

# SUPPLEMENTARY DATA

## CYP2E1 SUBSTRATE INHIBITION: MECHANISTIC INTERPRETATION THROUGH AN EFFECTOR SITE FOR MONOCYCLIC COMPOUNDS

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## DynaFit Script and Experimental Data - Model Discrimination for Catalytic Inhibition #1

;For two-site models (2a-d), substrate is always a homotypic effector  
;I represents either 4MP or IND

[task]

```
task = fit
data = velocities
model = Model 1 ?
```

[mechanism]

```
E + S <===> ES      : Ks  dissoc
ES + S <===> ESS     : Kss dissoc
E + I <===> EI       : Ki  dissoc
ES ----> E + P     : kcat
```

[constants]

```
Ks = 25 ?, Kss = 200 ?, kcat = 50 ?, Ki = 50 ?
```

[responses]

```
P = 1
```

[concentrations]

```
E = 0.025
```

[progress]

```
rapid equilibrium
```

[velocity]

```
directory    ./Mechanisms/CYP2E1/Ligands
extension    txt
variable     S
```

```
file 0uMI    | conc. I = 0
file 1uMI    | conc. I = 1
file 5uMI    | conc. I = 5
file 25uMI   | conc. I = 25
file 125uMI  | conc. I = 125
```

[output]

```
directory ./Mechanisms/CYP2E1/Ligands/Output/Inhibition
```

[settings]

```
<Marquardt>
interrupt = 200
```

```

;


---


[task]

    task = fit
    data = velocities
    model = Model 2a ?

;occupancy of one site affects binding at other = allosteric S and I

[mechanism]

    E + S <====> ES      : Ks   dissociation
    ES + S <====> ESS     : Kss  dissociation
    E + I <====> EI      : Ki   dissociation
    ES + I <====> ESI     : Ksi  dissociation
    EI + S <====> EIS     : Kis  dissociation
    ES ----> E + P      : kcat

[constants]

    Ks = 25 ?, Kss = 200 ?, kcat = 50 ?, Ki = 50 ?, Kis = 50 ?, Ksi = 50 ?
?
;


---


[task]

    task = fit
    data = velocities
    model = Model 2b ?

;substrate binding affects inhibitor binding = allosteric S

[mechanism]

    E + S <====> ES      : Ks   dissociation
    ES + S <====> ESS     : Kss  dissociation
    E + I <====> EI      : Ki   dissociation
    ES + I <====> ESI     : Ksi  dissociation
    EI + S <====> EIS     : Ks   dissociation
    ES ----> E + P      : kcat

[constants]

    Ks = 25 ?, Kss = 200 ?, kcat = 507 ?, Ki = 50 ?, Ksi = 50 ?
;


---


[task]

    task = fit
    data = velocities
    model = Model 2c ?

;inhibitor binding affects substrate binding = allosteric I

```

[mechanism]

```
E + S <====> ES      : Ks   dissoc
ES + S <====> ESS     : Kss  dissoc
E + I <====> EI       : Ki   dissoc
ES + I <====> ESI     : Ki   dissoc
EI + S <====> EIS     : Kis  dissoc
ES ----> E + P       : kcat
```

[constants]

```
Ks = 25 ?, Kss = 200 ?, kcat = 50 ?, Ki = 50 ?, Kis = 50 ?
;
```

---

[task]

```
task = fit
data = velocities
model = Model 2d ?
```

```
;occupancy of one site does not affect binding = no allosterism =
noncompetitive
```

[mechanism]

```
E + S <====> ES      : Ks   dissoc
ES + S <====> ESS     : Kss  dissoc
E + I <====> EI       : Ki   dissoc
ES + I <====> ESI     : Ki   dissoc
EI + S <====> ESI     : Ki   dissoc
ES ----> E + P       : kcat
```

[constants]

```
Ks = 25 ?, Kss = 200 ?, kcat = 50 ?, Ki = 50 ?
```

[end]

## **DynaFit Script - Model Discrimination for 4MP Inhibition #2**

```
;Variations of Model 2a in which ESI or EIS do not form
```

[task]

```
task = fit
data = velocities
model = Model 2a ?
```

```
;occupancy of one site affects binding at other
```

[mechanism]

```
E + S <====> ES      : Ks   dissoc
ES + S <====> ESS     : Kss  dissoc
E + I <====> EI       : Ki   dissoc
ES + I <====> ESI     : Ksi  dissoc
```

```
EI + S <====> EIS      : Kis  dissoc
ES ----> E + P        : kcat
```

[constants]

```
Ks = 20 ??, Kss = 200 ??, kcat = 50 ??, Ki = 2 ??, Kis = 125 ??, Ksi
= 500 ??
```

[responses]

```
P = 1
```

[concentrations]

```
E = 0.025
```

[progress]

```
rapid equilibrium
```

[velocity]

```
directory      ./Mechanisms/CYP2E1/Ligands/4MP
extension      txt
variable       S
```

```
file 0uM4MP    | conc. I = 0
file 1uM4MP    | conc. I = 1
file 5uM4MP    | conc. I = 5
file 25uM4MP   | conc. I = 25
file 125uM4MP  | conc. I = 125
```

[output]

```
directory ./Mechanisms/CYP2E1/Ligands/Output/4MP061207_2
```

[settings]

```
<Marquardt>
interrupt = 200
```

;

---

[task]

```
task = fit
data = velocities
model = Model 2a no ESI ?
```

```
;occupancy of one site affects binding at other
```

[mechanism]

```
E + S <====> ES      : Ks  dissoc
ES + S <====> ESS     : Kss  dissoc
E + I <====> EI       : Ki  dissoc
EI + S <====> EIS     : Kis  dissoc
```

```

    ES ----> E + P      :   kcat

[constants]

    Ks = 20 ??, Kss = 200 ??, kcat = 50 ??, Ki = 2 ??, Kis = 125 ??
; _____

[task]

    task = fit
    data = velocities
    model = Model 2a no EIS ?

;occupancy of one site affects binding at other

[mechanism]

    E + S <====> ES      :   Ks   dissoci
    ES + S <====> ESS     :   Kss  dissoci
    E + I <====> EI       :   Ki   dissoci
    ES + I <====> ESI     :   Ksi  dissoci
    ES ----> E + P      :   kcat

[constants]

    Ks = 20 ??, Kss = 200 ??, kcat = 50 ??, Ki = 2 ??, Ksi = 10 ??

[end]

```

### DynaFit Script - Model Discrimination for Binding of Heterocycles

```

[task]

    data = equilibria
    task = fit
    model = PL* ?

[components]

    P, L          ; P = P450, L = 4MP

[mechanism]

    P + L <====> P.L      :   K1   dissoci

[constants]

    K1 = 0.1 ??

[responses]

    P.L = .001 ??

[concentrations]

[data]

```

```

variable P, L
set alldata

[output]

    directory ./Mechanisms/BindingSites/Output/Binding
; _____

[task]

    data = equilibria
    task = fit
    model = PL*-PL2* ?

[mechanism]

    P + L <====> P.L      :    K1    dissoci
    P.L + L <====> P.L.L   :    K2    dissoci

[constants]

    K1 = 0.1 ??
    K2 = 0.1 ??

[responses]

    P.L = .001 ??
    P.L.L = 0.001 ??
; _____

[task]

    data = equilibria
    task = fit
    model = PL*-PL2 ?

[mechanism]

    P + L <====> P.L      :    K1    dissoci
    P.L + L <====> P.L.L   :    K2    dissoci

[constants]

    K1 = 0.1 ??
    K2 = 0.1 ??

[responses]

    P.L = .001 ??
; _____

[task]

    data = equilibria
    task = fit
    model = PL-PL2* ?

```

[mechanism]

P + L <===> P.L : K1 dissociation  
P.L + L <===> P.L.L : K2 dissociation

[constants]

K1 = 0.1 ??  
K2 = 0.1 ??

[responses]

P.L.L = 0.001 ??

[end]