\(_2\)), 40.5 (CH), 34.8 (CH\(_2\)), 25.8 (CH), 24.0 (CH\(_2\)), 22.1 (CH\(_3\)); HRMS (ESI): \(m/z\) [M+H]\(^+\) calculated for \(\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_2\text{S}\) 323.1715, found 323.1793.

### 1.5.2. General procedure for the preparation of 1,3-thiazines (16a–b)

A solution of thioureas \(15b\) or \(15d\) (0.31 mmol) in dry EtOH (1 mL) was added 22% HCl in EtOH (5 mL) and the mixture was stirred at room temperature for 4 h and then concentrated under vacuum. The residue was treated with 10% KOH in MeOH (20 mL) followed by evaporation, and the crude product was again dissolved in water (10 mL) and extracted with CHCl\(_3\) (3 × 20 mL). The combined organic layer was washed with saturated NaCl aqueous solution (15 mL), dried (Na\(_2\)SO\(_4\)) and concentrated under vacuum. The crude product was purified by column chromatography on silica gel with CHCl\(_3\):MeOH = 19:1.

**(1R,2S,5R)-5-Methyl-2-(((1R)-2-(phenylimino)-1,3-thiazinan-5-yl)cyclohexanol (16a):** Prepared from \(15b\). White crystals (71%); m.p. 73–75 °C; \([\alpha]_D^{20} = -46.0\) (c 0.9, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 7.31-7.28 (m, 2H), 7.16-7.09 (m, 3H), 3.58-3.55 (m, 1H), 3.43 (td, \(J = 4.1, 10.4\) Hz, 1H), 3.38-3.33 (m, 1H), 3.13 (t, \(J = 11.6\) Hz, 1H), 2.87-2.84 (m, 1H), 2.48-2.43 (m, 1H), 1.97-1.95 (m, 1H), 1.70-1.66 (m, 2H), 1.49-1.35 (m, 2H), 1.12-1.04 (m, 1H), 1.00 (q, \(J = 12.0\) Hz, 1H), 0.91 (d, \(J = 6.6\) Hz, 3H), 0.89-0.84 (m, 1H); \(^3\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 129.0 (CH), 124.6 (CH), 123.2 (CH), 71.3 (CH), 47.5 (CH\(_2\)), 45.3 (CH\(_2\)), 45.0 (CH\(_2\)), 34.2 (CH\(_2\)), 34.1 (CH), 31.3 (CH), 30.4 (CH\(_2\)), 26.3 (CH\(_2\)), 21.9 (CH\(_3\)); HRMS (ESI): \(m/z\) [M+H]\(^+\) calculated for \(\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_3\text{S}\) 305.1609, found 305.1679.

**(1S,2S,5R)-5-Methyl-2-(((1S)-2-(phenylimino)-1,3-thiazinan-5-yl)cyclohexanol (16b):** Prepared from \(15d\). White crystals (88%); m.p. 224–225 °C; \([\alpha]_D^{20} = +9.0\) (c 0.29, MeOH); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) = 7.56 (brs, 1H), 7.15 (t, \(J = 7.6\) Hz, 2H), 6.83-6.60 (m, 1H), 4.33 (d, \(J = 7.8\) Hz, 1H), 3.89 (s, 1H), 3.70 (brs, 1H), 3.11 (brs, 1H), 2.80 (brs, 1H), 1.75-1.51 (m, 5H), 1.38-1.35 (m, 1H), 1.20 (brs, 1H), 1.02-0.97 (m, 1H), 0.84-0.80 (m, 1H), 0.81 (d, 3H, \(J = 6.4\) Hz); \(^3\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) = 128.8 (CH), 128.7 (CH), 65.7
(CH), 43.1 (CH$_2$), 41.8 (CH$_2$), 35.1 (CH$_2$), 30.1 (CH$_2$), 25.7 (CH), 23.6 (CH$_2$), 22.8 (CH); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{17}$H$_{25}$N$_2$O$_2$ 305.1609, found 305.1686.

1.5.3. General procedure for the synthesis of 1,3-oxazines (17a–d)

To a solution of 15a–d (0.31 mmol) in MeOH (4 mL), MeI (1.50 mmol) was added. After 3 h stirring at room temperature, the mixture was evaporated, followed by adding 2.5 M KOH in MeOH (20 mL) and subsequently stirred for 1 h before evaporation. The residue was dissolved in water (20 mL) and extracted with CHCl$_3$ (3 × 20 mL). The organic phase was then dried with Na$_2$SO$_4$ and evaporated to dryness. The crude product was purified by column chromatography on silica gel (CHCl$_3$:MeOH = 19:1).

(1R,2R,5R)-5-Methyl-2-((S)-5-methyl-2-(phenylimino)oxazolidin-5-yl)cyclohexanol (17a): Prepared from 15a. White crystals (76%); m.p. 94–96 °C; $[\alpha]_D^20 = -5.0$ (c 0.28, MeOH); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) = 7.32-7.27 (m, 4H), 7.00-6.96 (m, 1H), 3.76-3.69 (m, 2H), 3.54 (d, $J = 11.4$ Hz, 1H), 3.22 (brs, 2H), 2.05-2.01 (m, 1H), 1.70-1.56 (m, 3H), 1.50-1.44 (m, 1H), 1.43 (s, 3H), 1.07-0.98 (m, 2H), 0.95-0.84 (m, 1H), 0.93 (d, $J = 6.5$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ (ppm) = 155.0 (C$_o$), 129.0 (CH), 122.7 (CH), 119.3 (CH), 89.6 (C$_q$), 71.1 (CH), 51.9 (CH), 43.6 (CH$_2$), 34.0 (CH$_2$), 31.1 (CH), 26.3 (CH$_2$), 21.9 (CH$_3$), 20.4 (CH$_3$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{17}$H$_{25}$N$_2$O$_2$ 289.1838, found 289.1909.

(1R,2S,5R)-5-Methyl-2-((R)-2-(phenylimino)-1,3-oxazinan-5-yl)cyclohexanol (17b): Prepared from 15b. White crystals (50%); m.p. 166–168 °C; $[\alpha]_D^20 = -37.0$ (c 0.25, MeOH); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) = 7.32-7.26 (m, 4H), 7.10-7.07 (m, 1H), 6.75 (s, 1H), 5.06 (t, $J = 5.4$ Hz, 1H), 3.99 (t, $J = 8.7$ Hz, 1H), 3.61 (t, $J = 7.9$ Hz, 1H), 3.42-3.37 (m, 1H), 3.28-3.23 (m, 1H), 3.19-3.15 (m, 1H), 2.16-2.03 (m, 2H), 1.92-1.89 (m, 1H), 1.71-1.68 (m, 1H), 1.51-1.41 (m, 1H), 1.15-1.00 (m, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 0.94-0.83 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ (ppm) = 155.9 (C$_q$), 138.4 (C$_q$), 129.3 (CH), 124.0 (CH), 121.2 (CH), 84.0 (CH), 71.0 (CH$_2$), 49.0 (CH), 43.8 (CH), 42.1 (CH$_2$), 39.7 (CH$_2$), 34.6 (CH$_2$), 31.2 (CH), 27.3 (CH$_2$), 22.0 (CH$_3$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{17}$H$_{25}$N$_2$O$_2$ 289.1838, found 289.1909.

(1S,2R,5R)-5-Methyl-2-((S)-5-methyl-2-(phenylimino)oxazolidin-5-yl)cyclohexanol (17c): Prepared from 15c. White crystals (90%); m.p. 94–95 °C; $[\alpha]_D^20 = -16.0$ (c 0.25, MeOH); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) = 7.31 (brs, 1H), 7.27 (t, $J = 7.4$ Hz, 2H), 7.00-6.96 (m, 1H), 4.30 (s, 1H), 3.89 (d, $J = 11.4$ Hz, 1H), 3.52-3.48 (m, 1H), 1.90-1.78 (m, 3H), 1.71-1.59 (m, 2H), 1.52 (s, 3H), 1.47-1.45 (m, 1H), 1.09-1.04 (m, 1H), 0.98-0.90 (m, 1H), 0.88-0.87 (d, $J = 6.1$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ (ppm) = 129.0 (CH), 122.4 (CH), 66.6 (CH), 49.0 (CH), 42.3 (CH$_2$), 34.7 (CH$_2$), 25.7 (CH), 24.8 (CH$_3$), 22.1 (CH$_3$), 20.3 (CH$_2$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{17}$H$_{25}$N$_2$O$_2$ 289.1838, found 289.1913.

(1S,2S,5R)-5-Methyl-2-((S)-2-(phenylimino)-1,3-oxazinan-5-yl)cyclohexanol (17d): Prepared from 15d. White crystals (83%); m.p. 171–172 °C; $[\alpha]_D^20 = +49.0$ (c 0.27, MeOH); $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ (ppm) = 8.13 (brs, 1H), 7.43 (s, 1H), 7.13 (t, $J = 7.5$ Hz, 2H), 6.78 (t, $J = 7.1$ Hz, 1H), 4.34 (d, $J = 4.1$ Hz, 1H), 4.30-4.27 (m, 1H), 3.86 (t, $J = 10.0$ Hz, 1H), 3.82 (s, 1H), 3.48-3.45 (m, 1H), 3.09-3.04 (m, 1H), 1.85-1.82 (m, 1H), 1.75-1.69 (m, 2H), 1.64-1.61 (m, 1H), 1.47-1.36 (m, 2H), 1.11-1.07 (m, 1H), 1.01-0.96 (m, 1H), 0.83-0.80 (m, 1H), 0.81 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ (ppm) = 128.6 (CH), 120.4 (CH), 68.5 (CH$_2$), 65.4 (CH), 42.9 (CH$_2$), 41.4 (CH), 40.5 (CH$_2$), 34.9 (CH$_2$), 33.4 (CH), 25.7 (CH), 23.4
(CH₂), 22.89 (CH); HRMS (ESI): m/z [M+H]^+ calculated for C₁₁H₂₅N₂O₂ 289.1838, found 289.1912.

1.5.4. General procedure for the preparation of pyrimidine analogues (19a–h), (21a–g) and (23a–g)

To a solution of aminodiols 2a–b, 6, 10, and 12 together with aminotriol 4 as well as aminoalcohols 8a–b, 14 (0.6 mmol) in EtOH (2 mL), 2,4-dichloro-5-fluoropyrimidine 18a, 2,4,5-trichloropyrimidine 18b or 4,6-dichloropyrimidine-5-amine 18c (0.6 mmol) and Et₃N (1.8 mmol, 182 mg) were added. After a treatment at reflux temperature for 24 h, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was dissolved in EtOAc (15 mL) and washed with H₂O (3 × 15 mL). The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with CHCl₃: MeOH = 19:1.

(1R,2R,5R)-2-(((S)-1-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (19a): Prepared from 2a and 18a. Brown crystals (94%); m.p. 66–69 °C; [α]₀₀° = +63.0 (c 0.13, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.86 (d, J = 2.7 Hz, 1H), 5.85 (s, 1H), 5.05 (bri, 1H), 3.85 (dd, J = 4.1, 10.4 Hz, 1H), 3.70 (dd, J = 6.7, 13.4 Hz, 1H), 3.32 (dd, J = 4.4, 13.5 Hz, 1H), 2.31 (bri, 1H), 1.95-1.91 (m, 1H), 1.78-1.73 (m, 1H), 1.69-1.65 (m, 1H), 1.53-1.43 (m, 2H), 1.24 (s, 3H), 1.10 (q, J = 11.9 Hz, 1H), 1.02-0.94 (m, 1H), 0.92 (d, J = 6.5 Hz, 3H), 0.89-0.79 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 154.1 (C₂), 146.3 (C₃), 144.3 (C₄), 139.4 (C₅), 75.2 (C₆), 73.0 (CH), 49.1 (CH), 49.2 (CH₂), 45.3 (CH₂), 34.1 (CH₂), 31.3 (CH), 26.4 (CH₂), 21.8 (CH₃), 21.1 (CH₃); HRMS (ESI): m/z [M+H]^+ calculated for C₁₄H₂₂ClF₆N₃O₂ 318.1306, found 318.1376.

(1R,2R,5R)-2-(((R)-1-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (19b): Prepared from 2b and 18a. White crystals (88%); m.p. 100–104 °C; [α]₀₀° = +4.0 (c 0.13, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.85 (d, J = 2.7 Hz, 1H), 5.95 (s, 1H), 4.78 (s, 1H), 3.85-3.80 (m, 1H), 3.74 (dd, J = 7.7, 13.3 Hz, 1H), 3.51 (dd, J = 3.1, 13.3 Hz, 1H), 2.61 (s, 1H), 1.95-1.92 (m, 1H), 1.87-1.82 (m, 1H), 1.75-1.72 (m, 1H), 1.61-1.55 (m, 1H), 1.53-1.45 (m, 1H), 1.20 (s, 3H), 1.16-1.05 (m, 2H), 0.97-0.86 (m, 1H), 0.94 (d, J = 6.8 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 154.5 (C₂), 146.4 (C₃), 144.4 (C₄), 139.4 (CH), 75.2 (C₅), 73.1 (CH), 52.4 (CH), 46.8 (CH₂), 45.6 (CH₂), 34.4 (CH₂), 31.5 (CH), 26.3 (CH₂), 24.9 (CH₃), 21.8 (CH₃); HRMS (ESI): m/z [M+H]^+ calculated for C₁₄H₂₂ClF₆N₃O₂ 318.1306, found 318.1376.

(S)-3-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-dirol (19c): Prepared from 4 and 18a. Yellow oil (94%); [α]₀₀° = +8.0 (c 0.1375, MeOH); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 7.86 (d, J = 2.6 Hz, 1H), 6.18 (s, 1H), 5.22 (s, 1H), 3.90-3.85 (m, 1H), 3.74-3.54 (m, 4H), 2.85 (s, 1H), 2.74 (s, 1H), 1.97-1.95 (m, 1H), 1.82-1.70 (m, 4H), 1.53-1.43 (m, 1H), 1.14-1.07 (m, 2H), 0.96-0.88 (m, 1H), 0.94 (d, J = 6.6 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 154.4 (C₂), 146.5 (C₃), 144.4 (C₄), 139.3 (CH), 76.0 (C₅), 72.4 (CH), 66.1 (CH₂), 48.0 (CH), 45.4 (CH₂), 43.9 (CH₂), 34.1 (CH₂), 31.2 (CH), 25.5 (CH₂), 21.8 (CH₃); HRMS (ESI): m/z [M+H]^+ calculated for C₁₄H₂₂ClF₆N₃O₃ 334.1255, found 334.1322.

(1R,2S,5R)-2-(((R)-1-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (19d): Prepared from 6 and 18a. White crystals (84%); m.p. 172–174 °C;
[α]20D = -55.0 (c 0.13, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 8.03 (d, J = 3.2 Hz, 1H); 8.01-7.97 (m, 1H), 4.60 (t, J = 4.9 Hz, 1H), 4.56 (d, J = 5.1 Hz, 1H), 3.49-3.47 (m, 2H), 3.45-3.40 (m, 1H), 3.28-3.22 (m, 1H), 2.19-2.17 (m, 1H), 1.86-1.84 (m, 1H), 1.60-1.57 (m, 2H), 1.37-1.33 (m, 2H), 1.13-1.04 (m, 1H), 0.89-0.72 (m, 2H), 0.86 (d, J = 6.8 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.0 (Cq), 146.7 (Cq), 144.7 (Cq), 139.6 (CH), 69.9 (CH), 62.1 (CH2), 45.5 (CH2), 45.2 (CH), 40.7 (CH2), 40.6 (CH), 34.9 (CH2), 31.4 (CH), 26.7 (CH2), 22.7 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClFN3O2 318.1306, found 318.1382.

(3S,3aR,6R,7aR)-3-(((2-Chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19e): Prepared from 8a and 18a. White crystals (53%); m.p. 175–177 °C; [α]20D = -3.0 (c 0.1150, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 8.09-8.07 (m, 2H), 4.86 (s, 1H), 3.93 (d, J = 9.7 Hz, 1H), 3.58 (dd, J = 6.5, 13.6 Hz, 1H), 3.51 (d, J = 9.6 Hz, 1H), 3.44-3.39 (m, 2H), 1.95-1.93 (m, 1H), 1.76-1.75 (m, 1H), 1.67-1.64 (m, 1H), 1.40-1.35 (m, 1H), 1.27-1.17 (m, 1H), 1.05-0.99 (m, 1H), 0.90 (d, J = 6.5 Hz, 3H), 0.88-0.84 (m, 1H), 0.83-0.74 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.3 (Cq), 153.8 (Cq), 146.6 (Cq), 144.5 (Cq), 140.4 (CH), 80.5 (CH), 79.2 (CH2), 77.9 (CH2), 51.5 (CH), 45.1 (CH2), 34.4 (CH2), 31.0 (CH), 22.4 (CH2), 22.3 (CH2); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClFN3O2 316.1149, found 316.1221.

(3R,3aR,6R,7aR)-3-(((2-Chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19f): Prepared from 8b and 18a. Colorless oil (70%); [α]20D = -3.0 (c 0.1425, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 7.90 (s, 1H), 5.72 (s, 1H), 3.87-3.79 (m, 2H), 3.70-3.60 (m, 2H), 3.35 (td, J = 3.8, 10.9 Hz, 1H), 2.67 (s, 1H), 2.17-2.11 (m, 1H), 1.95-1.91 (m, 1H), 180-1.76 (m, 1H), 1.61-1.56 (m, 1H), 1.52-1.43 (m, 1H), 1.21-1.11 (m, 2H), 0.99 (d, J = 6.6 Hz, 3H), 0.98-0.89 (m, 1H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 154.5 (Cq), 146.3 (Cq), 144.3 (Cq), 139.8 (CH), 82.2 (CH), 80.1 (Cq), 78.3 (CH2), 56.8 (CH), 45.4 (CH2), 40.1 (CH2), 34.6 (CH2), 30.9 (CH), 23.09 (CH2), 21.9 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClFN3O2 316.1149, found 316.1220.

(1S,2R,5R)-2-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (19g): Prepared from 10 and 18a. White crystals (75%); m.p. 158–160 °C; [α]20D = +36.0 (c 0.1450, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 7.85 (d, 1H), 5.93 (brs, 1H), 4.46 (s, 1H), 3.61-3.57 (m, 2H), 3.52-3.49 (m, 1H), 2.11 (s, 1H), 1.83-1.65 (m, 4H), 1.36-1.35 (m, 1H), 1.33 (s, 3H), 1.20-1.14 (m, 1H), 0.97-0.92 (m, 1H), 0.90 (d, J = 6.2 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 154.5 (Cq), 146.4 (Cq), 144.4 (Cq), 139.4 (CH), 74.0 (Cq), 68.3 (CH), 48.3 (CH2), 47.0 (CH), 42.9 (CH2), 34.5 (CH2), 25.7 (CH), 24.7 (CH3), 22.1 (CH3), 20.5 (CH2); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClFN3O2 318.1306, found 318.1378.

(1S,2S,5R)-2-((2-Chloro-5-fluoropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (19h): Prepared from 12 and 18a. White crystals (88%); m.p. 142–144 °C; [α]20D = +26.0 (c 0.1250, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 7.84 (s, 1H), 6.26 (s, 1H), 4.13 (s, 1H), 3.80-3.57 (m, 4H), 2.24 (brs, 1H), 1.91-1.75 (m, 4H), 1.65-1.47 (m, 3H), 1.29-1.17 (m, 1H), 1.03-0.90 (m, 1H), 0.89 (d, J = 6.8 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 146.4 (Cq), 144.3 (Cq), 139.2 (CH), 67.7 (CH), 62.0 (CH2), 42.9 (CH2), 42.7 (CH), 40.6 (CH2), 40.4 (CH), 34.8 (CH2), 25.8 (CH), 23.9 (CH2), 22.1 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClFN3O2 318.1306, found 318.1372.
(3R,3aR,6R,7aS)-3-(((2-Chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19i): Prepared from 14 and 18a. White crystals (72%); m.p. 156–158 °C; [α]D20 = −7.0 (c 0.1375, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 7.90 (d, J = 2.6 Hz, 1H), 5.65 (brs, 1H), 4.42-4.41 (m, 1H), 4.03 (d, J = 9.9 Hz, 1H), 3.78-3.73 (m, 2H), 3.66 (dd, J = 5.1, 13.9 Hz, 1H), 2.36 (s, 1H), 2.09-2.05 (m, 1H), 1.85-1.81 (m, 1H), 1.69-1.60 (m, 3H), 1.21-1.12 (m, 2H), 0.90 (d, J = 6.4 Hz, 3H), 0.86-0.81 (m, 1H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 154.5 (Cq), 146.3 (Cq), 144.3 (Cq), 140.0 (CH), 84.0 (Cq), 77.6 (CH), 76.6 (CH2), 46.9 (CH), 44.0 (CH2), 36.8 (CH2), 32.7 (CH2), 26.3 (CH), 24.2 (CH2), 22.1 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H20ClF3N3O2 316.1149, found 316.1220.

(1R,2R,5R)-2-((S)-1-(((2,5-Dichloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (21a): Prepared from 2a and 18b. White crystals (73%); m.p. 110–113 °C; [α]D20 = +66.0 (c 0.15, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 6.16 (s, 1H), 5.09 (brs, 1H), 3.85 (td, J = 4.3, 10.5 Hz, 1H), 3.70 (dd, J = 6.5, 13.4 Hz, 1H), 3.34 (dd, J = 4.3, 13.5 Hz, 1H), 2.43 (brs, 1H), 1.94-1.91 (m, 1H), 1.77-1.72 (m, 1H), 1.69-1.65 (m, 1H), 1.51-1.43 (m, 2H), 1.24 (s, 3H), 1.10 (q, J = 11.8 Hz, 1H), 1.02-0.94 (m, 1H), 0.92 (d, J = 6.5 Hz, 3H), 0.89-0.79 (m, 1H); 13C NMR (125MHz, CDCl3): δ (ppm) = 159.1 (Cq), 158.3 (Cq), 153.4 (CH), 113.4 (Cq), 75.2 (Cq), 72.9 (CH), 49.3 (CH2), 49.3 (CH2), 45.2 (CH2), 34.1 (CH2), 31.3 (CH), 26.4 (CH2), 21.8 (CH3), 21.1 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22Cl2N3O2 334.1011, found 334.1081.

(1R,2R,5R)-2-((R)-1-(((2,5-Dichloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (21b): Prepared from 2b and 18b. White crystals (75%); m.p. 94–96 °C; [α]D20 = +3.0 (c 0.1350, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 6.25 (s, 1H), 4.71 (s, 1H), 3.84-3.80 (m, 1H), 3.77-3.69 (m, 1H), 3.54-3.48 (m, 1H), 2.38 (m, 1H), 1.94-1.92 (m, 1H), 1.87-1.82 (m, 1H), 1.75-1.72 (m, 1H), 1.61-1.56 (m, 1H), 1.53-1.45 (m, 1H), 1.20 (s, 3H), 1.14-1.06 (m, 2H), 0.99-0.88 (m, 1H), 0.94 (d, J = 6.4 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 159.2 (Cq), 158.3 (Cq), 153.3 (CH), 113.5 (Cq), 75.1 (Cq), 73.2 (CH), 52.4 (CH), 47.2 (CH2), 45.6 (CH2), 34.4 (CH2), 31.5 (CH), 26.3 (CH2), 25.0 (CH3), 21.8 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22Cl2N3O2 334.1011, found 334.1082.

(S)-3-(((2,5-Dichloropyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol (21c): Prepared from 4 and 18b. White crystals (70%); m.p. 118–120 °C; [α]D20 = +1.0 (c 0.1150, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 6.46 (s, 1H), 5.15 (s, 1H), 3.90-3.86 (m, 1H), 3.66-3.65 (m, 2H), 3.59-3.55 (m, 2H), 2.57 (t, J = 6.3 Hz, 1H), 2.49 (s, 1H), 1.97-1.95 (m, 1H), 1.82-1.69 (m, 3H), 1.52-1.43 (m, 1H), 1.15-1.06 (m, 2H), 0.97-0.88 (m, 1H), 0.94 (d, J = 6.6 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 159.2 (Cq), 158.2 (Cq), 153.3 (CH), 113.63 (Cq), 75.9 (Cq), 72.5 (CH), 66.2 (CH2), 48.1 (CH), 45.4 (CH2), 44.4 (CH2), 34.1 (CH2), 31.2 (CH), 25.5 (CH2), 21.8 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22Cl2N3O4 350.1385, found 350.1386.

(1R,2S,5R)-2-((R)-1-(((2,5-Dichloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (21d): Prepared from 6 and 18b. White crystals (84%); m.p. 138–140 °C; [α]D20 = −45.0 (c 0.1150, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 8.14 (s, 1H), 7.95 (s, 1H), 5.75 (s, 1H), 4.80-4.78 (m, 1H), 4.68 (d, J = 5.0 Hz, 1H), 3.52-3.48 (m, 3H), 3.28-3.27 (m, 1H), 2.19-2.18 (m, 1H), 1.87-1.85 (m, 1H), 1.58-1.54 (m, 2H), 1.38-1.32 (m, 2H), 1.14-1.06 (m, 1H), 0.90-0.75 (m, 2H), 0.86 (d, J = 6.4 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ
(3S,3aR,6R,7aR)-3-(((2,5-Dichloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (21c): Prepared from 8a and 18b. Yellow crystals (88%); m.p. 90–91 °C; [α]_D^20 = −6.0 (c 0.13, MeOH); ^1H NMR (500 MHz, CDCl3): δ (ppm) = 8.05 (s, 1H), 5.94 (s, 1H), 4.05 (d, J = 10.4 Hz, 1H), 3.80–3.76 (m, 2H), 3.71 (dd, J = 5.1, 13.9 Hz, 1H), 3.57 (td, J = 3.9, 10.9 Hz, 1H), 2.48 (s, 1H), 2.16–2.13 (m, 1H), 1.80–1.69 (m, 2H), 1.55–1.44 (m, 1H), 1.42–1.33 (m, 1H), 1.30–1.25 (m, 1H), 1.08 (q, J = 11.4 Hz, 1H), 0.99 (d, J = 6.6 Hz, 3H), 0.97–0.91 (m, 1H); ^13C NMR (125 MHz, CDCl3): δ (ppm) = 159.2 (Cq), 158.3 (Cq), 153.8 (CH), 113.4 (Cq), 81.4 (CH), 79.8 (CH2), 79.4 (CH2), 53.1 (CH), 48.6 (CH2), 39.9 (CH2), 34.1 (CH2), 30.9 (CH), 22.4 (CH2), 21.9 (CH3); HRMS (ESI): m/z [M+H]^+ calculated for C_{14}H_{18}Cl_{2}N_{3}O_{2} 334.0854, found 334.0927.

(1S,2R,5R)-2-((S)-1-(((2,5-Dichloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (21f): Prepared from 10 and 18b. Yellow oil (84%); [α]_D^20 = +17.0 (c 0.1375, MeOH); ^1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 6.17 (brs, 1H), 4.46 (s, 1H), 3.62–3.48 (m, 3H), 2.08 (brs, 1H), 1.83–1.67 (m, 5H), 1.37–1.34 (m, 1H), 1.33 (s, 3H), 1.20–1.14 (m, 1H), 0.97–0.86 (m, 1H), 0.90 (d, J = 6.1 Hz, 3H); ^13C NMR (125 MHz, CDCl3): δ (ppm) = 159.2 (Cq), 158.3 (Cq), 153.4 (CH), 113.4 (Cq), 74.0 (Cq), 68.3 (CH), 48.7 (CH2), 46.9 (CH), 42.9 (CH2), 34.5 (CH2), 25.7 (CH), 24.7 (CH3), 22.1 (CH3), 20.5 (CH2); HRMS (ESI): m/z [M+H]^+ calculated for C_{14}H_{19}Cl_{2}N_{3}O_{2} 334.1011, found 334.1083.

(1S,2S,5R)-2-((S)-1-(((2,5-Dichloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (21g): Prepared from 12 and 18b. White crystals (85%); m.p. 184–186 °C; [α]_D^20 = +6.0 (c 0.1425, MeOH); ^1H NMR (500 MHz, CDCl3): δ (ppm) = 7.99 (s, 1H), 6.51 (s, 1H), 4.11 (s, 1H), 3.75–3.58 (m, 4H), 2.29 (brs, 1H), 1.88–1.85 (m, 2H), 1.79–1.75 (m, 2H), 1.60–1.46 (m, 3H), 1.26–1.17 (m, 1H), 0.98–0.84 (m, 1H), 0.98 (d, J = 6.3 Hz, 3H); ^13C NMR (125 MHz, CDCl3): δ (ppm) = 159.2 (Cq), 158.4 (Cq), 153.3 (CH), 113.4 (Cq), 67.7 (CH), 62.1 (CH2), 42.7 (CH), 42.7 (CH2), 41.1 (CH2), 40.4 (CH), 34.8 (CH2), 25.8 (CH2), 23.9 (CH2), 22.14 (CH3); HRMS (ESI): m/z [M+H]^+ calculated for C_{14}H_{21}Cl_{2}N_{3}O_{2} 334.1011, found 334.1077.

(3R,3aR,6R,7aS)-3-(((2,5-Dichloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (21h): Prepared from 14 and 18b. White crystals (80%); m.p. 185–186 °C; [α]_D^20 = +3.0 (c 0.13, MeOH); ^1H NMR (500 MHz, CDCl3): δ (ppm) = 8.04 (s, 1H), 5.95 (s, 1H), 4.42–4.41 (m, 1H), 4.03 (d, J = 10.0 Hz, 1H), 3.79–3.73 (m, 2H), 3.69–3.65 (dd, J = 5.0, 13.9 Hz, 1H), 2.39 (s, 1H), 2.08–2.05 (m, 1H), 1.86–1.81 (m, 1H), 1.69–1.60 (m, 3H), 1.21–1.11 (m, 2H), 0.90 (d, J = 6.58 Hz, 3H), 0.87–0.81 (m, 1H); ^13C NMR (125 MHz, CDCl3): δ (ppm) = 159.0 (Cq), 158.4 (Cq), 153.8 (CH), 113.4 (Cq), 84.0 (Cq), 77.6 (CH), 76.6 (CH2), 46.8 (CH), 44.4 (CH2), 36.8 (CH2), 32.8 (CH2), 26.3 (CH), 24.2 (CH2), 22.1 (CH3); HRMS (ESI): m/z [M+H]^+ calculated for C_{14}H_{25}Cl_{2}N_{3}O_{2} 332.0854, found 332.0926.

(1R,2R,5R)-2-((S)-1-(((5-Amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (23a): Prepared from 2a and 18c. Yellow oil (82%); [α]_D^20 = −10.0 (c 0.13, MeOH); ^1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 5.72 (brs, 1H), 5.50–5.48 (m, 1H), 3.83 (td, J = 4.3, 10.4 Hz, 1H), 3.68 (dd, J = 6.6, 13.7 Hz, 1H), 3.41 (dd, J = 4.5, 13.8 Hz, 1H), 1.96–1.94 (m, 1H), 1.72–1.62 (m, 4H), 1.57–1.51 (m, 1H), 1.49–1.41 (m, 1H), 1.24 (s, 3H), 1.09 (q, J = 11.7 Hz, 1H), 1.02–0.93 (m, 1H), 0.91 (d, J = 5.7 Hz, 3H), 0.88–0.78 (m, 1H); ^13C
NMR (125 MHz, CDCl3): δ (ppm) = 155.1 (Cq), 148.9 (CH), 142.7 (Cq), 122.1 (Cq), 76.5 (Cq), 72.5 (CH), 50.3 (CH2), 49.0 (CH), 44.8 (CH2), 34.2 (CH2), 31.2 (CH), 26.6 (CH2), 21.9 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClN2O2 315.1509, found 315.1578.

(1R,2R,5R)-2-(((R)-1-(((5-Amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol) (23b): Prepared from 2b and 18c. White crystals (80%); m.p. 213–214 °C; [αl]D = −20.0 (c 0.12, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 7.69 (s, 1H), 6.58-6.56 (m, 1H), 5.41 (d, J = 4.1 Hz, 1H), 5.30 (s, 1H), 5.13 (s, 2H), 3.88 (dd, J = 7.3, 13.6 Hz, 1H), 3.61-3.55 (m, 1H), 3.41 (dd, J = 4.0, 13.5 Hz, 1H), 1.82-1.77 (m, 2H), 1.63-1.60 (m, 1H), 1.40-1.32 (m, 2H), 1.03 (s, 3H), 0.93-0.89 (m, 1H), 0.86 (d, J = 6.3 Hz, 3H), 0.83-0.75 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 153.3 (Cq), 145.9 (CH), 137.4 (Cq), 124.0 (Cq), 75.3 (Cq), 71.6 (CH), 52.6 (CH), 48.6 (CH2), 45.8 (CH2), 34.9 (CH2), 31.4 (CH), 26.3 (CH2), 23.9 (CH3), 22.5 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClN2O2 315.1509, found 315.1579.

(S)-3-(((5-Amino-6-chloropyrimidin-4-yl)amino)-2-(((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diyl)-5-methylcyclohexanol (23c): Prepared from 4 and 18c. Yellow crystals (79%); m.p. 160–162 °C; [αl]D = −6.0 (c 0.1325, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.02 (s, 1H), 5.64 (s, 1H), 5.16 (s, 1H), 3.87-3.82 (m, 1H), 3.75-3.65 (m, 2H), 3.61-3.51 (m, 4H), 3.03-2.98 (m, 2H), 1.97-1.95 (m, 1H), 1.88-1.84 (m, 1H), 1.77-1.68 (m, 2H), 1.50-1.42 (m, 1H), 1.15-1.04 (m, 2H), 0.93-0.88 (m, 1H), 0.92 (d, J = 6.4 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 155.2 (Cq), 149.1 (CH), 76.5 (Cq), 72.4 (CH), 66.2 (CH2), 48.6 (CH), 45.4 (CH2), 44.9 (CH2), 34.2 (CH2), 31.3 (CH), 25.5 (CH2), 21.8 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H24ClN2O3 331.1458, found 331.1526.

(1R,2S,5R)-2-(((R)-1-(((5-Amino-6-chloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (23d): Prepared from 6 and 18c. Yellow crystals (88%); m.p. 187–189 °C; [αl]D = −32.0 (c 0.1375, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 7.71 (s, 1H), 6.73-6.71 (m, 1H), 5.01 (s, 2H), 4.56 (d, J = 5.1 Hz, 1H), 4.47 (t, J = 5.1 Hz, 1H), 3.53-3.35 (m, 4H), 3.27-3.23 (m, 1H), 2.25-2.18 (m, 1H), 1.88-1.85 (m, 1H), 1.67-1.58 (m, 2H), 1.44-1.37 (m, 2H), 1.05-1.02 (m, 1H), 0.91-0.84 (m, 1H), 0.87 (d, J = 6.4 Hz, 3H), 0.80-0.77 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 152.7 (Cq), 146.1 (CH), 137.0 (Cq), 123.9 (Cq), 70.0 (CH), 61.7 (CH2), 45.5 (CH2), 44.6 (CH), 40.4 (CH2), 40.3 (CH), 35.0 (CH2), 31.5 (CH), 26.0 (CH2), 22.7 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H24ClN2O3 331.1509, found 315.1581.

(3S,3aR,6R,7aR)-3-(((5-Amino-6-chloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzo[b]furan-3-ol (23e): Prepared from 8a and 18c. White crystals (88%); m.p. 171–172 °C; [αl]D = −30.0 (c 0.1425, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.03 (s, 1H), 5.42-5.40 (m, 1H), 4.15 (brs, 1H), 4.06 (d, J = 9.9 Hz, 1H), 3.77 (d, J = 10.1 Hz, 1H), 3.75-3.65 (m, 2H), 3.57 (td, J = 3.7, 10.8 Hz, 1H), 3.48 (s, 2H), 2.16-2.08 (m, 1H), 1.77-1.74 (m, 1H), 1.70-1.66 (m, 1H), 1.55-1.46 (m, 1H), 1.45-1.37 (m, 1H), 1.25-1.19 (m, 1H), 1.05 (q, J = 11.2 Hz, 1H), 0.98 (d, J = 6.5 Hz, 3H), 0.95-0.89 (m, 1H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 155.6 (Cq), 149.1 (CH), 143.7 (Cq), 122.1 (Cq), 81.5 (CH), 80.2 (Cq), 79.7 (CH2), 53.5 (CH), 50.2 (CH2), 40.0 (CH2), 34.2 (CH2), 31.0 (CH), 22.6 (CH2), 22.0 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C14H22ClN2O2 313.1353, found 313.1424.
(1S,2R,5R)-2-((S)-1-((5-Amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (23f): Prepared from 10 and 18c. Brown oil (83%); [α]_{D}^{20} = -7.0 (c 0.1825, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.00 (s, 1H), 5.63 (brs, 1H), 4.56 (brs, 1H), 4.52 (brs, 1H), 4.44 (s, 1H), 3.69 (dd, J = 4.0, 13.7 Hz, 1H), 3.57 (s, 2H), 3.49-3.41 (m, 1H), 3.02 (brs, 1H), 1.86-1.67 (m, 5H), 1.32-1.30 (m, 4H), 1.15-1.08 (m, 1H), 0.97-0.84 (m, 1H), 0.88 (d, J = 6.3 Hz, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 155.4 (Cq), 149.0 (CH), 142.7 (Cq), 122.1 (Cq), 75.0 (Cq), 68.0 (CH), 49.8 (CH2), 47.6 (CH), 42.6 (CH2), 34.7 (CH2), 25.7 (CH), 24.4 (CH3), 22.1 (CH3), 20.4 (CH2); HRMS (ESI): m/z [M+H]+ calculated for C_{14}H_{23}ClN_{4}O_{2} 315.1509, found 315.1579.

(1S,2S,5R)-2-((S)-1-((5-Amino-6-chloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (23g): Prepared from 12 and 18c. Yellow crystals (90%); m.p. 182–183 °C; [α]_{D}^{20} = +19.0 (c 0.1150, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 7.70 (s, 1H), 6.71 (t, J = 5.1 Hz, 1H), 4.97 (s, 2H), 4.69 (t, J = 5.0 Hz, 1H), 4.56 (d, J = 3.4 Hz, 1H), 3.91 (s, 1H), 3.52-3.38 (m, 4H), 1.74-1.63 (m, 4H), 1.49-1.39 (m, 3H), 1.04-0.99 (m, 1H), 0.86-0.84 (m, 1H), 0.81 (d, J = 6.4 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 152.9 (Cq), 146.1 (CH), 137.2 (Cq), 123.9 (Cq), 67.1 (CH), 59.9 (CH3), 43.0 (CH3), 43.0 (CH), 40.7 (CH2), 40.6 (CH), 35.4 (CH2), 25.9 (CH), 23.2 (CH2), 22.9 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C_{14}H_{23}ClN_{4}O_{2} 315.1575.

(3R,3aR,6R,7aS)-3-((5-Amino-6-chloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (23h): Prepared from 14 and 18c. Brown oil (86%); [α]_{D}^{20} = -6.0 (c 0.1250, MeOH); 1H NMR (500 MHz, CDCl3): δ (ppm) = 8.04 (s, 1H), 5.34-5.29 (m, 1H), 4.43 (m, 1H), 4.01 (d, J = 9.5 Hz, 1H), 3.75-3.71 (m, 3H), 3.50 (brs, 2H), 2.08-2.04 (m, 1H), 1.87-1.81 (m, 1H), 1.67-1.58 (m, 3H), 1.20-1.11 (m, 2H), 0.89 (d, J = 6.7 Hz, 3H), 0.86-0.81 (m, 1H); 13C NMR (125 MHz, CDCl3): δ (ppm) = 155.4 (Cq), 149.2 (CH), 143.5 (Cq), 122.1 (Cq), 84.4 (Cq), 77.6 (CH), 76.7 (CH2), 47.1 (CH), 45.3 (CH2), 36.8 (CH2), 32.9 (CH2), 26.3 (CH), 24.4 (CH2), 22.1 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C_{14}H_{23}ClN_{4}O_{2} 313.1353, found 313.1422.

1.5.5. General procedure for the preparation of N^2-(p-trifluoromethyl)aniline substituted pyrimidines (20a–i) and (22a–h)

A mixture of pyrimidines 19a–i or 21a–h (0.16 mmol) and 4-trifluoromethylaniline (0.24 mmol) in EtOH (200 μL) was heated in microwave reactor at 150 °C, 200 W, 19 bar for 80 min. The formed precipitate was filtered off and washed with CH2Cl2 to afford the desired product in pure form without further purification.

(1R,2R,5R)-2-((S)-1-((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20a): Prepared from 19a. White crystals (72%); m.p. 154–156 °C; [α]_{D}^{20} = +43.0 (c 0.1150, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 9.82 (s, 1H), 8.04 (d, J = 4.1 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.54 (brs, 1H), 3.66-3.61 (m, 1H), 3.54-3.45 (m, 2H), 1.84-1.82 (m, 1H), 1.72-1.69 (m, 1H), 1.53-1.51 (m, 1H), 1.41-1.36 (m, 2H), 1.12 (s, 3H), 1.00-0.88 (m, 2H), 0.85 (d, J = 6.4 Hz, 3H), 0.80-0.73 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 153.8 (Cq), 143.9 (Cq), 141.9 (Cq), 140.0 (Cq), 135.4 (CH), 126.1 (CH), 119.5 (CH), 75.8 (Cq), 71.4 (CH), 49.6 (CH), 49.2 (CH2), 45.2 (CH3), 34.5 (CH2), 31.1 (CH), 26.3 (CH2), 22.4 (CH3), 21.8 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C_{21}H_{27}F_{4}N_{4}O_{2} 443.1992, found 443.2061.
(1R,2R,5R)-2-((R)-1-((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20b): Prepared from 19b. Yellow crystals (87%); m.p. 154–156 °C; [α]D 10 = +16.0 (c 0.14, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.38 (s, 1H), 8.29 (brs, 1H), 8.13 (d, J = 4.9 Hz, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 3.92-3.88 (m, 1H), 3.58-3.49 (m, 2H), 1.86-1.84 (m, 1H), 1.79-1.76 (m, 1H), 1.61-1.59 (m, 1H), 1.41-1.33 (m, 2H), 1.03 (s, 3H), 0.97-0.90 (m, 1H), 0.87 (d, J = 6.4 Hz, 3H), 0.85-0.78 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.5 (Cq), 152.0 (Cq), 142.9 (Cq), 141.3 (Cq), 139.3 (Cq), 130.0 (CH), 126.3 (CH), 120.1 (CH), 75.3 (Cq), 71.4 (CH), 52.8 (CH2), 48.8 (CH2), 45.6 (CH2), 34.8 (CH2), 31.4 (CH), 26.1 (CH2), 23.5 (CH3), 22.4 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H23F4N4O2 443.1992, found 443.2062.

(S)-3-((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol hydrochloride (20c): Prepared from 19c. White crystals (84%); m.p. 158–160 °C; [α]D 10 = +18.0 (c 0.1075, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.07 (1H, s), 8.07 (d, J = 4.4 Hz, 1H), 7.92 (brs, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 3.78-3.74 (m, 1H), 3.69-3.64 (m, 1H), 3.62-3.58 (m, 1H), 3.41-3.54 (2H, m), 1.88-1.75 (m, 2H), 1.65-1.54 (m, 2H), 1.38-1.36 (m, 1H), 1.09-1.00 (m, 1H), 0.97-0.90 (m, 1H), 0.87 (d, J = 6.4 Hz, 3H), 0.83-0.75: 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.0 (Cq), 143.4 (Cq), 141.8 (Cq), 139.8 (Cq), 132.7 (CH), 126.3 (CH), 119.8 (CH), 77.2 (CH2), 70.9 (CH), 65.2 (CH2), 48.3 (CH), 45.7 (CH2), 45.2 (CH2), 34.8 (CH2), 31.2 (CH), 25.8 (CH2), 22.5 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H27F4N4O3 459.1941, found 459.2004.

(1R,2S,5R)-2-((R)-1-((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20d): Prepared from 19d. Yellow crystals (94%); m.p. 179–181 °C; [α]D 10 = −23.0 (c 0.1375, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 9.46 (s, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 3.6 Hz, 1H), 7.54 (d, J = 8.8 Hz, 2H), 7.36-7.34 (m, 1H), 4.62-4.60 (m, 1H), 4.54 (d, J = 5.6 Hz, 1H), 3.54-3.49 (m, 3H), 3.40-3.35 (m, 2H), 2.22-2.20 (m, 1H), 1.87-1.84 (m, 1H), 1.61-1.57 (m, 2H), 1.40-1.33 (m, 2H), 1.16-1.13 (m, 1H), 0.91-0.89 (m, 1H), 0.87 (d, J = 6.5 Hz, 3H), 0.91-0.78 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 155.8 (Cq), 152.7 (Cq), 145.4 (Cq), 142.8 (Cq), 140.8 (Cq), 138.6 (CH), 126.0 (CH), 120.4 (Cq), 118.0 (CH), 70.0 (CH), 62.4 (CH2), 45.6 (CH2), 45.3 (CH), 40.9 (CH), 40.6 (CH2), 34.9 (CH2), 31.5 (CH), 27.0 (CH2), 22.7 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H27F4N4O3 459.1941, found 459.2004.

(3S,3aR,6R,7aR)-3-((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20e): Prepared from 19e. White crystals (87%); m.p. 150–152 °C; [α]D 10 = −33.0 (c 0.1350, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.58 (s, 1H), 8.71 (brs, 1H), 8.19 (d, J = 4.9 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 3.98 (d, J = 9.3 Hz, 1H), 3.72 (dd, J = 6.6, 13.6 Hz, 1H), 3.55-3.50 (m, 2H), 3.46-3.41 (m, 1H), 1.95-1.93 (m, 1H), 1.59 (d, J = 11.1 Hz, 2H), 1.43-1.33 (m, 1H), 1.26-1.19 (m, 1H), 1.13-1.04 (m, 1H), 0.89 (d, J = 6.48 Hz, 3H), 0.85-0.72 (m, 2H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.6 (Cq), 151.8 (Cq) 142.6 (Cq), 141.2 (Cq), 139.2 (Cq), 131.0 (CH), 126.4 (CH), 120.3 (CH), 80.6 (CH), 79.1 (CH2), 52.1 (CH), 46.7 (CH2), 40.4 (CH2), 34.4 (CH2), 30.9 (CH), 22.4 (CH3), 22.3 (CH2); HRMS (ESI): m/z [M+H]+ calculated for C21H23F4N4O2 441.1835, found 441.1906.
(3R,3aR,6R,7aR)-3-(((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20f): Prepared from 19f. Yellow crystals (90%); m.p. 156–158 °C; [α]D20 = −40.0 (c 0.1450, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.38 (s, 1H), 8.27 (brs, 1H), 8.15 (d, J = 4.6 Hz, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 3.79–3.73 (m, 2H), 3.57 (d, J = 9.17 Hz, 1H), 3.44 (dd, J = 4.6, 13.6 Hz, 1H), 3.27–3.21 (m, 1H), 1.94–1.92 (m, 1H), 1.83–1.80 (m, 1H), 1.71–1.69 (m, 1H), 1.55–1.50 (m, 1H), 1.38–1.36 (m, 1H), 1.19–1.11 (m, 1H), 1.03 (q, J = 11.2 Hz, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.91–0.83 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.5 (Cα), 143.0 (Cβ), 141.4 (Cγ), 139.4 (Cδ), 131.9 (CH), 126.2 (CH), 120.4 (CH), 81.3 (CH), 79.3 (CH2), 76.9 (CH2), 55.9 (CH), 45.9 (CH2), 34.7 (CH2), 31.0 (CH), 22.7 (CH2), 22.3 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H23F3N2O2 441.1835, found 441.1908.

(1S,2R,5R)-2-(((S)-1-(((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20g): Prepared from 19g. White crystals (80%); m.p. 166–168 °C; [α]D20 = +18.0 (c 0.1350, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.26 (brs, 1H), 8.44 (brs, 1H), 8.10 (d, J = 4.6 Hz, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 4.18 (s, 1H), 3.54 (d, J = 5.7 Hz, 2H), 1.79–1.65 (m, 3H), 1.60–1.57 (m, 2H), 1.34–1.31 (m, 1H), 1.17 (s, 3H), 1.04–0.97 (m, 1H), 0.90–0.84 (m, 1H), 0.82 (d, J = 6.4 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 126.4 (CH), 119.8 (CH), 73.9 (Cγ), 65.6 (CH), 49.0 (CH), 48.5 (CH2), 43.2 (CH2), 35.3 (CH2), 25.8 (CH), 25.4 (CH3), 22.8 (CH3), 21.2 (CH2); HRMS (ESI): m/z [M+H]+ calculated for C21H23F3N2O2 443.1992, found 443.2062.

(1S,2S,5R)-2-(((S)-1-(((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20h): Prepared from 20h. Yellow crystals (96%); m.p. 145–147 °C; [α]D20 = +15.0 (c 0.1, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.26 (s, 1H), 8.44 (s, 1H), 8.08 (d, J = 4.3 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 3.83 (s, 1H), 3.57–3.52 (m, 2H), 3.46–3.43 (m, 1H), 3.17 (s, 1H), 1.86–1.85 (m, 1H), 1.76–1.62 (m, 3H), 1.49–1.39 (m, 3H), 1.05–0.99 (m, 1H), 0.88–0.83 (m, 1H), 0.81 (d, J = 6.3 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 153.9 (Cα), 143.2 (Cβ), 141.5 (Cγ), 139.5 (Cδ), 126.3 (CH), 119.7 (CH), 67.2 (CH), 60.7 (CH2), 43.0 (CH2), 43.0 (CH), 41.8 (CH2), 41.0 (CH), 35.4 (CH2), 25.9 (CH), 23.2 (CH2), 22.8 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H23F3N2O2 443.1992, found 443.2058.

(3R,3aR,6R,7aS)-3-(((5-Fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20i): Prepared from 19i. Yellow crystals (87%); m.p. 238–240 °C; [α]D20 = −61.0 (c 0.1250, MeOH); 1H NMR (500 MHz, DMSO-d6): δ (ppm) = 10.22 (s, 1H), 8.11 (d, J = 4.6 Hz, 1H), 8.05 (brs, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.7 Hz, 2H), 4.28–4.27 (m, 1H), 3.85 (d, J = 9.2 Hz, 1H), 3.81–3.77 (m, 1H), 3.54–3.46 (m, 2H), 1.87–1.84 (m, 1H), 1.79–1.75 (m, 1H), 1.65–1.62 (m, 1H), 1.55–1.52 (m, 1H), 1.45–1.43 (m, 1H), 1.12–0.94 (m, 2H), 0.83 (d, J = 6.5 Hz, 3H), 0.83–0.77 (m, 1H); 13C NMR (125 MHz, DMSO-d6): δ (ppm) = 154.0 (Cα), 143.3 (Cβ), 139.7 (Cγ), 133.4 (CH), 126.2 (CH), 120.0 (CH), 83.0 (Cγ), 76.9 (CH), 75.8 (CH2), 46.9 (CH2), 44.9 (CH2), 37.1 (CH2), 33.1 (CH2), 26.5 (CH), 24.2 (CH2), 22.6 (CH3); HRMS (ESI): m/z [M+H]+ calculated for C21H23F3N2O2 441.1835, found 441.1909.

(1R,2R,5R)-2-(((S)-1-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22a): Prepared from 21a. White crystals (80%); m.p. 167–169 °C; [α]D20 = +54.0 (c 0.1225, MeOH); 1H NMR
(500 MHz, CDCl₃): δ (ppm) = 10.21 (s, 1H), 8.17 (s, 1H), 7.89 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.12 (s, 1H), 3.64 (td, J = 4.1, 10.3 Hz, 1H), 3.59-3.45 (m, 2H), 1.85 (d, J = 12.1 Hz, 1H), 1.69-1.66 (m, 1H), 1.54-1.52 (m, 1H), 1.38-1.31 (m, 2H), 1.13 (s, 3H), 0.98-0.91 (m, 2H), 0.85 (d, J = 6.5 Hz, 3H), 0.80-0.72 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 158.6 (C₆), 148.5 (CH) 143.4 (C₅), 126.2 (CH), 123.9 (C₆) 120.0 (CH), 105.3 (C₄), 75.4 (C₅), 71.3 (CH), 49.9 (CH), 49.6 (CH₂), 45.2 (CH₂), 34.5 (CH₂), 31.1 (CH), 26.1 (CH₂), 22.4 (CH₃), 22.0 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₂₁H₂₇ClF₃N₄O₂ 459.1696, found 459.1770.

((1R,2R,5R)-2-((R)-1-((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22b): Prepared from 21b. White crystals (82%); m.p. 82–84 °C; [α]²⁰D = +12.0 (c 0.1325, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 9.95 (s, 1H), 8.10 (s, 1H), 7.90 (d, J =8.7 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H). 7.20 (brs, 1H), 3.95 (dd, J = 7.3, 13.4 Hz, 1H), 3.55 (td, J = 3.9, 10.3 Hz, 1H), 3.47 (dd, J = 4.2, 13.3 Hz, 1H), 1.87-1.79 (m, 2H), 1.63-1.60 (m, 1H), 1.42-1.34 (m, 2H), 1.00 (s, 3H), 0.99-0.90 (m, 1H), 0.88 (d, J = 6.4 Hz, 3H), 0.86-0.78 (m, 1H); ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) = 158.6 (C₆), 149.5 (CH), 143.7 (C₅), 126.2 (CH), 123.9 (C₄), 119.8 (CH), 105.3 (C₅), 74.9 (C₄), 71.3 (CH), 52.7 (CH), 49.3 (CH₂), 45.7 (CH₂), 34.8 (CH₂), 31.4 (CH), 26.0 (CH₂), 23.2 (CH₃), 22.4 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₂₁H₂₇ClF₃N₄O₂ 459.1696, found 459.1770.

(S)-3-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol hydrochloride (22c): Prepared from 21c. Yellow crystals (98%); m.p. 163–165 °C; [α]³⁰D = −4.0 (c 0.1300, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 10.10 (1H, s), 8.12 (s, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.7 Hz, 2H), 7.37 (s, 1H), 3.80-3.76 (m, 1H), 3.68-3.59 (m, 2H), 3.41 (s, 2H), 1.89-1.76 (m, 2H), 1.59-1.54 (m, 2H), 1.38-1.33 (m, 1H), 1.09-1.00 (m, 1H), 0.97-0.90 (m, 1H), 0.87 (d, J = 6.6 Hz, 3H), 0.83-0.75 (m, 1H); ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) = 158.5 (C₆), 148.7 (CH), 143.6 (C₅), 126.2 (CH), 119.8 (CH), 105.4 (C₄), 76.5 (C₅), 70.9 (CH), 65.6 (CH₂), 48.6 (CH), 45.9 (CH₂), 45.7 (CH₂), 34.7 (CH₂), 31.3 (CH), 25.7 (CH₂), 22.5 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₂₁H₂₇ClF₃N₄O₂ 475.1645, found 475.1712.

((1R,2S,5R)-2-((R)-1-((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22d): Prepared from 21d. Yellow crystals (98%); m.p. 173–175 °C; [α]³⁰D = −29.0 (c 0.1250, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 10.48 (brs, 1H), 8.29 (brs, 1H), 8.17 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 3.65-3.61 (m, 1H), 3.53 (d, J = 6.7 Hz, 2H), 3.46-3.41 (m, 1H), 3.28 (td, J = 3.9, 10.3 Hz, 1H), 2.22-2.19 (m, 1H), 1.86-1.84 (m, 1H), 1.57-1.52 (m, 2H), 1.36-1.31 (m, 2H), 1.18-1.10 (m, 1H), 0.90-0.88 (m, 1H), 0.86 (d, J = 6.4 Hz, 3H), 0.82-0.74 (m, 1H); ¹³C NMR (125MHz, DMSO-d₆): δ (ppm) = 158.2 (C₆), 153.9 (C₅), 145.6 (CH), 142.8 (C₄), 126.3 (CH), 123.8 (C₅), 120.3 (CH), 105.3 (C₆), 69.9 (CH), 62.4 (CH₂), 45.5 (CH), 45.5 (CH₂), 42.4 (CH₂), 40.8 (CH), 34.9 (CH₂), 31.4 (CH), 27.2 (CH₂), 22.6 (CH₃); HRMS (ESI): m/z [M+H]+ calculated for C₂₁H₂₇ClF₃N₄O₂ 459.1696, found 459.1769.

(3S,3aR,6R,7aR)-3-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (22e): Prepared from 21e. White crystals (85%); m.p. 188–190 °C; [α]³⁰D = −23.0 (c 0.1350, MeOH); ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 10.46 (s, 1H), 8.20 (s, 1H), 7.87 (d, J = 8.5 Hz, 3H), 7.66 (d, J = 8.5 Hz,
2H), 3.98 (d, J = 9.6 Hz, 1H), 3.72 (dd, J = 6.3, 13.4 Hz, 1H), 3.59 (dd, J = 6.0, 13.4 Hz, 1H), 3.53 (d, J = 9.8 Hz, 1H), 3.46-3.40 (m, 1H), 1.96-1.93 (m, 1H), 1.61-1.59 (m, 2H), 1.38-1.37 (m, 1H), 1.27-1.17 (m, 1H), 1.12-1.08 (m, 1H), 0.89 (d, J = 6.5 Hz, 3H), 0.87-0.82 (m, 1H), 0.79-0.71 (m, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ (ppm) = 158.9 (C$_q$), 154.2 (C$_q$), 147.8 (CH), 142.9 (C$_q$), 126.3 (CH), 123.8 (C$_q$), 120.3 (CH), 105.4 (C$_q$), 80.7 (CH), 79.2 (CH$_2$), 78.6 (CH$_2$), 52.5 (CH), 47.4 (CH$_2$), 40.4 (CH$_2$), 34.4 (CH$_2$), 30.9 (CH), 22.5 (CH$_2$), 22.4 (CH$_2$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{21}$H$_{25}$Cl$_3$F$_3$N$_4$O$_2$ 457.1540, found 457.1615.

(1S,2R,5R)-2-((S)-1-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22f): Prepared from 21f. White crystals (87%); m.p. 137–140 °C; [α]$_D^{20}$ = +29.0 (c 0.1325, MeOH); $^1$H NMR (500 MHz, DMSO-$d_6$): δ (ppm) = 10.45 (s, 1H), 8.17 (s, 1H), 8.08 (brs, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 4.19 (s, 1H), 3.61-3.50 (m, 2H), 1.80-1.55 (m, 5H), 1.33-1.23 (m, 1H), 1.16 (s, 3H), 1.03-0.98 (m, 1H), 0.91-0.82 (m, 1H), 0.83 (d, J = 6.3 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ (ppm) = 158.6 (C$_q$), 154.1 (C$_q$), 145.6 (CH), 143.0 (C$_q$), 126.4 (CH), 120.1 (CH), 105.4 (C$_q$), 73.5 (C$_q$), 65.4 (CH), 49.9 (CH), 49.0 (CH$_2$), 43.2 (CH$_2$), 35.4 (CH$_2$), 31.2 (CH), 25.8 (CH$_3$), 22.7 (CH$_3$), 21.4 (CH$_2$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{21}$H$_{25}$Cl$_3$F$_3$N$_4$O$_2$ 459.1696, found 459.1769.

(1S,2S,5R)-2-((S)-1-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22g): Prepared from 21g. White crystals (95%); m.p. 162–164 °C; [α]$_D^{20}$ = +2.0 (c 0.1150, MeOH); $^1$H NMR (500 MHz, DMSO-$d_6$): δ (ppm) = 10.15 (brs, 1H), 8.10 (s, 1H), 8.02 (brs, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 3.83 (s, 1H), 3.62-3.56 (m, 3H), 3.49-3.45 (m, 1H), 1.84-1.63 (m, 4H), 1.50-1.45 (m, 3H), 1.07-0.98 (m, 1H), 0.89-0.84 (m, 1H), 0.81 (d, J = 6.4 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ (ppm) = 158.2 (C$_q$), 143.5 (C$_q$), 148.5 (CH), 126.3 (CH), 119.7 (CH), 105.3 (C$_q$), 66.9 (CH), 61.6 (CH$_2$), 43.0 (CH$_2$), 42.8 (CH$_2$), 42.5 (CH), 40.9 (CH), 35.4 (CH$_2$), 25.8 (CH), 23.6 (CH$_2$), 22.8 (CH$_3$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{21}$H$_{27}$Cl$_3$F$_3$N$_4$O$_2$ 459.1696, found 459.1761.

(3R,3aR,6R,7aS)-3-(((5-Chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methylcyclohexa-3,5-dien-1-yl hydrobenzofuran-3-ol hydrochloride (22h): Prepared from 21h. Yellow crystals (85%); m.p. 223–225 °C; [α]$_D^{20}$ = −30.0 (c 0.1350, MeOH); $^1$H NMR (500 MHz, DMSO-$d_6$): δ (ppm) = 10.08 (s, 1H), 8.13 (s, 1H), 7.88 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 4.29-4.28 (m, 1H), 3.87 (d, J = 9.4 Hz, 1H), 3.75 (dd, J = 5.6, 13.4 Hz, 1H), 3.58 (dd, J = 5.3, 13.5 Hz, 1H), 3.49 (d, J = 9.4 Hz, 1H), 1.87-1.85 (m, 1H), 1.77-1.72 (m, 1H), 1.61-1.52 (m, 2H), 1.45-1.44 (m, 1H), 1.13-0.99 (m, 1H), 0.83 (d, J = 6.5 Hz, 3H), 0.83-0.77 (m, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ (ppm) = 158.4 (C$_q$), 150.0 (CH),143.6 (C$_q$), 126.2 (CH), 119.9 (CH), 105.3 (C$_q$), 82.8 (CH$_2$), 76.8 (CH), 75.9 (CH$_2$), 46.9 (CH), 45.1 (CH$_2$), 37.0 (CH$_2$), 33.0 (CH$_2$), 26.5 (CH), 24.1 (CH$_2$), 22.6 (CH$_3$); HRMS (ESI): m/z [M+H]$^+$ calculated for C$_{21}$H$_{25}$Cl$_3$F$_3$N$_4$O$_2$ 457.1540, found 457.1613.

1.6 Determination of antiproliferative effect

The growth-inhibitory effects of the presented heterocyclic compounds were determined by a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on a panel of human adherent cancer cell lines of gynecological origin containing HeLa and SiHa (cervical cancers), A2780 (ovarian cancer) and MDA-MB-231 (breast cancer) cells. All cell lines were purchased from the European Collection of Cell Cultures (Salisbury, UK) except the SiHa, obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultivated in minimal essential medium (MEM) supplemented with fetal bovine serum.
(10%), non-essential amino acids, and penicillin-streptomycin (1% each) at 37 °C in a humidified atmosphere containing 5% CO₂. All media and supplements were obtained from Lonza Group Ltd. (Basel, Switzerland). Cancer cells were plated into 96-well plates at the density of 5000 cells/well. After overnight incubation, the test compound was added in two concentrations (10 µM and 30 µM) and incubated for 72 h under cell-culturing conditions. Then, MTT solution (5 mg/mL, 20 µL) was added to each well and incubated for four h. Finally, the medium was removed, and the precipitated formazan was dissolved in DMSO during 60 min of shaking at 37 °C. The absorbance was measured at 545 nm using a microplate reader (SpectroStarNano, BMG Labtech, Ortenberg, Germany). Two independent experiments were carried out with five wells for each condition. Cisplatin (Ebewe GmbH, Unterach, Austria) was used as a positive control. Calculations were performed utilising the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA).
2. **Investigation of antiproliferative activity of the (−)-isopulegol-based heterocyclic compounds**

Table 1. Antiproliferative properties of the heterocyclic derivatives

| Compound | Chemical structure | Conc. (μM) | Growth Inhibition (%) ± SEM | HeLa | SiHa | A2780 | MDA-MB-231 |
|----------|-------------------|-----------|-----------------------------|-----|------|-------|-----------|
| 16a      | ![Chemical Structure](image) | 10        | -                           | -   | -    | -     | -         |
|          |                   | 30        | 18.49 ± 3.50                | 12.55 ± 11.74 | -     | -     | -         |
| 16b      | ![Chemical Structure](image) | 10        | 31.45 ± 1.55                | 47.12 ± 1.72  | 55.02 ± 1.27 | -     |
|          |                   | 30        | 83.37 ± 0.46                | 65.69 ± 1.19  | 82.06 ± 0.28 | 41.58 ± 1.09 |
|   | 17a | 10  | 17.07 ± 1.74 | 22.41 ± 1.96 | - | - |
|---|-----|-----|--------------|--------------|---|---|
|   |     | 30  | 19.03 ± 4.25 | 32.40 ± 1.49 | 12.01 ± 0.38 | 13.60 ± 4.66 |
| 17b | 10  | - | - | - | - | - |
|   | 30  | - | 12.06 ± 7.06 | - | - | - |
| 17c | 10  | - | 12.66 ± 2.97 | 25.21 ± 1.92 | - | - |
|   | 30  | 19.22 ± 2.38 | 17.75 ± 1.58 | 37.98 ± 2.82 | - | - |
|     | Chemical Structure | Time | Value 1  | Value 2  | Value 3  | Value 4  |
|-----|-------------------|------|----------|----------|----------|----------|
| 17d | ![Chemical Structure](image1) | 10   | 26.44 ± 1.24 | -        | -        | -        |
|     |                   | 30   | 23.04 ± 1.82 | 43.07 ± 1.25 | 23.21 ± 2.43 | -        |
| 19a | ![Chemical Structure](image2) | 10   | -        | -        | -        | -        |
|     |                   | 30   | 26.06 ± 2.24 | 34.63 ± 1.55 | -        | -        |
| 20a | ![Chemical Structure](image3) | 10   | 75.38 ± 0.53 | 54.14 ± 1.05 | 75.64 ± 2.25 | 32.06 ± 1.94 |
|     |                   | 30   | 86.75 ± 0.41 | 89.19 ± 0.81 | 92.14 ± 0.49 | 67.79 ± 1.09 |
|    |                  | 10       | 20       | 30       | 40       |
|----|-----------------|----------|----------|----------|----------|
| 20b| ![Molecule](image) | 94.62 ± 0.35 | 84.94 ± 1.11 | 91.50 ± 0.36 | 55.65 ± 1.26 |
|    | ![Molecule](image) | 96.90 ± 0.46 (IC$_{50}$: 7.20 μM) | 95.89 ± 0.74 (IC$_{50}$: 2.62 μM) | 96.58 ± 0.76 (IC$_{50}$: 4.98 μM) | 99.82 ± 1.61 (IC$_{50}$: 9.91 μM)** |
| 20c| ![Molecule](image) | 28.69 ± 1.54 | 41.01 ± 0.99 | 21.14 ± 1.22 | - |
|    | ![Molecule](image) | 94.95 ± 0.45 | 84.07 ± 1.20 | 94.83 ± 0.10 | 63.52 ± 1.17 |
| 20d| ![Molecule](image) | 19.49 ± 3.01 | 31.56 ± 0.93 | 79.18 ± 0.49 | - |
|    | ![Molecule](image) | 92.06 ± 0.36 | 92.45 ± 0.54 | 94.42 ± 0.30 | 74.72 ± 2.11 |
| 20e |   |   |   |   |   |
|-----|---|---|---|---|---|
|     | ![Molecule](image) | 10 | 12.59 ± 1.39 | 26.37 ± 2.64 | 28.11 ± 3.60 |
|     |   | 30 | 50.98 ± 3.52 | 37.05 ± 0.77 | 70.09 ± 1.19 |
|     | ![Molecule](image) | 10 | 13.77 ± 2.29 | 24.96 ± 0.39 | - |
|     |   | 30 | 92.44 ± 1.47 | 74.26 ± 3.53 | 89.04 ± 2.28 | 74.60 ± 2.78 |
|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| **20g** | ![Chemical Structure](image) | 10 | $37.11 \pm 3.39$ | $32.92 \pm 0.91$ | $31.49 \pm 2.40$ | - |
|     | ![Chemical Structure](image) | 30 | $70.69 \pm 1.19$ | $58.90 \pm 0.48$ | $98.36 \pm 0.29$ | $56.98 \pm 2.27$ |
| **20h** | ![Chemical Structure](image) | 10 | $30.15 \pm 1.99$ | $11.05 \pm 2.92$ | $11.29 \pm 1.11$ | - |
|     | ![Chemical Structure](image) | 30 | $59.09 \pm 2.27$ | $37.91 \pm 1.40$ | $56.94 \pm 1.11$ | $34.49 \pm 1.63$ |
| **20i** | ![Chemical Structure](image) | 10 | $65.72 \pm 1.07$ | $61.55 \pm 1.14$ | $35.26 \pm 1.38$ | $22.45 \pm 1.11$ |
|     | ![Chemical Structure](image) | 30 | $68.08 \pm 0.82$ | $61.61 \pm 1.77$ | $53.75 \pm 3.31$ | $37.80 \pm 1.32$ |
|     | Structure | 10          | 30          | 29.03 ± 2.20 |       |
|-----|-----------|-------------|-------------|--------------|-------|
| 22a |           | 27.66 ± 0.60| 35.35 ± 1.00| 78.25 ± 0.75 |       |
|     |           | 93.40 ± 0.26| 89.59 ± 0.54| 92.12 ± 0.32 | 88.09 ± 0.54 |
| 22b |           | 96.00 ± 0.39| 76.02 ± 2.05| 96.26 ± 0.58 | 40.32 ± 2.18 |
|     |           | 96.55 ± 0.43| 95.32 ± 0.43| 96.69 ± 0.23 | 96.16 ± 1.09 |
|     |           | (IC<sub>50</sub>: 9.03 μM)** | (IC<sub>50</sub>: 5.69 μM) | (IC<sub>50</sub>: 4.13 μM) | (IC<sub>50</sub>: 11.47 μM) |
| 22c |           | 18.42 ± 3.17| 21.84 ± 2.29| 28.15 ± 2.32 |       |
|     |           | 92.99 ± 0.27| 72.33 ± 2.40| 93.16 ± 0.40 | 74.01 ± 0.88 |
|   | 22d | 22e | 22f |
|---|-----|-----|-----|
|   | ![Molecule 22d](image) | ![Molecule 22e](image) | ![Molecule 22f](image) |
| 10 | ![Table 1](table) | ![Table 2](table) | ![Table 3](table) |
| 30 | ![Table 4](table) | ![Table 5](table) | ![Table 6](table) |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 22g |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 22h |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 23a |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 23b | ![Chemical Structure](image) | 10 | 52.61 ± 1.56 | - | - | 10.68 ± 2.36 |
|-----|------------------------------|----|---------------|---|---|---------------|
|     |                              | 30 | 58.33 ± 0.21  | 21.55 ± 2.37 | 36.23 ± 3.13 | 11.82 ± 3.71 |
| 23c | ![Chemical Structure](image) | 10 | 57.62 ± 1.48  | - | 18.39 ± 1.03 | - |
|     |                              | 30 | 72.53 ± 2.44  | 17.93 ± 2.00 | 43.50 ± 0.72 | - |
| 23d | ![Chemical Structure](image) | 10 | 48.75 ± 3.01  | - | 35.06 ± 1.27 | - |
|     |                              | 30 | 69.46 ± 2.70  | - | 46.78 ± 2.75 | - |
|   | 23e |   | 23f |   | 23h |   | Cisplatin |
|---|-----|---|-----|---|-----|---|-----------|
|   | ![23e](image) | 10 | 58.49 ± 1.05 | 20.33 ± 0.35 | 29.57 ± 0.56 | - |           |
|   | ![23e](image) | 30 | 60.88 ± 4.23 | 31.47 ± 1.20 | 42.16 ± 1.12 | 11.47 ± 0.24 |
|   | ![23f](image) | 10 | 50.49 ± 1.19 | - | 36.18 ± 3.29 | - |           |
|   | ![23f](image) | 30 | 83.37 ± 1.91 | 14.29 ± 3.03 | 44.75 ± 3.08 | 11.51 ± 1.94 |
|   | ![23h](image) | 10 | 60.12 ± 1.61 | - | 20.71 ± 2.02 | - |           |
|   | ![23h](image) | 30 | 63.35 ± 1.14 | 17.49 ± 4.25 | 42.90 ± 2.06 | 18.79 ± 1.76 |
|   | Cisplatin | 10 | 42.61 ± 2.33 | 60.98 ± 0.92 | 83.57 ± 2.21 | 42.72 ± 2.68 |
|   |   | 30 | 99.93 ± 0.26 | 88.95 ± 0.53 | 95.02 ± 0.28 | 86.44 ± 0.42 |
* Cancer cell growth inhibition values less than 10% were considered insignificant and are not given numerically

** In the case of the most effective test compounds (20b and 22b) and reference agent cisplatin, the viability assays were repeated with a set of dilutions (0.1–30 μM), and the IC₅₀ values were determined using GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA).

During our antiproliferative work we intended to use a practical investigational approach, therefore, we focused on the present treatment guidelines of gynecological cancers to choose a long time available, well-characterised positive control molecule which is recommended for the therapy of all cancer types included in our experiment (namely, breast, cervical and ovarian cancer). According to the online available guidelines of the European Society of Medical Oncology (ESMO) platinum compounds, like cisplatin, are effective in the clinical treatment of different gynecological cancers.

Platinum-based therapy is still the first-line treatment option in local cervical cancer and it can be beneficial in combination with paclitaxel or topotecan in the therapy of advanced/metastatic cervical cancer. Moreover, patients suffering from either primary (early stage or epithelial form) or recurrent ovarian cancer can be treated with platinum derivatives, in most cases as part of combination chemotherapy. Among numerous different types of breast cancer according to their pathophysiological or developmental origin, for most triple negative breast cancer (defined by the absence of expression of estrogen and progesterone receptors and of overexpression of HER2 or amplification of HER2neu) chemotherapy included platinum derivatives remains the standard treatment. MDA-MB-231 cell line used in our work is an experimentally applicable triple negative breast cancer cell line.
3. $^1$H-,$^{13}$C- NMR spectra of new heterocyclic compounds

S1: $^1$H- and $^{13}$C-NMR of compound (3S,3aR,6R,7aR)-6-methyl-3-(((S)-1-phenylethyl)amino)methyl)octahydrobenzofuran-3-ol (8a)
S2: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aR)-6-Methyl-3-(((S)-1-phenylethyl)amino)methyl)octahydrobenzofuran-3-ol (8b)
S3: $^1$H- and $^{13}$C-NMR of compound (3$S$,3$a$R,6$R$,7$a$R)-3-(aminomethyl)-6-methyloctahydrobenzofuran-3-ol (8c)
S4: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aR)-3-(aminomethyl)-6-methyloctahydrobenzofuran-3-ol (8d)
S5: $^1$H- and $^{13}$C-NMR of compound 1-((S)-2-hydroxy-2-(((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15a)
**S6:** $^1$H- and $^{13}$C-NMR of compound 1-((R)-3-hydroxy-2-((1S,2R,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15b)
S7: $^1$H- and $^{13}$C-NMR of compound 1-((S)-2-hydroxy-2-((1R,2S,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15c)
S8: $^1$H- and $^{13}$C-NMR of compound 1-((S)-3-hydroxy-2-((1S,2S,4R)-2-hydroxy-4-methylcyclohexyl)propyl)-3-phenylthiourea (15d)
S9: $^1$H- and $^{13}$C-NMR of compound $(1R,2S,5R)$-5-methyl-2-((R)-2-(phenylimino)-1,3-thiazinan-5-yl)cyclohexanol (16a)
S10: $^1$H- and $^{13}$C-NMR of compound $(1S,2S,5R)$-5-methyl-2-((S)-2-(phenylimino)-1,3-thiazinan-5-yl)cyclohexanol (16b)
**S11**: $^1$H- and $^{13}$C-NMR of compound $(1R,2R,5R)$-5-methyl-2-((S)-5-methyl-2-(phenylimino)oxazolidin-5-yl)cyclohexanol (17a)
S12: $^1$H- and $^{13}$C-NMR of compound (1$R$,2$S$,5$R$)-5-methyl-2-((R)-2-(phenylimino)-1,3-oxazinan-5-yl)cyclohexanol (17b)
S13: $^1$H- and $^{13}$C-NMR of compound (1$S$,2$R$,5$R$)-5-methyl-2-((S)-5-methyl-2-(phenylimino)oxazolidin-5-yl)cyclohexanol ($17c$)
S14: $^1$H- and $^{13}$C-NMR of compound (1S,2S,5R)-5-methyl-2-((S)-2-(phenylimino)-1,3-oxazinan-5-yl)cyclohexanol (17d)
$^{1}H$- and $^{13}C$-NMR of compound (1$R$,2$R$,5$R$)-2-((S)-1-((2-chloro-5-fluoropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (19a)
S16: $^1$H- and $^{13}$C-NMR of compound (1$R$,2$R$,5$R$)-2-(((R)-1-((2-chloro-5-fluoropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (19b)
S17: $^1$H- and $^{13}$C-NMR of compound (S)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol (19c)
S18: $^1$H- and $^{13}$C-NMR of compound (1R,2S,5R)-2-((R)-1-((2-chloro-5-fluoropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (19d)
S19: $^1$H- and $^{13}$C-NMR of compound (3$S$,3a$R$,6$R$,7a$R$)-3-(((2-chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19e)
S20: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aR)-3-(((2-chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19f)
S21: $^1$H- and $^{13}$C-NMR of compound $(1S,2R,5R)-2-((S)-1-((2$-chloro-5$-fluoropyrimidine-4$-yl)amino)-2$-hydroxypropan-2$-yl)-5$methylcyclohexanol (19g)
S22: $^1$H- and $^{13}$C-NMR of compound (1$S$,2$S$,5$R$)-2-((S)-1-((2-chloro-5-fluoropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (19h)
S23: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aS)-3-(((2-chloro-5-fluoropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (19i)
S24: $^1$H- and $^{13}$C-NMR of compound (1R,2R,5R)-2-((S)-1-((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20a)
S25: $^1$H- and $^{13}$C-NMR of compound (1R,2R,5R)-2-((R)-1-((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20b)
S26: $^1$H- and $^{13}$C-NMR of compound (S)-3-((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol hydrochloride (20c)
S27: $^1$H- and $^{13}$C-NMR of compound (1R,2S,5R)-2-[(R)-1-[(5-fluoro-2-[(4-trifluoromethyl)phenyl]aminopyrimidin-4-yl]amino)-3-hydroxypropan-2-yl]-5-methylcyclohexan-1-ol hydrochloride (20d)
S28: $^1$H- and $^{13}$C-NMR of compound (3S,3aR,6R,7aR)-3-(((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20e)
S29: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aR)-3-(((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20f)
S30: $^1$H- and $^{13}$C-NMR of compound \((1S,2R,5R)-2-((S)-1-((5\text{-}fluoro-2-((4\text{-}trifluoromethyl)phenyl)amino)pyrimidin\text{-}4\text{-}yl)amino)-2\text{-}hydroxypropan\text{-}2\text{-}yl)-5\text{-}methylcyclohexan\text{-}1\text{-}ol\) hydrochloride (20g)
S31: $^1$H- and $^{13}$C-NMR of compound (1S,2S,5R)-2-((S)-1-(5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (20h)
S32: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aS)-3-(((5-fluoro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (20i)
S33: $^1$H- and $^{13}$C-NMR of compound $(1R,2R,5R)-2-(((S)-1-((2,5$-dichloropyrimidin-4-yl)$amino)$)-2$-hydroxypropan-2-yl)-5$-methylcyclohexanol (21a)
S34: $^1$H- and $^{13}$C-NMR of compound $(1R,2R,5R)-2-(((R)-1-((2,5$-dichloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (21b)
S35: $^1$H- and $^{13}$C-NMR of compound (S)-3-((2,5-Dichloropyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol (21c)
S36: $^1$H- and $^{13}$C-NMR of compound (1R,2S,5R)-2-((R)-1-((2,5-dichloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (21d)
S37: $^1$H- and $^{13}$C-NMR of compound (3S,3aR,6R,7aR)-3-(((2,5-dichloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (21e)
S38: $^1$H- and $^{13}$C-NMR of compound (1$S,2R,5R$)-2-((S)-1-(2,5-dichloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (21f)
S39: $^{1}$H- and $^{13}$C-NMR of compound (1S,2S,5R)-2-((S)-1-((2,5-dichloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (21g)
S40: $^1$H- and $^{13}$C-NMR of compound (3R,3aR,6R,7aS)-3-(((2,5-dichloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (21h)
S41: $^1$H- and $^{13}$C-NMR of compound $^{1R,2R,5R}$-$2$-$((S)$-$1$-$(5$-chloro-$2$-$(4$-(trifluoromethyl)phenyl)amino)pyrimidin-$4$-yl)amino)-2-hydroxypropan-$2$-yl)-$5$-methylcyclohexan-$1$-ol hydrochloride (22a)
S42: $^1$H- and $^{13}$C-NMR of compound $((1R,2R,5R)-2-((R)-1-((5$-chloro$-2-((4$-(trifluoromethyl)$)phenyl)amino$)pyrimidin$-4$-yl)amino$)-2$-hydroxypropan$-2$-yl)5$-methylcyclohexan$-1$-ol hydrochloride (22b)
S33: $^1$H- and $^{13}$C-NMR of compound (S)-3-((5-chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol hydrochloride (22c)
S34: $^1$H- and $^{13}$C-NMR of compound $(1R,2S,5R)$-$2-((R)-1-((5$-chloro$-2-((4$-$|(trifluoromethyl)|$phenyl)$amino$)pyrimidin$-4$-$yl$)amino$)-3$-hydroxypropan$-2$-$yl$)-5$-methylicyclohexan$-1$-$ol$ hydrochloride (22d)
S35: $^1$H- and $^{13}$C-NMR of compound (3S,3aR,6R,7aR)-3-(((5-chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (22e)
S36: $^1$H- and $^{13}$C-NMR of compound (1S,2R,5R)-2-((S)-1-((5-chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22f)
**S37:** $^1$H- and $^{13}$C-NMR of compound $(1S,2S,5R)$-2-((S)-1-((5-chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexan-1-ol hydrochloride (22g)
S38: \(^1\)H- and \(^{13}\)C-NMR of compound (3R,3aR,6R,7aS)-3-(((5-chloro-2-((4-(trifluoromethyl)phenyl)amino)pyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol hydrochloride (22h)
S39: $^1$H- and $^{13}$C-NMR of compound $(1R,2R,5R)$-2-((S)-1-((5-amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (23a)
S40: 

$^1$H- and $^{13}$C-NMR of compound ($1R,2R,5R$)-2-((R)-1-((5-amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (23b)
S41: $^1$H- and $^{13}$C-NMR of compound (S)-3-((5-amino-6-chloropyrimidin-4-yl)amino)-2-((1R,2R,4R)-2-hydroxy-4-methylcyclohexyl)propane-1,2-diol (23c)
$^{1}$H- and $^{13}$C-NMR of compound (1$R$,2$S$,5$R$)-2-((R)-1-((5-amino-6-chloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (23d)
S43: $^1$H- and $^{13}$C-NMR of compound (3$S$,3$aR$,6$R$,7$aR$)-3-(((5-amino-6-chloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (23e)
S44: $^1$H- and $^{13}$C-NMR of compound (1S,2R,5R)-2-((S)-1-((5-Amino-6-chloropyrimidin-4-yl)amino)-2-hydroxypropan-2-yl)-5-methylcyclohexanol (23f)
S45: $^{1}$H- and $^{13}$C-NMR of compound (1$S$,2$S$,5$R$)-2-((S)-1-((5-amino-6-chloropyrimidin-4-yl)amino)-3-hydroxypropan-2-yl)-5-methylcyclohexanol (23g)
S46: $^1$H- and $^{13}$C-NMR of compound (3$R$,3a$R$,6$R$,7a$S$)-3-(((5-amino-6-chloropyrimidin-4-yl)amino)methyl)-6-methyloctahydrobenzofuran-3-ol (23h)
4. Docking study

According to the validation study, the RMSD values showed that the crystal structure 4DEE (ADP bound to Aurora A active site) is valid and could be confidently used for docking study, where the RMSD values of all ADP generated conformations were less than 2 Å (Table 2).

| Entry  | RMSD (Å)  |
|--------|-----------|
| 4DEE 1 | 0.8808    |
| 4DEE 2 | 1.9663    |
| 4DEE 3 | 0.9532    |
| 4DEE 4 | 0.8146    |
| 4DEE 5 | 0.8178    |
| 4DEE 6 | 1.1551    |
| 4DEE 7 | 1.1506    |
| 4DEE 8 | 0.8264    |
| 4DEE 9 | 0.9767    |
| 4DEE 10| 0.9962    |

Figure 1. A: Compound 20b bound to Aurora A active site (PDB entry 4DEE), B: Overlay of compound 20b (carbons shown blue) and ADP (carbons shown Red), C: Structure of the compound 20b.

4.1. Determination of Molecular properties of 20b

Based upon the truth that oral bioavailability is highly affected by the molecular properties of the drug molecule, we have calculated the molecular properties of compound 20b by using Accelrys Discovery Studio 2.5 software. The Lipinski's rules of five shows that the absorption of a ligand is higher when: molecular weight less than 500 D, calculated log P value less than
5, no more than 5 hydrogen bond donor (HBD) groups, no more than 10 hydrogen bond acceptor (HBA) groups, and no more than 10 rotatable bonds.

As shown in table (3), it is obvious that compound 20b in accordance with Lipinski’s rules of five, so it might have good oral bioavailability.

| Compound | ALogP | Molecular Weight | Num. HBA | Num. HBD | Num. Rotable bounds |
|----------|-------|------------------|----------|----------|---------------------|
| 20b      | 3.41  | 442.45           | 6        | 4        | 7                   |

4.2. In-Silico ADMET analysis

ADMET refers to absorption, distribution, metabolism, excretion and toxicity properties for a molecule. They were predicted for compound 20b by using ADMET descriptors in Accelrys Discovery studio 2.5. There are six mathematical models are used to quantitatively predict properties of a set of compounds. These models contain: aqueous solubility (predict solubility in water at 25°C), blood brain barrier (BBB) penetration, cytochrome P450 (CYP450) 2D6 inhibition, hepatotoxicity, human intestinal absorption (HIA) and plasma protein binding. An ADMET model was also generated to predict the human intestinal absorption (HIA) and Blood Brain Barrier (BBB) penetration of tested compound. The model includes 95 and 99% confidence ellipses in the ADMET_PSA_2D and ADMET_ALogP98 plan as shown in figure (2).

![Figure 2](image)

**Figure 2.** Plot of Polar Surface Area (PSA) vs. LogP for a standard and test set showing the 95% and 99% confidence limit ellipses corresponding to the Blood Brain Barrier and Intestinal Absorption models.

| Compound | BBB level | Absorption level | Solubility level | Hepatotoxicity level | CYP2D6 inhibition level | PPB level | AlogP98 | PSA 2D |
|----------|-----------|------------------|------------------|----------------------|-------------------------|-----------|---------|--------|
| 20b      | 3 (low)   | 0 (good)         | 1 (possible)     | 0 (non-toxic)        | 0 (non-inhibitor)       | 1 (>90%)  | 4.52    | 89.77  |

As shown in Table 4, all these values are within the standard range shown in figure (2), which confirm that this compound has good absorption through human intestinal. Additionally, it exhibits low ability to penetrate blood brain barrier (BBB).
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