molecules

# Supplementary Material

**Table S1.** The topological polar surface area (TPSA) and parameters of Lipinski´s rule of five for vemurafenib and its derivatives (VEM-1, VEM-2, VEM-3).

| Compound | TPSA | MWt | LogP | HBD | HBA |
|---|---|---|---|---|---|
| | expected values | | | | |
| | (<140Å$^2$) | (≤500 g mol$^{-1}$) | (≤5) | (≤5) | (≤8) |
| vemurafenib | 91.92 | 489.93 | 5.17 | 2 | 5 |
| VEM-1 | 177.14 | 645.64 | 3.26 | 4 | 11 |
| VEM-2 | 177.14 | 659.67 | 3.59 | 4 | 11 |
| VEM-3 | 177.14 | 673.69 | 3.96 | 4 | 11 |

**Table S2.** Mode of action (inhibition/substrate) and intrinsic hepatic clearance ($Cl_{int}$) for selected cytochrome P450 isoforms for vemurafenib and its derivatives (VEM-1, VEM-2, VEM-3).

| Compound | CYP | | | | | |
|---|---|---|---|---|---|---|
| | Mode of action | | | $C_{lint}$ (µL/min/mg) | | |
| | 1A2 | 2C9 | 3A4 | 1A2 | 2C9 | 3A4 |
| vemurafenib | S/I | S | S | 4.84 | 220.15 | 76.64 |
| VEM-1 | S/I | NS | S | 15.26 | - | 1238.95 |
| VEM-2 | S/I | NS | S/I | 13.97 | - | 903.74 |
| VEM-3 | S/I | NS | S/I | 10.38 | - | 832.65 |

I-indicates inhibition of CYP isoforms; S-indicates substrate for CYP isoforms; NS-indicates non substrate for isoforms.

**Table S3.** Mode of action (inhibition/substrate) of selected transporters models (likelihood of the renal organic anion transporters (OAT1 and OAT3) and hepatic organic cation transporters (OCT1 and OCT2)) for vemurafenib and its derivatives (VEM-1, VEM-2, VEM-3).

| Compound | OAT1 substrate/ inhibitor | OAT3 substrate/ inhibitor | OCT1 substrate/ inhibitor | OCT2 substrate/ inhibitor |
|---|---|---|---|---|
| vemurafenib | No/Yes | No/Yes | No/Yes | No/Yes |
| VEM-1 | No/Yes | No/Yes | No/Yes | No/Yes |
| VEM-2 | No/Yes | No/Yes | No/Yes | No/Yes |
| VEM-3 | No/Yes | No/Yes | No/Yes | No/Yes |

**Table S4.** The comparison between predicted and observed pharmacokinetic parameters of vemurafenib administered at three different doses.

| Parameters | Vemurafenib | | | | | |
|---|---|---|---|---|---|---|
| | Predicted | | | Observed [1] | | |
| Dose [mg] | 240 mg | 480 mg | 960 mg | 240 mg | 480 mg | 960 mg |
| $C_{max\ 0\text{-}8h}$ [ng/mL] | 1.1 | 2.1 | 3.8 | 1.9 | 2.6 | 4.8 |
| $T_{max\ 0\text{-}8h}$ [h] | 5.0 | 5.0 | 5.9 | 4.0 | 4.0 | 5.0 |
| $AUC_{0\text{-}8h}$ [ng-h/mL] | 6.2 | 11.8 | 21.3 | 8.3 | 13.8 | 27.0 |
| $AUC_{0\text{-}24h}$ [ng-h/mL] | 18.4 | 35.0 | 63.3 | 40.9 | 62.4 | 130.6 |
| $t_{1/2}$ [h] | 15.1 | 15.0 | 15.0 | 31.5 | 38.4 | 34.1 |

**Table S5.** Predicted pharmacokinetic parameters for VEM-1, VEM-2 and VEM-3 administered at three different doses.

| Parameters | VEM-1 | | | VEM-2 | | | VEM-3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose [mg] | 240 mg | 480 mg | 960 mg | 240 mg | 480 mg | 960 mg | 240 mg | 480 mg | 960 mg |
| $C_{max\ 0\text{-}8h}$ [ng/mL] | 13.9 | 19.1 | 23.6 | 6.1 | 8.1 | 9.7 | 3.0 | 3.6 | 4.2 |
| $T_{max\ 0\text{-}8h}$ [h] | 5.8 | 5.8 | 6.0 | 6.3 | 6.3 | 6.5 | 6.3 | 6.4 | 6.6 |
| $AUC_{0\text{-}8h}$ [ng-h/mL] | 80.0 | 110.0 | 135.3 | 34.5 | 45.5 | 54.3 | 16.6 | 20.2 | 23.7 |
| $AUC_{0\text{-}24h}$ [ng-h/mL] | 230.3 | 316.9 | 393.0 | 107.6 | 141.7 | 170.1 | 51.7 | 62.7 | 73.45 |
| $t_{1/2}$ [h] | 14.0 | 13.9 | 13.8 | 17.5 | 17.3 | 17.2 | 16.7 | 16.5 | 16.38 |

**Table S6.** Toxicity risk for vemurafenib and its derivatives (VEM-1, VEM-2, VEM-3) employing mutagenicity models (*in silico* AMES test).

| Compound | $MUT_{Risk}$ | $MUT_{xRisk}$ | $TOX_{Risk}$ | $MUT_{Code}$ |
|---|---|---|---|---|
| | expected values | | | |
| | (>2) | (0-4) | (0-7) | |
| vemurafenib | 0.6 | 0.35 | 1 | S_97 |
| VEM-1 | 0 | 0 | 1.5 | - |
| VEM-2 | 0.6 | 0.35 | 1.5 | S_97 |
| VEM-3 | 0.6 | 0.35 | 2 | S_97 |

Filters provide a qualitative estimate of potential toxicity concerns. Approximately 16% of commercial drugs within the focused subset of the WDI receive a $MUT_{Risk}$ score > 1 and approximately 4% have a score > 2. $TOX_{Risk}$ evaluates overall toxicological concerns and is greater than 3.3 for ~10% of the focused WDI.

**Table S7.** Intermolecular interactions of vemurafenib and its derivatives (VEM-1, VEM-2, VEM-3) in the active site of the $BRAF_{V600E}$ kinase.

| Compound | Hydrogen bond | Distance H-A [Å] | Hydrophobic interaction | Distance [Å] | Electrostatic and halogen interactions | Distance [Å] |
|---|---|---|---|---|---|---|
| Vemurafenib | Cys-532 | 2.93 | His-539 | 5.09 | Lys-483 | 2.87 |
| | Gln-530 | 2.88 | Trp-531 | 4.63; 5.72; 5.83 | Ala-481 | 3.57 |
| | Asp-594 | 2.14; 2.21 | Phe-583 | 4.94 | | |
| | Gly-569 | 3.36 | Leu-514 | 5.09 | | |
| | Phe-595 | 3.13 | Leu-505 | 4.24 | | |
| | Ser-535 | 2.92 | | | | |
| VEM-1 | Cys-532 | 3.25 | Val-471 | 5.02; 5.23 | Phe-596 | 3.63 |
| | Gly-534 | 2.60 | Ala-481 | 4.33 | Cys-532 | 5.63 |
| | Ile-527 | 3.14 | Leu-567 | 5.34 | Asp-594 | 3.65 |
| | Lys-483 | 2.19 | Ile-572 | 4.18 | | |
| | Asp-594 | 2.96 | Lys-507 | 4.88 | | |
| | Gly-593 | 3.13 | Phe-595 | 5.30; 5.69 | | |
| | Gly-596 | 3.08 | | | | |
| | Leu-505 | 2.97 | | | | |
| | Phe-595 | 2.98 | | | | |
| | TIPW-3748 | 3.80 | | | | |
| | TIPW-5244 | 2.42 | | | | |
| | TIPW-1760 | 2.01 | | | | |
| | TIPW-2605 | 2.25 | | | | |
| VEM-2 | Asp-594 | 2.77 | Phe-583 | 4.11 | - | - |
| | Gly-596 | 3.28 | Ala-481 | 4.26; 4.37 | | |
| | Phe-595 | 3.26 | Val-471 | 5.01; 5.29 | | |
| | Thr-529 | 3.98; 3.65 | Lys-483 | 3.63; 4.12 | | |
| | TIPW-4050 | 2.60 | Leu-515 | 4.74 | | |
| | TIPW-2420 | 2.25 | Phe-595 | 4.64 | | |
| | TIPW-4803 | 2.27 | | | | |
| | TIPW-1538 | 2.05 | | | | |
| VEM-3 | Cys-532 | 2.85; 2.35 | Phe-583 | 4.44 | Lys-483 | 4.57 |
| | Ile-527 | 2.82 | Trp-531 | 5.63 | Leu-514 | 3.12 |
| | Gly-596 | 3.09 | Ala-481 | 4.48; 4.43 | | |
| | Phe-595 | 2.97 | Val-471 | 5.13; 5.24 | | |
| | Thr-508 | 1.52 | Lys-483 | 3.78; 3.94 | | |
| | Leu-514 | 1.77 | Leu-515 | 5.28 | | |
| | Asp-594 | 2.81 | Arg-509 | 4.28 | | |
| | Leu-505 | 2.84; 2.46 | Phe-595 | 4.22 | | |
| | Gly-593 | 3.03 | | | | |
| | Ser-536 | 3.40 | | | | |

| | |
|---|---|
| Gly-534 | 3.60 |
| Arg-603 | 2.99 |
| TIPW-1936 | 2.87 |
| TIPW-990 | 3.60 |

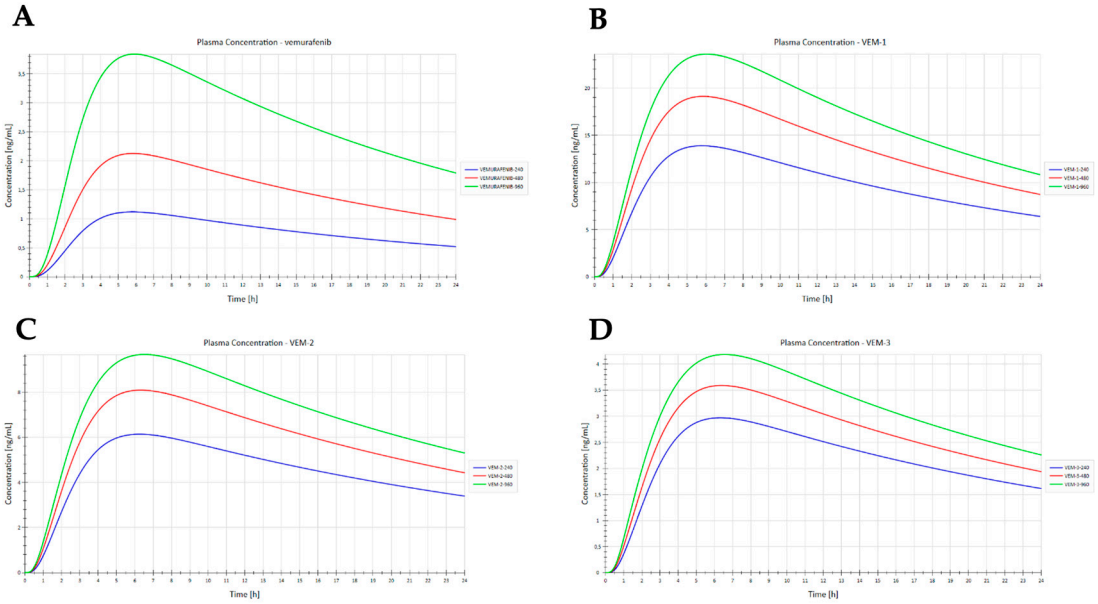


**Figure S1.** Simulated plasma concentration-time profiles in humans after oral administration of vemurafenib (A) and VEM-1 (B), VEM-2 (C) and VEM-3 (D) at doses of 240 mg, 480 mg and 960 mg.

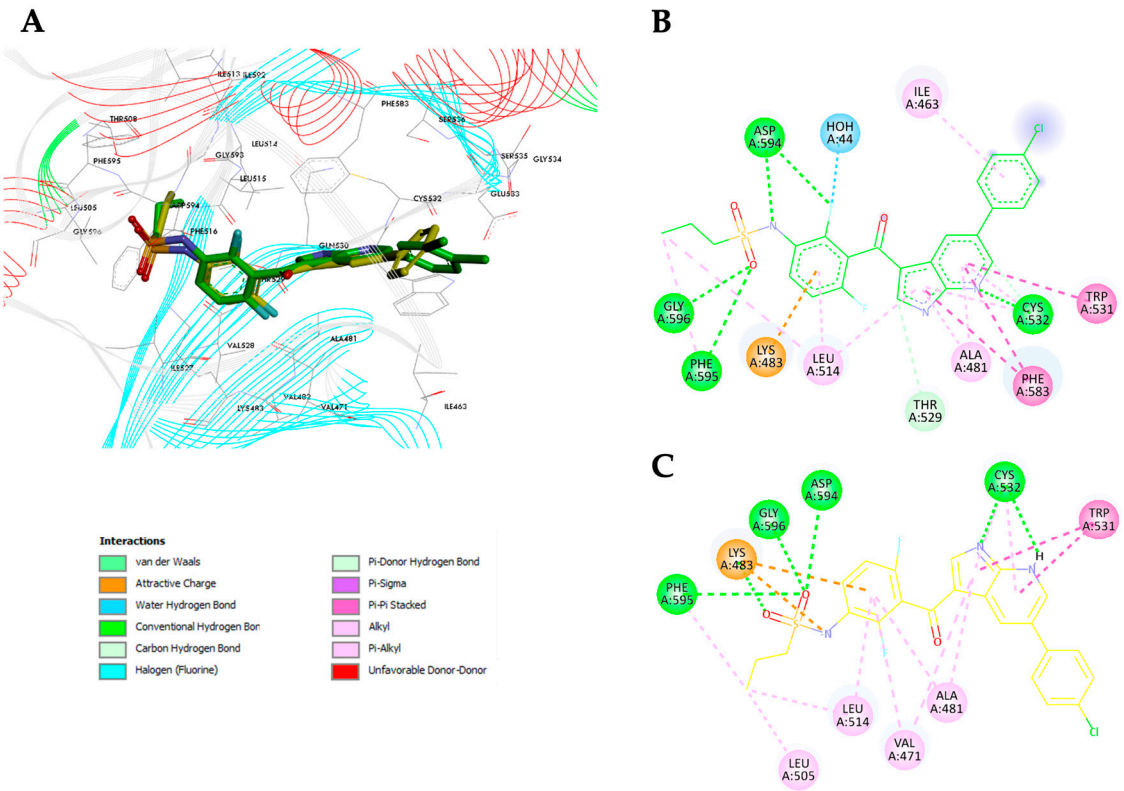


**Figure S2.** Simulated and crystal structure of the $BRAF_{V600E}$-vemurafenib complex. (A) Comparison of the position between the docked structure (yellow color) and the X-ray crystal structure (green color) of vemurafenib in the ATP binding site. The binding mode of vemurafenib (reference molecule) in the crystal structure of BRAF kinase (B), and vemurafenib in the structure of BRAF kinase after docking (C).

## Reference

1. Zhang, W.; Heinzmann, D.; Grippo, J.F. Clinical Pharmacokinetics of Vemurafenib. *Clin Pharmacokinet* **2017**, *56*, 1033-1043, doi:10.1007/s40262-017-0523-7.