# Computational methods for the identification of molecular targets of toxic food additives. Butylated hydroxytoluene as a case study

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**Supplementary Methods**

## 1. ChEMBL compounds virtual screening

The list of tested compounds has been retrieved from ChEMBL using the official python client named *ChEMBL webresource client* [1]. The ChEMBL IDs used were: CHEMBL221 for hNET; CHEMBL222 for COX-1; CHEMBL2095172 for GABA-AR $\alpha$-1/$\beta$-2/$\gamma$-2 receptor; CHEMBL2094121 for GABA-AR $\alpha$-1/$\beta$-3/$\gamma$-2 receptor; CHEMBL225 for 5-$HT_{2C}$R; CHEMBL1833 for 5-$HT_{2B}$R. Where possible *Ki* data were used, with the exception of COX-1 and GABA-AR $\alpha$-1/$\beta$-2/$\gamma$-2 for which the IC50 values were used. Affinity values not expressed in nM were filtered out. For each target a sampling procedure of the compounds has been performed as follows: all affinity data were converted in *pKi*/*pIC50*; the data have been then divided into bins on the basis of the integer part of their value. For each bin, if possible, 30 compounds were randomly sampled. If a bin contained less than 30 compounds, all the compounds were taken instead. The total number of compounds for each target is reported in table S1. For each target-compounds pair library the docking simulations were performed using the same settings chosen for BHT and AutoDock Vina. Compounds were divided in strong binders if their *pKi*/*pIC50* values were greater than 6, otherwise they were defined weak binders. Due to the issues already discussed in the main text, GABA-AR $\beta$3+$\alpha$- interface was not taken into consideration in these further tests. The results of this analysis are illustrated in Figures 1SM-6SM.

| Target | Compounds number |
|---|---|
| GABA-AR $\alpha$-1/$\beta$-2/$\gamma$-2 | 149 |
| GABA-AR $\alpha$-1/$\beta$-3/$\gamma$-2 | 75 |
| 5-$HT_{2B}$R | 148 |
| 5-$HT_{2C}$R | 165 |
| COX-1 | 180 |
| hNET | 212 |

**Table 1SM.** Total number of compounds used for the virtual screening against each target.

## 2. ADME analysis

Assessment of absorption, distribution, metabolism and excretion (ADME) is a crucial part of drug development. Computational approaches can be used in place of experimental methodology to cut both time and cost. SwissADME is a web server for the prediction of small molecules physicochemical properties such as, but not limited to, pharmacokinetics properties and drug-likeness [2]. It employs several predictive models and when possible, it adopts a consensus approach (e.g. for lipophilicity prediction). In this work, ADME analysis has been performed in order to predict if BHT could reach the identified targets (Figure 7SM).

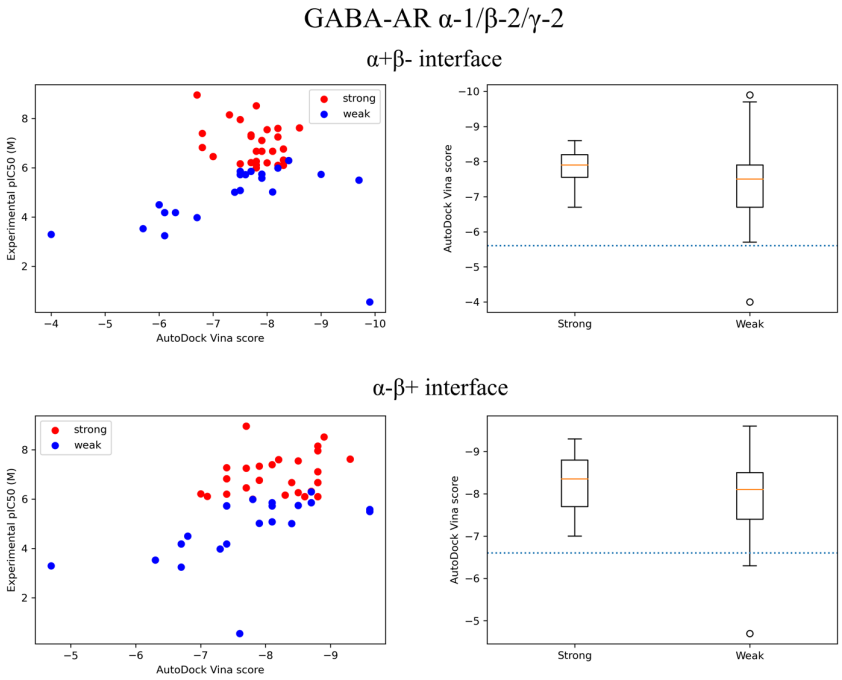


**Figure 1SM.** Virtual screening results for $\alpha$+β2- (above) and $\alpha$-β2+ (below) interfaces of the GABA-AR $\alpha$-1/β-2/$\gamma$-2. In the scatterplots (on the left) are plotted the experimental results (*pIC50*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plots (on the right) are depicted the AutoDock Vina results distribution for strong and weak binders. The score for BHT (-5.6 kcal/mol for $\alpha$+β2- and -6.6 kcal/mol for $\alpha$-β2+) is represented as a dotted blue line.

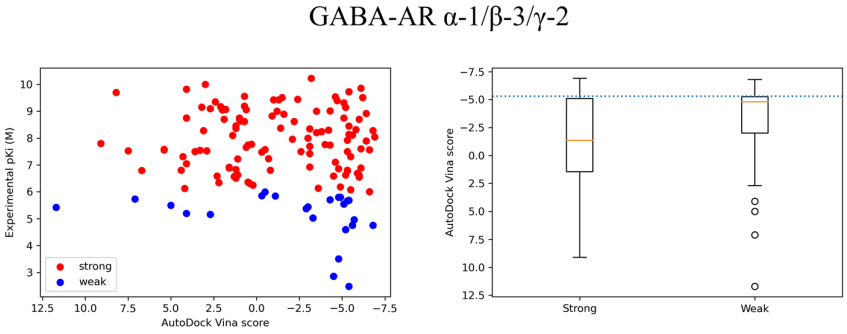


**Figure 2SM.** Virtual screening results for $\alpha$+β3- interface of the GABA-AR $\alpha$-1/β-3/$\gamma$-2. In the scatterplot (on the left) are plotted the experimental results (*pKi*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plot (on the right) are depicted the AutoDock Vina results distribution for strong and weak binders. The score for BHT (-5.3 kcal/mol for $\alpha$+β3-) is represented as a dotted blue line.

5-HT2BR

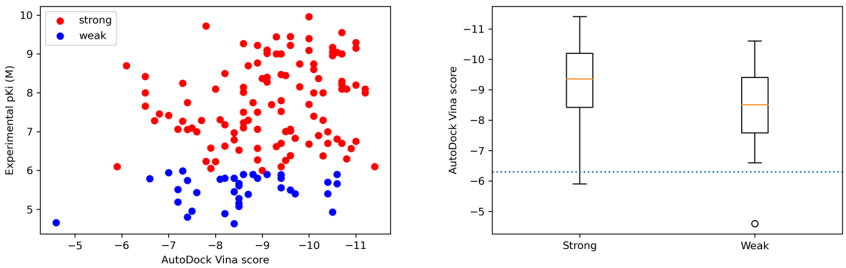


**Figure 3SM.** Virtual screening results for 5-HT$_{2B}$R. In the scatterplot (on the left) are plotted the experimental results (*pKi*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plot (on the right) are depicted the AutoDock Vina results distribution for strong and weak binders. The score for BHT (-7.0 kcal/mol) is represented as a dotted blue line.

5-HT2CR

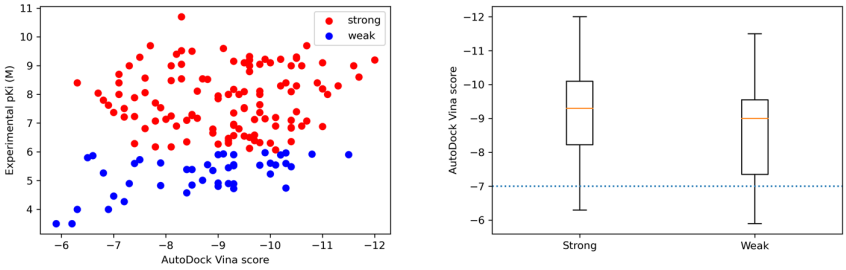


**Figure 4SM.** Virtual screening results for 5-HT$_{2C}$R. In the scatterplot (on the left) are plotted the experimental results (*pKi*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plot (on the right) are depicted the AutoDock Vina results distribution for strong and weak binders. The score for BHT (-7.7 kcal/mol) is represented as a dotted blue line.

COX-1

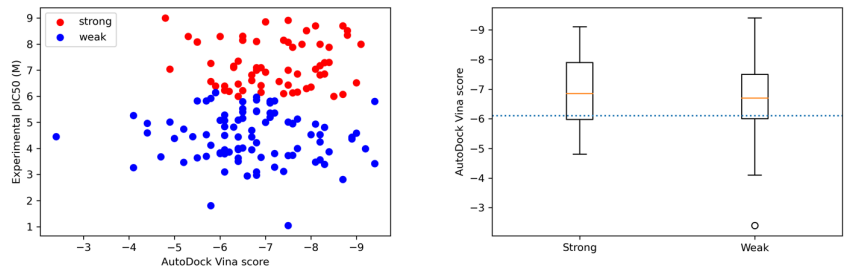


**Figure 5SM.** Virtual screening results for COX-1. In the scatterplot (on the left) are plotted the experimental results (*pIC50*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plot (on the right) are depicted the AutoDock Vina results

distribution for strong and weak binders. The score for BHT (-6.1 kcal/mol) is represented as a dotted blue line.

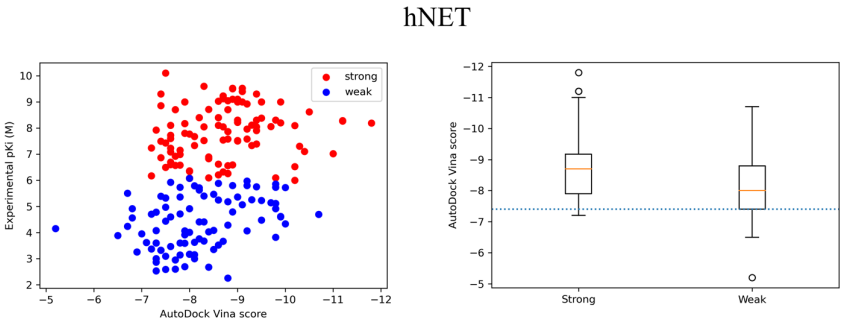


**Figure 6SM.** Virtual screening results for hNET. In the scatterplot (on the left) are plotted the experimental results (*pKi*) against the AutoDock Vina score results for the weak (blue) and strong (red) binders; in the box plot (on the right) are depicted the AutoDock Vina results distribution for strong and weak binders. The score for BHT (-7.4 kcal/mol) is represented as a dotted blue line.

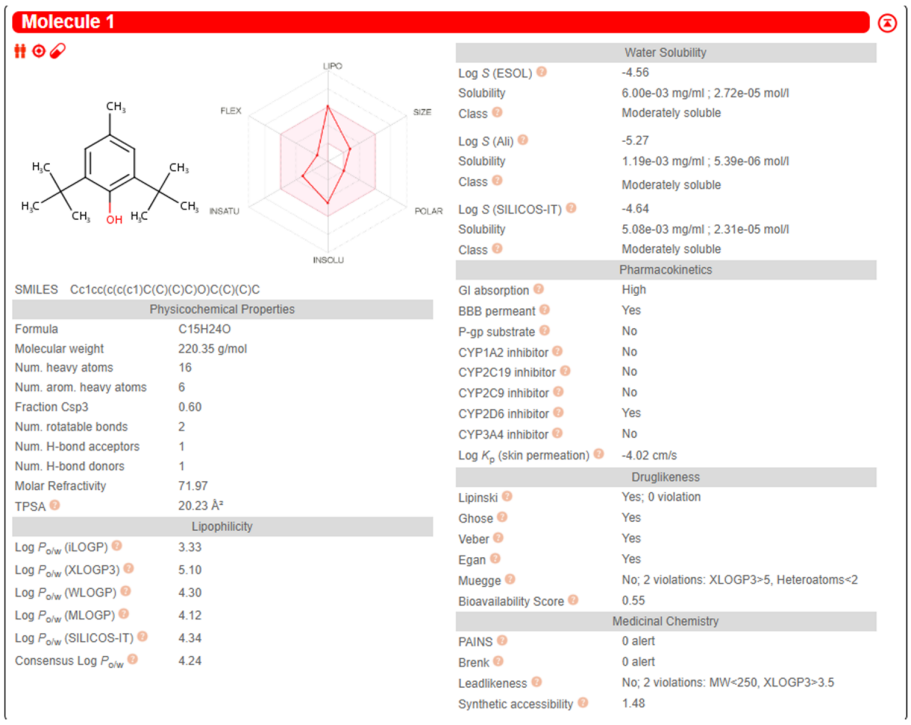


**Figure 7SM.** ADME analysis performed with SwissADME.

**References**

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2. Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* **2017**, *7*, 42717.