# Supplemental Data

# Evaluation of oral anticoagulants with vitamin K epoxide reductase in its native milieu

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**The Supplemental Data includes:**

Supplemental Tables 1 to 6

Supplemental Figure 1 to 3

## Supplemental Tables

### Supplemental Table 1 Comparison of inhibition efficiency of clinical used VKAs on VKOR activity

| VKAs | $IC_{50}$ (nM) | R square |
|---|---|---|
| Warfarin | 5.1 ± 0.5 | 0.9963 |
| Phenprocoumon | 4.2 ± 0.6 | 0.9934 |
| Fluindione | 6.6 ± 0.7 | 0.9964 |
| Acenocoumarol | 0.81 ± 0.11 | 0.9936 |

**$IC_{50}$** is calculated by GraphPad Prism and presented as mean ± SD (Standard Deviation).

**R square:** correlation coefficient.

**Supplemental Table 2 $EC_{50}$ of KO for naturally occurring VKOR mutations**

| VKOR mutations | $EC_{50}$ ± SD (nM) | VKOR mutations | $EC_{50}$ ± SD (nM) |
|---|---|---|---|
| Wide-type | 27.6 ± 1.9 | S56F | 24.4 ± 1.9 |
| A26P | 40.8 ± 4.9 | R58G | 26.6 ± 0.8 |
| A26T | 22.2 ± 2.7 | W59R | 42.0 ± 5.3 |
| L27V | 28.8 ± 1.6 | W59C | 43.9 ± 6.9 |
| H28Q | 20.4 ± 2.4 | W59L | 35.2 ± 1.6 |
| V29L | 37.3 ± 3.7 | V66G | 37.3 ± 3.1 |
| A34P | 32.2 ± 4.8 | V66M | 29.1 ± 3.3 |
| D36G | 28.1 ± 2.5 | H68Y | 26.1 ± 1.7 |
| D36Y | 26.6 ± 3.4 | G71A | 26.1 ± 3.8 |
| A41S | 22.3 ± 2.0 | N77S | 30.0 ± 2.8 |
| V45A | 24.1 ± 2.4 | N77Y | 27.9 ± 3.4 |
| S52L | 35.8 ± 4.4 | I123N | 25.5 ± 4.1 |
| S52W | 31.4 ± 4.8 | L128R | 55.2 ± 5.1 |
| V54L | 26.2 ± 2.4 | Y139H | 63.5 ± 5.3 |

**Supplemental Table 3 Apparent Vmax and Km of VKOR reducing KO under different fluindione concentrations determined by cell-based assay**

| Fluindione (nM) | Vmax (nM/min, x$10^{-5}$) | Km (nM) |
|---|---|---|
| 30 | 109.1 ± 1.5 | 626.6 ± 24.8 |
| 15 | 127.0 ± 2.7 | 544.8 ± 34.9 |
| 7.5 | 137.1 ± 2.5 | 486.9 ± 28.1 |
| 3.75 | 133.3 ± 4.5 | 322.6 ± 37.4 |
| 1.88 | 127.2 ± 5.9 | 163.1 ± 30.1 |
| 0.94 | 126.7 + 3.2 | 69.5 ± 8.1 |
| 0 | 146.9 ± 2.3 | 53.6 ± 4.0 |

**Supplemental Table 4 R square of fitting fluindione inhibition of VKOR by non-linear regression with different inhibition models**

| Fluindione (nM) | Inhibition model | | | |
|---|---|---|---|---|
| | Competitive | Noncompetitive | Uncompetitive | Mixed type |
| 30 | **0.9344** | 0.8246 | 0.7411 | - |
| 15 | **0.9823** | 0.8908 | 0.8327 | - |
| 7.5 | **0.995** | 0.8995 | 0.8731 | - |
| 3.75 | **0.9622** | 0.9095 | 0.9158 | - |
| 1.88 | **0.9392** | 0.9347 | 0.9469 | - |
| 0.94 | **0.9751** | 0.8913 | 0.8594 | - |
| 0 | **0.9597** | 0.7294 | 0.6388 | - |
| **Global (shared)** | **0.9722** | **0.892** | **0.8613** | **Ambiguous** |

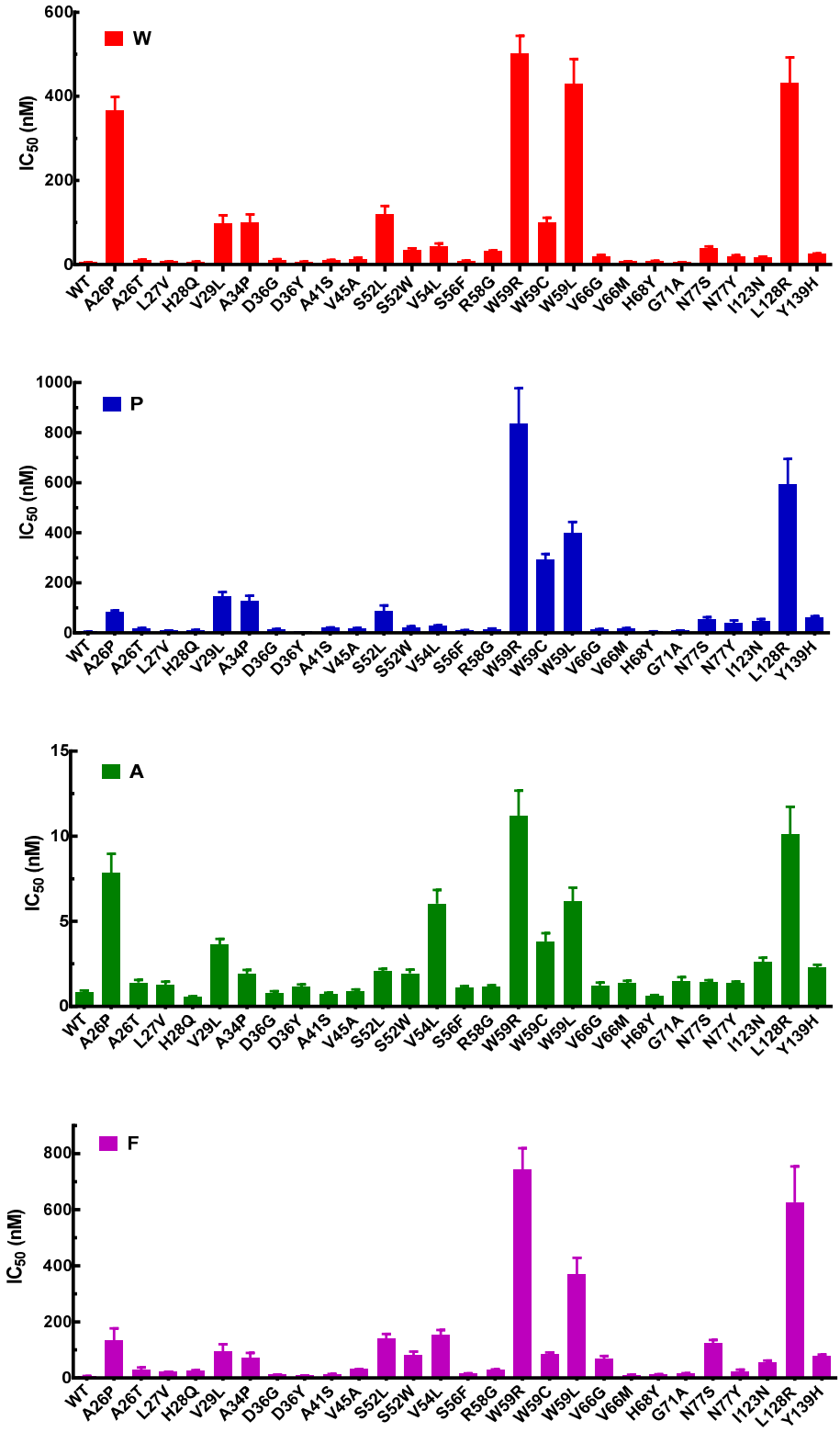
**Supplemental Table 5 Apparent Vmax and Km of VKOR reducing KO under different warfarin concentrations determined by cell-based assay**

| Warfarin (nM) | Vmax (nM/min, x$10^{-5}$) | Km (nM) |
|---|---|---|
| 20 | 80.3 ± 1.4 | 970.0 ± 45.0 |
| 10 | 112.1 ± 3.8 | 851.6 ± 78.1 |
| 5 | 139.0 ± 5.3 | 616.6 ± 69.0 |
| 2.5 | 140.0 ± 8.7 | 364.4 ± 76.1 |
| 1.25 | 152.8 ± 8.8 | 168.5 ± 38.2 |
| 0.625 | 172.7 ± 6.2 | 79.7 ± 12.7 |
| 0 | 188.7 ± 2.2 | 49.7 ± 2.7 |

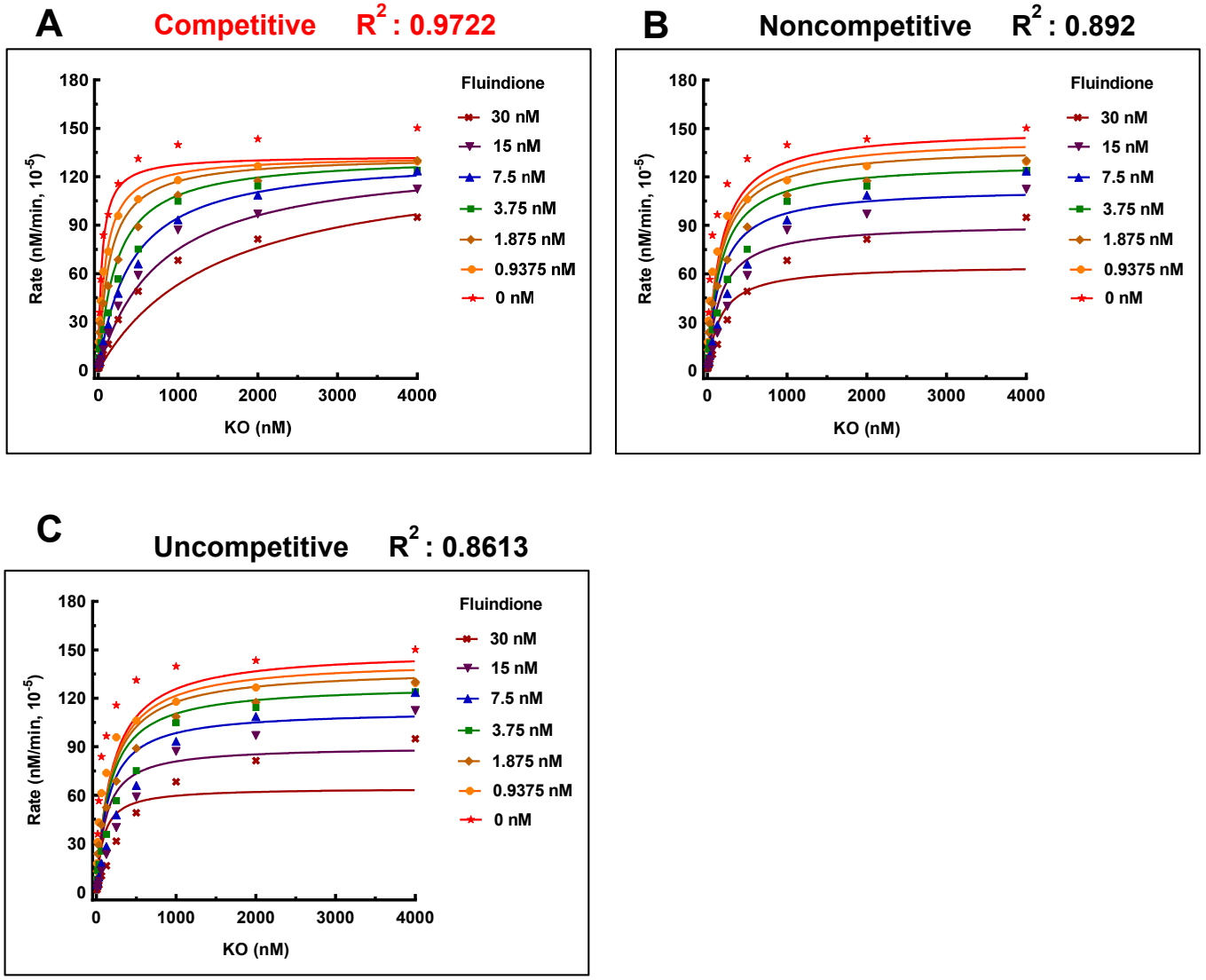
**Supplemental Table 6 R square of fitting warfarin inhibition of VKOR by non-linear regression with different inhibition models**

| Warfarin (nM) | Inhibition model | | | |
|---|---|---|---|---|
| | Competitive | Noncompetitive | Uncompetitive | Mixed type |
| 20 | 0.8562 | 0.5966 | 0.3454 | **0.9517** |
| 10 | 0.9204 | 0.708 | 0.4868 | **0.9852** |
| 5 | 0.9638 | 0.8035 | 0.6526 | **0.9892** |
| 2.5 | 0.9026 | 0.8239 | 0.7486 | **0.9191** |
| 1.25 | 0.9266 | 0.9117 | 0.9068 | **0.9367** |
| 0.62 | 0.8737 | 0.953 | 0.9452 | **0.9373** |
| 0 | 0.9686 | 0.8898 | 0.8268 | **0.9911** |
| **Global (shared)** | **0.9545** | **0.9178** | **0.8741** | **0.9752** |

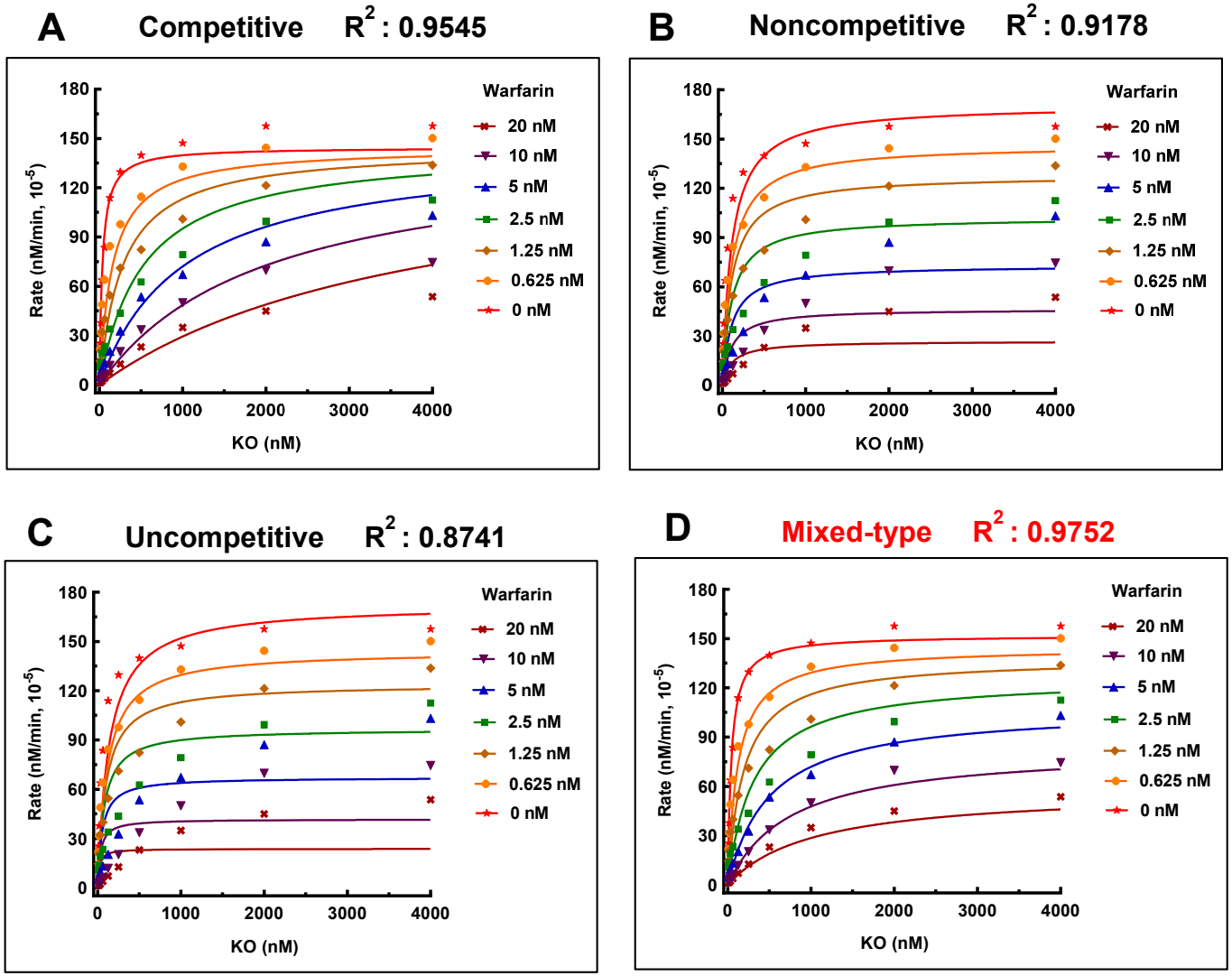
## Supplemental Figure




**Supplemental Figure 1** Half-maximal inhibition ($IC_{50}$) of clinically used VKAs for the naturally occurring VKOR mutations. For a better visualization, data from Table 1 were presented as column charts for side-by-side comparison. W: warfarin, P: phenprocoumon, A: acenocoumarol, F: fluindione.




**Supplemental Figure 2** Fitting kinetics data of fluindione inhibition of VKOR to different inhibition models by non-linear regression using GraphPad Prism. Experimental data are the same as in Figure 5A.




**Supplemental Figure 3** Fitting kinetics data of warfarin inhibition of VKOR to different inhibition models by non-linear regression using GraphPad Prism. Experimental data are the same as in Figure 5D.