**Supporting Information**

# Design, Synthesis, and Inhibitory Activity of Hydroquinone Ester Derivatives against Mushroom Tyrosinase

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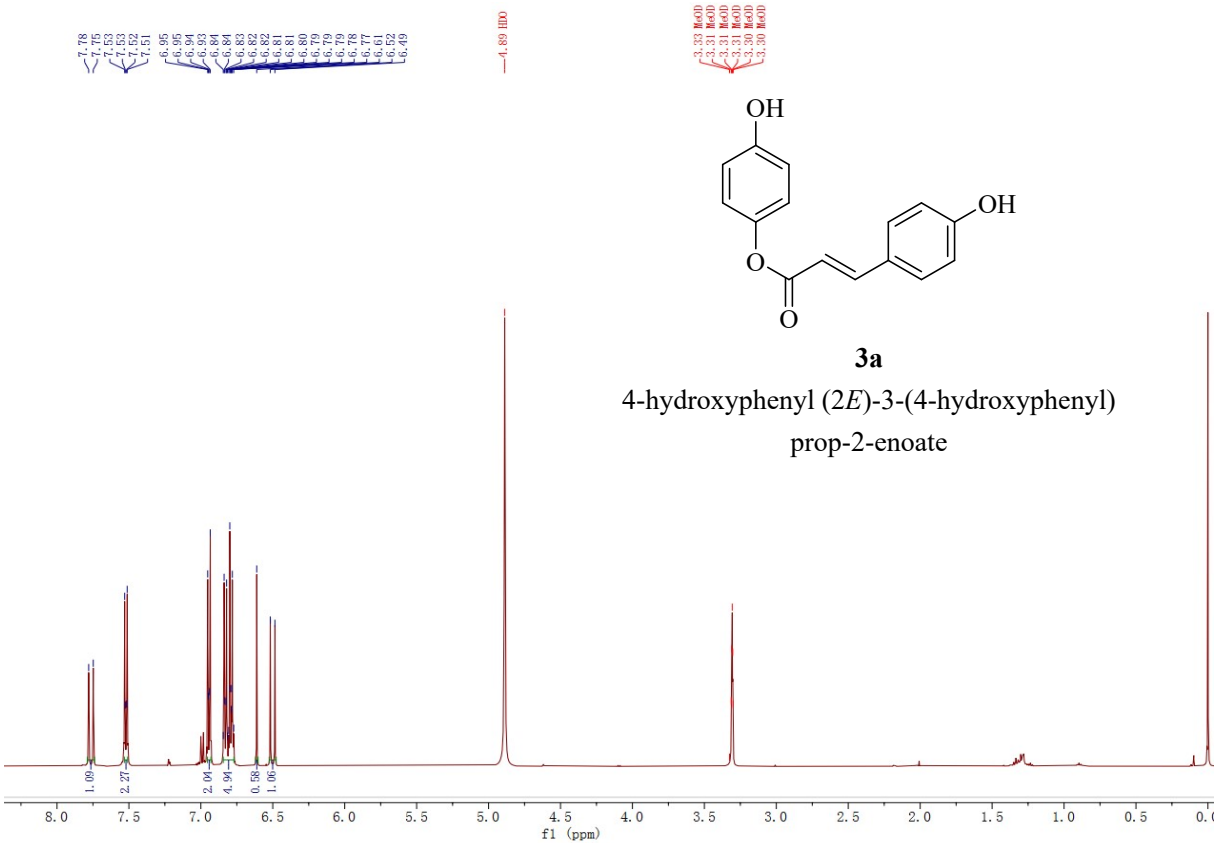
This file includes 7 Figures and 2 Tables

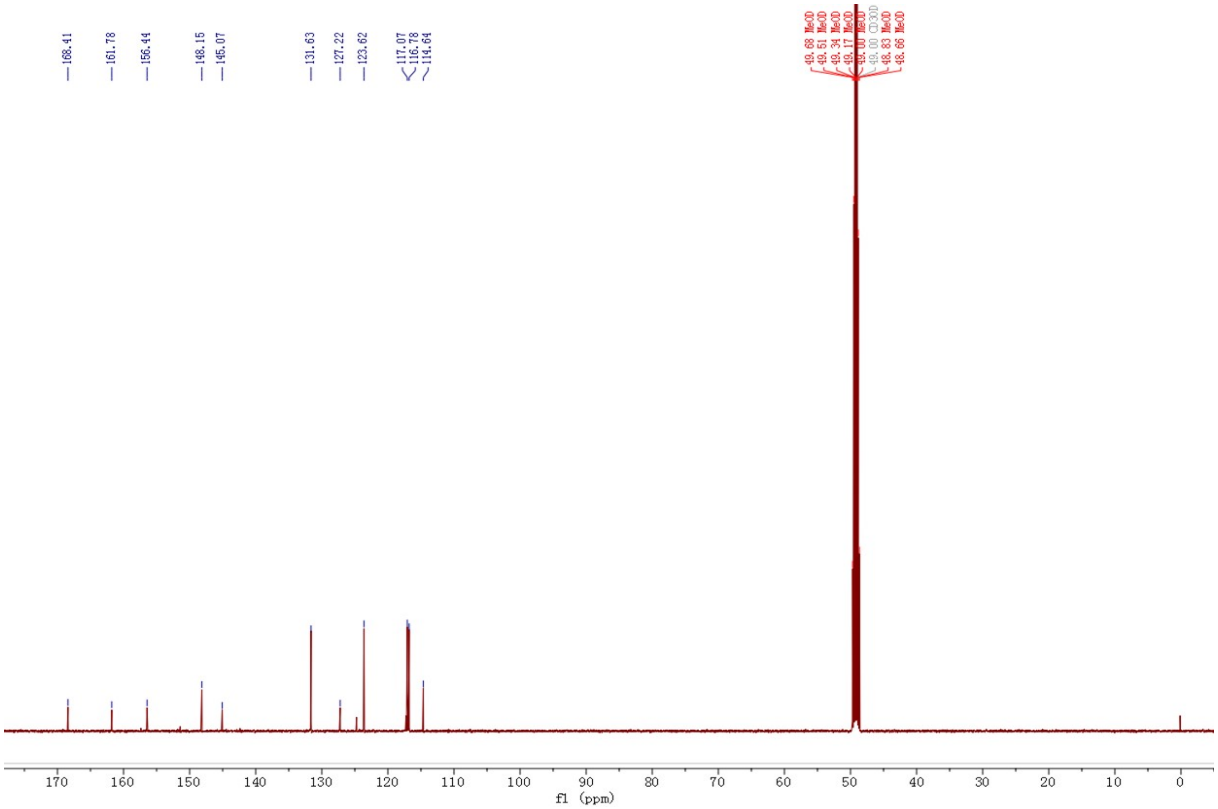
Contents of the Figures and the Tables



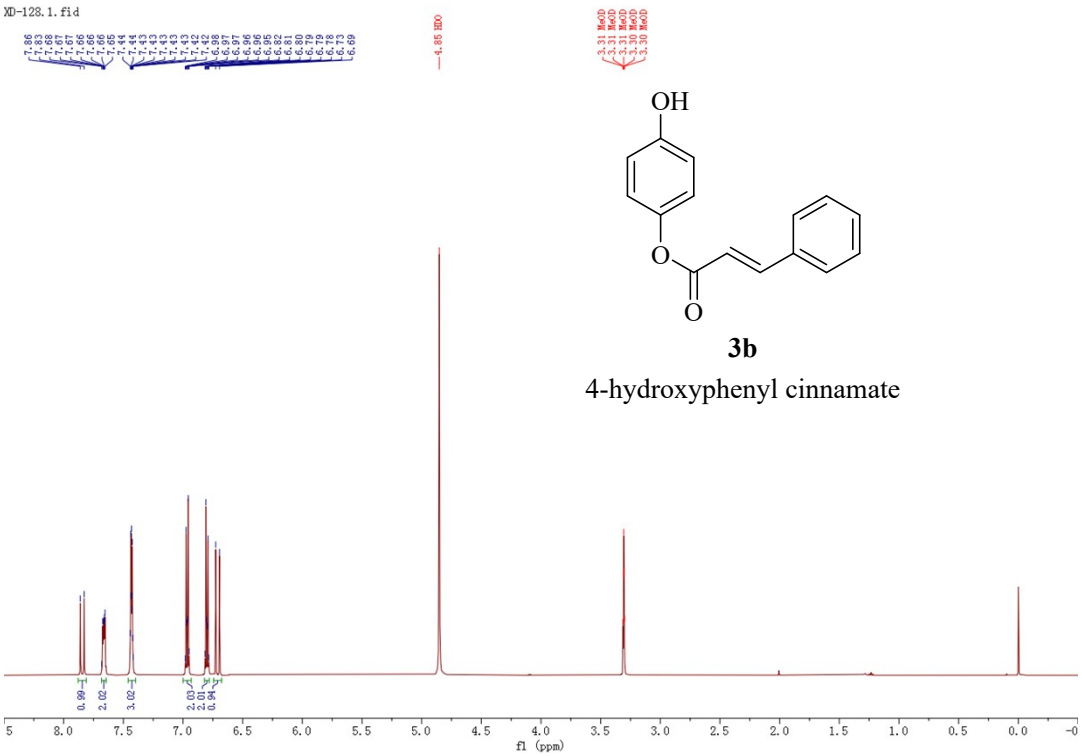

**3a**

4-hydroxyphenyl (2*E*)-3-(4-hydroxyphenyl) prop-2-enoate

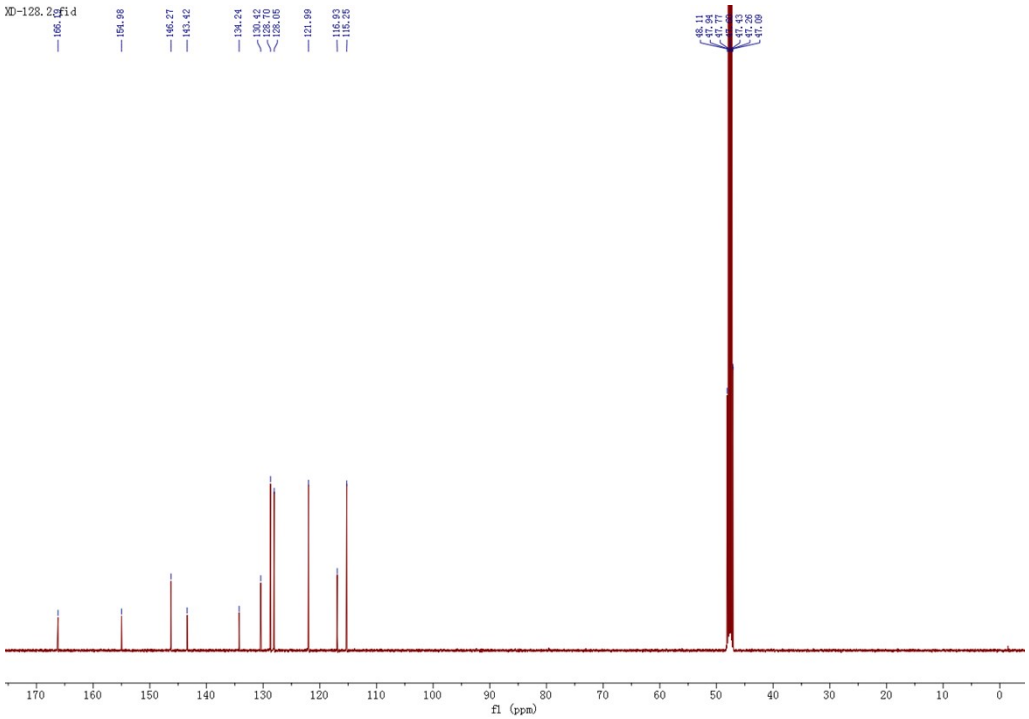
(1) $^{1}$H NMR for compound 3a



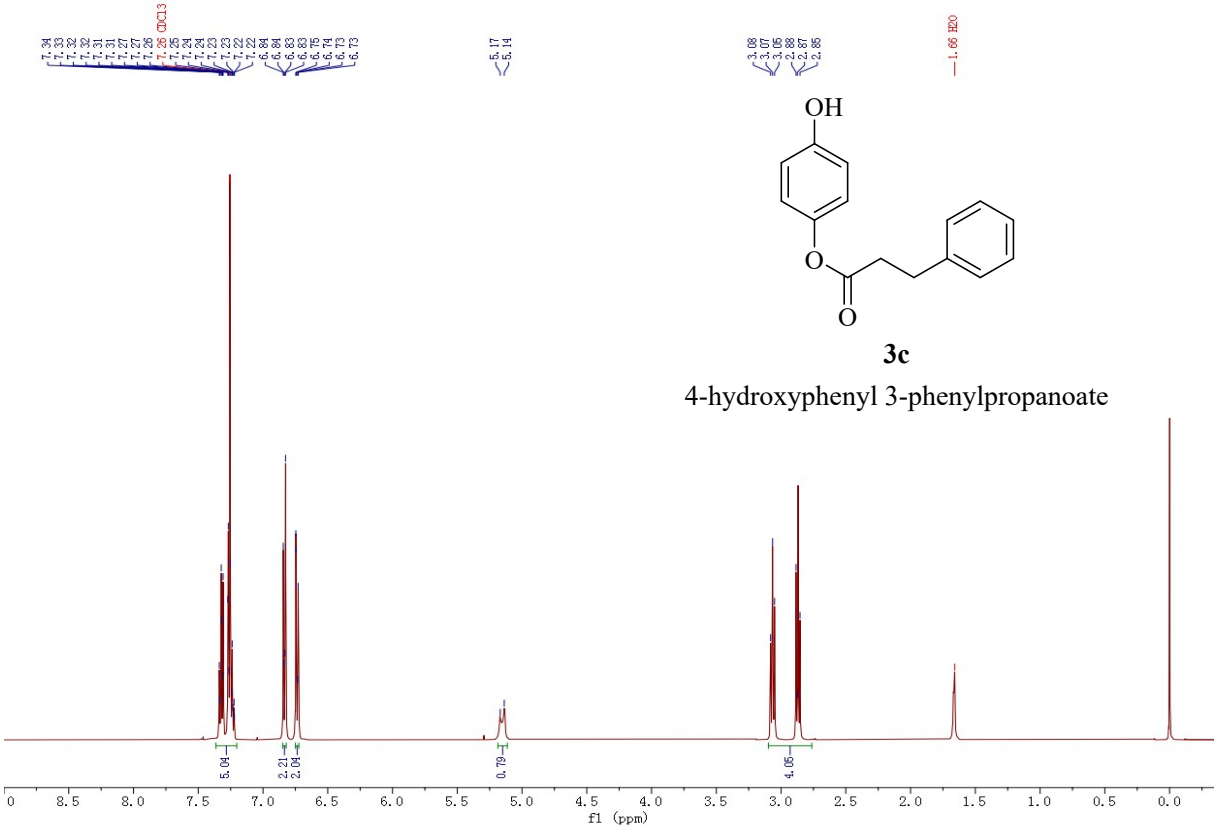

(2) $^{13}$C NMR for compound 3a



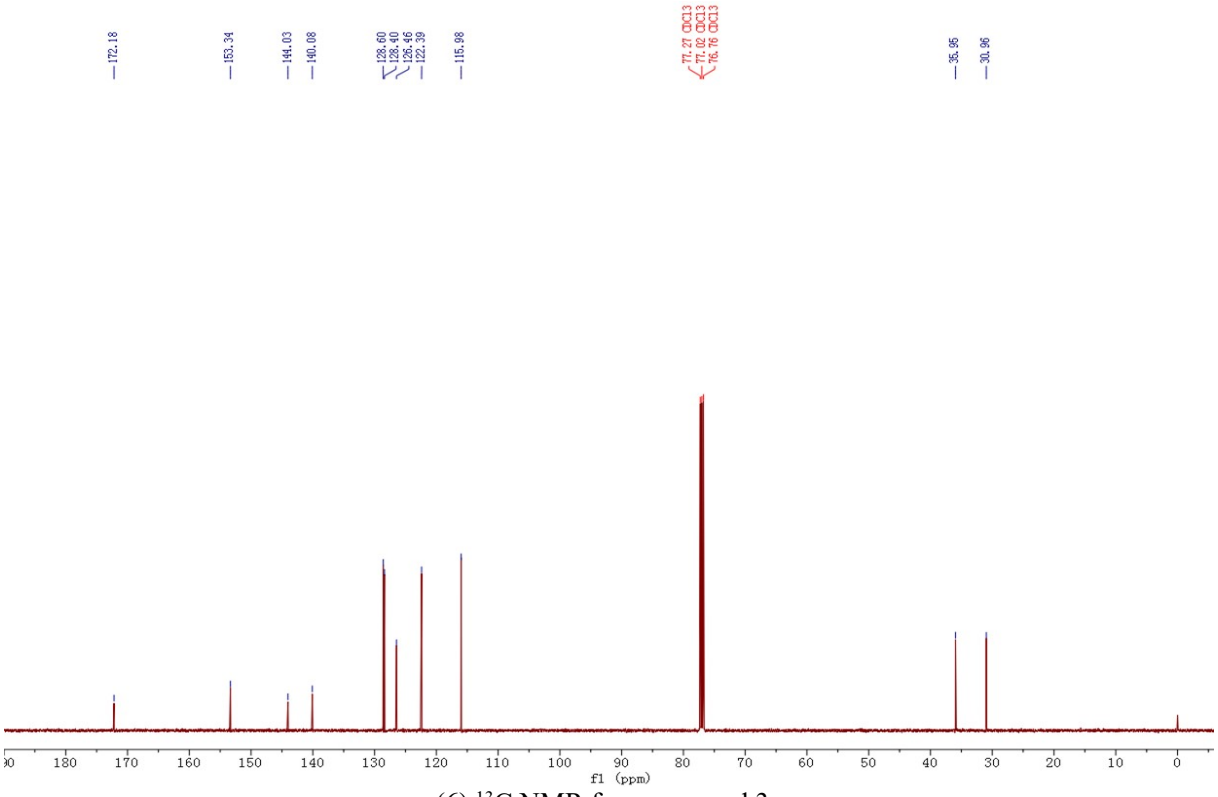

(3) $^{1}$H NMR for compound 3b



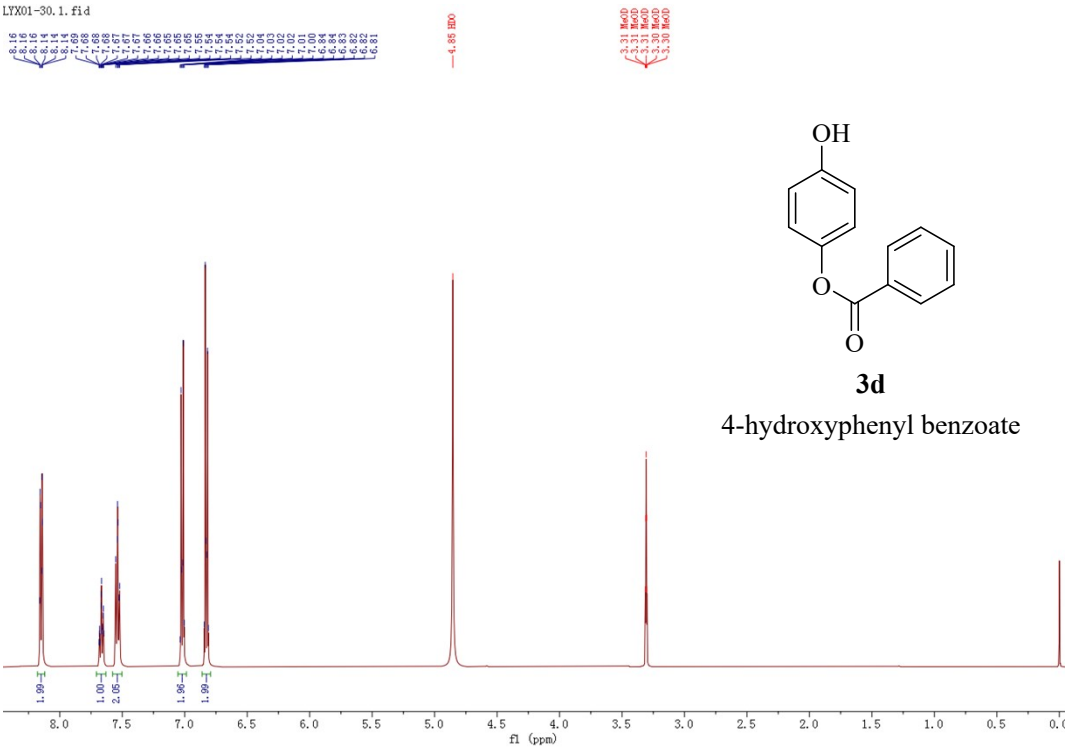

(4) $^{13}$C NMR for compound 3b



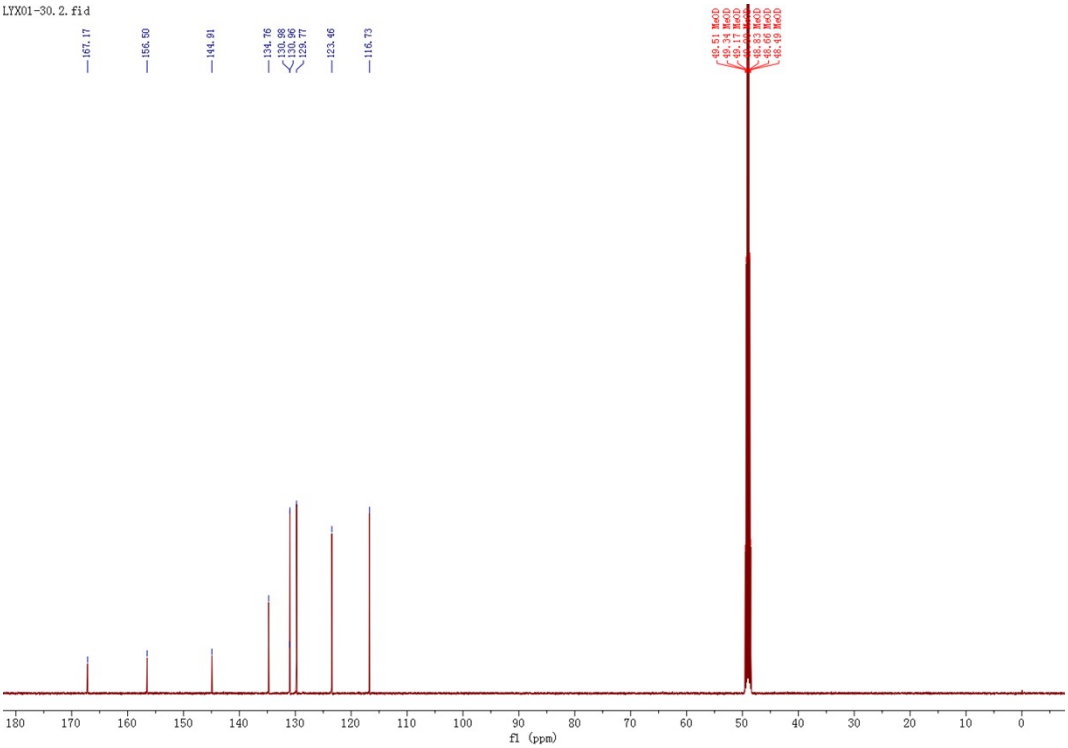

(5) $^{1}$H NMR for compound 3c



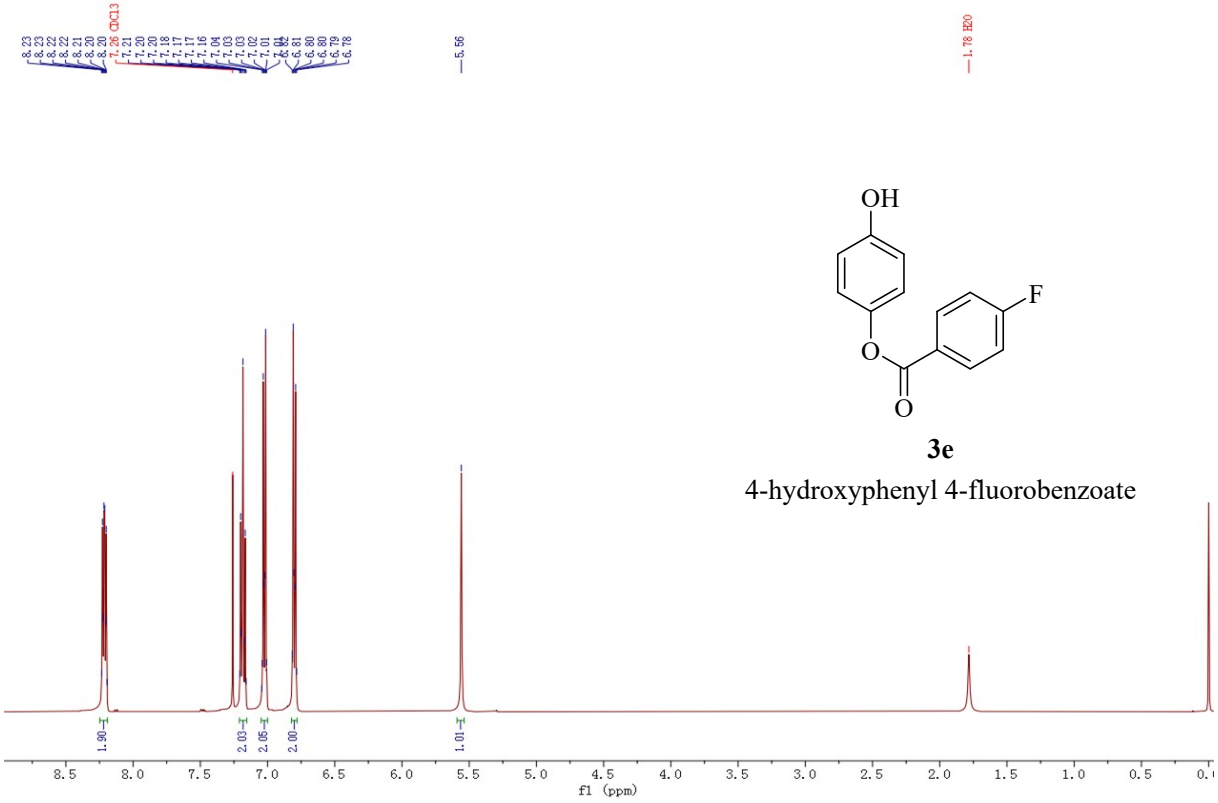

(6) $^{13}$C NMR for compound 3c



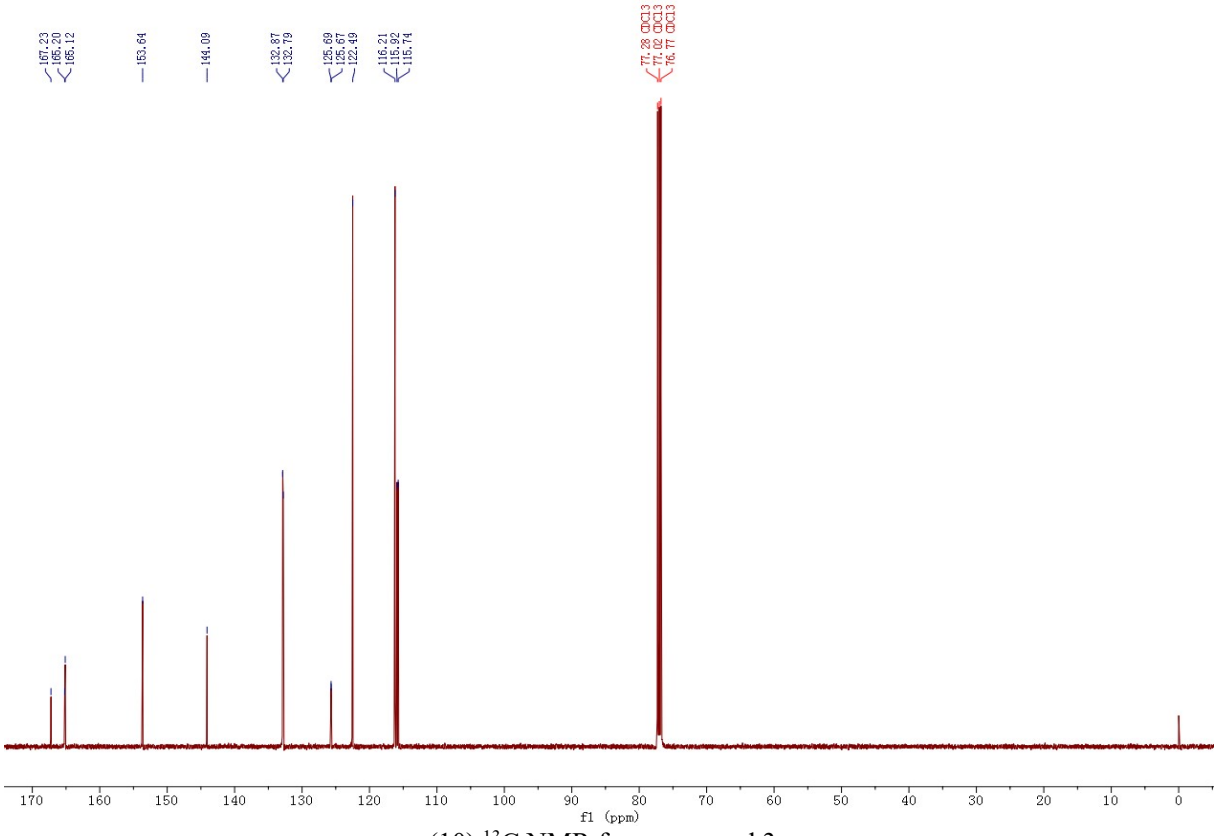

(7) $^{1}$H NMR for compound 3d



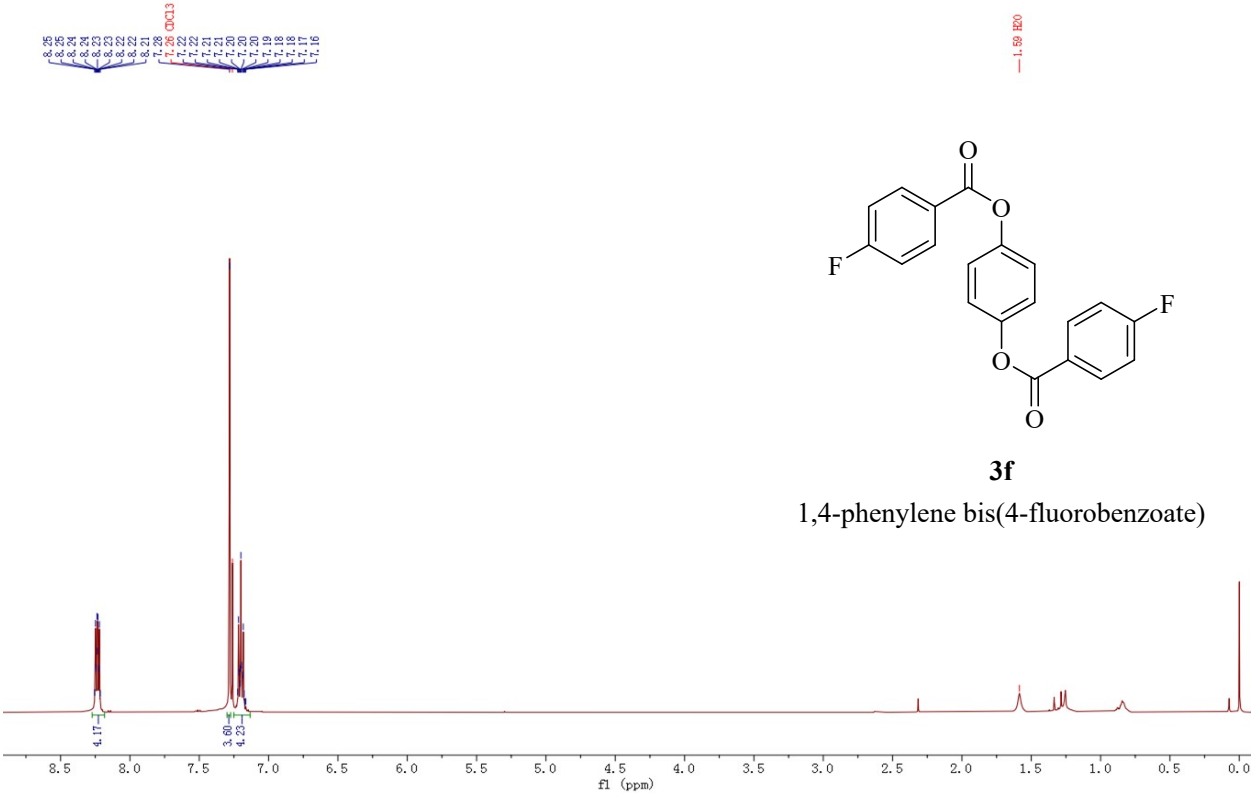

(8) $^{13}$C NMR for compound 3d




(9) $^{1}$H NMR for compound 3e



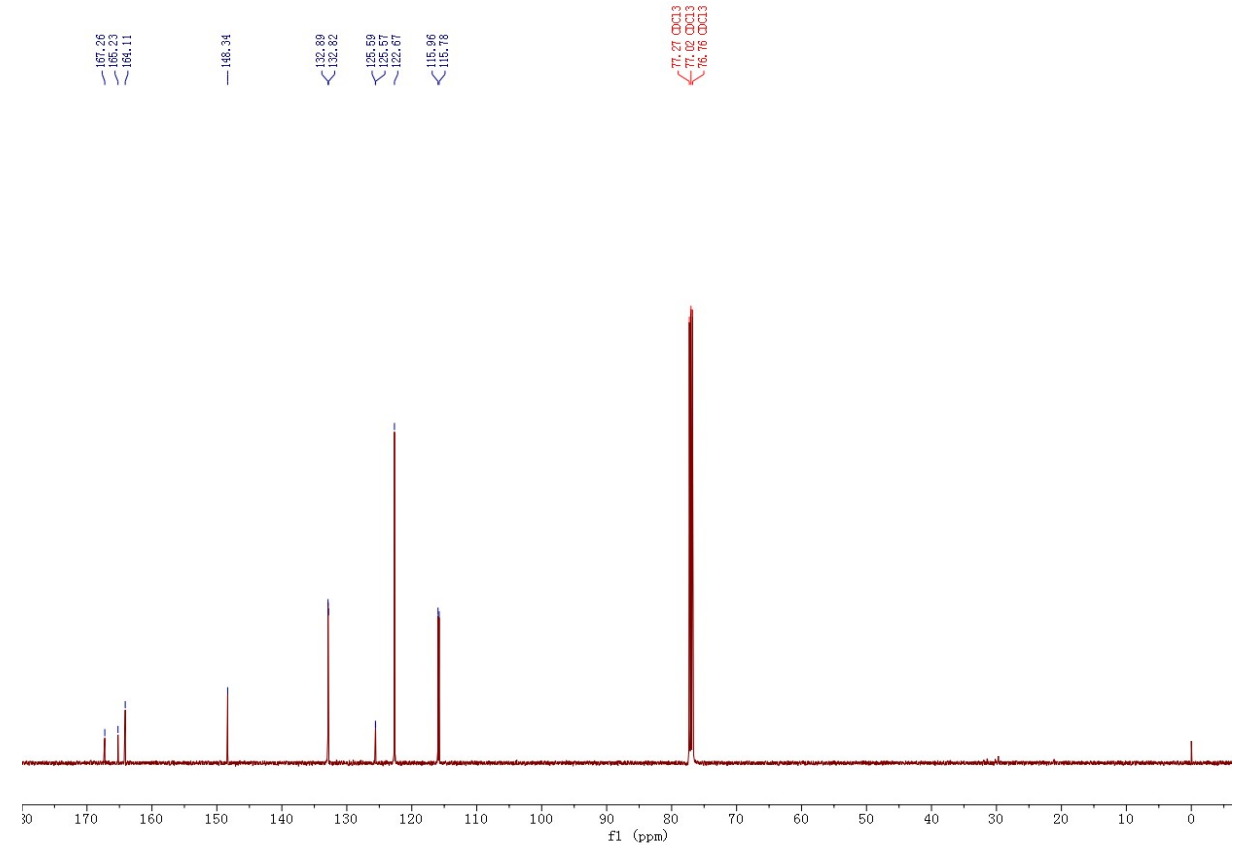

(10) $^{13}$C NMR for compound 3e



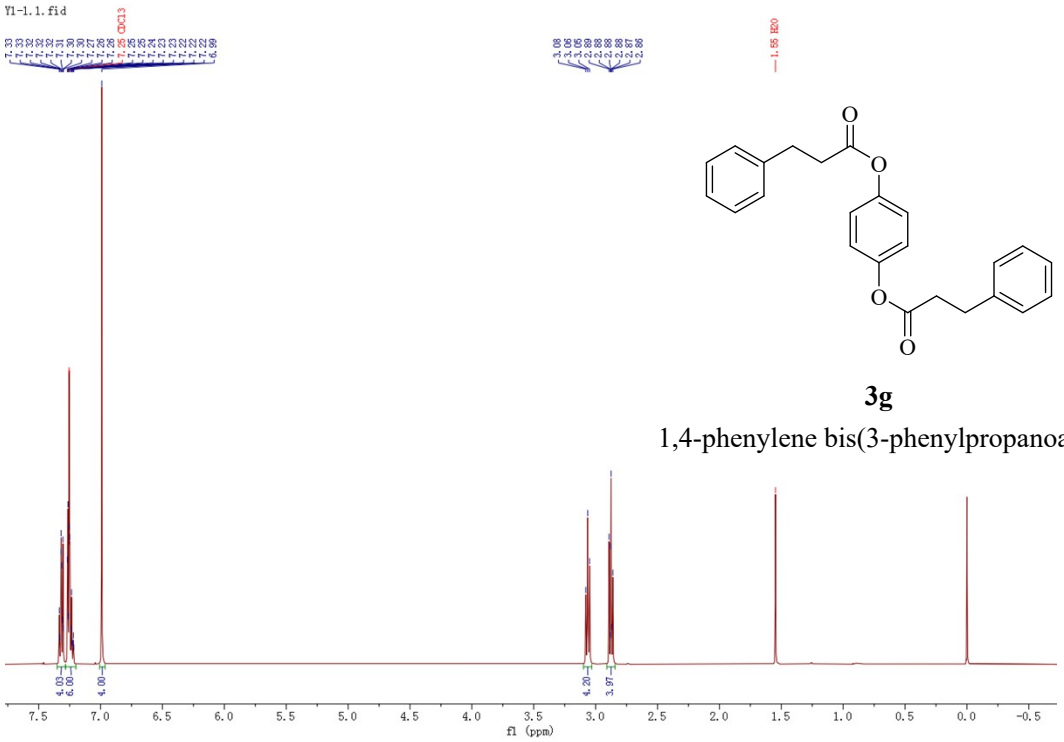

**3f**

1,4-phenylene bis(4-fluorobenzoate)

(11) $^{1}$H NMR for compound 3f




(12) $^{13}$C NMR for compound 3f

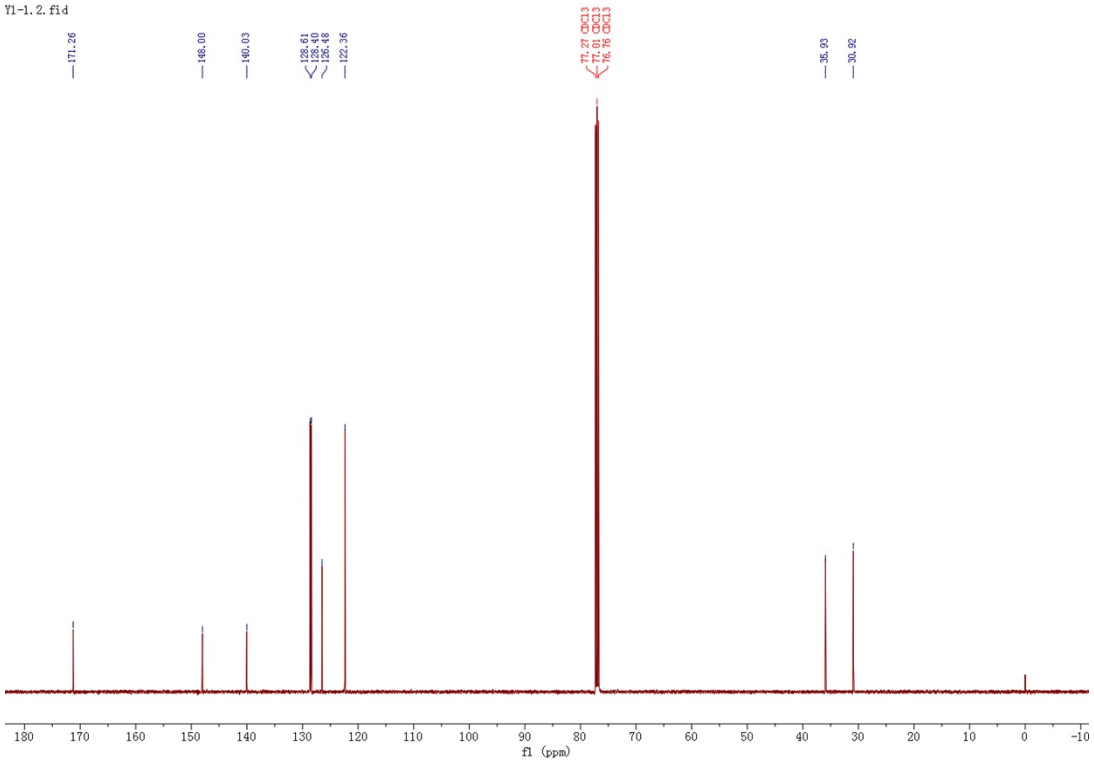





**3g**

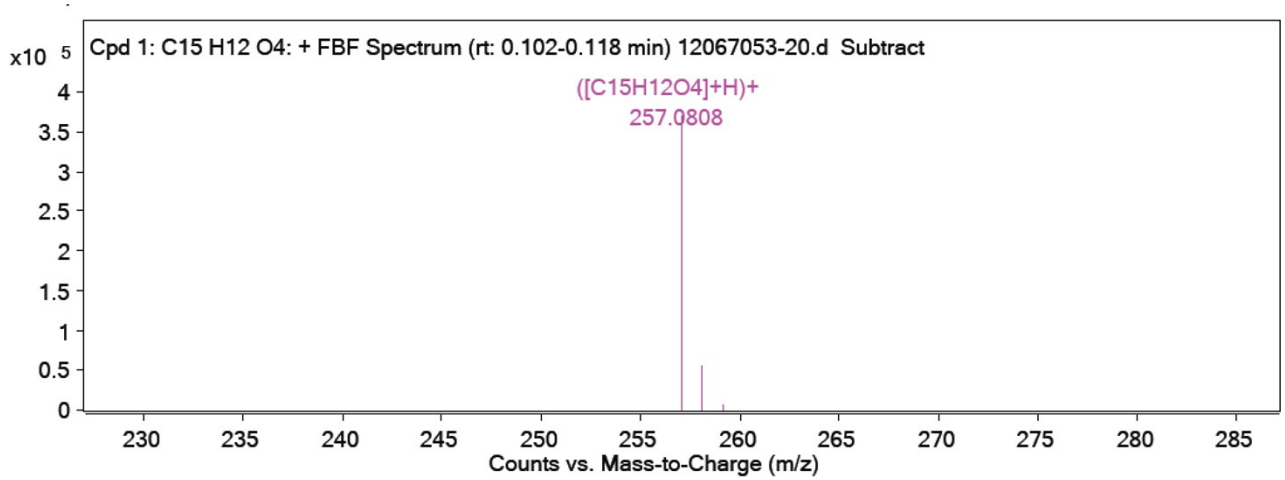
1,4-phenylene bis(3-phenylpropanoate)

(13) $^{1}$H NMR for compound 3g

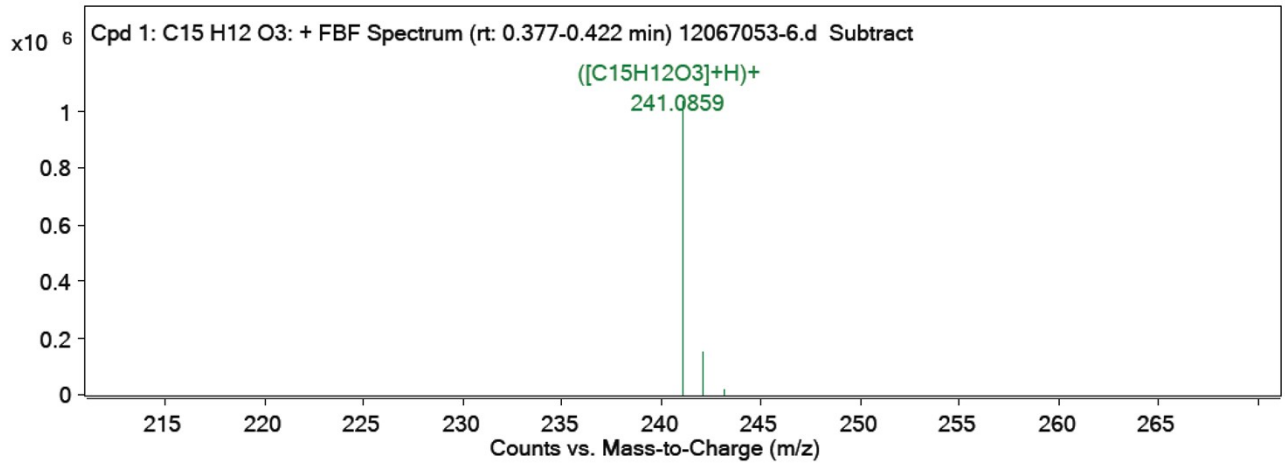



(14) $^{13}$C NMR for compound 3g

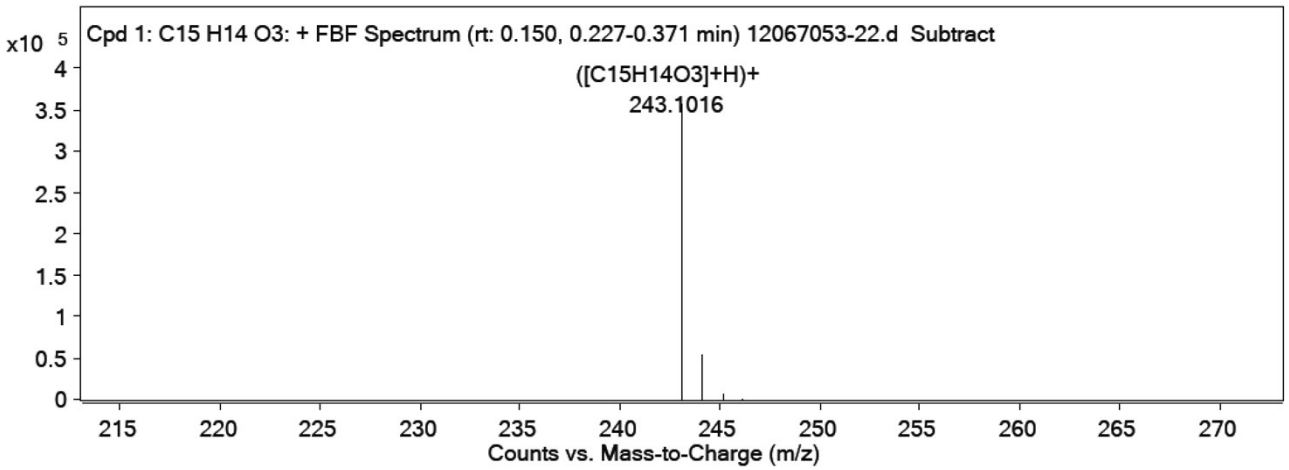
Figure S1. $^{1}$H NMR and $^{13}$C NMR for compound 3a-3g



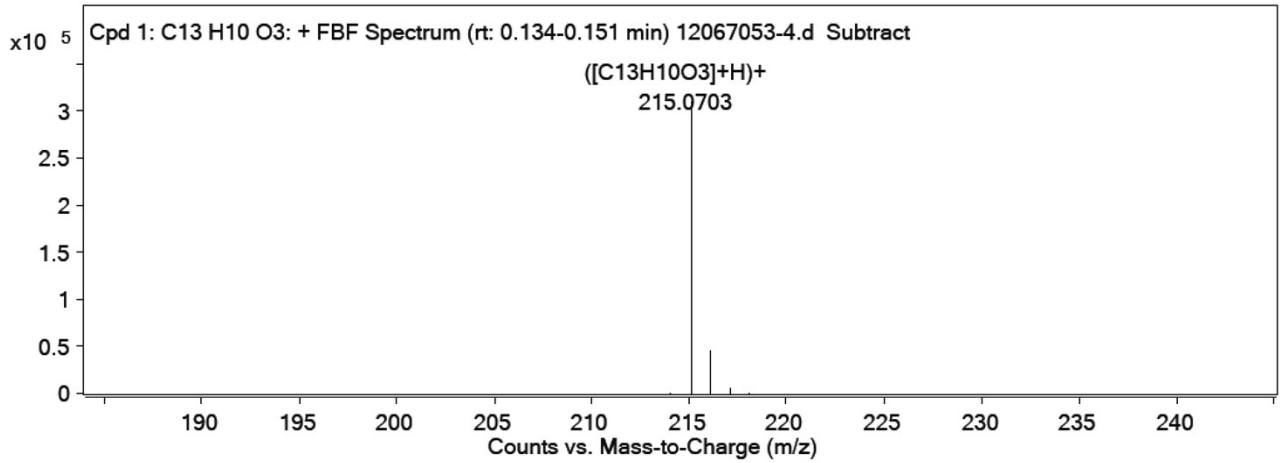

(1) HRMS for compound 3a



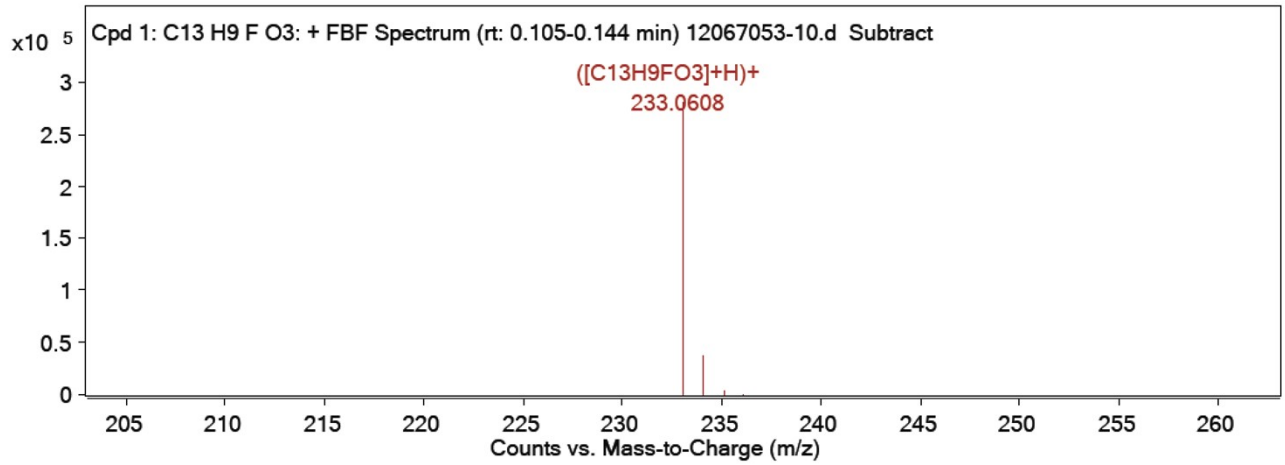

(2) HRMS for compound 3b



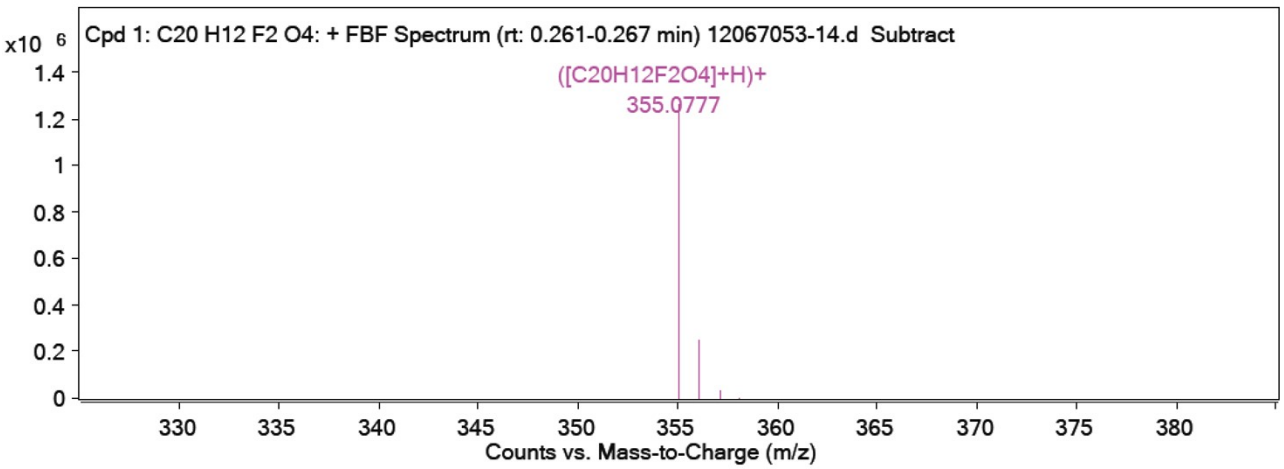

(3) HRMS for compound 3c



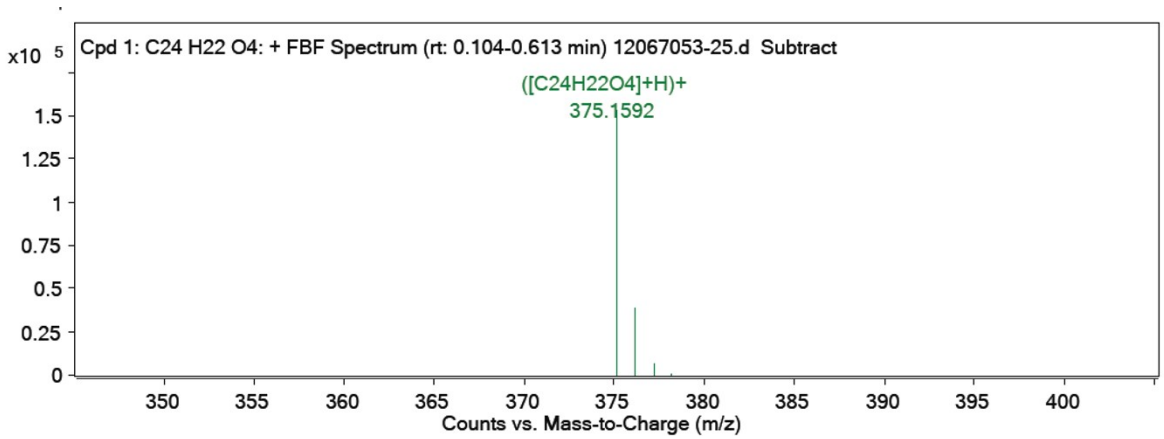

(4) HRMS for compound 3d




(5) HRMS for compound 3e

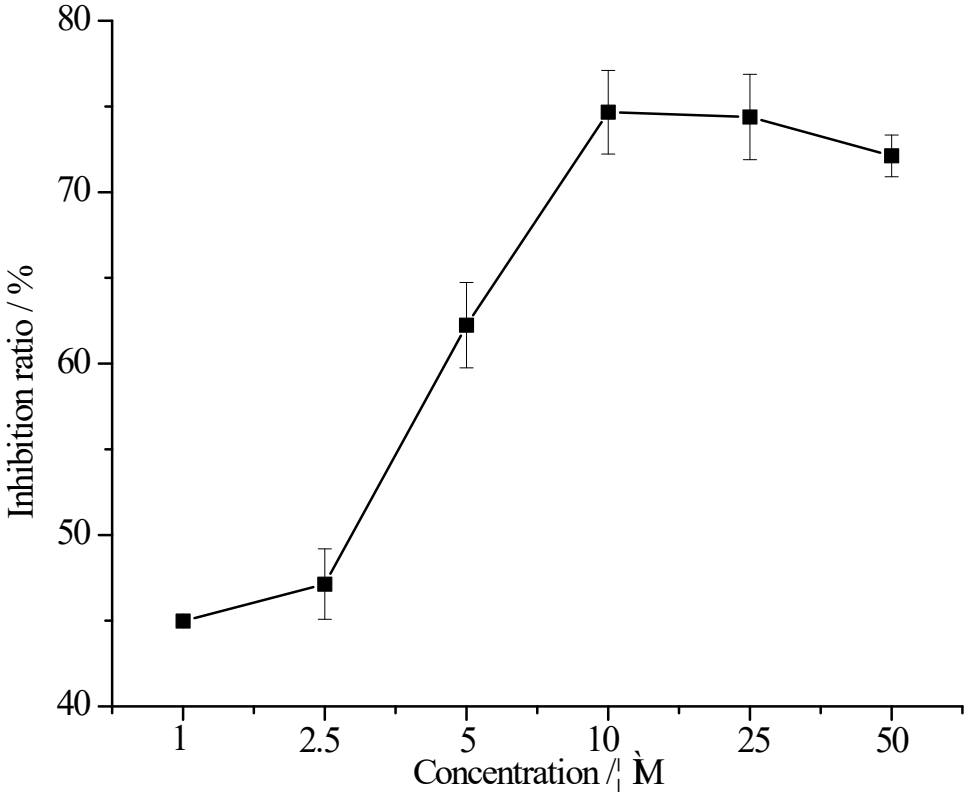



(6) HRMS for compound 3f

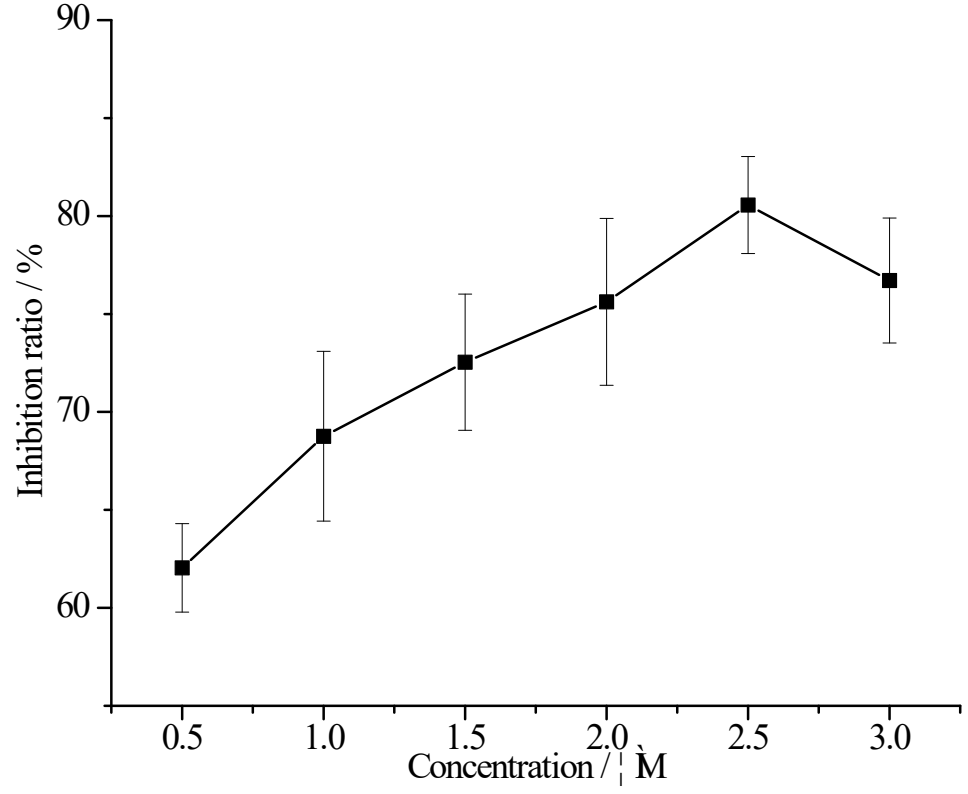



(7) HRMS for compound 3g

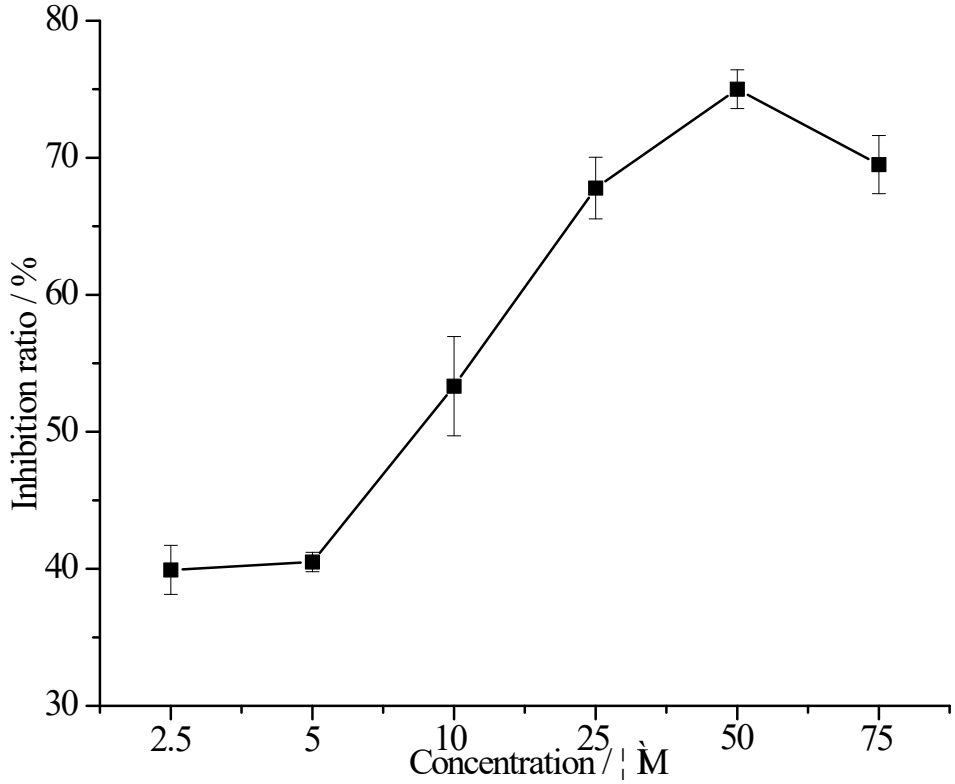
Figure S2. HRMS for compounds 3a-3g



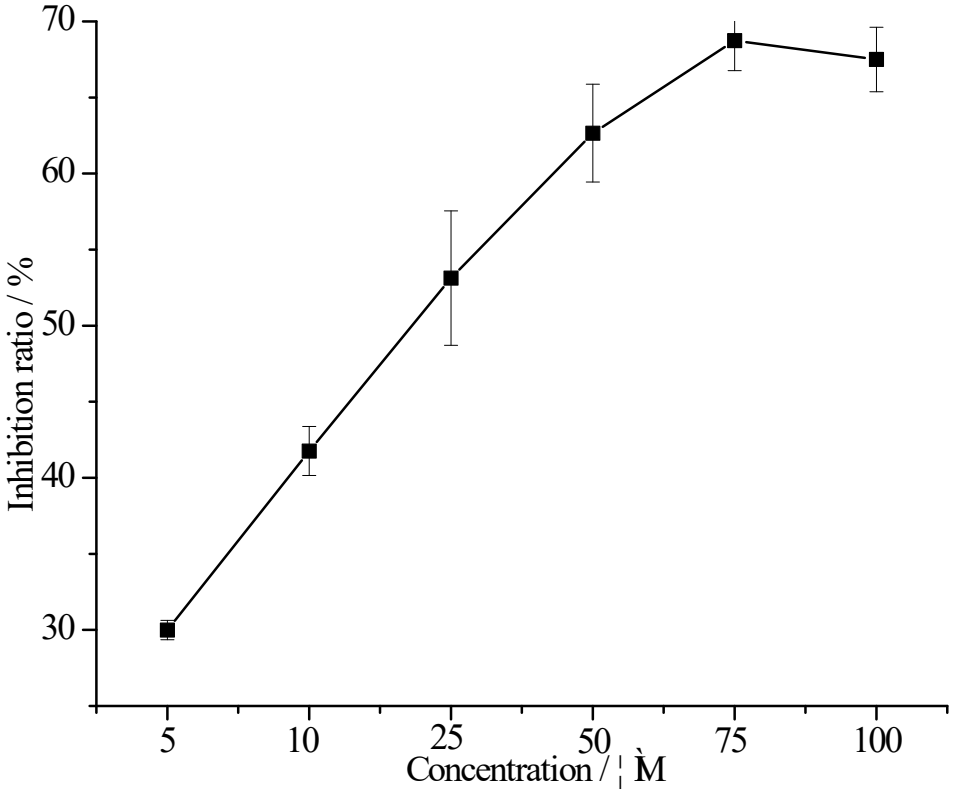

(1) The inhibitory activity of compound 3a on mTyr



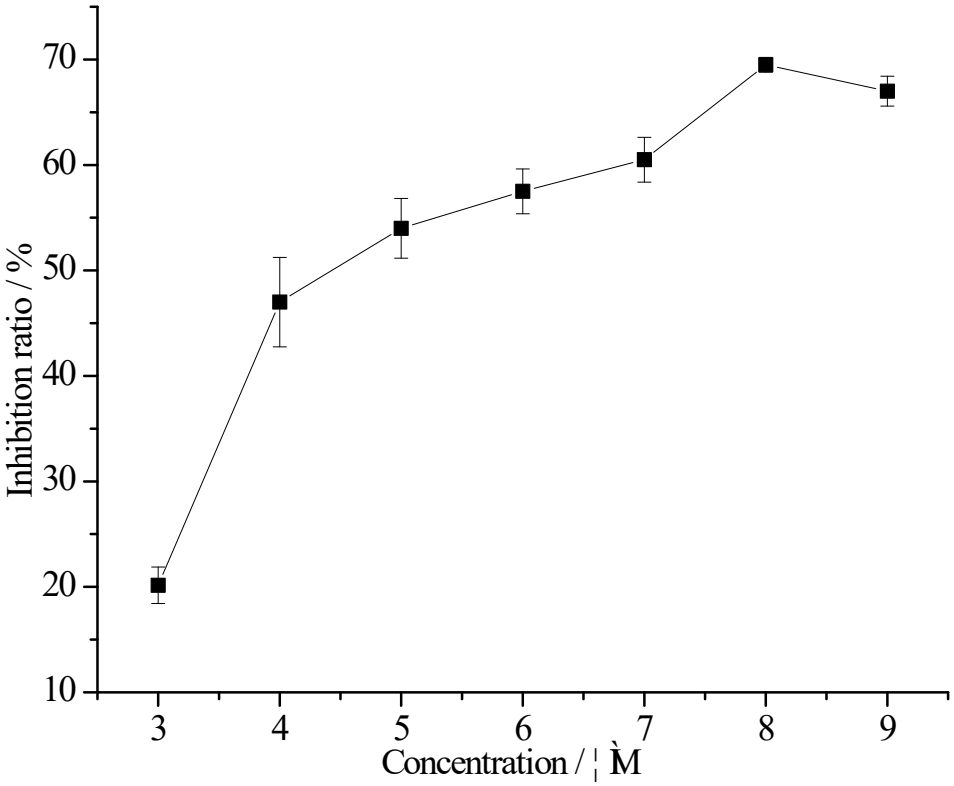

(2) The inhibitory activity of compound 3b on mTyr



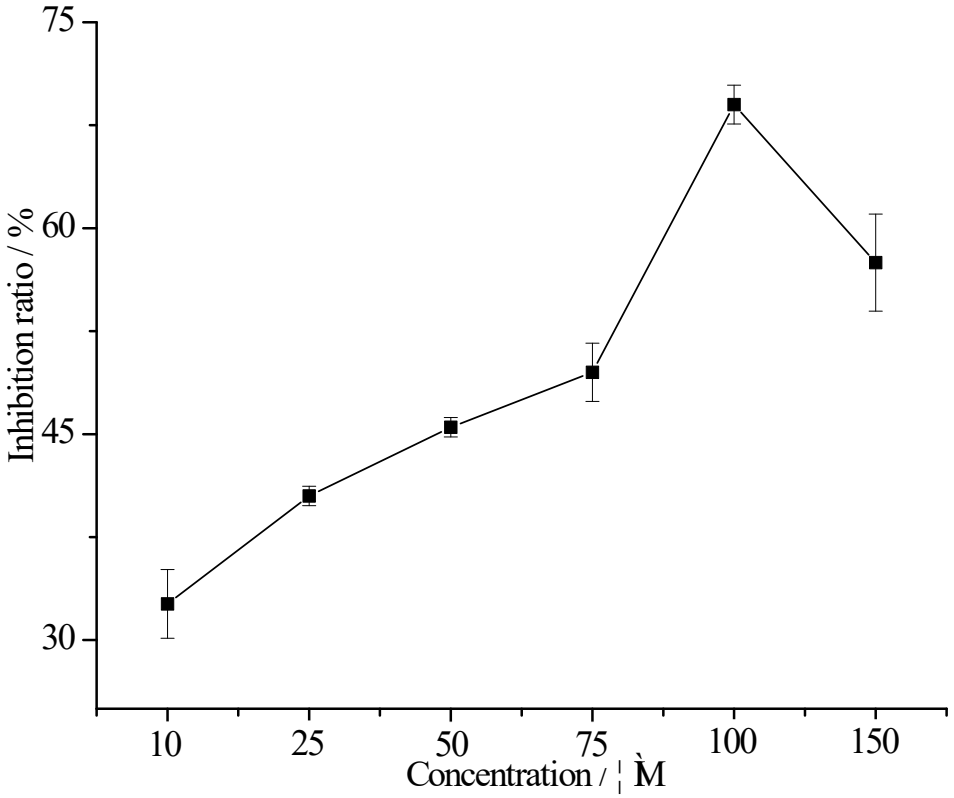

(3) The inhibitory activity of compound 3c on mTyr



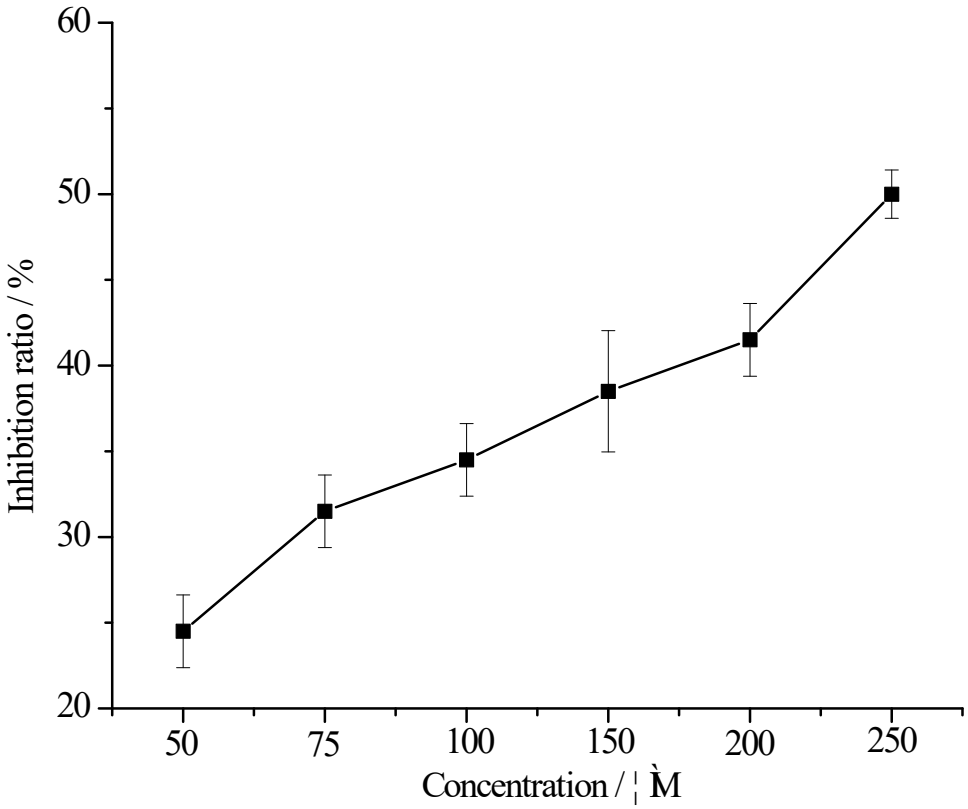

(4) The inhibitory activity of compound 3d on mTyr




(5) The inhibitory activity of compound 3e on mTyr

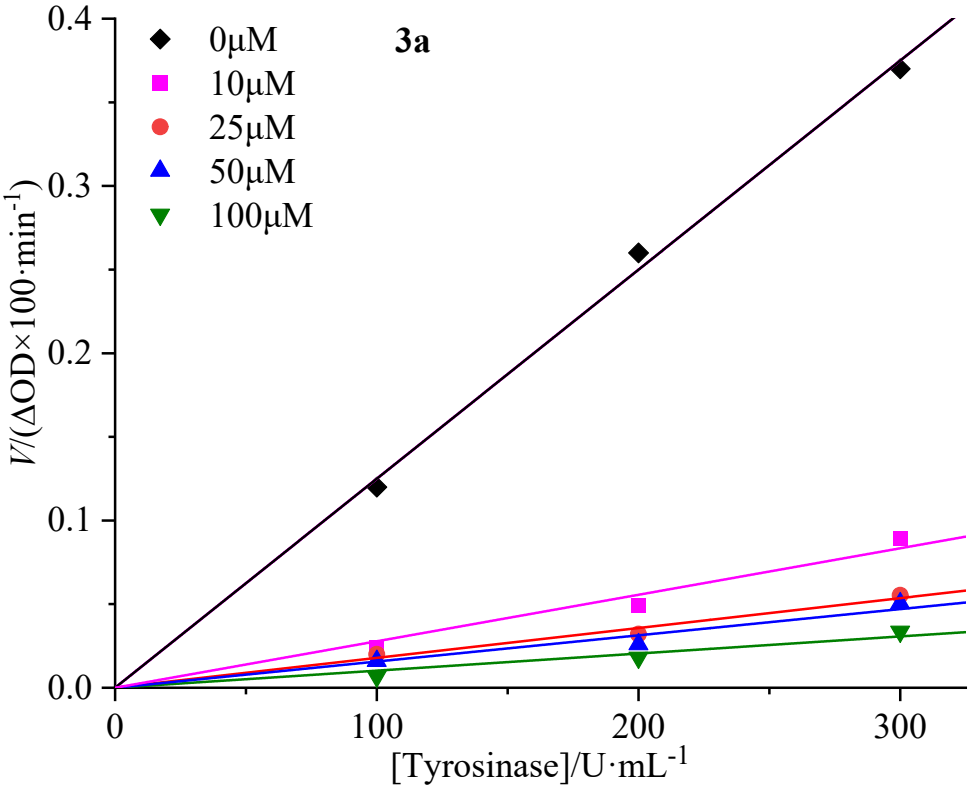



(6) The inhibitory activity of compound 3f on mTyr

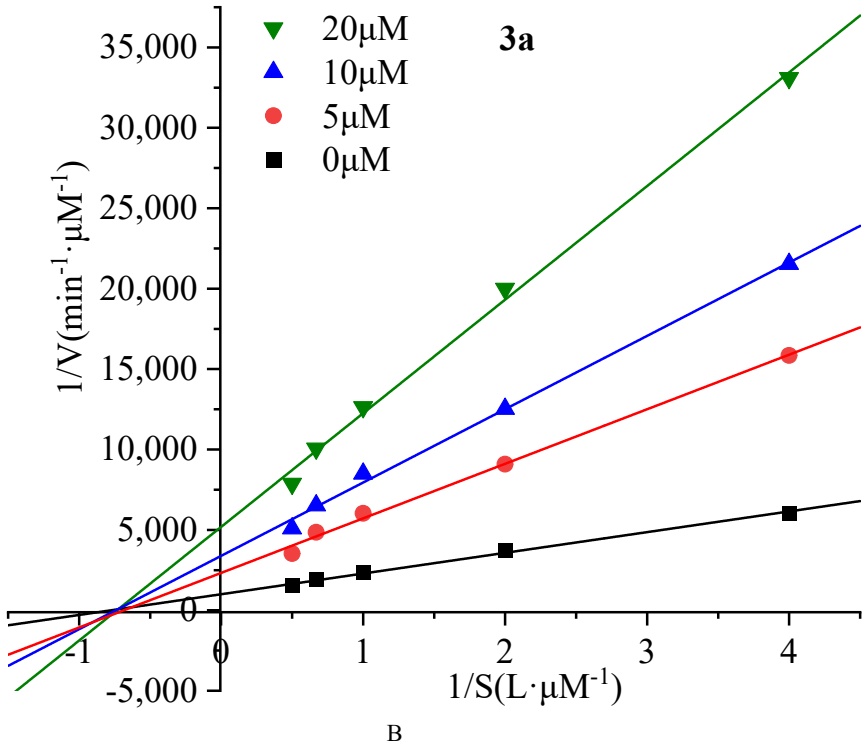



(7) The inhibitory activity of compound 3g on mTyr

Figure S3. The inhibitory activity of compounds 3a-3g on mTyr

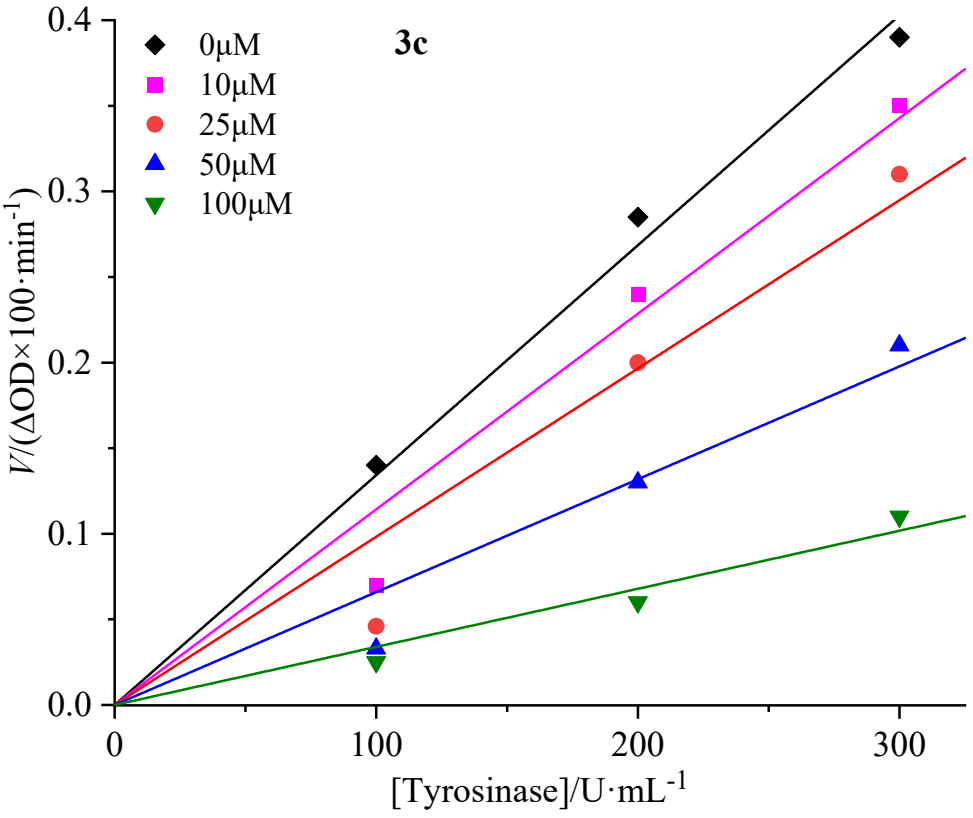



A

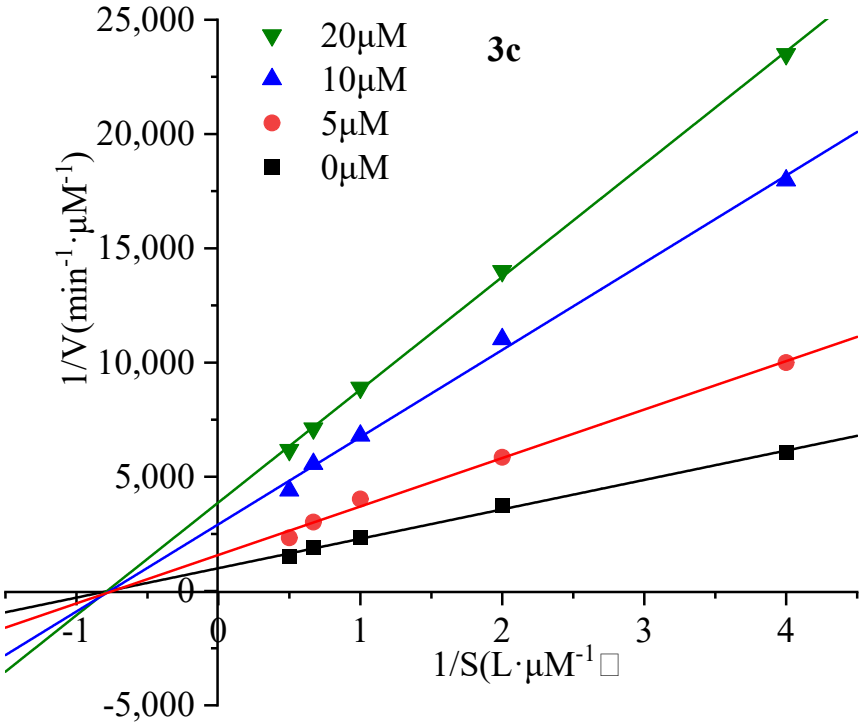


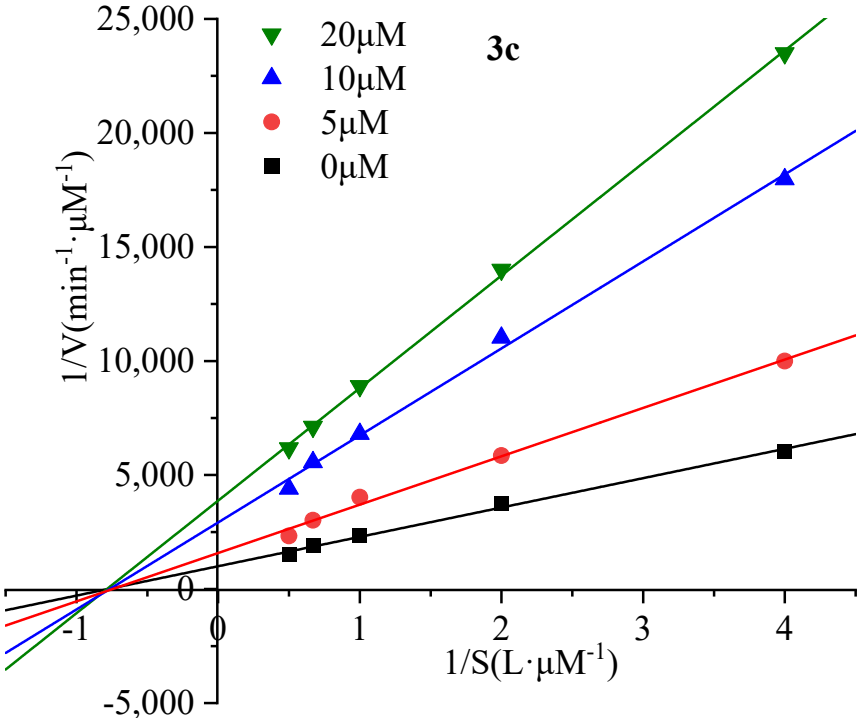

B

（1）Compound 3a


3c
0μM
10μM
25μM
50μM
100μM
V/(ΔOD×100·min-1)
[Tyrosinase]/U·mL-1
0.0
0.1
0.2
0.3
0.4
0
100
200
300

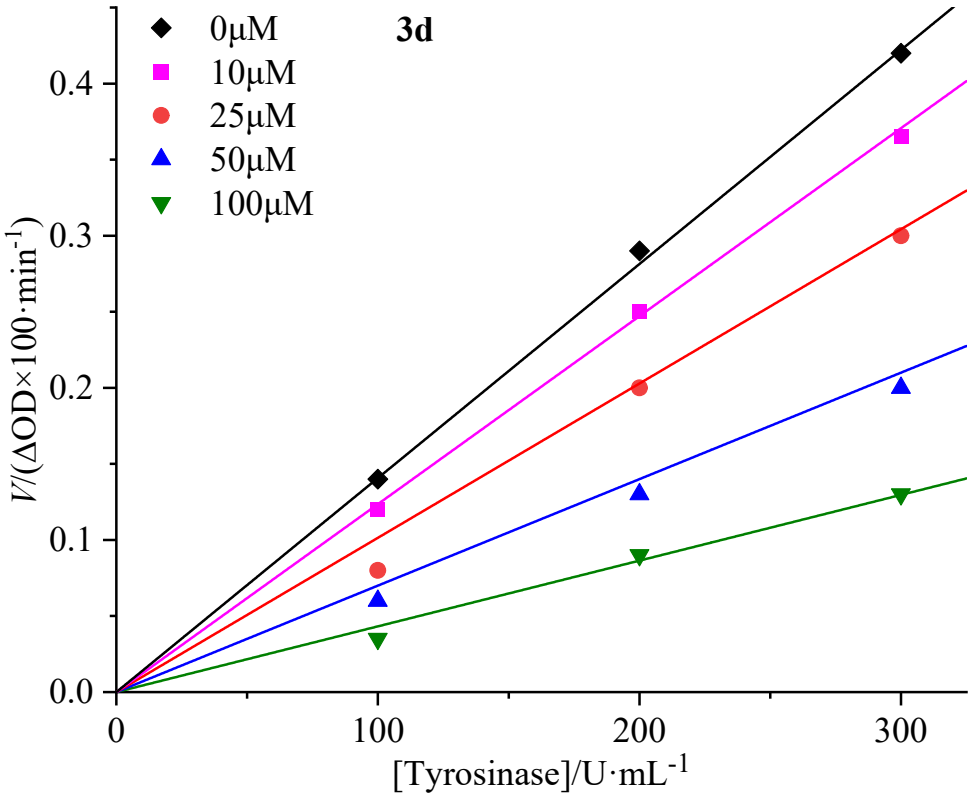
A


3c
20μM
10μM
5μM
0μM
1/V(min-1·μM-1)
1/S(L·μM-1)
25,000
20,000
15,000
10,000
5,000
0
-5,000
-1
0
1
2
3
4

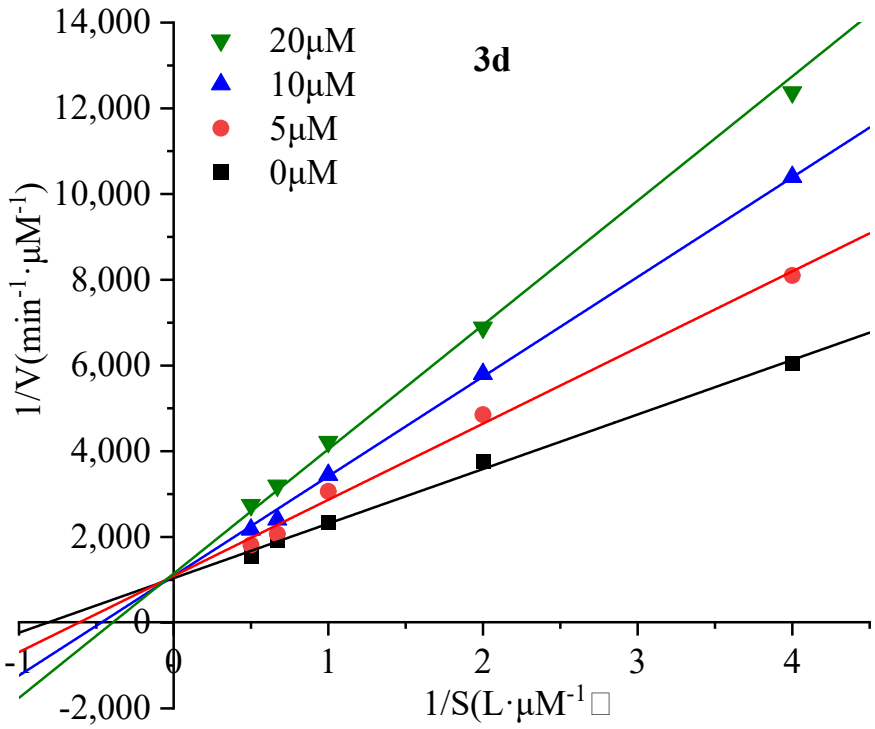


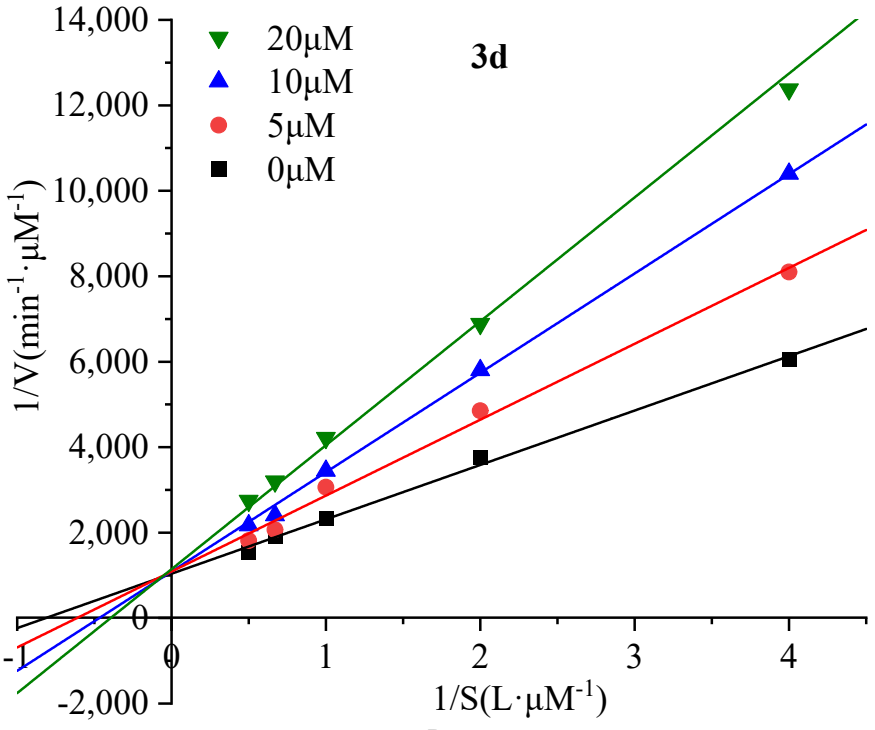

B

（2）Compound 3c



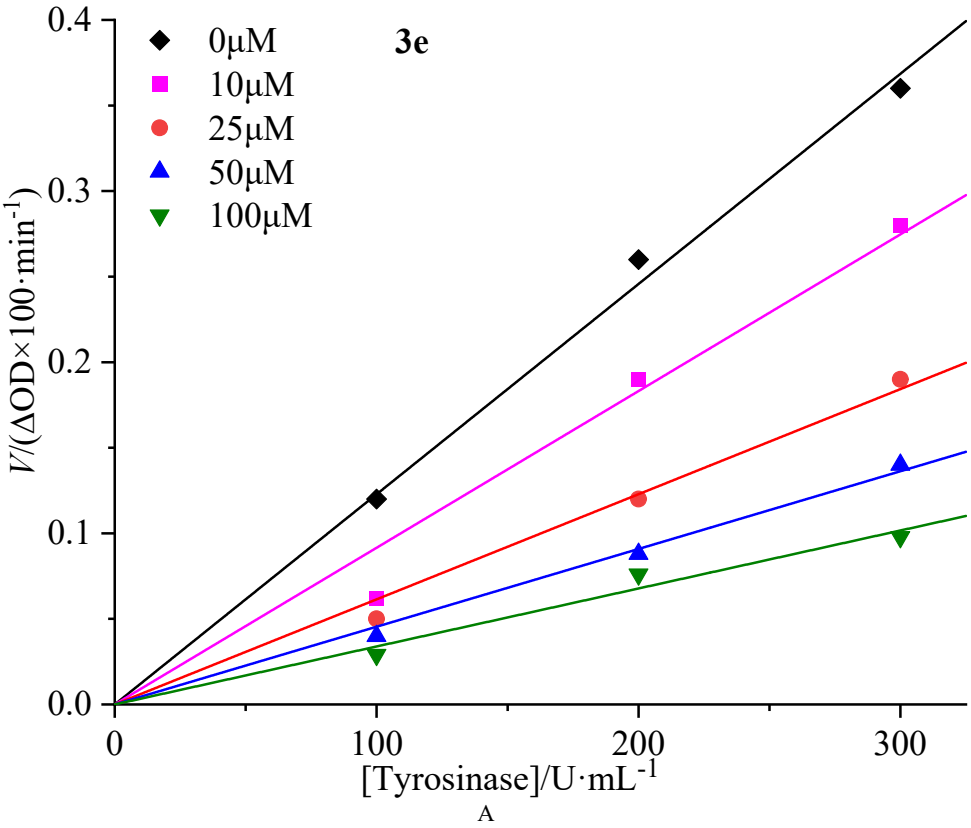

A



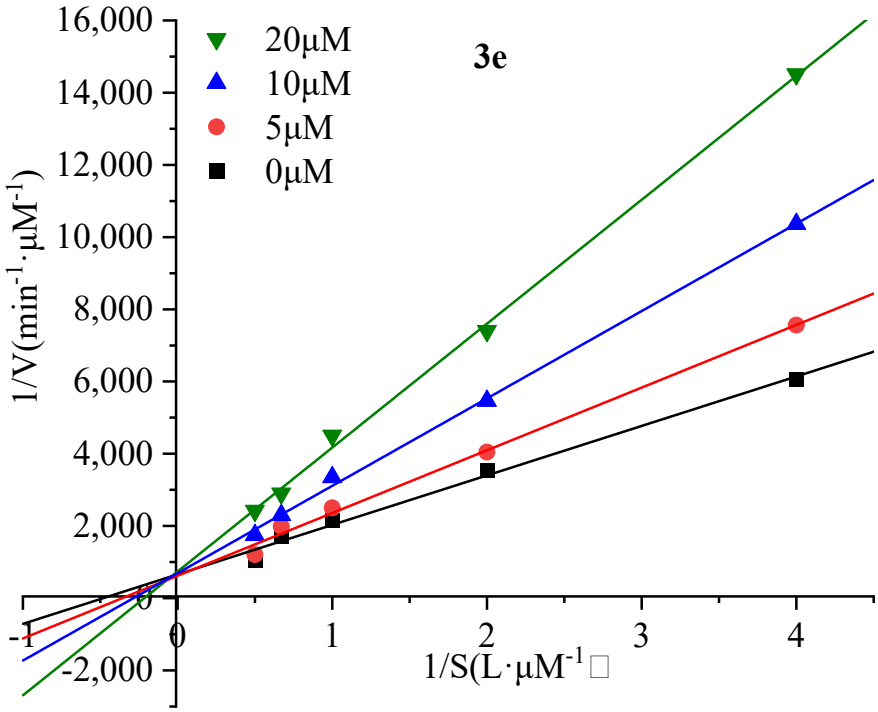



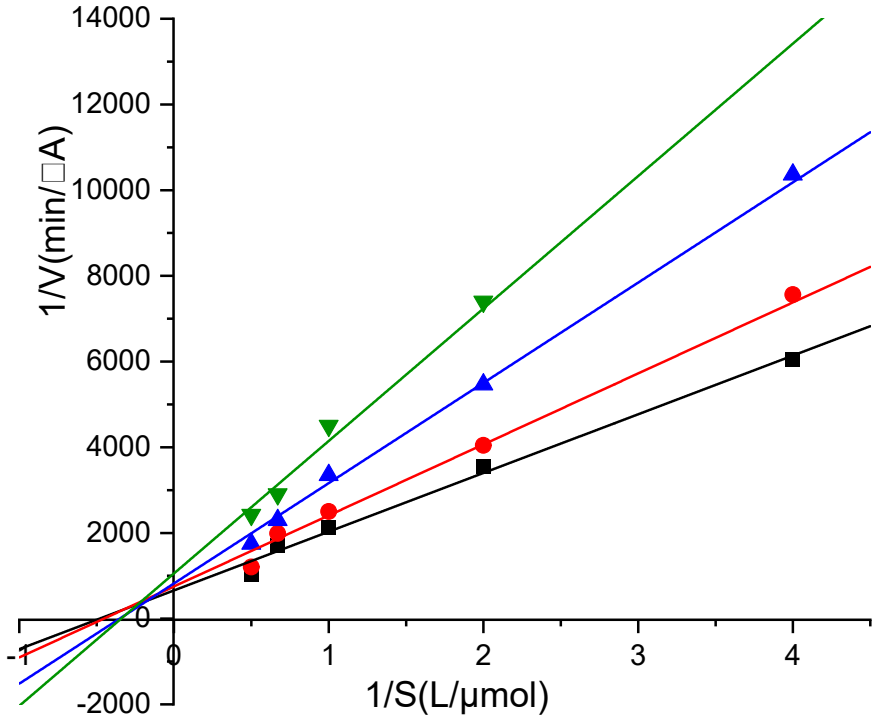

B

（3）Compound 3d


0μM
10μM
25μM
50μM
100μM
3e
V/(ΔOD×100·min-1)
[Tyrosinase]/U·mL-1
0.0
0.1
0.2
0.3
0.4
0
100
200
300

A

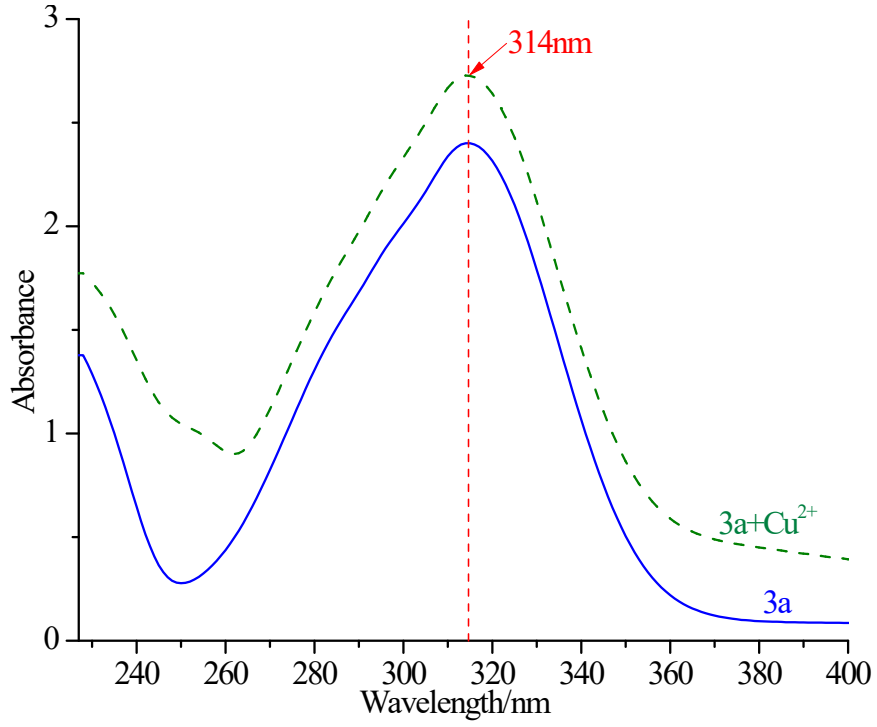

20μM
10μM
5μM
0μM
3e
1/V(min-1·μM-1)
1/S(L·μM-1)
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
-2,000
-1
0
1
2
3
4



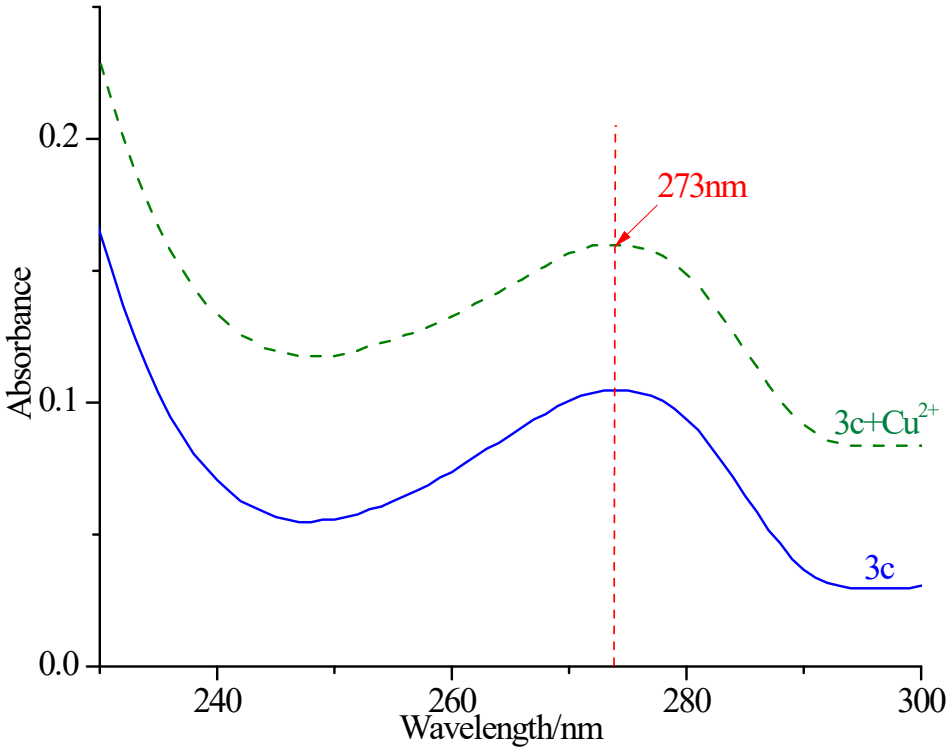

B

（4）Compound 3e

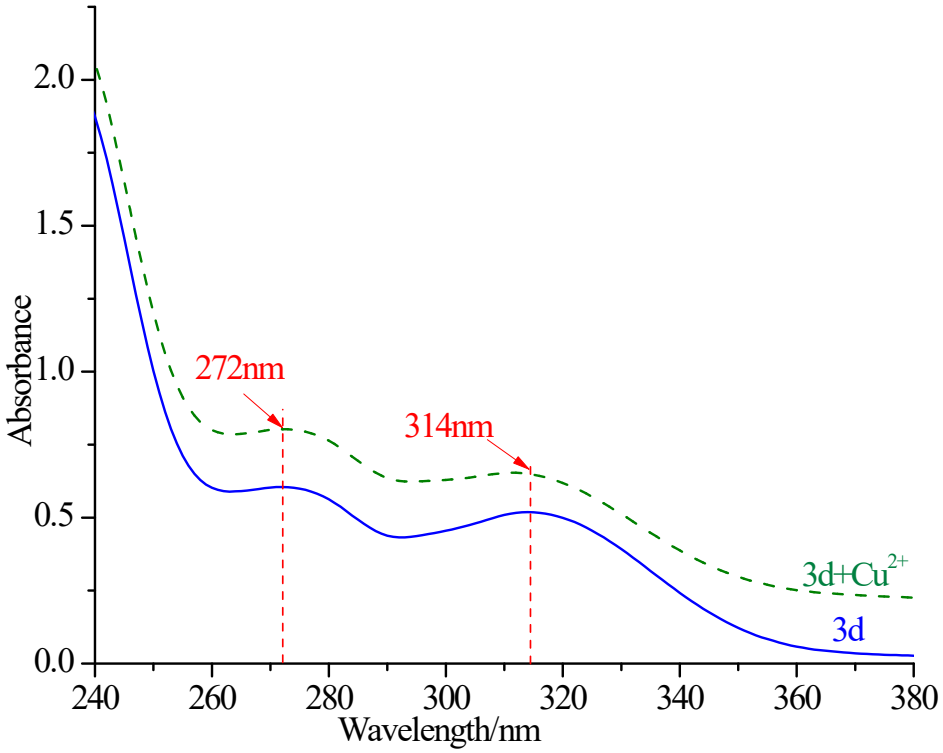
Figure S4 Inhibition reversibility (A) and inhibition type (B) of compound 3a, 3c-3e on mTyr.



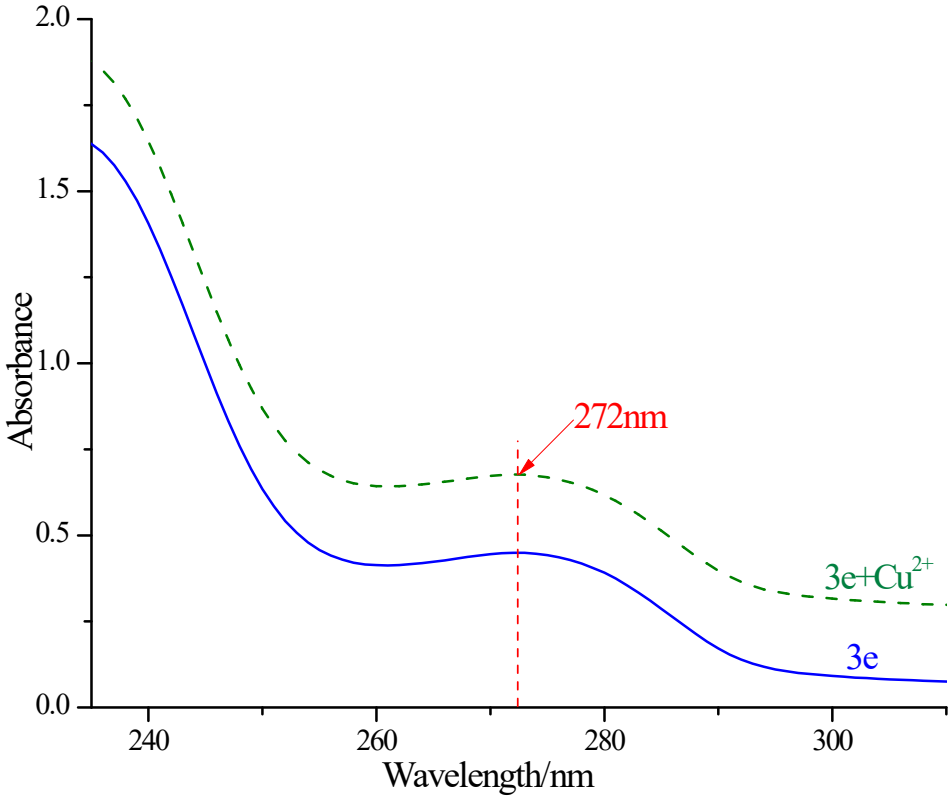

Compound 3a




Compound 3c




Compound 3d




Compound 3e

Figure S5. UV Spectra of compound 3a, 3c-3e before and after interaction with mTyr

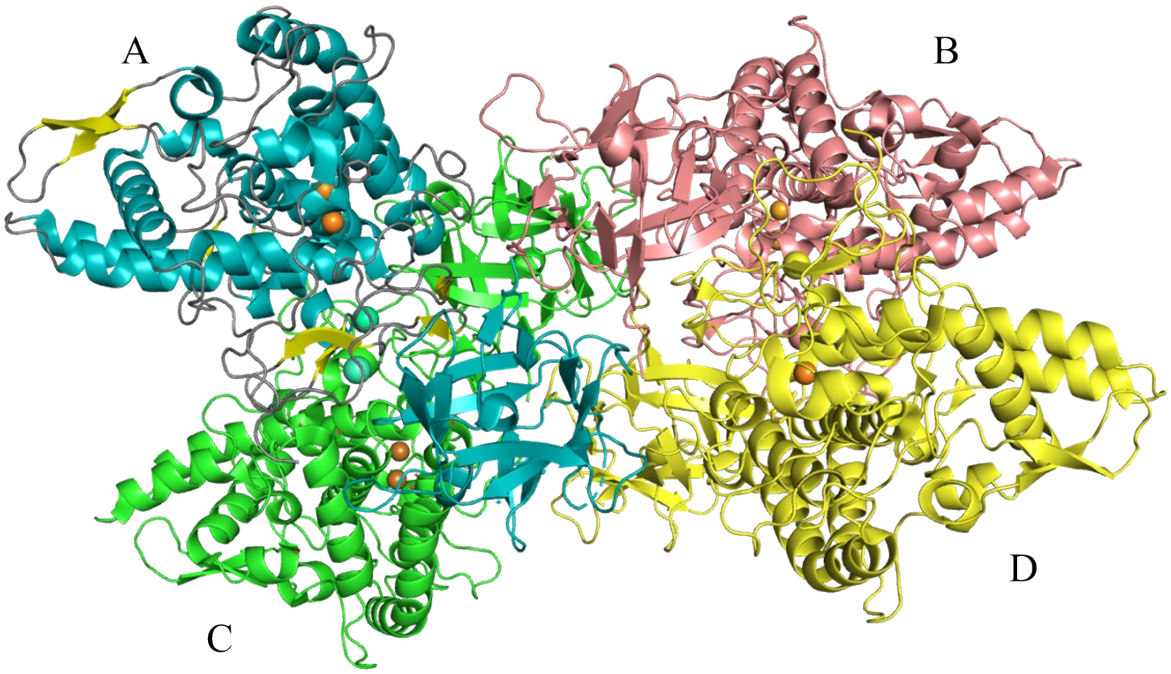
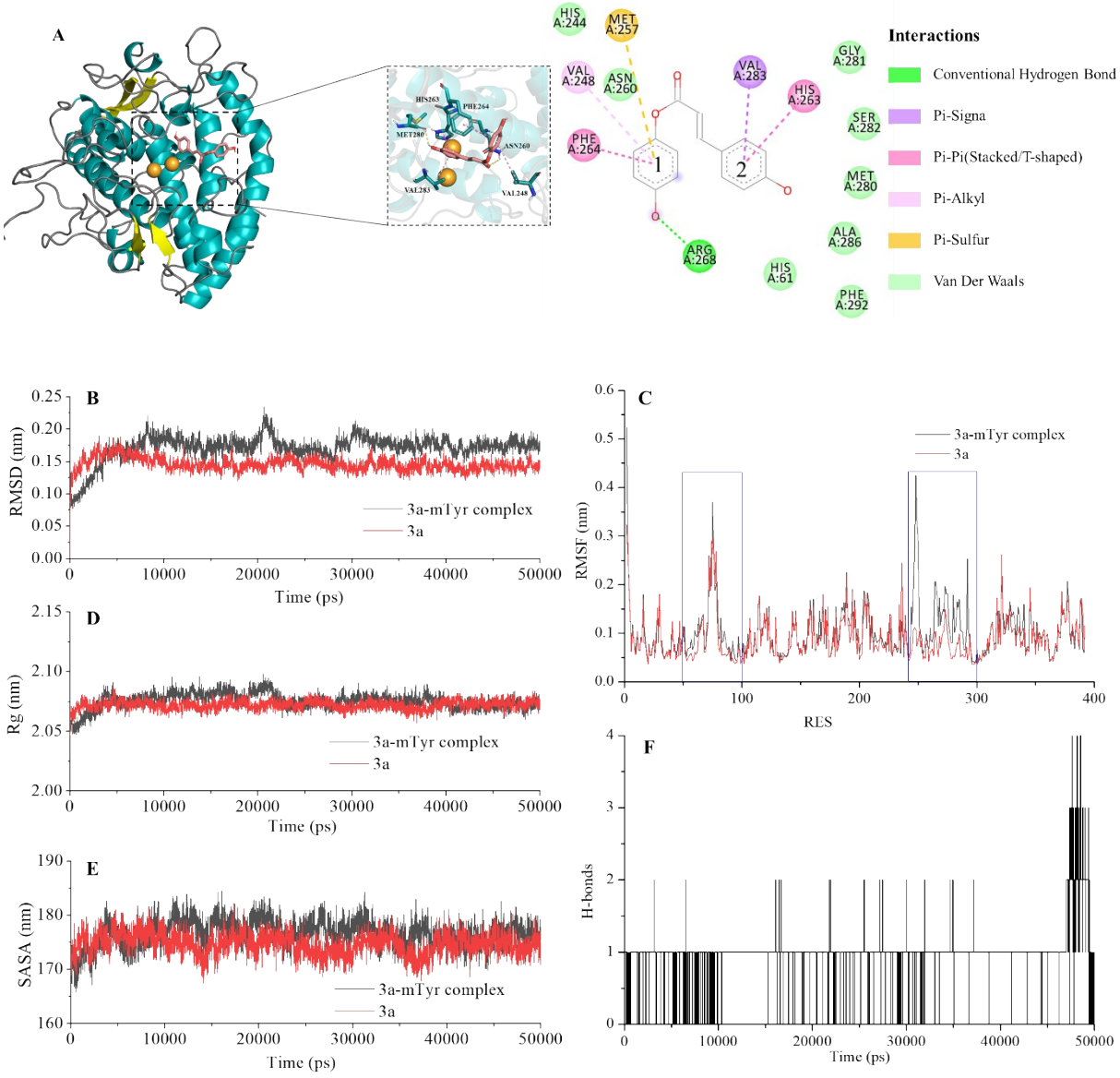
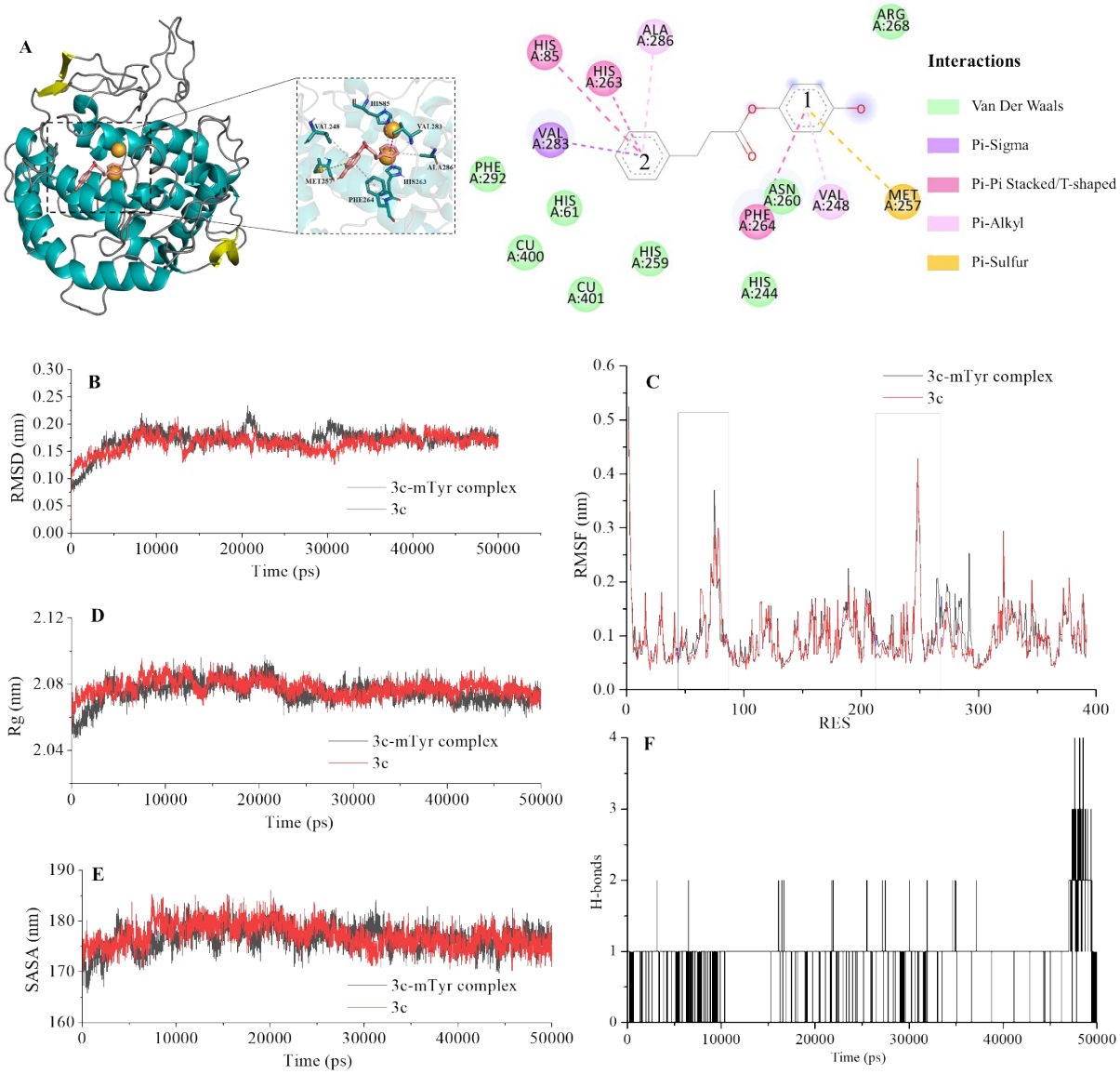


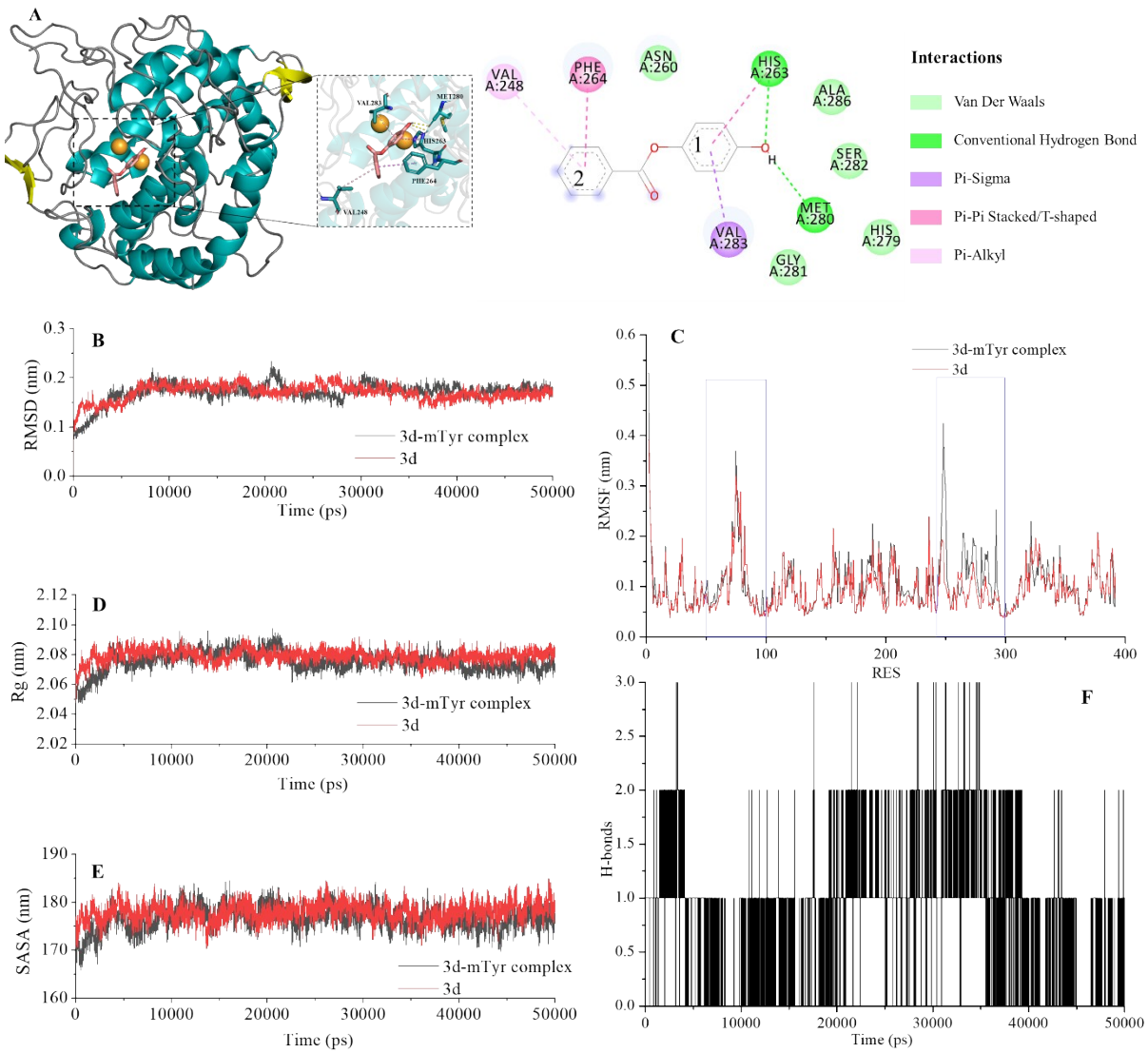

Figure S6. The crystal structure of mTyr (PDB ID: 2Y9X)


A
HIS A:244
MET A:257
VAL A:248
ASN A:260
VAL A:283
HIS A:263
GLY A:281
SER A:282
PHE A:264
MET A:280
ALA A:286
ARG A:268
HIS A:61
PHE A:292
1
2
Interactions
Conventional Hydrogen Bond
Pi-Signa
Pi-Pi(Stacked/T-shaped)
Pi-Alkyl
Pi-Sulfur
Van Der Waals
B
RMSD (nm)
Time (ps)
3a-mTyr complex
3a
C
RMSF (nm)
RES
D
Rg (nm)
E
SASA (nm)
F
H-bonds

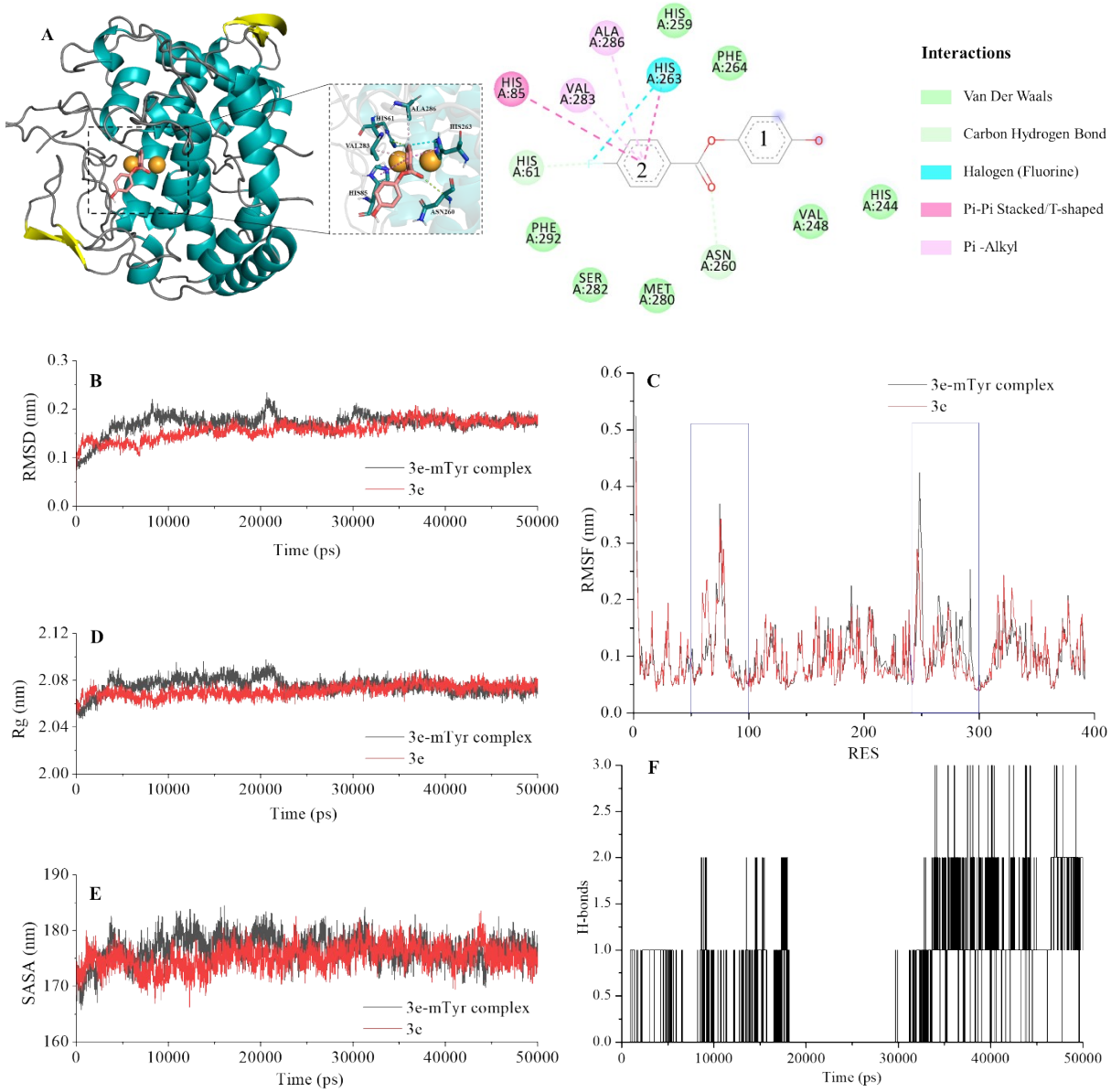
Compound 3a


A
HIS A:85
HIS A:263
ALA A:286
ARG A:268
VAL A:283
PHE A:292
HIS A:61
CU A:400
CU A:401
HIS A:259
HIS A:244
PHE A:264
ASN A:260
VAL A:248
MET A:257
Interactions
Van Der Waals
Pi-Sigma
Pi-Pi Stacked/T-shaped
Pi-Alkyl
Pi-Sulfur
B
RMSD (nm)
Time (ps)
3c-mTyr complex
3c
C
RMSF (nm)
RES
D
Rg (nm)
E
SASA (nm)
F
H-bonds

Compound 3c


A
VAL A:248
PHE A:264
ASN A:260
HIS A:263
ALA A:286
SER A:282
MET A:280
HIS A:279
GLY A:281
VAL A:283
Interactions
Van Der Waals
Conventional Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked/T-shaped
Pi-Alkyl
B
RMSD (nm)
Time (ps)
3d-mTyr complex
3d
C
RMSF (nm)
RES
D
Rg (nm)
E
SASA (nm)
F
H-bonds

Compound 3d




Compound 3e

Figure S7. Docking model for compound 3a, 3c-3e with mTyr (A) and Molecular dynamics results of compound 3a, 3c-3e and (3a, 3c-3e) -mTyr complex with: B. RMSD, C. RMSF, D. Rg, E. SASA, and F. H-bonds

Table S1. Linear fitting equation, Michaelis constant ($K_m$), maximum reaction rate ($V_m$), and inhibition type for mTyr at varying concentrations of compounds 3a-3e

| Compound | Concentration (μM) | Linear fitting equation | $R^2$ | $K_m$ (μM) | $V_m$ ($\times 10^3$, $min^{-1}$) | Inhibition type |
|---|---|---|---|---|---|---|
| 3a | 0 | Y=1000.25+1287.41X | 0.9991 | 1.2871 | 0.9998 | Noncompetitive |
| | 5 | Y=2340.91+3012.40X | 0.9958 | 1.2870 | 0.4272 | |
| | 10 | Y=3516.19+4525.44X | 0.9963 | 1.2867 | 0.2843 | |
| | 20 | Y=5446.87+7064.94X | 0.9965 | 1.2869 | 0.1836 | |
| 3b | 0 | Y=333.72+1645.85X | 0.9902 | 4.9318 | 2.9965 | Mix-noncompetitive |
| | 1 | Y=1320.62+2456.41X | 0.9949 | 2.6173 | 0.7572 | |
| | 2 | Y=2889.49+3833.45X | 0.9964 | 1.3267 | 0.3461 | |
| | 4 | Y=4662.26+5183.79X | 0.9982 | 1.1119 | 0.2145 | |
| 3c | 0 | Y=1000.25+1287.41X | 0.9991 | 1.2871 | 0.9998 | Noncompetitive |
| | 5 | Y=1580.45+2034.21X | 0.9990 | 1.2870 | 0.6327 | |
| | 10 | Y=2920.18+3759.70X | 0.9991 | 1.2873 | 0.3424 | |
| | 20 | Y=3875.12+4950.54X | 0.9999 | 1.2774 | 0.2581 | |
| 3d | 0 | Y=1084.97+1272.89X | 0.9961 | 1.1732 | 0.9217 | Competitive |
| | 5 | Y=1085.12+1776.46X | 0.9985 | 1.6371 | 0.9216 | |
| | 10 | Y=1086.06+2324.89X | 0.9996 | 2.1407 | 0.9208 | |
| | 20 | Y=1085.95+2900.15X | 0.9967 | 2.6706 | 0.9209 | |
| 3e | 0 | Y=679.35+1370.31X | 0.9904 | 2.0171 | 1.472 | Competitive |
| | 5 | Y=678.55+1736.97X | 0.9943 | 2.5598 | 1.474 | |
| | 10 | Y=680.49+2419.72X | 0.9983 | 3.5558 | 1.470 | |
| | 20 | Y=681.12+3431.14X | 0.9983 | 5.0375 | 1.468 | |

Table S2 Docking energy and bonding condition of compounds 3a-3e with mTyr

| Compounds | Docking energy (kcal·mol$^{-1}$) | Bonding condition | | | |
|---|---|---|---|---|---|
| | | Hydrogen Bonds | Hydrophobic | Miscellaneous (Pi-Sulfur) | Halogen (Fluorine) |
| 3a | -7.6 | Ph1O←Arg 268 (6.66 Å, Conventional Hydrogen Bond) | Ph1←Phe264 (6.68Å, Pi-Pi Stacked/T-shaped); Ph1←Val248 (5.16Å, Pi-alkyl); Ph2←His263 (5.57Å, Pi-Pi Stacked/T-shaped); Ph2←Val283 (5.11Å, Pi-Sigma). | Ph1←Met257 (6.50 Å) | |
| 3b | -7.2 | Ph1OH←Met280(5.01 Å, Conventional Hydrogen Bond) | Ph1←His263 (5.32Å, Pi-Pi Stacked); Ph1←Val283 (4.89Å, Pi- Sigma); Ph2←Val248 (5.31 Å, Pi-Sigma). | Ph2←Met257 (6.57 Å) | |
| 3c | -6.7 | | Ph1←Val248 (5.57Å, Pi-Alkyl); Ph1←Phe264 (6.74Å, Pi-Pi Stacked/T-shaped); Ph2←Val283 (4.26Å, Pi-Sigma); Ph2←His85/His263 (5.47/5.48Å, Pi-Pi Stacked/T-shaped); Ph2←Ala 286 (6.53Å, Pi-Alkyl). | Ph1←Met257( 7.58 Å) | |
| 3d | -7.0 | Ph1OH←Met280(4.88 Å, Conventional)Hydrogen Bond; Ph1O←His 263 (4.06 Å, Conventional Hydrogen Bond) | Ph1←His 263 (5.52Å, Pi-Pi Stacked/T-shaped); Ph1←Val283 (4.82Å, Pi-Sigma); Ph2←Val248 (6.29Å, Pi-Alkyl); Ph2←Phe264 (6.62Å, Pi-Pi Stacked/T-shaped). | | |
| 3e | -6.7 | Ph2F←His61 (5.50 Å, Carbon Hydrogen Bond) | Ph2←Val283 (4.42Å, Pi-Signa); Ph2← Val283/Ala286 (4.63/6.95Å, Pi-Alkyl); Ph2←His85/His263 (7.42/4.57Å, Pi-Pi Stacked/T-shaped). | | Ph2F←His 263 (5.41 Å Halogen (Fluorine)) |