Supporting Information

Mechanism of Inactivation of GABA Aminotransferase by (*E*)- and (*Z*)-(1*S*,3*S*)-3-Amino-4-fluoromethylenyl-1-cyclopentanoic Acid

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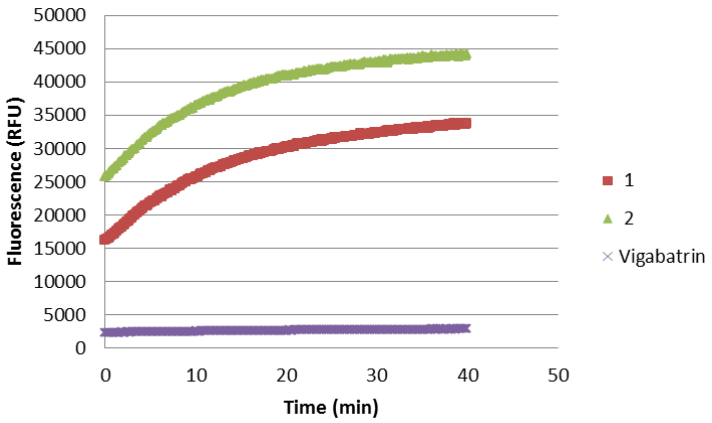


Figure S1. Glutamic acid formation after pre-incubation of GABA-AT with **1**, **2**, and vigabatrin (control) for 5 min

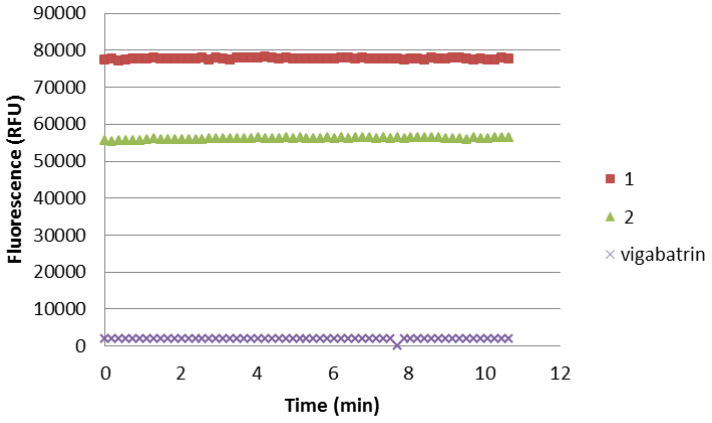


Figure S2. Glutamic acid formation after inactivation of GABA-AT with **1**, **2**, and vigabatrin (control) for 24 h

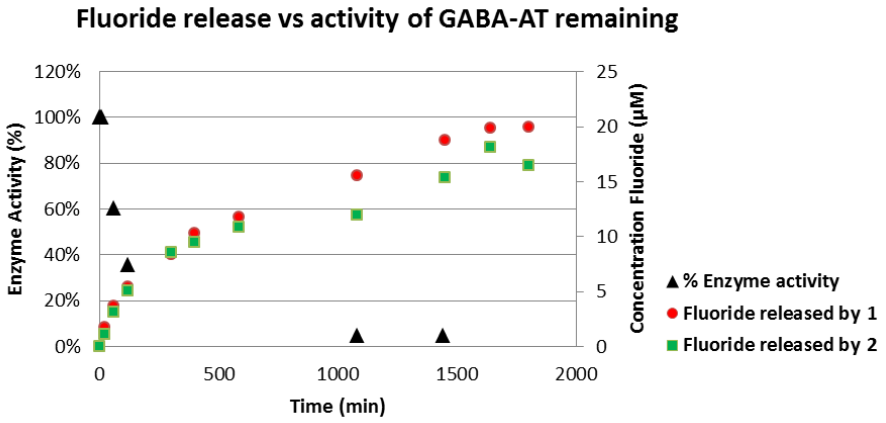


Figure S3. Fluoride ion release and measurement of GABA-AT activity by 2 mM **1** and **2**

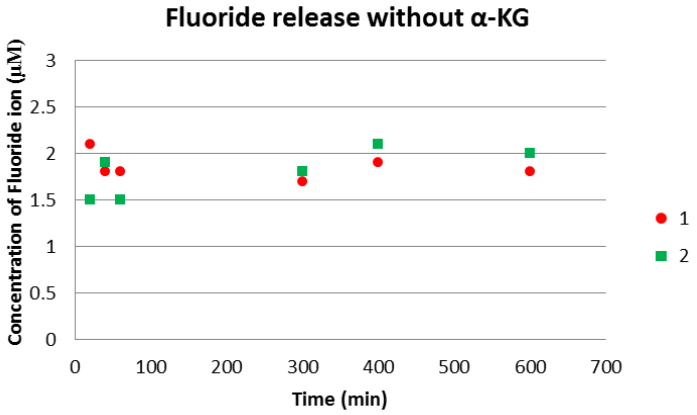


Figure S4. Fluoride ion release from **1** and **2** in the absence of α-ketoglutarate

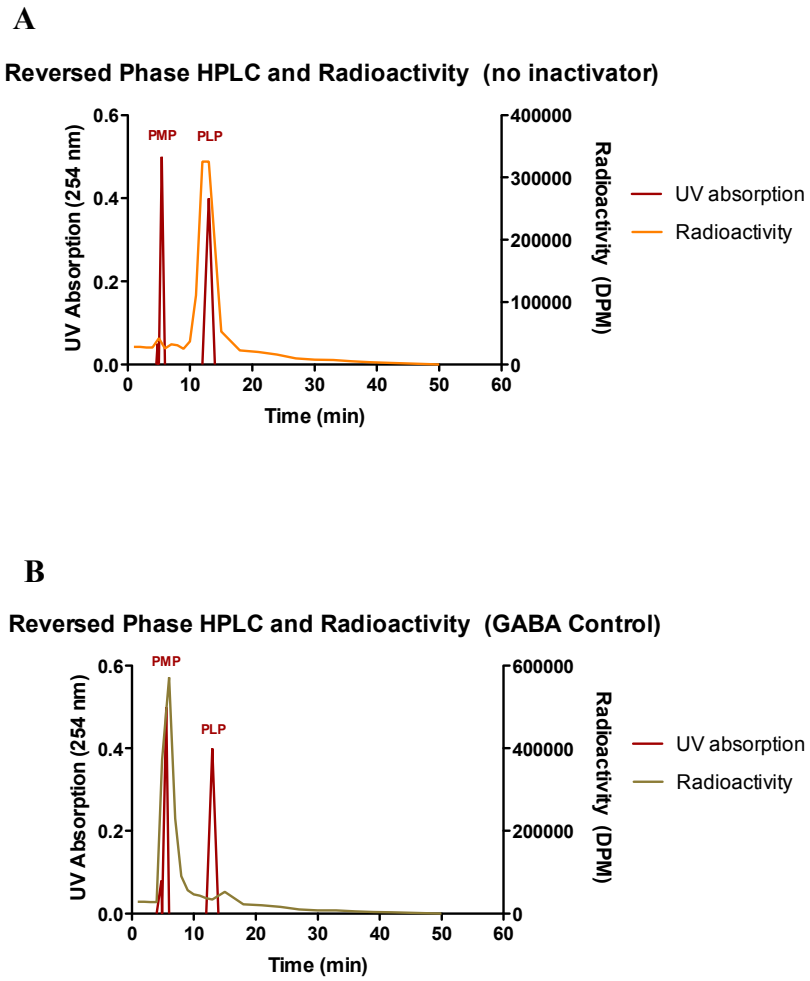


Figure S5. (A) Negative control run with the same concentrations of each reagent, excluding inactivator (**B**) Positive control run with 40 mM GABA containing no inactivator or α-ketoglutarate

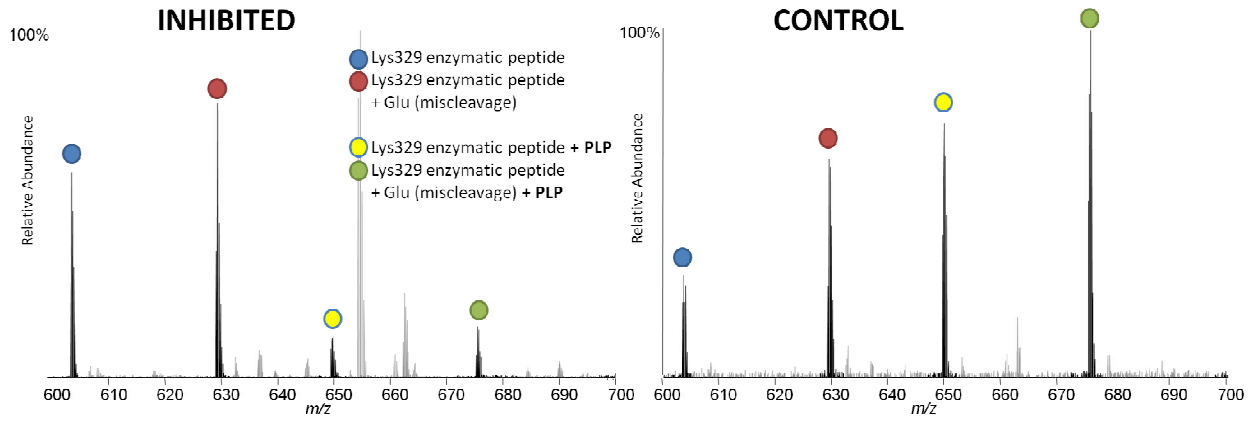


Figure S6. Peptide of the inactivated GABA-AT with **1** and a control. The samples were reduced with $NaBH_4$ before treating with protease. Blue indicates the peptide, red is a peptide with an extra glutamate residue (because of the missed cleavage), yellow is the peptide plus PLP, and green is the peptide with an extra glutamate residue plus PLP.

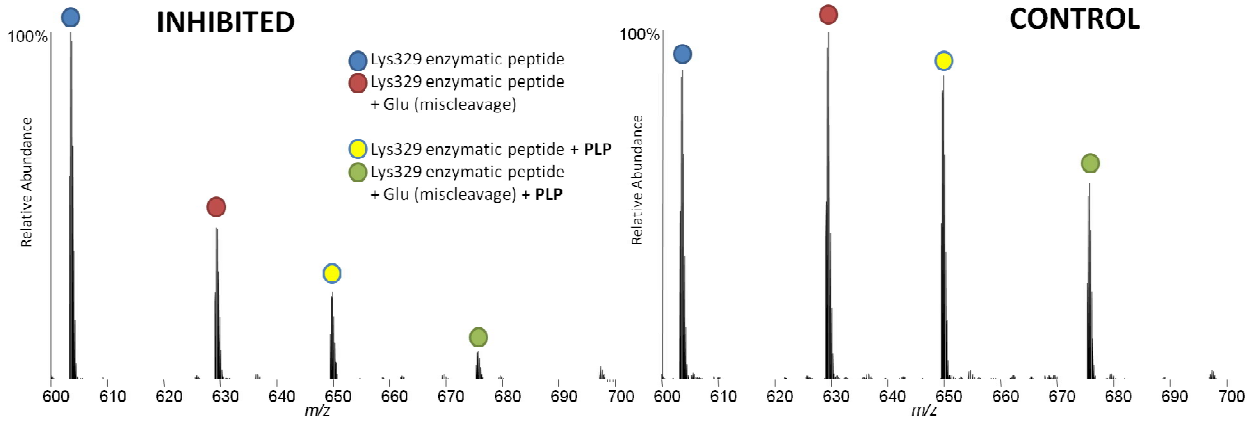
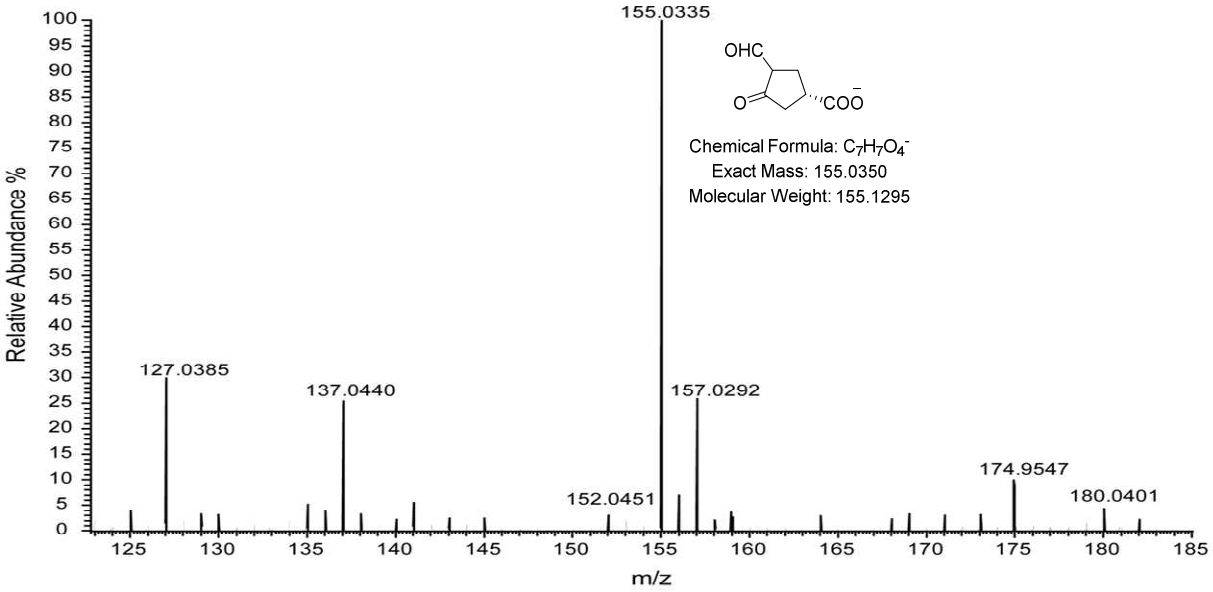


Figure S7. Peptide of the inactivated GABA-AT with **2** and a control. The samples were reduced with $NaBH_4$ before treating with protease. Blue indicates the peptide, red is a peptide with an extra glutamate residue (because of the missed cleavage), yellow is the peptide plus PLP, and green is the peptide with an extra glutamate residue plus PLP.

A



B

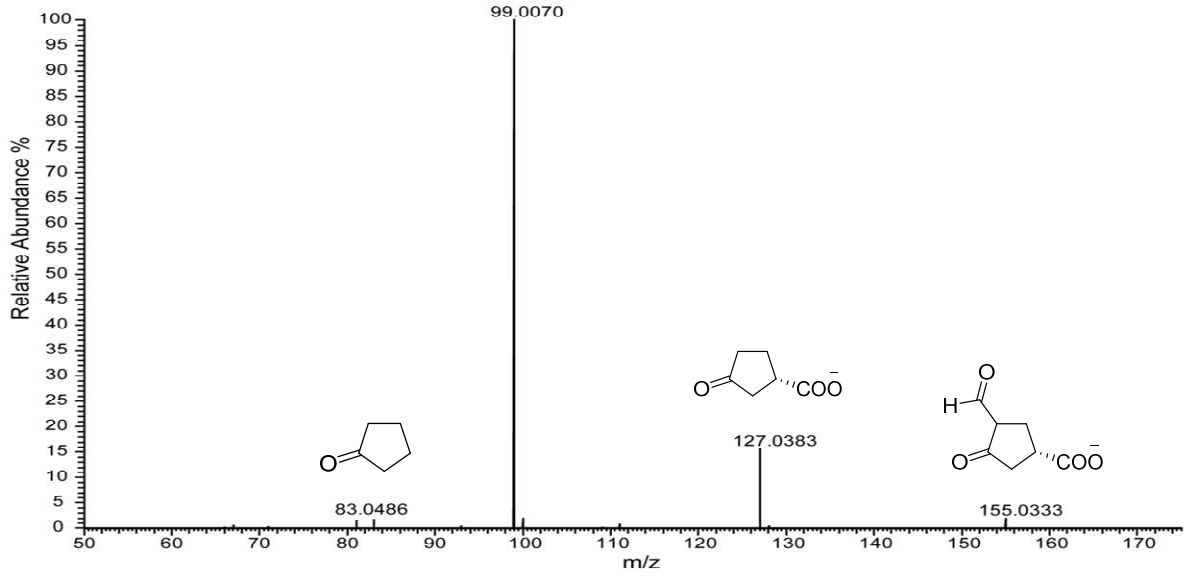


Figure S8. (**A**) High resolution mass spectrum of metabolite released from a reaction incubation of **2** and GABA-AT. (**B**) Fragmentation and assigned structures of peak *m/z* 155 from a reaction incubation of **2** and GABA-AT.

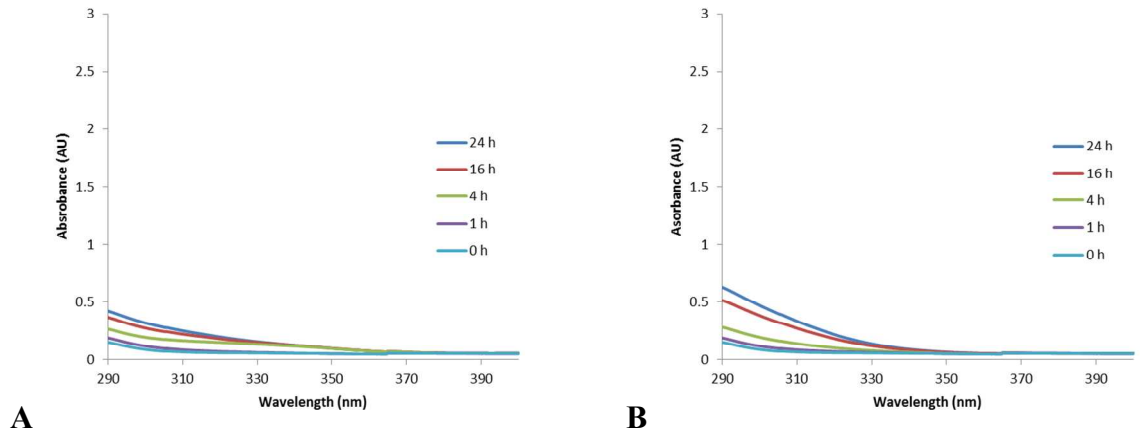


Figure S9. UV absorption spectra of control experiments: (A) GABA-AT and GABA and α-ketoglutarate overtime, (B) GABA-AT and GABA and no α-ketoglutarate overtime.

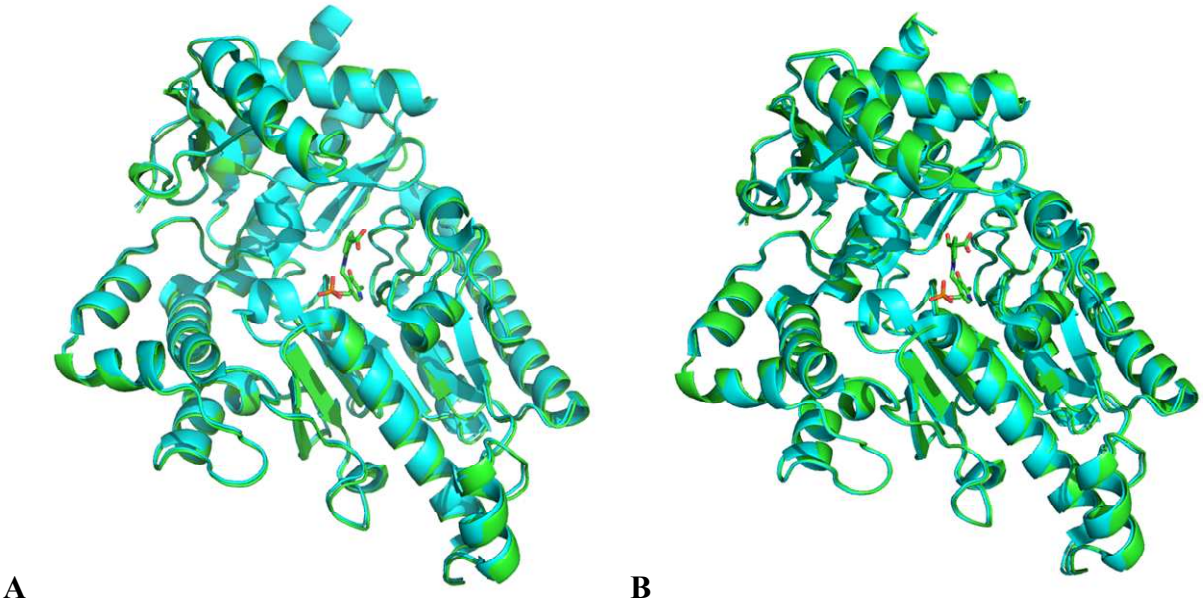


Figure S10. A) Ribbon diagram of the superimposed native GABA-AT (cyan) and **1**-inactivated GABA-AT (green). B) Ribbon diagram of the superimposed native GABA-AT (cyan) and **2**-inactivated GABA-AT (green)

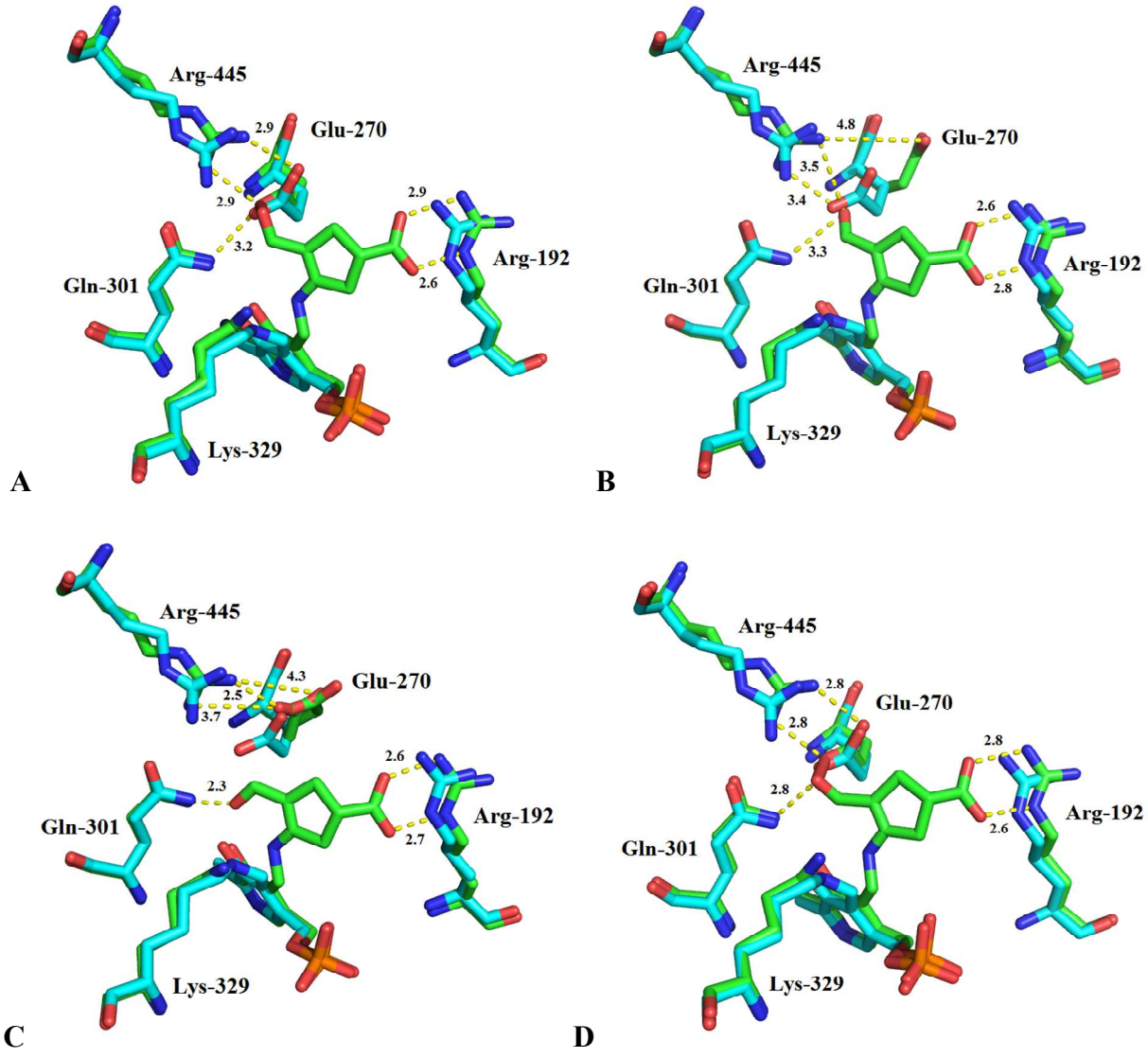


Figure S11. Superimposition of the crystal structures of four **2**-inactivated GABA-AT (green) and native GABA-AT (cyan) monomers.

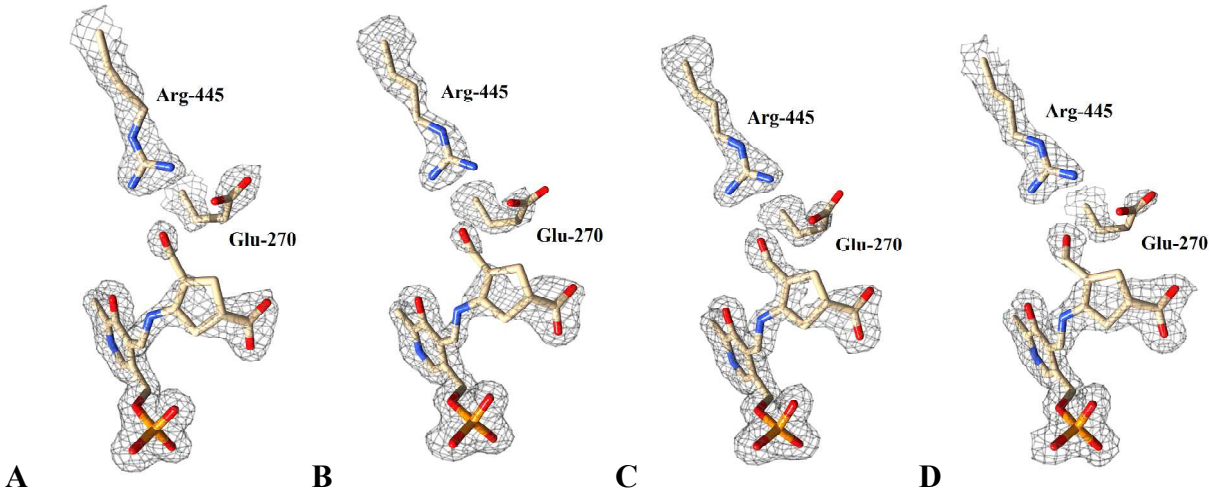


Figure S12 Stereoview of four **1**-inactivated GABA-AT monomers. The $2F_o-F_c$ electron-density map is shown as light gray mesh at 1.0σ level.

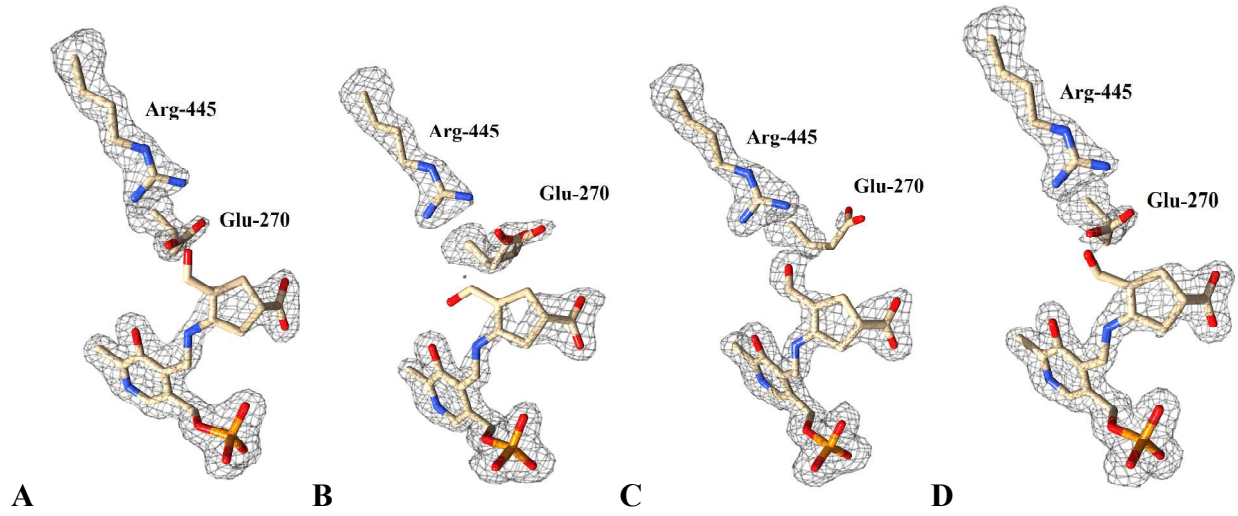


Figure S13. Stereoview of four **2**-inactivated GABA-AT monomers. The $2F_o-F_c$ electron-density map is shown as light gray mesh at 1.0σ level. The occupancies for atoms in the aldehyde group in monomers A, B, and C were set to 0 because the aldehyde groups of the adducts clashed with the carboxylate of the Glu-270. The side chain of Glu-270 in monomer B is modeled in two alternate conformations.

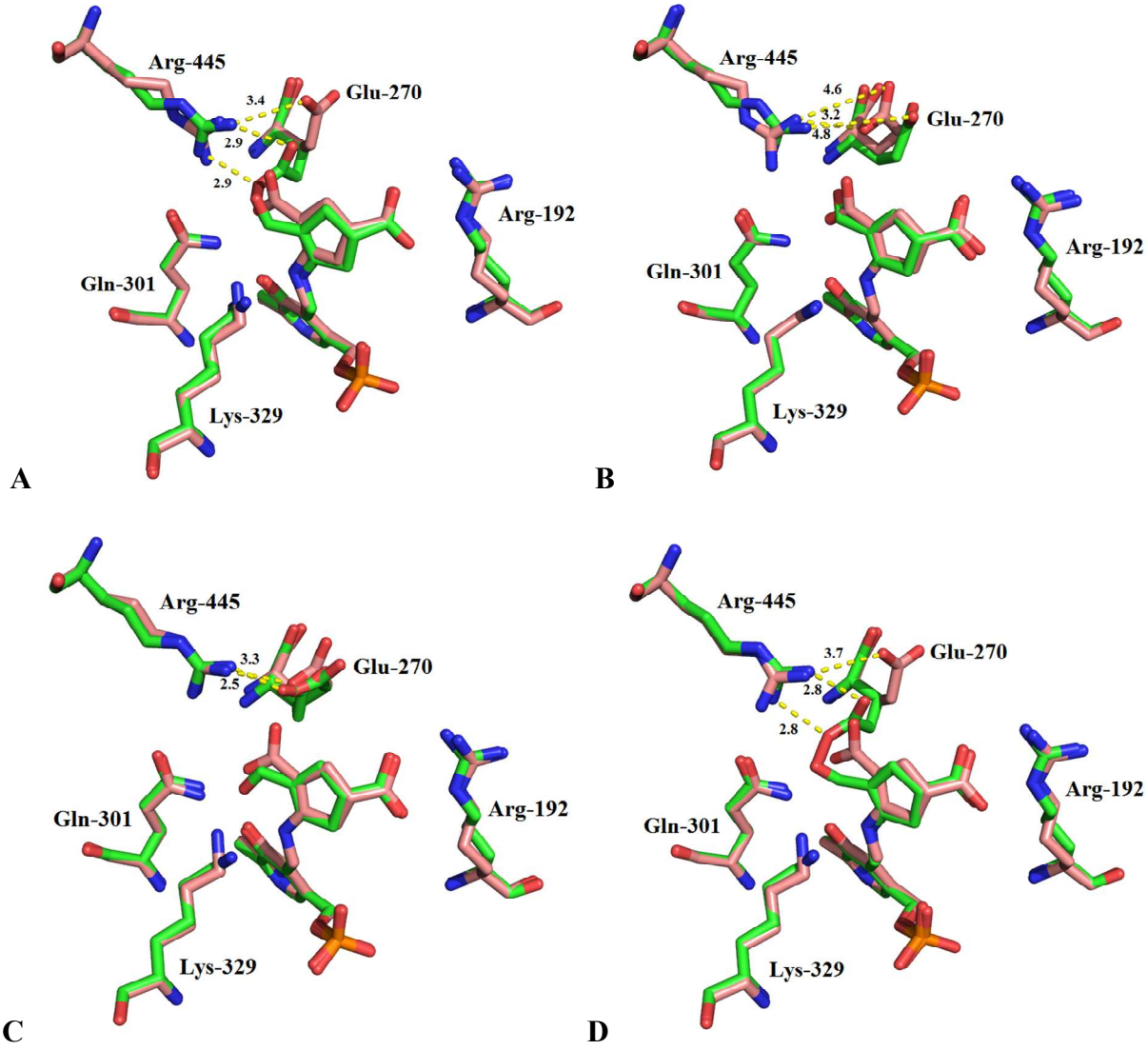


Figure S14. Superimposition of the crystal structures of four **2**-inactivated GABA-AT (green) and CPP-115-inactivated GABA-AT (pink) monomers.

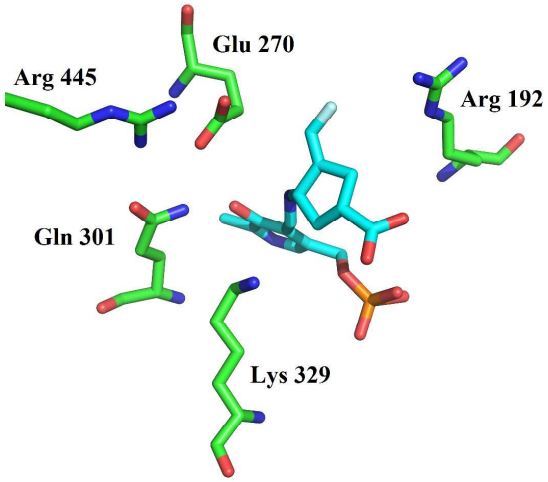


Figure S15. GOLD predicted pose of (*E*)-**5** (Scheme S1) in the GABA-AT active site that preserves the Arg445 – Glu270 salt bridge. Only selected residues are shown in green. Note that the orientation of the γ-carboxylic acid is away from Arg192.

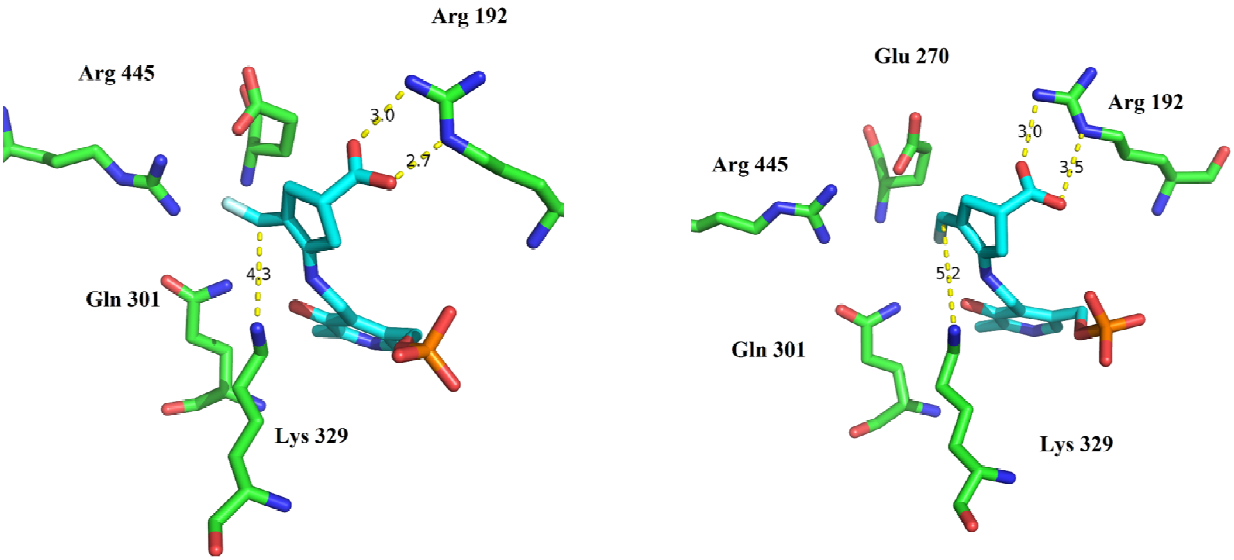


Figure S16. GOLD predicted pose of **6-*E*-isomer** (left) and **6-*Z*-isomer (right)**. The ligands are colored in cyan, whereas the enzyme is colored in green. The distance between Lys 329 and the electrophilic center, as well as the H-bonding with Arg 192 is shown in yellow lines.

Table S1. Crystallographic Data Collection and Refinement Statistics

Data set	**GABA-AT inactivated by 1**	**GABA-AT inactivated by 2**
PDB code	xxxx	yyyy
Space Group	$P2_1$	$P2_1$
Cell Dimensions		
a (Å)	69.6	69.6
b (Å)	227.6	227.2
c (Å)	71.3	71.3
$\alpha = \gamma$ (°)	90	90
β (°)	109.0	108.8
Resolution (Å)	50.00-1.70	50.00-1.70
[a]R_{merge} (%)	4.0 (70.6)	6.8(84.3)
I/sigma	15.5 (1.2)	19.7(1.4)
Completeness (%)	97.7 (94.7)	97.5(95.3)
Redundancy	3.0 (2.7)	3.2(2.9)
No. Total reflections	681840	712557
No. Unique reflections	225037	224347
[b]R_{cryst} / [c]R_{free} (%)	15.8/19.5	16.4/19.0
No. of Solvent Atoms	1787	1842
No. of Atoms	16699	16824
B-factors (Å^2)		
Overall	26.6	27.7
Ligand	9.81/48.17	13.22/41.38
[d]RMSD Bond Length (Å)	0.017	0.005
[d]RMSD Bond Angles (°)	1.650	1.027
Ramachandran		
Favored (%)	96.0	95.9
Allowed (%)	3.70	3.71
Outlier (%)	0.30	0.30

The values for the highest resolution bin are in parentheses.

[a]Linear $R_{merge} = \Sigma|I_{obs}-I_{avg}|/ \Sigma I_{avg}$

[b]$R_{cryst} = \Sigma|F_{obs}-F_{calc}|/ \Sigma F_{obs}$

[c]Five percent of the reflection data were selected at random as a test set and only these data were used to calculate R_{free}

[d]RMSD, root mean square deviation