Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary Material

# Investigating Natural Compounds against Oncogenic RET Tyrosine Kinase using Pharmacoinformatic Approaches for Cancer Therapeutics

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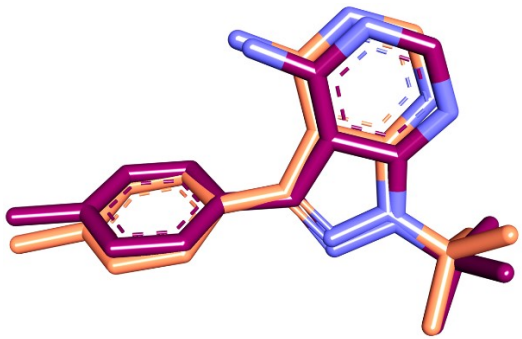

**Figure S1.** Overlay of the docked pose (mauve) of bound co-crystallized ligand, PP1 with its conformation (orange) in the crystal structure (PDB ID: 2IVV).

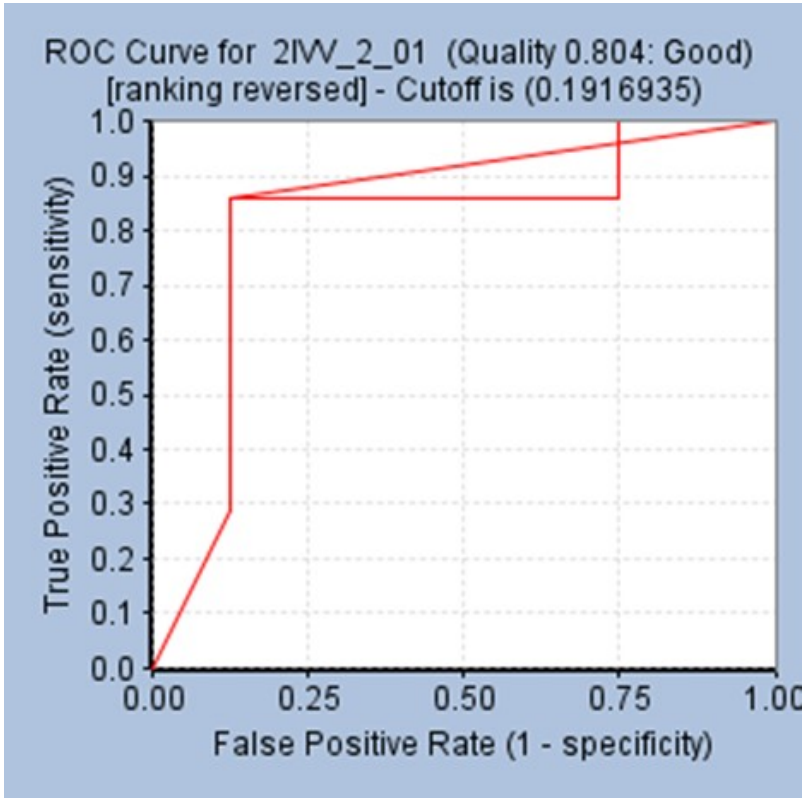


**Figure S2.** The ROC (Receiver Operating Characteristic) curve predicted for the receptor-ligand pharmacophore model, *Pharmacophore_01*.

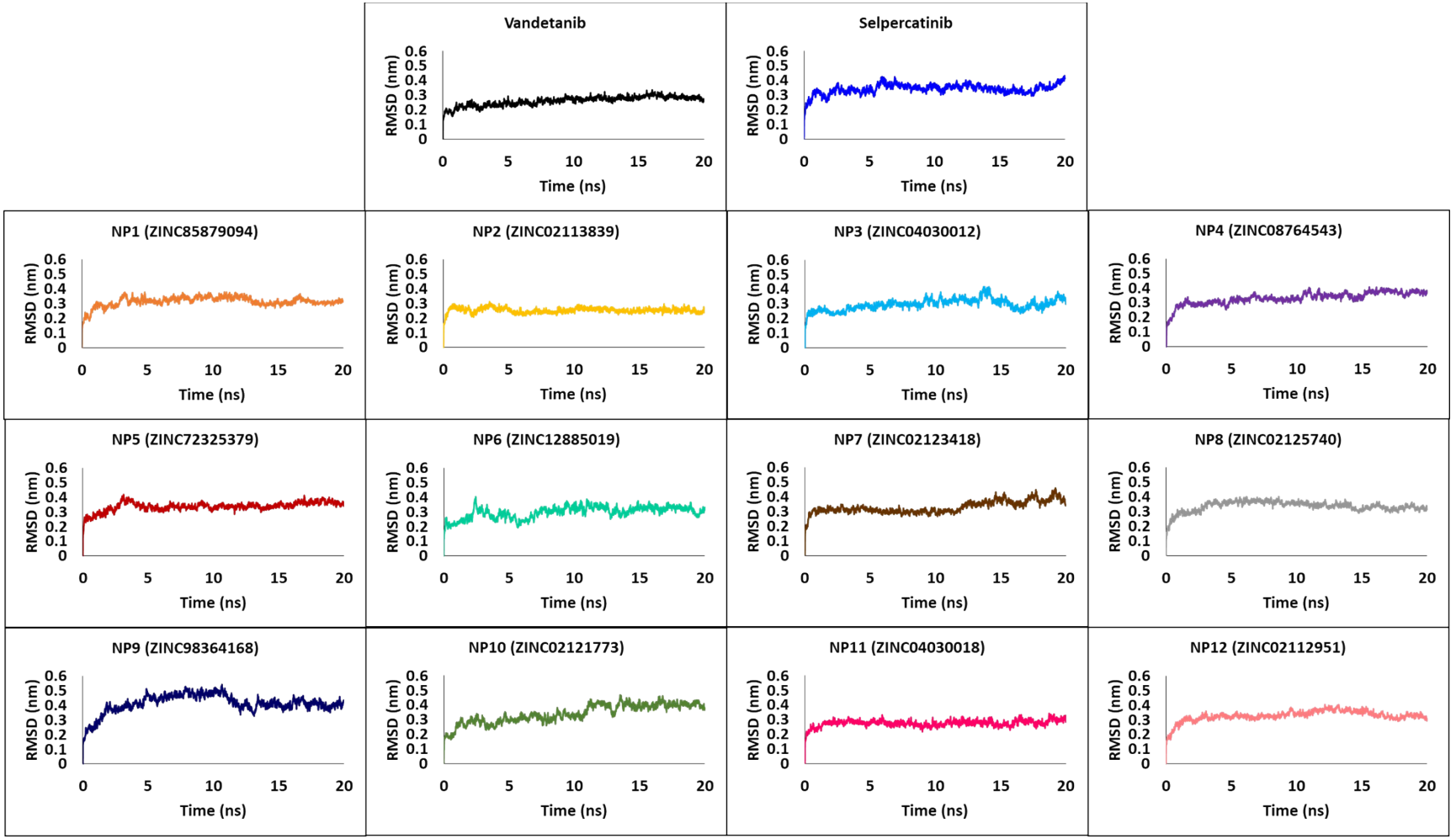


**Figure S3.** Backbone RMSDs are shown as a function of time for wild-type (WT) RET protein structures with the acquired 12 natural products (NPs) and reference inhibitors (vandetanib and selpercatinib).

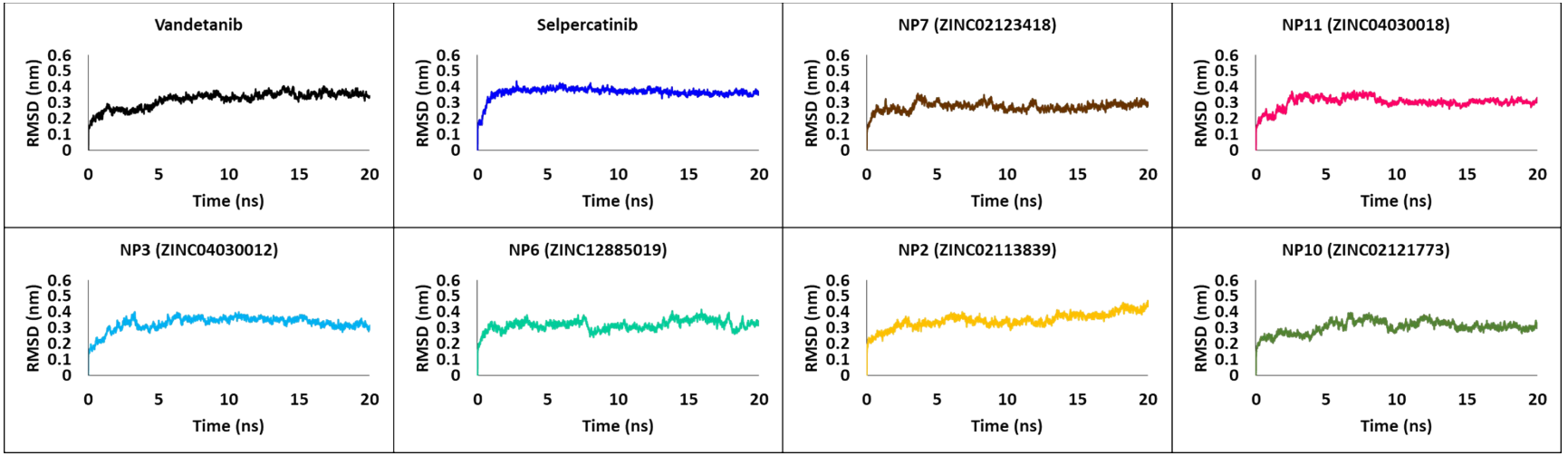


**Figure S4.** Backbone RMSDs are shown as a function of time for gate-keeper (V804M) mutant RET protein structures with the acquired 6 natural products (NPs) and reference inhibitors (vandetanib and selpercatinib).

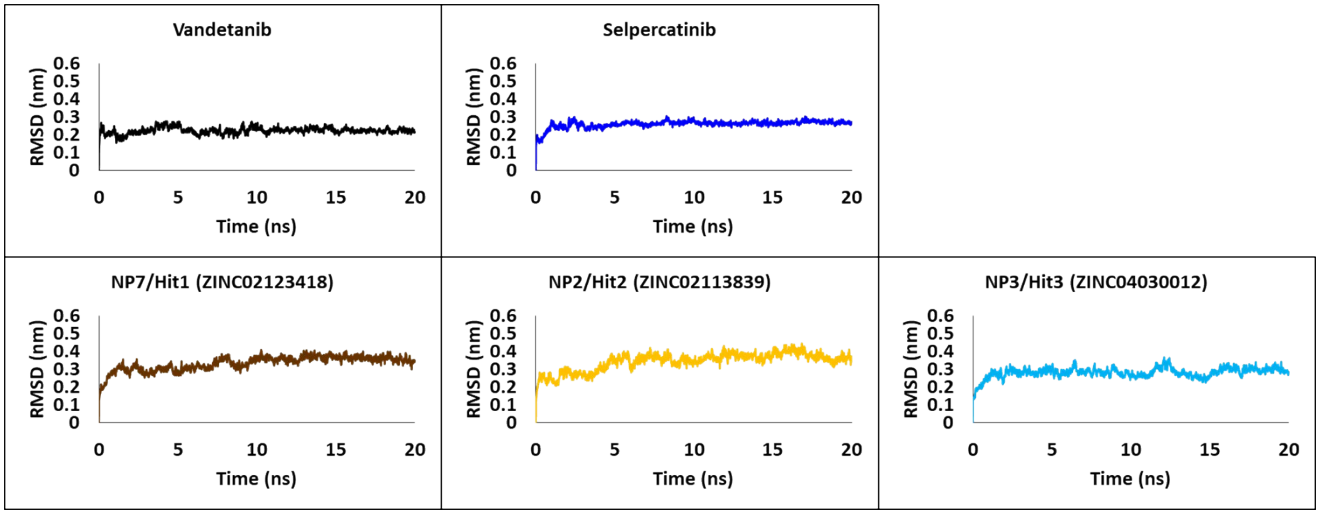


**Figure S5.** Backbone RMSDs are shown as a function of time for gate-keeper (V804L) mutant RET protein structures with the acquired 3 hits and reference inhibitors (vandetanib and selpercatinib).

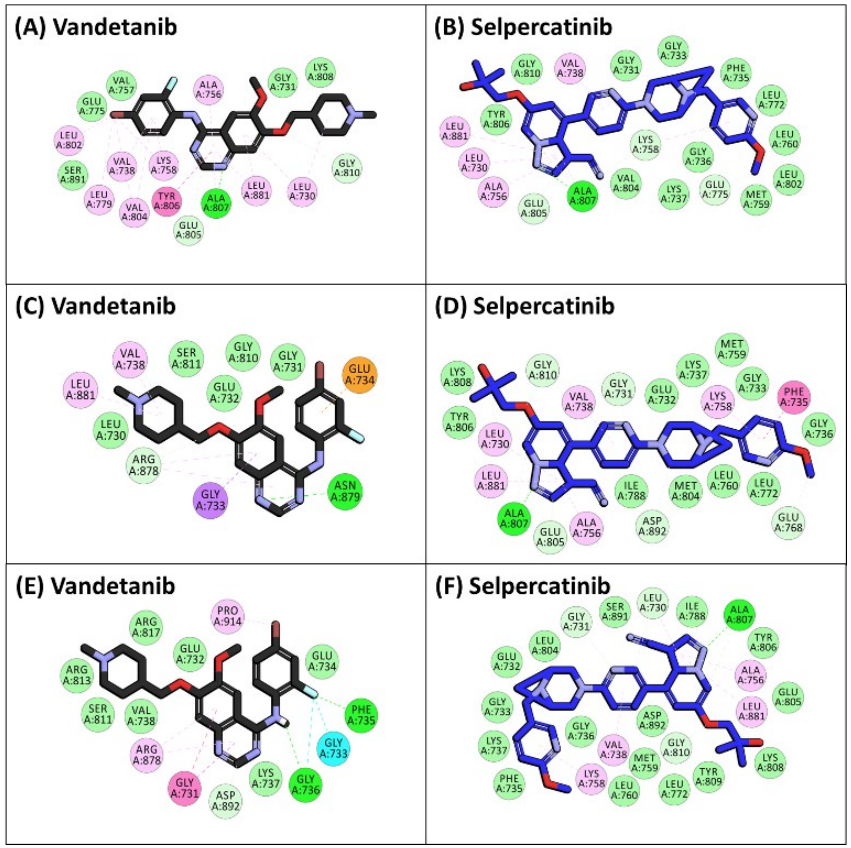


**Figure S6.** The 2D molecular interactions of reference inhibitors- Vandetanib (black) and Selpercatinib (blue) with key residues of wild-type (A and B), V804M (C and D), and V804L (E and F) RET kinase domain, respectively.

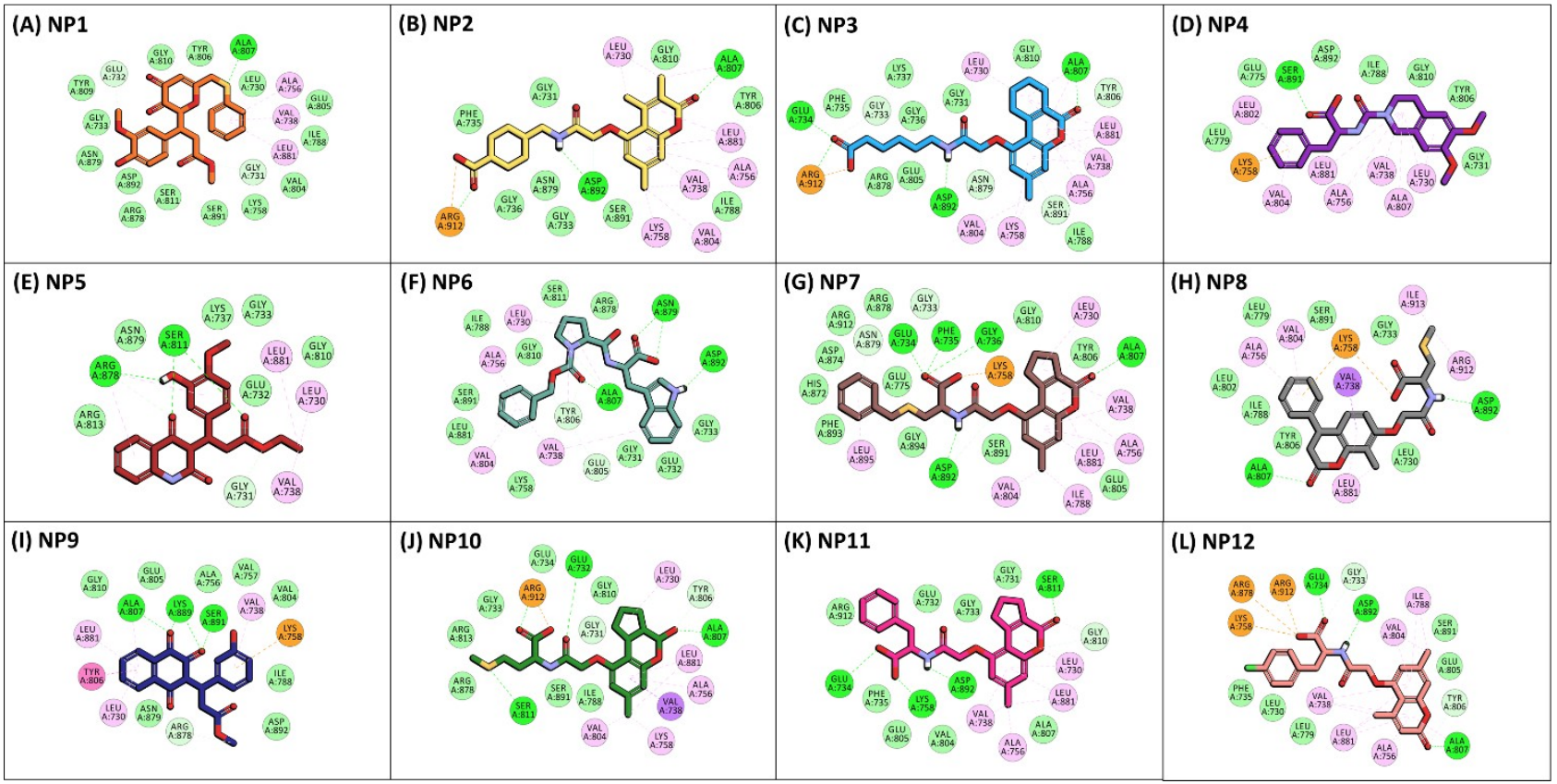


**Figure S7.** The 2D molecular interactions of 12 acquired natural products (NPs) with key residues of wild-type RET kinase domain. The hydrogen bonds are shown as green dashed lines, van der Waals interactions are shown as light green spheres and the hydrophobic bonds are displayed as pink, purple, and orange spheres.

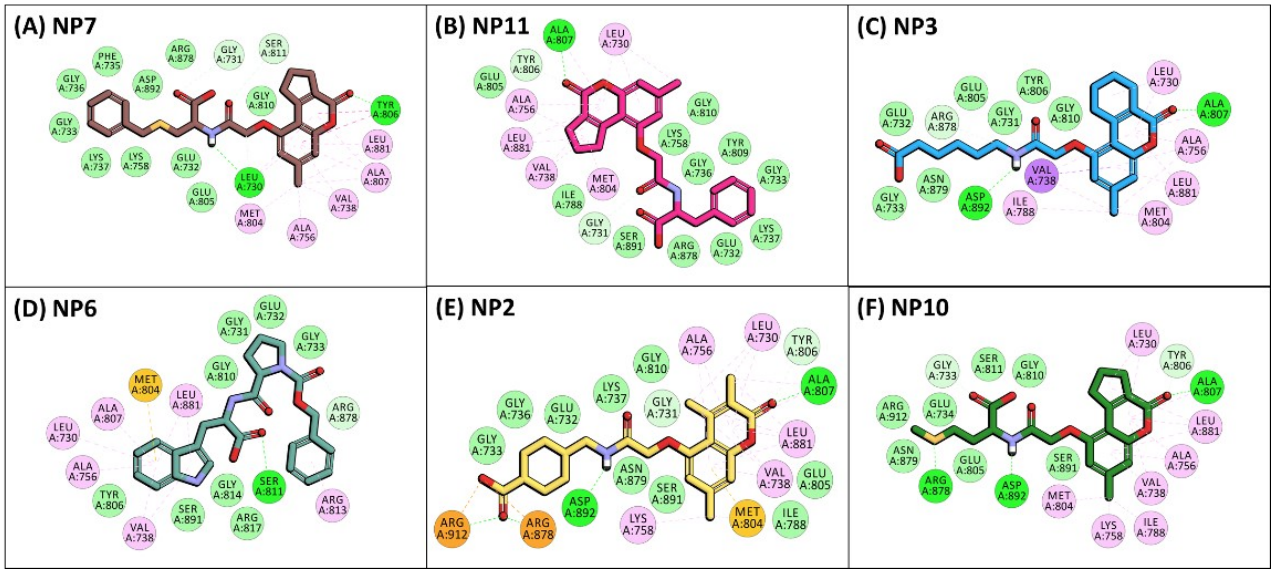


**Figure S8.** The 2D molecular interactions of 6 acquired natural products (NPs) with key residues of gate-keeper (V804M) mutant RET kinase domain. The hydrogen bonds are shown as green dashed lines, van der Waals interactions are shown as light green spheres and the hydrophobic bonds are displayed as pink, purple, and orange spheres.

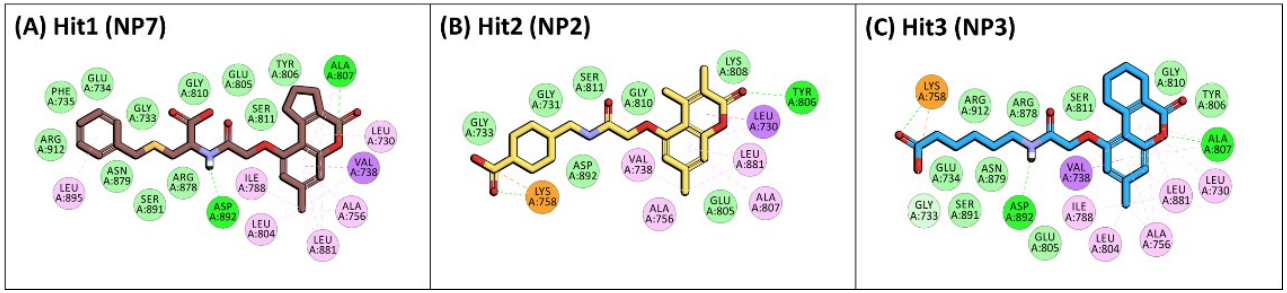


**Figure S9.** The 2D molecular interactions of 3 acquired hits with key residues of gate-keeper (V804L) mutant RET kinase domain. The hydrogen bonds are shown as green dashed lines, van der Waals interactions are shown as light green spheres and the hydrophobic bonds are displayed as pink, purple, and orange spheres.

**Table S1.** The molecular docking scores and binding free energy ($\Delta G_{bind}$) values of drug-like natural products (NPs) from ZINC database with RET wild-type (WT) tyrosine kinase domain. A total of 27 compounds demonstrated better dock scores than reference inhibitors (vandetanib and selpercatinib), while 12 NPs exhibited better $\Delta G_{bind}$ values.

| ZINC ID | GoldScore | ChemScore | $\Delta G_{bind}$ |
|---|---|---|---|
| ZINC02105755 | 72.877 | -31.261 | -22.14 +/- 18.323 |
| **ZINC02123418** | **71.278** | **-31.781** | **-157.41 +/- 20.002** |
| ZINC00940539 | 71.216 | -31.828 | 3.62 +/- 24.266 |
| **ZINC02125740** | **70.205** | **-30.759** | **-155.95 +/- 33.849** |
| ZINC70666480 | 69.750 | -33.147 | -66.69 +/- 16.894 |
| **ZINC85879094** | **69.010** | **-31.624** | **-212.39 +/- 30.783** |
| ZINC22468274 | 66.819 | -32.337 | 65.41 +/- 19.942 |
| ZINC85876830 | 65.816 | -29.814 | -65.00 +/- 20.359 |
| **ZINC02113839** | **65.073** | **-30.138** | **-197.55 +/- 21.070** |
| **ZINC04030018** | **64.727** | **-29.145** | **-129.02 +/- 20.563** |
| **ZINC02121773** | **64.722** | **-29.273** | **-134.62 +/- 27.939** |
| **ZINC72325379** | **64.376** | **-30.846** | **-182.00 +/- 33.852** |
| ZINC22468267 | 64.371 | -30.211 | 58.91 +/- 23.987 |
| **ZINC04030012** | **64.092** | **-28.399** | **-189.68 +/- 23.350** |
| ZINC85877411 | 63.971 | -29.839 | -68.59 +/- 14.801 |
| ZINC22468256 | 63.943 | -29.919 | 73.33 +/- 22.479 |
| **ZINC08764543** | **63.386** | **-29.256** | **-185.95 +/- 20.154** |
| **ZINC12885019** | **63.384** | **-30.701** | **-168.33 +/- 28.872** |
| ZINC85876206 | 63.286 | -30.002 | -74.24 +/- 17.243 |
| **ZINC98364168** | **63.270** | **-32.993** | **-154.52 +/- 36.901** |
| ZINC20590657 | 63.121 | -38.209 | 73.12 +/- 22.355 |
| ZINC22934508 | 62.299 | -31.763 | 70.47 +/- 32.344 |
| ZINC14589738 | 62.139 | -30.320 | -84.29 +/- 15.523 |
| ZINC20533280 | 60.854 | -33.777 | 77.27 +/- 22.217 |
| **ZINC02112951** | **60.537** | **-29.619** | **-125.58 +/- 33.252** |
| ZINC22468251 | 59.632 | -31.819 | 85.39 +/- 20.430 |
| ZINC20590553 | 58.605 | -31.637 | 65.56 +/- 30.077 |
| **Selpercatinib** | **57.563** | **-28.584** | **-95.76 +/- 21.511** |
| **Vandetanib** | **55.874** | **-28.271** | **-115.97 +/- 17.341** |

*Drug-like compounds with better dock scores as well as $\Delta G_{bind}$ values than reference inhibitors are highlighted in bold.

**Table S2.** The molecular docking scores and binding free energy ($\Delta G_{bind}$) values of natural products (NPs) from ZINC database with RET gate-keeper mutant (V804M) structure. A total of 6 NPs demonstrated both better dock scores and $\Delta G_{bind}$ values than reference inhibitors (vandetanib and selpercatinib) with the mutant kinase domain.

| ZINC ID | GoldScore | ChemScore | $\Delta G_{bind}$ |
|---|---|---|---|
| **ZINC04030012** | **76.060** | **-32.400** | **-222.16 +/- 48.294** |
| **ZINC02113839** | **75.266** | **-31.192** | **-177.67 +/- 25.168** |
| **ZINC02123418** | **74.794** | **-32.776** | **-232.44 +/- 31.621** |
| **ZINC12885019** | **73.758** | **-32.029** | **-190.33 +/- 32.931** |
| **ZINC02121773** | **67.104** | **-29.690** | **-170.17 +/- 31.811** |
| **ZINC04030018** | **66.202** | **-30.197** | **-228.39 +/- 29.790** |
| ZINC02112951 | 61.874 | -28.997 | -229.71 +/- 27.457 |
| ZINC02125740 | 60.246 | -27.368 | -140.75 +/- 27.788 |
| ZINC85879094 | 59.246 | -25.753 | -193.80 +/- 27.451 |
| ZINC08764543 | 57.186 | -25.735 | -151.01 +/- 17.654 |
| ZINC72325379 | 52.941 | -16.962 | -176.62 +/- 28.823 |
| ZINC98364168 | 51.357 | -20.172 | -101.41 +/- 30.242 |
| **Selpercatinib** | **63.682** | **-29.079** | **-103.53 +/- 28.664** |
| **Vandetanib** | **50.606** | **-21.445** | **-82.01 +/- 17.181** |

*Drug-like compounds with better dock scores as well as $\Delta G_{bind}$ values than reference inhibitors are highlighted in bold.

**Table S3.** The molecular docking scores and binding free energy ($\Delta G_{bind}$) values of hits from ZINC database with RET gate-keeper mutant (V804L) structure. A total of 3 hits demonstrated both better dock scores and $\Delta G_{bind}$ values than reference inhibitors (vandetanib and selpercatinib) with the mutant kinase domain.

| ZINC ID | GoldScore | ChemScore | $\Delta G_{bind}$ |
|---|---|---|---|
| **ZINC04030012** | **74.348** | **-34.474** | **-146.40 +/- 19.385** |
| **ZINC02123418** | **74.206** | **-32.961** | **-194.31 +/- 32.373** |
| **ZINC02113839** | **73.766** | **-30.960** | **-171.98 +/- 27.358** |
| ZINC02125740 | 69.780 | -27.337 | -150.77 +/- 23.313 |
| ZINC02121773 | 61.608 | -24.673 | -217.40 +/- 32.298 |
| ZINC12885019 | 66.773 | -31.159 | -175.25 +/- 50.752 |
| ZINC04030018 | 63.613 | -23.953 | -114.98 +/- 30.894 |
| ZINC02112951 | 59.551 | -24.609 | -229.47 +/- 39.821 |
| ZINC85879094 | 61.496 | -25.421 | -210.20 +/- 29.391 |
| ZINC08764543 | 55.605 | -26.057 | -168.27 +/- 48.356 |
| ZINC98364168 | 54.331 | -25.267 | -111.60 +/- 36.711 |
| ZINC72325379 | 51.814 | -16.051 | -188.80 +/- 40.351 |
| **Selpercatinib** | **73.704** | **-29.035** | **-105.83 +/- 25.589** |
| **Vandetanib** | **50.853** | **-24.674** | **-93.36 +/- 22.040** |

*Drug-like compounds with better dock scores as well as $\Delta G_{bind}$ values than reference inhibitors are highlighted in bold.

**Table S4.** The average backbone RMSD (root mean square deviation) values (in nm) of acquired natural products and reference inhibitors (vandetanib and selpercatinib) with wild-type (WT), V804M, and V804L RET variant structures.

| ZINC ID | Average RMSD (WT) | Average RMSD (V804M) | Average RMSD (V804L) |
|---|---|---|---|
| ZINC85879094 (NP1) | **0.31** | N/A | N/A |
| ZINC02113839 (NP2) | **0.25** | **0.34** | **0.34** |
| ZINC04030012 (NP3) | **0.29** | **0.32** | **0.28** |
| ZINC08764543 (NP4) | **0.33** | N/A | N/A |
| ZINC72325379 (NP5) | **0.33** | N/A | N/A |
| ZINC12885019 (NP6) | **0.29** | **0.31** | N/A |
| ZINC02123418 (NP7) | **0.32** | **0.27** | **0.33** |
| ZINC02125740 (NP8) | **0.33** | N/A | N/A |
| ZINC98364168 (NP9) | **0.41** | N/A | N/A |
| ZINC02121773 (NP10) | **0.34** | **0.30** | N/A |
| ZINC04030018 (NP11) | **0.27** | **0.30** | N/A |
| ZINC02112951 (NP12) | **0.32** | N/A | N/A |
| Selpercatinib | **0.34** | **0.36** | **0.25** |
| Vandetanib | **0.26** | **0.32** | **0.22** |

*N/A: The RMSD values were only reported for the identified natural products with the respective RET structures.

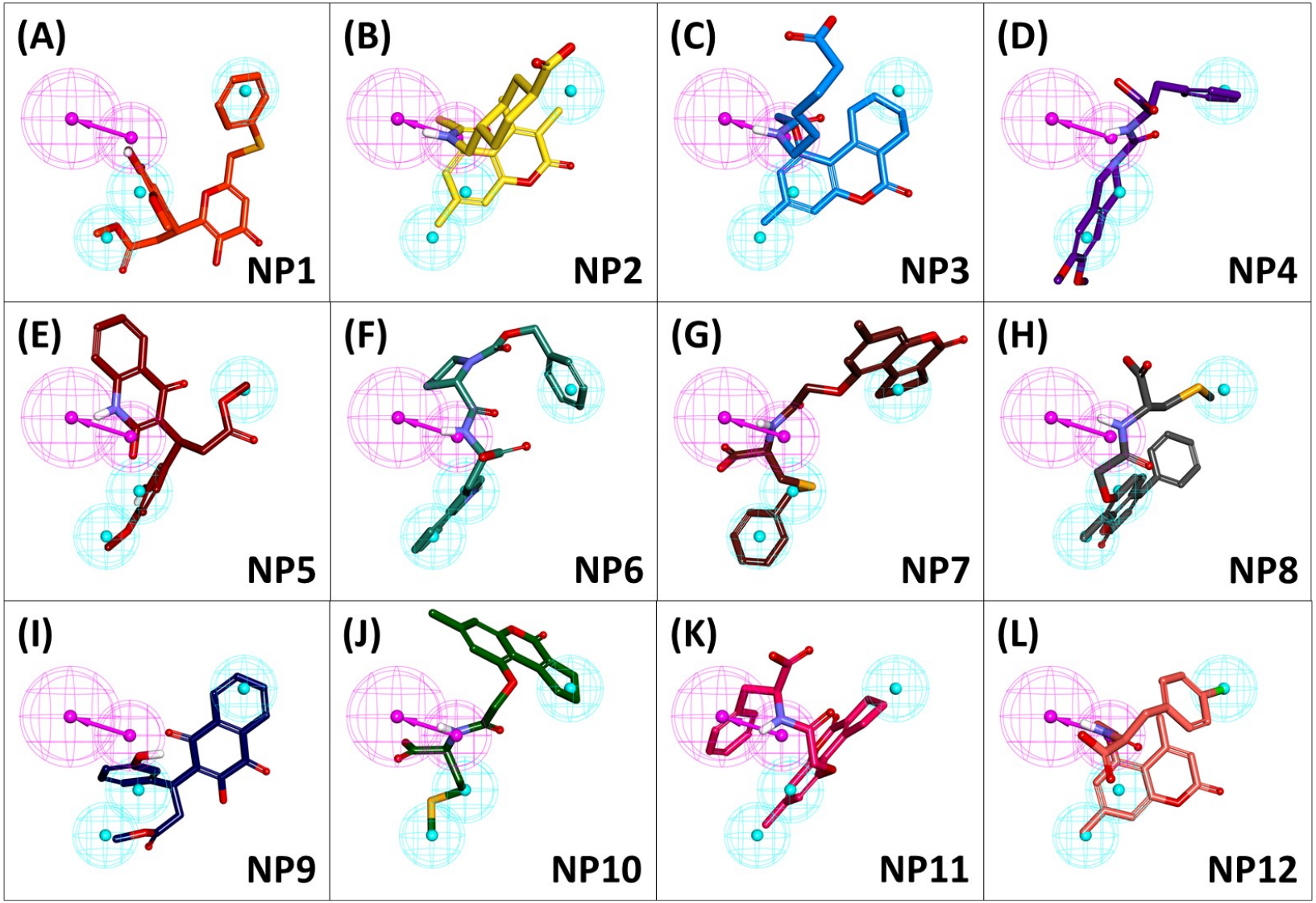


**Figure S10.** The mapping of the 12 acquired natural products with the pharmacophoric features. All 12 compounds represent the HBD (hydrogen bond donor) and Hy (hydrophobic) feature.