SUPPORTING INFORMATION:

A Critical Assessment of Mushroom Tyrosinase-based Enzyme Inhibition Assays: Are they Suitable for Bioactivity-Guided Fractionation of Extracts?

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SI – Table of content

S1 – SciFinder query for mh-Tyr

When querying the keywords ‘mushroom tyrosinase’ in SciFinder, 2246 entries were retrieved (see Figure S 1). From this list, 325 entries (14 %) were published in the decade of 1990 to 1999, 577 entries (26 %) from 2000 to 2009, and already 878 entries (39 %) from 2010 to 2018. This query was conducted 3rd August 2018.

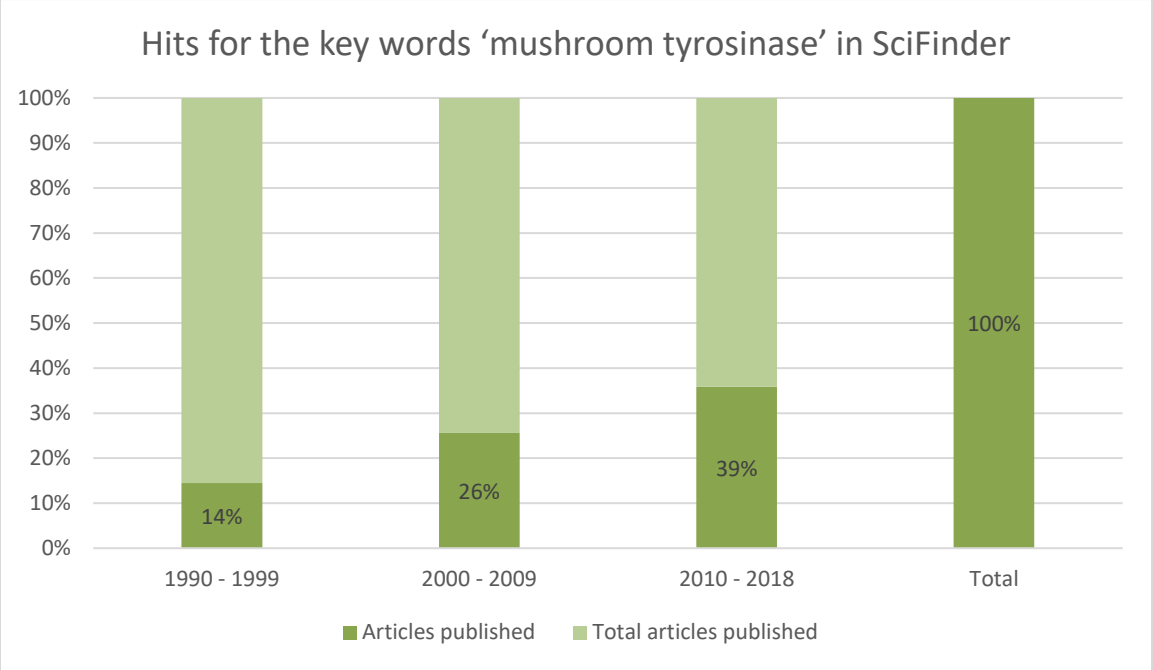


Figure S 1: Hits for the key words ‘mushroom tyrosinase’ in SciFinder quieried on 08.03.2018.

S2 – In silico target prediction

Target prediction, in literature often referred to as target fishing or inverse virtual screening, is a computational technique that allows the medicinal chemist to estimate novel protein targets for chemical compounds. This principle is widely used for classical ‘target fishing’, drug repositioning, screening for off-target effects, or in computational toxicology. In classical target fishing, the aim is to predict protein targets for ‘orphan’ compounds, e.g. compound for which it

is not clear what the molecular target is. An orphan compound could be a newly synthesized compound where bioactivities still need to be found, or a compound that is bioactive in a cell-based assay. In the latter case, it is often not clear what the protein interaction partner is. In drug repositioning, the aim is to find novel targets for an already established drug. The screening for off-target effects aims to find interactions that can cause unwanted side-effects in the human body. In computational toxicology, the aim is to flag compounds that may exert toxic effects in humans. Here, the aim is to map novel compounds on targets that are known to cause side effects like e.g. the human *Ether-à-go-go*-Related Gene (hERG).

Within the present study, the in silico target prediction was performed using two publicly available web servers: Similarity ensemble approach (SEA, http://sea.bkslab.org/) and SwissTargetPrediction (STP, http://www.swisstargetprediction.ch/). Both web-servers predict novel protein targets based on the molecular similarity of know binders (derived from the ChEMBL in both cases) to the queried compound. While SEA fully relies on 2-dimensional similarity by calculating ECFP4 fingerprints, STP combines two and three dimensional similarity of the respective molecules.[1, 2] In Table S 1 it is shown that seven out of eleven compounds were predicted as tyrosinase inhibitors by SEA, whereas STP predicted four out of eleven as tyrosinase inhibitors. Interestingly, all four STP predictions are consensus hits with SEA, meaning that the predictions of both webservers were consistent. The situation that many of the DHCs described as competitive inhibitors in literature are actually substrates of mh-Tyr, complicates a proper evaluation. Nevertheless, we think that also substrates can be considered as bioactive compounds. Even more, it is much more unlikely to meet the requirements for being a substrate as for being an inhibitor. Therefore, we classified a DHC as bioactive on mh-Tyr if we identified it as a substrate

or it is a known inhibitor from literature. A summary of the performance evaluation of the in silico target prediction is provided in Table S 2.

Table S 1. In silico predicted activity vs. in vitro assessed biological activity of the eleven investigated compounds on mh-Tyr.

Compound	Predicted bioactivity in silico		Identified as substrate in vitro	Described as inhibitor in literature	Prediction accuracy
	SEA [a]	STP [b]			
trilobatin (**1**)	yes	yes	yes	no	TP [c]
sieboldin (**2**)	yes	yes	yes	no	TP
phloretin (**3**)	yes	no	yes	yes[3-5]	TP
3-OH-phloretin (**4**)	no	no	yes	yes[6]	FN [d]
asebogenin (**5**)	yes	no	yes	yes[7]	TP
phloridzin (**6**)	yes	yes	yes	yes[3, 8, 9]	TP
3-OH-phloridzin (**7**)	yes	yes	yes	yes[6]	TP
phlorein 2′-xyloglucoside (**8**)	no	no	yes	no	FN
neohesperidin dihydrochalcone (**9**)	no	no	yes	no	FN
calomelanen (**10**)	no	no	no	no	TN[e]
2′,6′-dihydroxy-4′-methoxy DHC (**11**)	yes	no	no	yes[7]	TP

[a] Similarity ensemble approach; [b] SwissTargetPrediction; [c] true positive; [d] false negative; [e] true negative.

Table S 2. Performance summary.

Metric	**SEA**	**STP**	**Consensus hits of SEA and STP**
TP	7	4	7
FP [a]	0	0	0
TN	0	0	1
FN	4	7	3

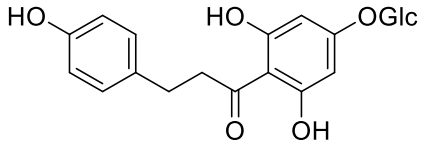
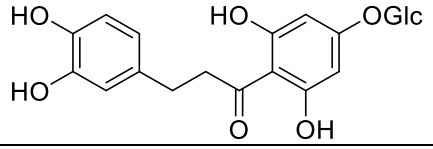
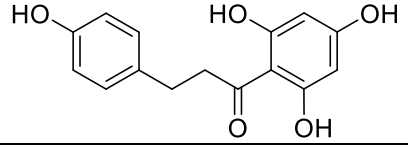
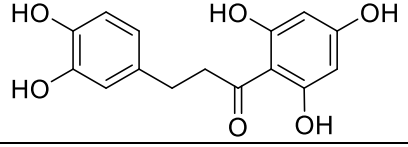
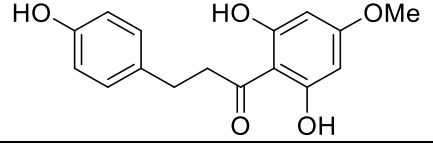
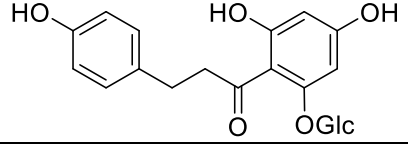
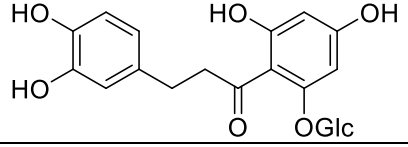
[a] false positive

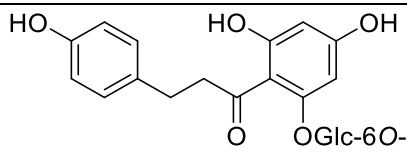
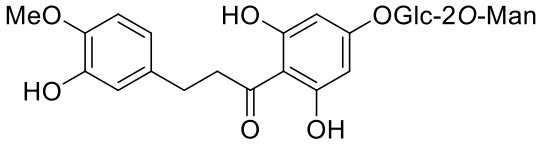
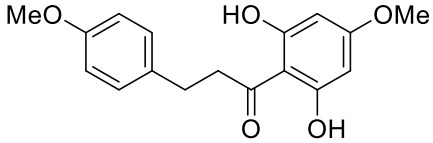
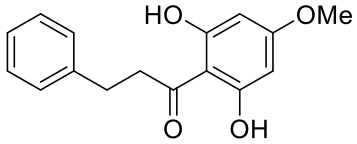
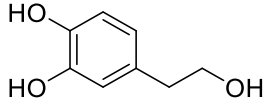
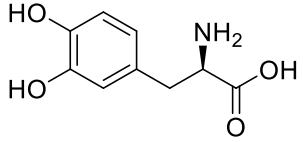
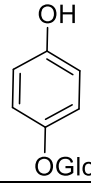
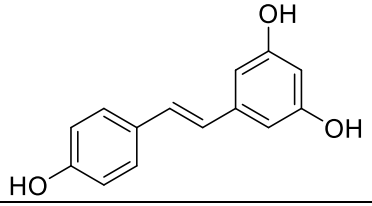
Moreover, negative predictions (true negative and false negative) should not have a big influence on the method's validation, because this simply reflects the fact that the model's design did not include this exact chemical space. When using pharmacophore models for target prediction this could just mean that e.g. no tyrosinase model was used for screening. When using 2D similarity searches like e.g. SEA or STP, this could mean that similar compounds were not present in the tyrosinase training set. In both cases, this phenomenon can be addressed to the mere richness of potential protein targets. This could only be achieved by a model collection that covers all druggable protein targets of the human body, which is of course utopic.

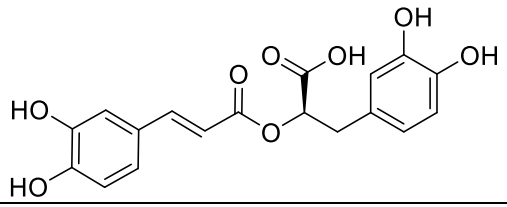
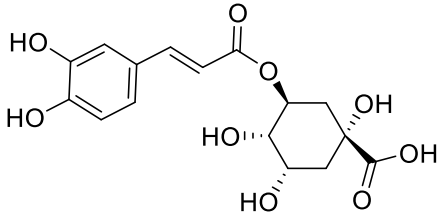
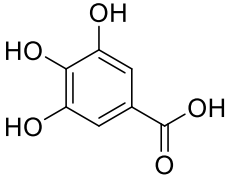
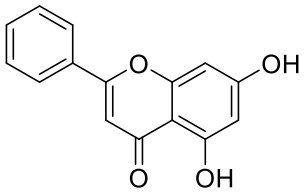
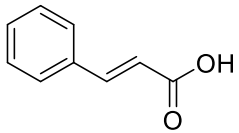
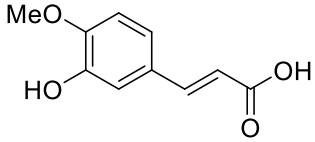
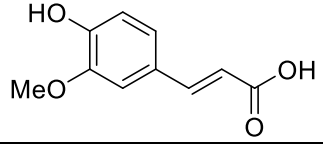
S3 – Full dataset

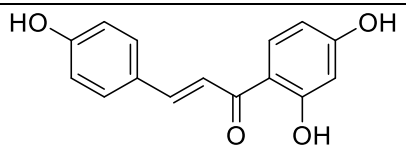
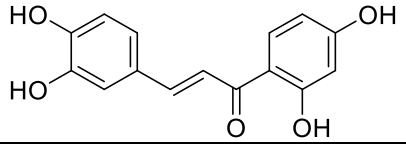
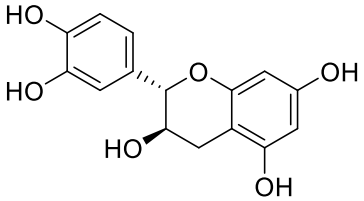
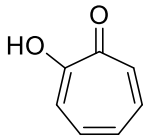
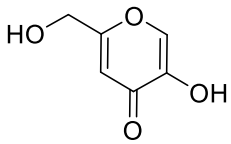
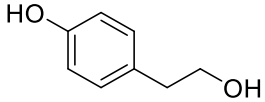
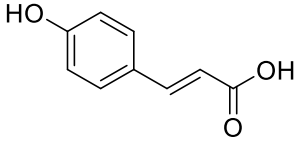
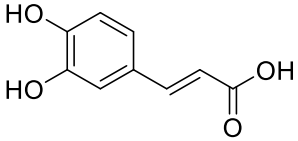
The full dataset is shown in Table S 3. Please note that compounds **1** to **25** are summarized in the subset shown in the manuscript.

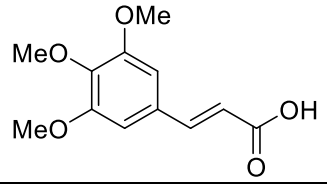
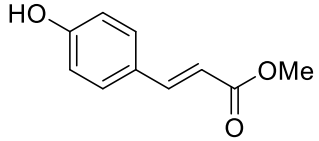
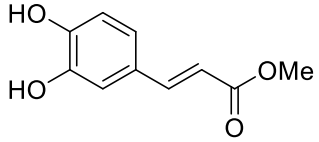
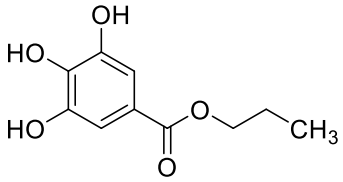
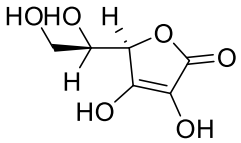
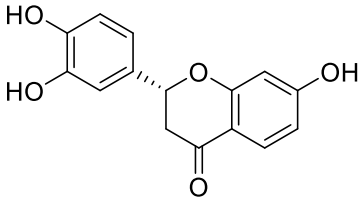
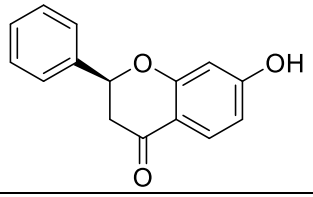
Table S 3. Full dataset of investigated polyphenols. All compounds had a purity above 95 %, with the exception of **49** which had a purity of ≥ 90 %.

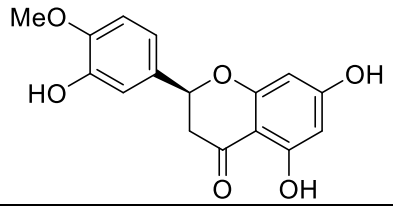
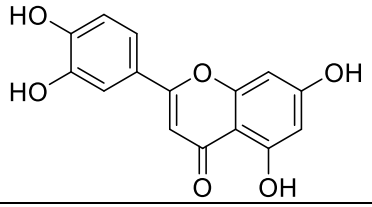
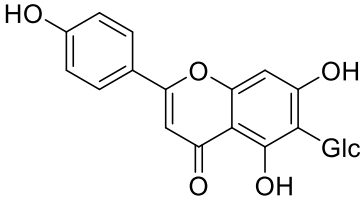
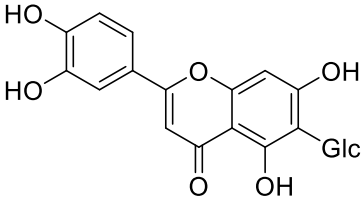
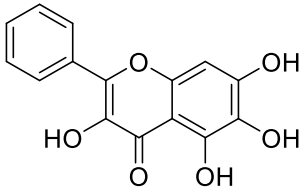
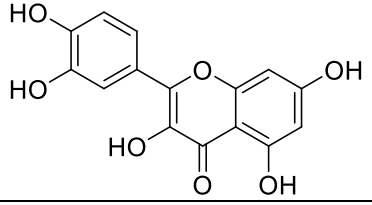
No.	Molecular structure	Name	Scaffold	CAS	Origin/Reference	Purity	Assay interference
1		trilobatin	dihydro-chalcone	4192-90-9	TransMIT GmbH, PlantMetaChem Prod [a].: T 017	> 98 %	yes
2		sieboldin	dihydro-chalcone	18777-73-6	TransMIT GmbH, PlantMetaChem Prod.: S 025	> 98 %	yes
3		phloretin	dihydro-chalcone	60-82-2	TransMIT GmbH, PlantMetaChem Prod.: P 036	> 98 %	yes
4		3-OH-phloretin	dihydro-chalcone	57765-66-9	TransMIT GmbH, PlantMetaChem Prod.: H 031	> 98 %	yes
5		asebogenin	dihydro-chalcone	520-42-3	TransMIT GmbH, PlantMetaChem Prod.: A 020	> 98 %	yes
6		phloridzin	dihydro-chalcone	60-81-1	TransMIT GmbH, PlantMetaChem Prod.: P 037	> 98 %	yes
7		3-OH-phloridzin	dihydro-chalcone	30779-02-3	Isolated as published in [10]	≥ 97 % [e]	yes

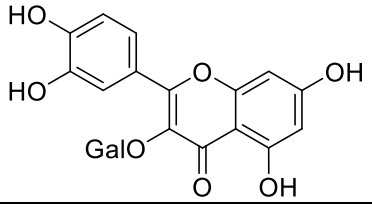
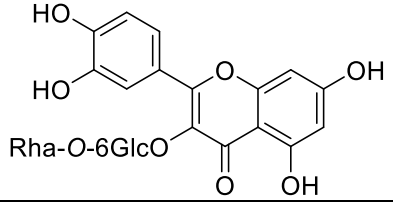
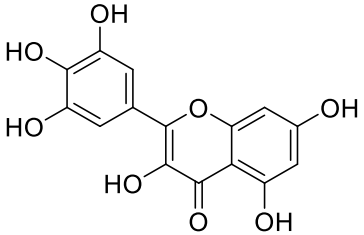
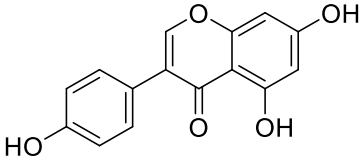
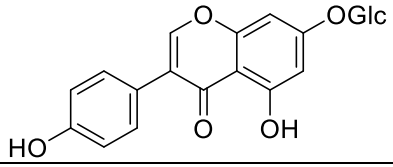
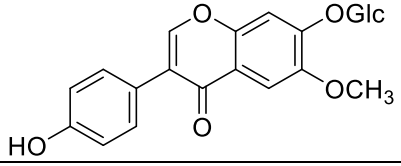
8		phlorein 2′-xyloglucoside	dihydro-chalcone	145758-09-4	TransMIT GmbH, PlantMetaChem Prod.: P 064	> 98 %	yes
9		neohesperidin dihydrochalcone	dihydro-chalcone	20702-77-6	TransMIT GmbH, PlantMetaChem Prod.: N 019	> 98 %	yes
10		calomelanen	dihydro-chalcone	35241-54-4	TransMIT GmbH, PlantMetaChem Prod.: D 018	> 98 %	no
11		2′,6′-dihydroxy-4′-methoxy dihydrochalcone	dihydro-chalcone	35241-55-5	TransMIT GmbH, PlantMetaChem Prod.: D 017	> 98 %	no
12		3-OH-tyrosol	phenyl-ethanoid	10597-60-1	Sigma-Aldrich, Inc. Prod.: H4291 Ba [b].: SLBF6846	≥ 98 %	yes
13		L-DOPA	α-amino acid	59-92-7	Sigma-Aldrich, Inc. Prod.: D9628 Ba.: SLBF6724F	≥ 98 %	yes
14		arbutin	established positive control	497-76-7	Alfa Aesar Prod.: L14945 Ba.: 10203662	≥ 98 %	yes
15		resveratrol	stilbene	501-36-0	TCI europe N.V. Prod.: R0071 Ba.: EFEBD-LG	≥ 99 %	yes

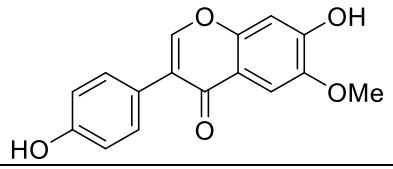
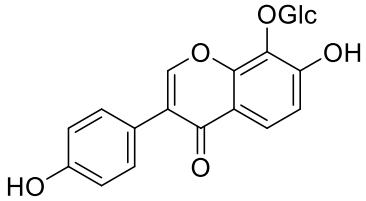
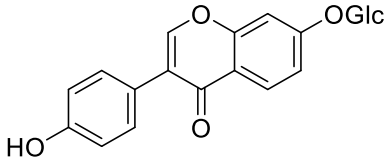
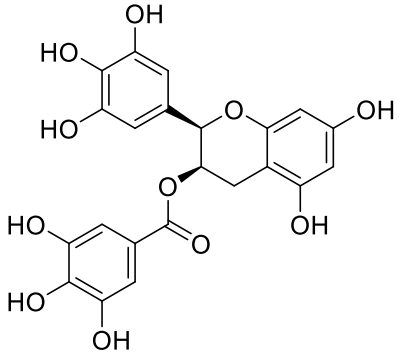
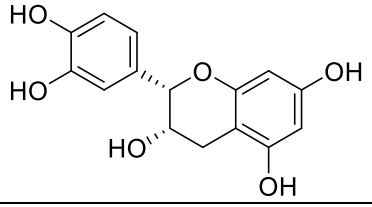
16		rosmarinic acid	organic acid	20283-92-5	Sigma-Aldrich, Inc. Prod.: 95082 Ba.: BCBF5239V	≥ 98 %	yes
17		chlorogenic acid	organic acid	327-97-9	Carl Roth GmbH + Co. KG Prod.: 6385.2 Ba.: 1521633	≥ 97 %	yes
18		gallic acid	organic acid	149-91-7	Sigma-Aldrich, Inc. Prod.: 398225 Ba.: LKBP6646V	≥ 98 %	yes
19		chrysin	flavone	480-40-0	Sigma-Aldrich, Inc. Prod.: 95082 Ba.: BCBF5239V	≥ 98 %	no
20		cinnamic acid [f]	organic acid	621-82-9	Schering Kahlbaum AG Prod.: 05136 Ba.: A3125		no
21		isoferulic acid [f]	organic acid	537-73-5	Serva Prod.: 26462 Ba.:		no
22		ferulic acid	organic acid	1135-24-6	Sigma-Aldrich, Inc. Prod.: 12870-8 Ba.: STBB0939	99 %	yes

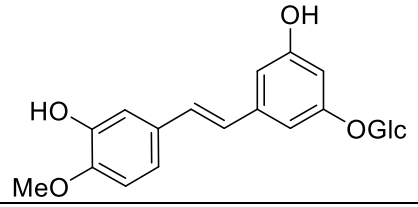
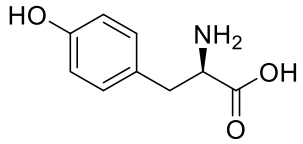
23		isoliquiritigenin	chalcone	961-29-5	TCI europe N.V. Prod.: I0822 Ba.: ZXSKH-TG	≥ 97 %	yes
24		butein	chalcone	487-52-5	Isolated as published in [11]	≥ 95 % [e]	yes
25		2*R*,3*S*-catechin	catechin	154-23-4	Extrasynthese Prod.: 0976S Ba.: 09110211	≥ 99 %	yes
26		tropolone	established positive control	533-75-5	TCI europe N.V. Prod.: T0606 Ba.: ILX8J-GN	> 98.0 %	no
27		kojic acid	established positive control	501-30-4	Sigma-Aldrich, Inc. Prod.: 95197 Ba.: BCBN6618V	≥ 99.0 %	no
28		tyrosol	phenyl-ethanoid	501-94-0	Sigma-Aldrich, Inc. Prod.: 188255 Ba.: MKBK6451V	≥ 98 %	Yes
29		*p*-coumaric acid	organic acid	7400-08-0	Merck KGaA Prod.: 800237 Ba.: S5328037	≥ 98 %	yes
30		caffeic acid	organic acid	331-39-5	Sigma-Aldrich, Inc. Prod.: C0625 Ba.: 059K1009	≥ 98 %	yes

31		3,4,5-trimethoxy cinnamic acid	organic acid	90-50-6	Fluka, now Sigma-Aldrich, Inc. Prod.: 92200 Ba.: 27825	97 %	no
32		methyl coumarate	organic acid	3943-97-3	Merck KGaA Prod.: 800237 Ba.: S5328037 [g]		yes
33		methyl caffeate	organic acid	3843-74-1	Sigma-Aldrich, Inc. Prod.: C0625 Ba.: 059K1009 [g]		yes
34		Propyl gallate [f]	organic acid	121-79-9			yes
35		ascorbic acid	organic acid	50-81-7	Merck KGaA Prod.: 1831 Ba.: 5000740100	≥ 99 %	no
36		7*R*-butin	flavan	492-14-8	Isolated as published in [11]	≥ 95 % [e]	yes
37		2*R*-7-hydroxy flavanone	flavan	6515-36-2	INDOFINE Chemical Company, Inc. Prod.: H-024	≥ 99 %	no

38		2*R*-hesperetin	flavan	520-33-2	INDOFINE Chemical Company, Inc. Prod.: 021115S	≥ 99 %	no
39		luteolin	flavone	491-70-3	Extrasynthese Prod.: 1125 S Ba.: 12032223	≥ 99 %	no
40		isovitexin	flavone	38953-85-4	Extrasynthese Prod.: 1235S Ba.: 12032217	≥ 99 %	no
41		isoorientin	flavone	4261-42-1	Sigma-Aldrich, Inc. Prod.: 02187 Ba.: BCBG4794V	≥ 98 %	yes
42		baicalein	flavonol	491-67-8	Sigma-Aldrich, Inc. Prod.: 465119 Ba.: MKBC2199V	98 %	no
43		quercetin	flavonol	117-39-5	Sigma-Aldrich, Inc. Prod.: Q-0125 Ba.: 42H0565	≥ 98 %	yes

44		hyperoside	flavonol	482-36-0	Isolated as published in [12]	≥ 95 % [e]	yes
45		rutin	flavonol	153-18-4	Carl Roth GmbH + Co. KG Prod.: 5154.1	≥ 96 %	yes
46		myricetin	flavonole	529-44-2	Carl Roth GmbH + Co. KG Prod.: 4187.1 Ba.: 02252625	≥ 99 %	yes
47		genistein	isoflavone	446-72-0	PhytoLab Prod.: 89199 Ba.: 1877	≥ 95 %	yes
48		genistin	isoflavone	529-59-9	PhytoLab Prod.: 89200 Ba.: 7051	≥ 95 %	no
49		glycitin	isoflavone	40246-10-4	PhytoLab Prod.: 89693 Ba.: 5680	≥ 95 %	yes

50		glycitein	isoflavone	40957-83-3	PhytoLab Prod.: 89692 Ba.: 6246	≥ 95 %	no
51		puerarin	isoflavone	3681-99-0	PhytoLab Prod.: 89318 Ba.: 748	≥ 95 %	yes
52		daidzin	isoflavone	552-66-9	PhytoLab Prod.: 89182 Ba.: 7049	≥ 95 %	yes
53		epigallocatechin gallate	catechin	989-51-5	Teavigo, Taiyo Kagaku Co., Ltd. 0071 UQ81146020	≥ 90 %	yes
54		2*R*,3*R*-epicatechin	catechin	154-23-4	Extrasynthese Prod.: 0977S Ba.: 09110212	≥ 99 %	yes

55		rhaponticin	stilbene	155-58-8	Carl Roth GmbH + Co. KG Prod.: 5804.1 Ba.: 490164896	n.a.	no
56		L-tyrosine [d]	α-amino acid	60-18-4	n.a. [c]	n.a.	yes

[a] product number; [b] batch number; [c] not applicable; [d] L-tyrosine was not investigated experimentally, but included in this dataset since it is known to be a physiological substrate of mh-TYR; [e] the respective chromatograms and NMR-spectra are attached to the quoted publication, if not otherwise detailed; [f] origin could not be reproduced. To verify the compound's identity and purity ^{1}H NMR spectra were measured which are shown in S6 – Spectroscopic data, Supporting Information; [g] these products served as starting materials for the final products specified. The synthesis was carried out analogously to [13]. In brief: carboxylic acids were dissolved in MeOH and a few drops of concentrated sulfuric acid (98 %) was added. After refluxing for 24 hours, the mixture's pH was neutralized with $NaHCO_3$. The aqueous phase was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was removed. ^{1}H-NMR spectra of the final products are attached in S6 – Spectroscopic data, Supporting Information.

S4 – Time-dependent absorption plots

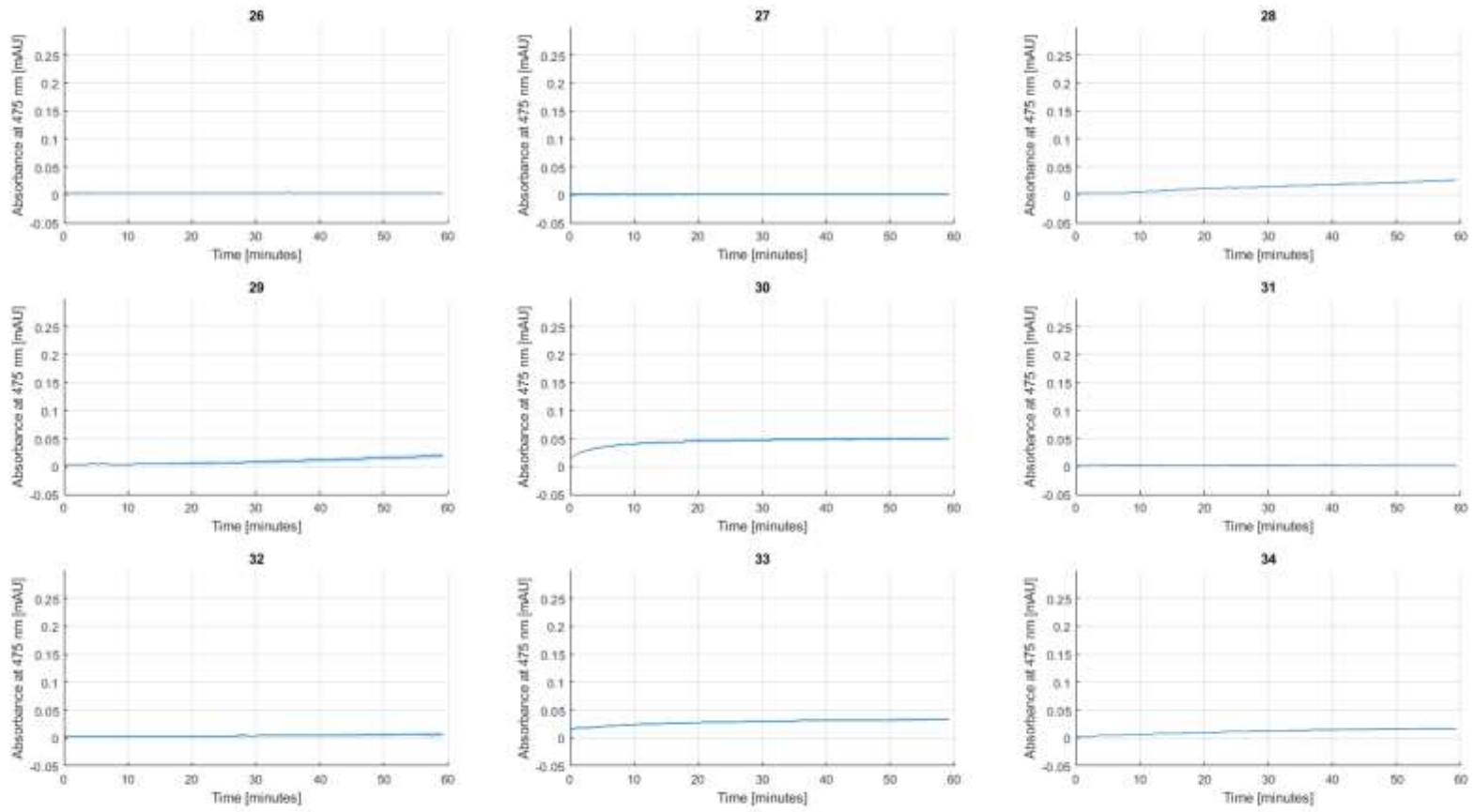


Figure S 2. Time-dependent absorption plots of **26** - **34**. Shown is the mean of the triplicates of each compound with 95% confidence intervals.

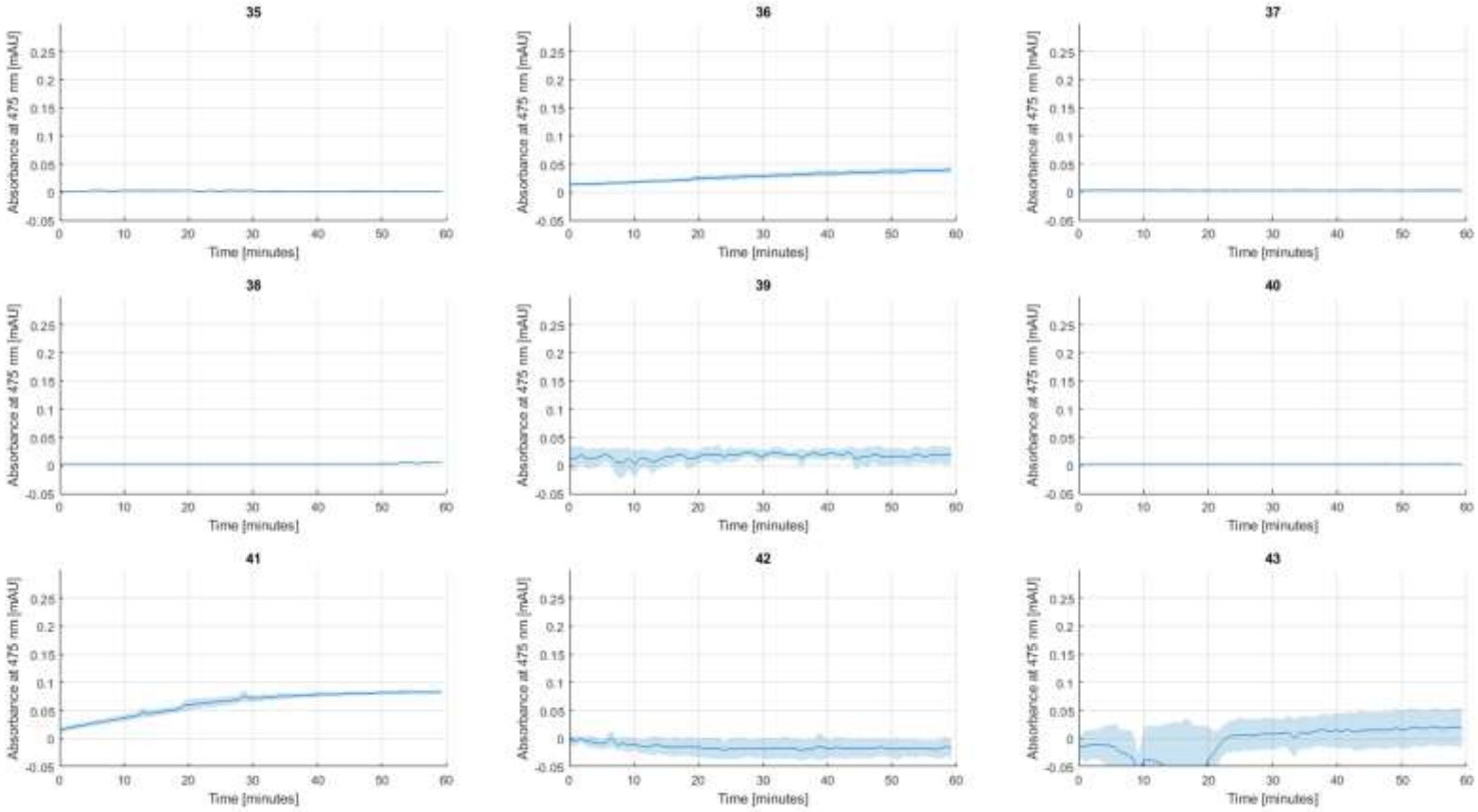


Figure S 3. Time-dependent absorption plots of **35 - 43**. Shown is the mean of the triplicates of each compound with 95% confidence intervals.

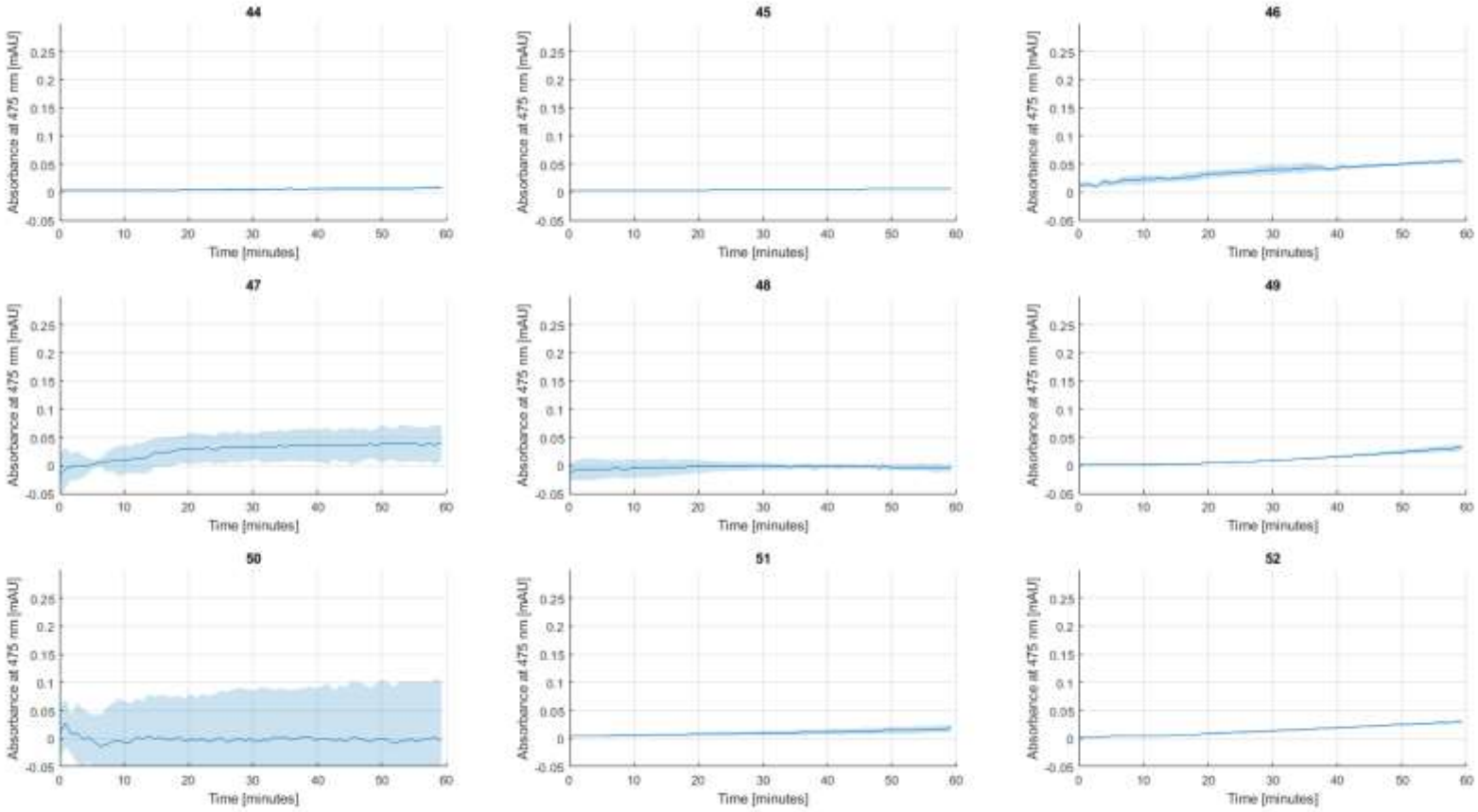


Figure S 4. Time-dependent absorption plots of **44 - 52**. Shown is the mean of the triplicates of each compound with 95% confidence intervals.

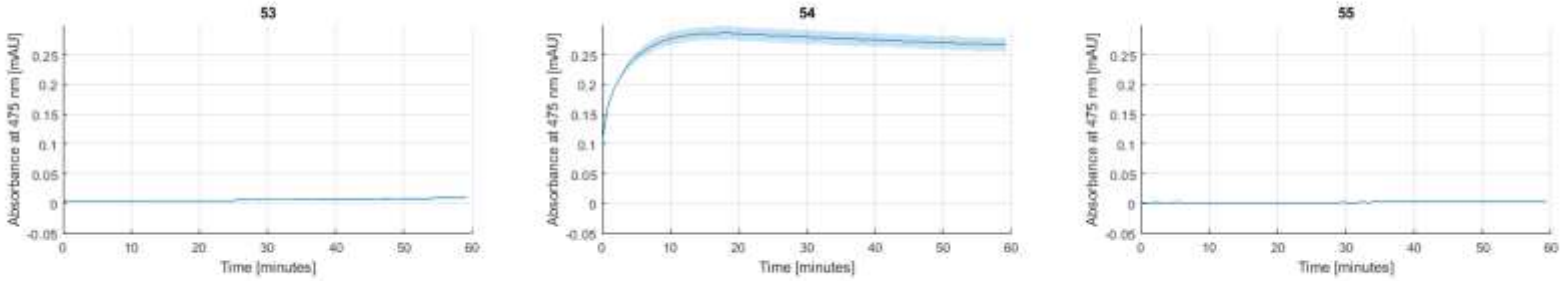


Figure S 5. Time-dependent absorption plots of **53 - 55**. Shown is the mean of the triplicates of each compound with 95% confidence intervals.

S5 – Pharmacophore modeling

In model 1, the HBD feature (-3.81, -8.46, 4.61) was set to optional. The hydrophobic feature's (-2.18, -7.16, 2.31) tolerance was increased by 0.75 Å.

The combined pharmacophore models 1 and 2 were validated using the full dataset (Table S 3). In total, our two pharmacophore models reported 47 compounds as hits, from which 41 were indeed true AIs (true positive, 65 % of the whole dataset) and 6 were NIs (false positive, 9 % of the whole dataset). Six true AIs were missed (false negative, 9 % of the whole dataset) and eleven compounds were correctly predicted as NIs (true negative, 17 % of the whole dataset). This results in an AU_{ROC} of 0.86, a relative enrichment factor (rel. EF) of 0.87, an accuracy (Acc) of 0.81, a yield of actives (Ya) of 0.87, a sensitivity of 0.87, and a specificity of 0.64. The ROC curve is provided in Figure S 5. A comprehensive summary of the quantitative metrics is provided in Table S 4.

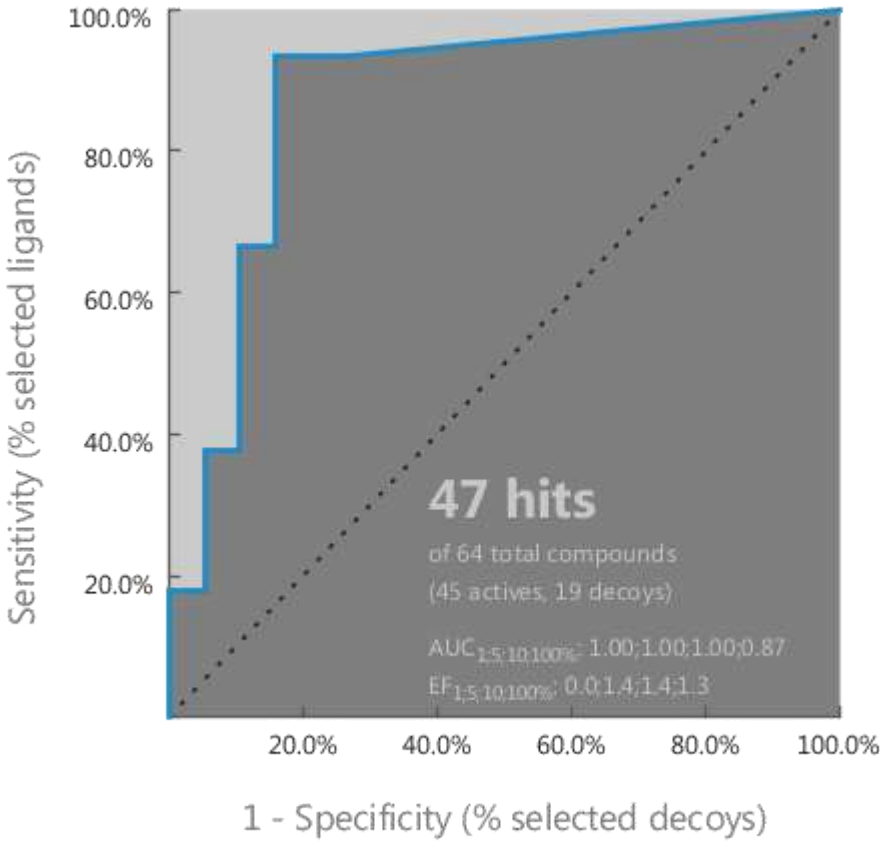


Figure S 6. ROC-curve yielded from the pharmacophore models 1 and 2 used in cooperative mode.

Table S 4. Quantitive Performace Metrics of Models 1 and 2 used in Cooperative Mode.

Metric	Score	Short explanation
General data		
Total dataset size	64	
AI	45	
NI	19	
Performance metrics		
Hit rate	72 %	Percentage of hits reported relative to the total dataset (0-100)
True positive hits (TP)	42	AI correctly classified as AIs
True negative hits (TN)	14	NI correctly classified as NIs
False positive hits (FP)	5	NI incorrectly classified as AIs
False negative hits (FN)	3	AI incorrectly classified as NIs
Sensitivity (Se)	0.93	The model's capability to correctly classify AIs (0-1)
Specificity (Sp)	0.74	The model's capability to correctly classify NIs (0-1)
Enrichment factor (EF)	1.27	Enrichment of TPs on the hitlist, relative to a random selection (dataset specific)
Relative Enrichment factor (rel. EF)	0.89	Enrichment factor relative to the maximum enrichment factor (0-1)
Yield of actives (Ya)	0.89	Ratio of TPs to the total number of retrieved hits (0-1)
Accuracy (Acc)	0.88	Overall accuracy of correctly classified compounds (0-1)
AUC_{ROC}	0.87	'Area under the receiver operating curve'. ROCs should run as vertical as possible, while a completely random model creates a ROC similar to the dotted line in Figure S 6 (0-1)

[a] The numbers in brackets represent the numeric minima and maxima of the respective metric.

Table S 5. Compounds that were Incorrectly Classified by the in silico Tool.

Compound	Virtual hit
tropolone (**26**)	FP
hesperetin (R) (**38**)	FP
luteolin (**39**)	FP
isovitexin (**40**)	FP
rutin (**45**)	FN

genistein (**47**)	FN
genistin (**48**)	FP
puerarin (**51**)	FN

The described in silico workflow for the rapid identification of mh-Tyr substrates was designed to be more sensitive than specific, meaning that for the intended purpose it is more important to detect as many AIs as possible, even with the risk of giving rise to 'false alarms'. Thus, the FPs are – considering the scope of this tool – accepted, while the FNs require a detailed search for the root cause, why they were incorrectly classified. The FN compounds **45**, **47**, and **51** are all glucosides. The respective sugar moieties interfere with the exclusion volumes coat of at least one of the pharmacophore models, leading to a mismatch.

We retrieved a dataset from ChEMBL using a workflow custom-built in the KNIME analytics platform.[14] The workflow is illustrated in Figure S 6, and provided as file 'chembl.knwf'. This workflow outputs the dataset in two ways: First as .sdf-file ('tyrosinase_inhibitors_chembl.sdf') that was used for virtual screening and as .csv-file ('tyrosinase_inhibitors_chembl.csv'). The dataset as sd-file was converted to a multiconformational screening library in the same manner as described in the experimental section (OMEGA 'BEST' settings). The resulting hitlist obtained from pharmacophore models 1 and 2 in parallel is as well provided as 'hitlist.sdf'.

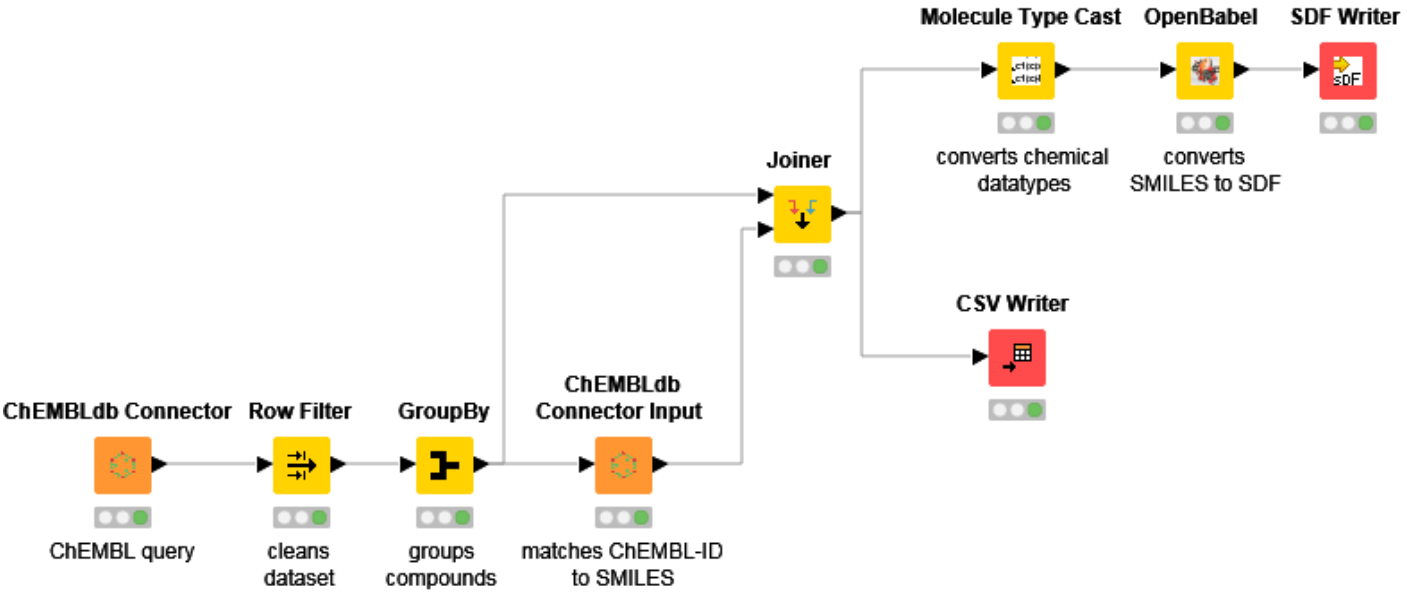


Figure S 7: KNIME workflow to retrieve and filter mh-Tyr inhibitors from ChEMBL.

S6 – Spectroscopic data

*Cinnamic acid (**20**)*: ^{1}H NMR (600.19 MHz, MeOH-d_4) δ 7.67 (1H, d, J = 16.0, H-8), 7.38 – 7.6 (5H, m, J = 6.6 Hz, H-2, H-3, H-4, H-5, H-6, H-7).

^{1}H NMR spectrum is provided in Figure S 7. Data was in good agreement with literature.[15]

*Isoferulic acid (**21**)*: ^{1}H NMR (600.19 MHz, MeOH-d_4) δ 7.55 (1H, d, J = 15.9, H-8), 6.27 (1H, d, J = 15.9, H-7), 7.07 (1H, d, J = 2.1, H-2), 7.04 (1H, dd, J = 8.3, 2.0, H-6), 6.94 (1H, d, J = 8.3, H-5), 3.31 (3H, d, H-10, H-11, H-12).

^{1}H NMR spectrum is provided in Figure S 8. Data was in good agreement with literature.[16]

*Methyl coumarate (**32**)*: ^{1}H NMR (600.19 MHz, MeOH-d_4) δ 7.61 (1H, d, J = 15.9 Hz, H-7), 7.44 (2H, d, J = 8.6 Hz, H-2, H-4), 6.80 (2H, d, J = 8.6 Hz, H-3, H-5), 6.32 (1H, d, J = 15.9 Hz, H-8), 3.75 (3H, s, H-10).

^{1}H NMR spectrum is provided in Figure S 9. Data was in good agreement with literature.[17]

*Methyl caffeate (**33**)*: ^{1}H NMR (600.19 MHz, MeOH-d_4) δ 7.54 (1H, d, J = 15.9 Hz, H-7), 7.03 (1H, d, J = 2.0 Hz, H-2), 6.93 (1H, dd, J = 8.2, 2.0 Hz, H-6), 6.77 (1H, d, J = 8.2 Hz, H-5), 6.25 (1H, d, J = 15.9 Hz, H-8), 3.75 (3H, s, H-10).

^{1}H NMR spectrum is provided in Figure S 10. Data was in good agreement with literature.[18]

*Propyl gallate (**34**)*: ^{1}H NMR (600.19 MHz, MeOH-d_4) δ 7.06 (2H, s, H-2, H-6), 4.18 (2H, t, J = 6.6 Hz, H-8), 1.87 – 1.59 (2H, m, H-9), 1.02 (3H, t, J = 7.4 Hz, H-10).

^{1}H NMR spectrum is provided in Figure S 11. Data was in good agreement with literature.[19]

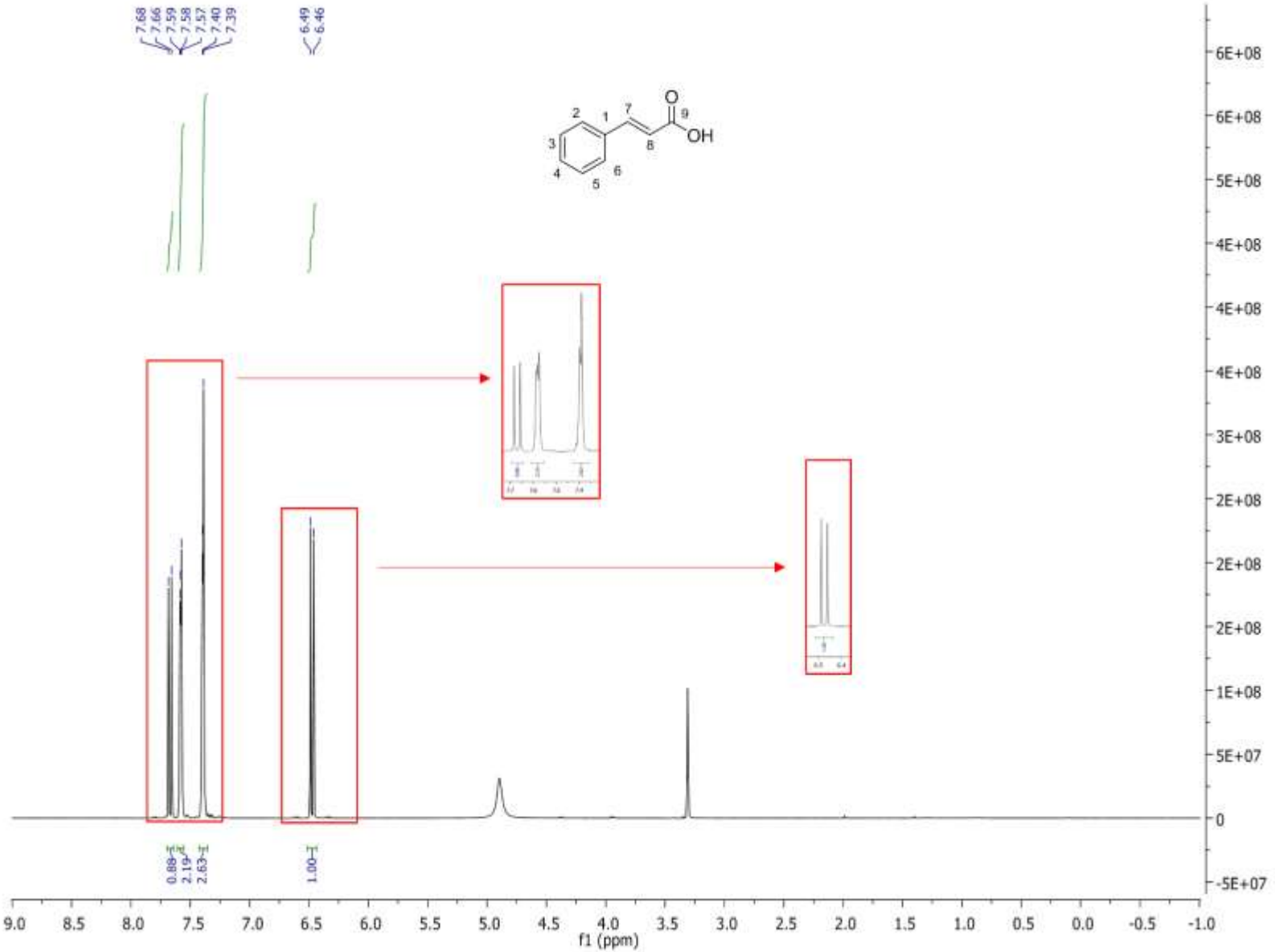


Figure S 8. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of **20**.

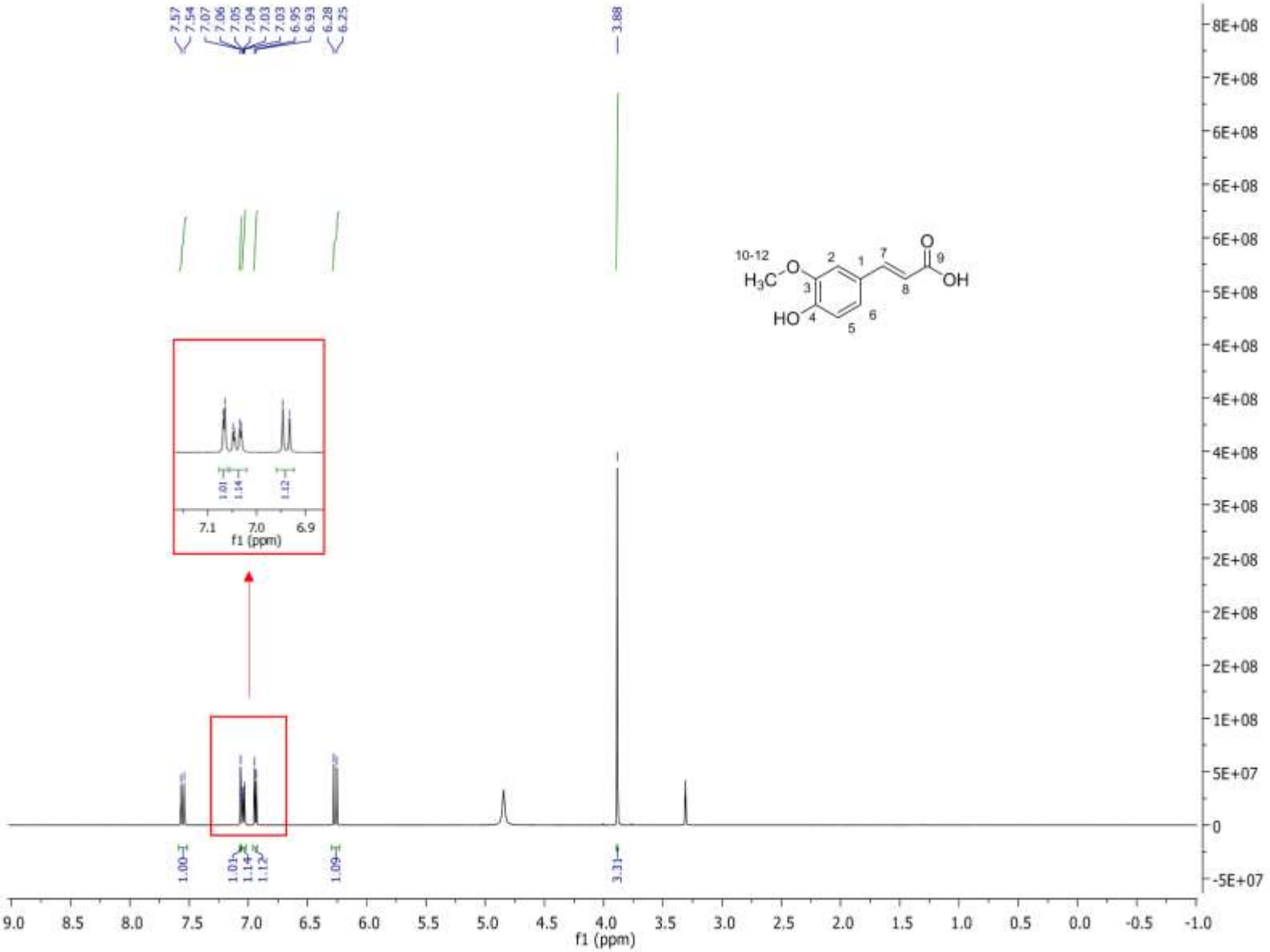


Figure S 9. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of **21**.

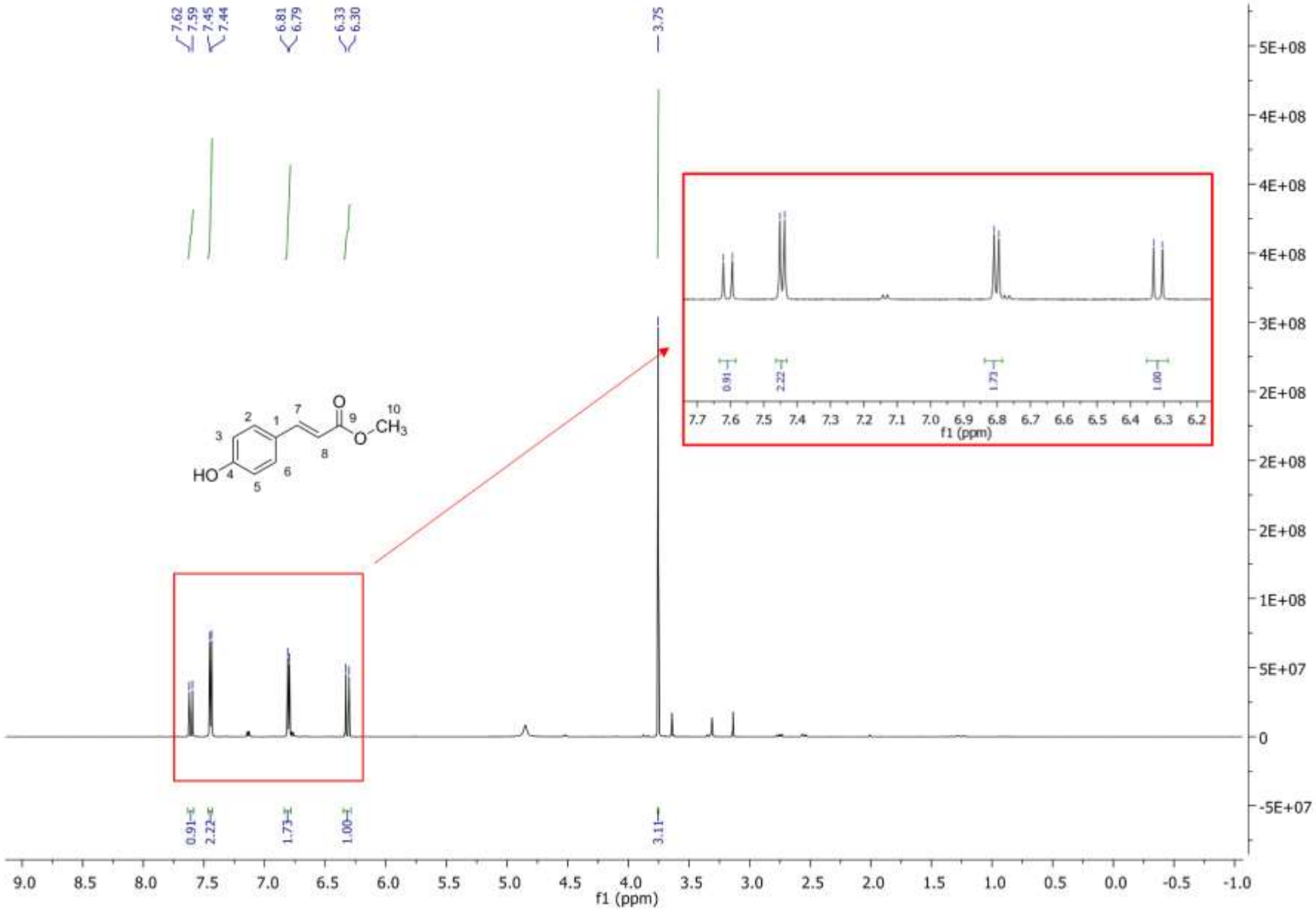


Figure S 10. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of **32**.

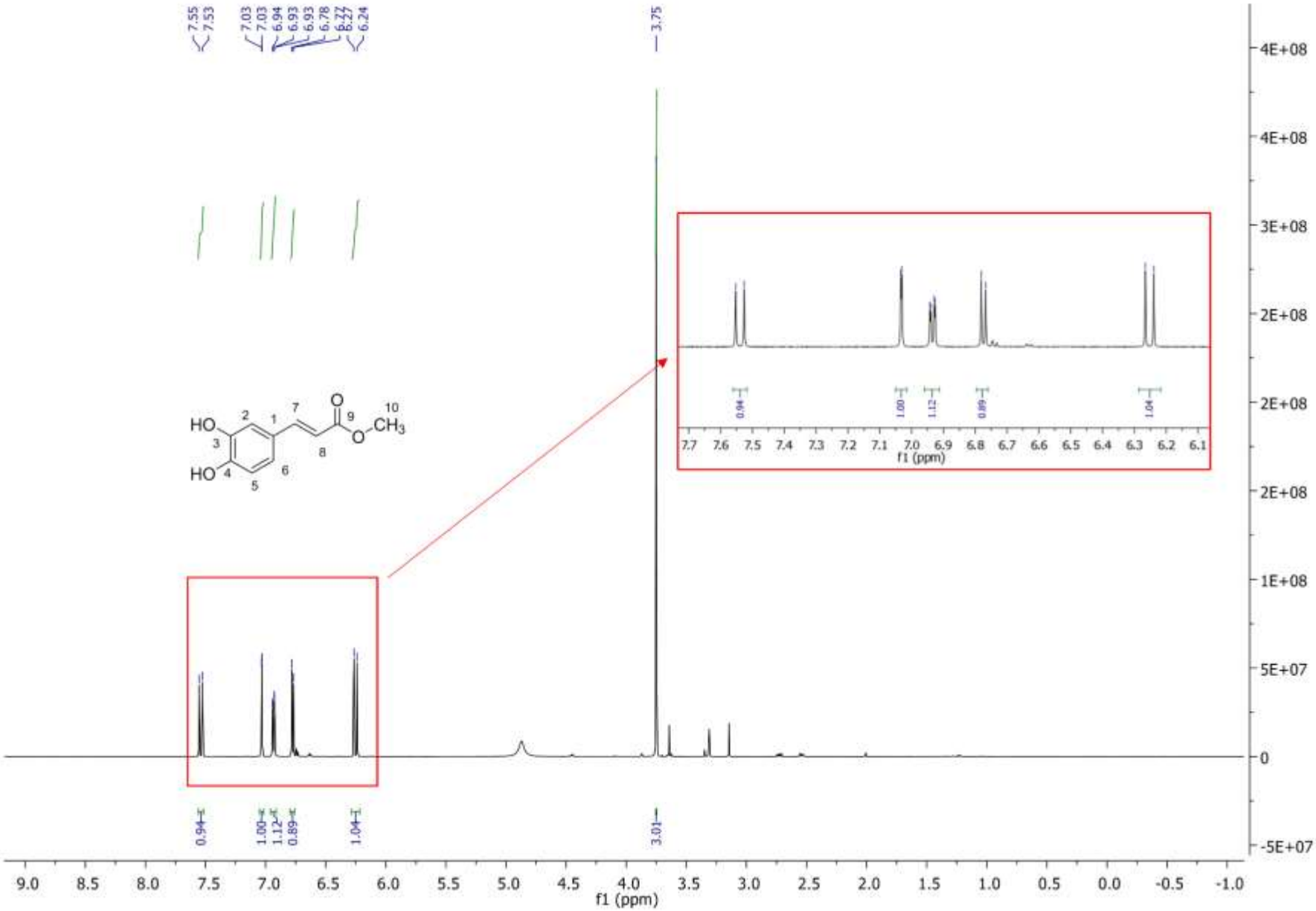


Figure S 11. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of **33**.

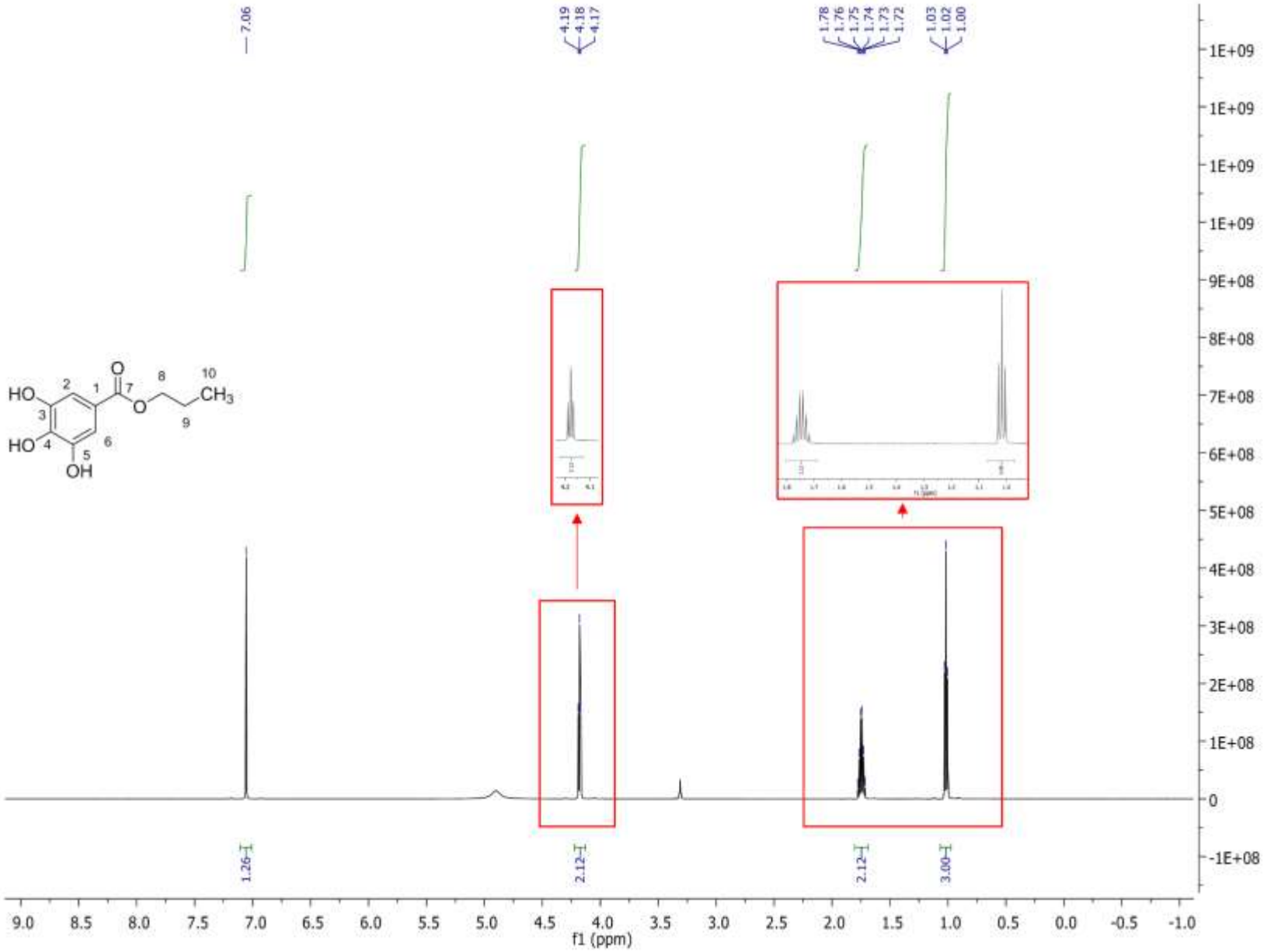


Figure S 12. ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of **34**.

S7 – Literature search for compounds **1** to **25**

Table S 6. Summary of Literature Data regarding mh-Tyr Inhibition or Substrate Specificity.

Compound	Type of inhibition identified by the authors	Year	Ref.
trilobatin (**1**)	[a]		
sieboldin (**2**)	[a]		
phloretin (**3**)	alternative substrate	2015	[20]
	inhibitor (no mode determined)	2014	[4]
	competitive−uncompetitive mixed type inhibitor	2014	[5]
	competitive inhibitor	2011	[3]
3-OH-phloretin (**4**)	competitive inhibitor	2007	[6]
asebogenin (**5**)	inhibitor (no mode determined)	2003	[7]
phloridzin (**6**)	alternative substrate	2017	[21]
	alternative substrate	2015	[20]
	competitive inhibitor	2011	[3]
	inhibitor (no mode determined), unstable results	2009	[9]
	alternative substrate	2007	[22]
	competitive inhibitor	2007	[8]
3-OH-phloridzin (**7**)	[a]		
phlorein 2′-xyloglucoside (**8**)	[a]		
neohesperidin dihydrochalcone (**9**)	[a]		
calomelanen (**10**)	[a]		
2′,6′-dihydroxy-4′-methoxy dihydrochalcone (**11**)	inhibitor (no mode determined)	2003	[7]
3-OH-tyrosol (**12**)	alternative substrate	2016	[23]
L-DOPA (**13**)	natural substrate		
arbutin (**14**) [b]	hydroquinone is an alternative substrate	1981	[24]
	arbutin and hydroquinone as positive control	2002	[25]
	arbutin as positive control (‘competitive inhibitor’)	2002	[26]
	arbutin is an alternative substrate	2003	[27]
	arbutin is probably ‘a poor substrate’	2004	[28]

	arbutin as positive control (incl. IC_{50})	2004	[29]
	inhibitor (no mode determined) (incl. IC_{50})	2007	[30]
	arbutin as positive control	2008	[31]
	hydroquinone as positive control	2010	[19]
	[c] arbutin is an established mh-Tyr inhibitor	2017	[32]
	[c] arbutin is an established mh-Tyr inhibitor	2018	[33]
resveratrol (**15**)	inhibitor (no mode determined) (incl. IC_{50})	2002	[34]
	inhibitor (no mode determined) (incl. IC_{50})	2007	[30]
	alternative substrate	2008	[31]
	inhibitor (no mode determined) (incl. IC_{50})	2010	[35]
	inhibitor (no mode determined) (incl. IC_{50})	2010	[35]
	inhibitor (no mode determined) (incl. IC_{50})	2012	[36]
	alternative substrate	2012	[37]
	inhibitor (no mode determined) (incl. IC_{50})	2012	[38]
	alternative substrate	2015	[39]
	inhibitor (no mode determined) (incl. IC_{50})	2010	[40]
rosmarinic acid (**16**)	alternative substrate	2011	[41]
chlorogenic acid (**17**)	inhibitor (no mode determined)	2007	[42]
gallic acid (**18**)	alternative substrate	1981	[24]
	inhibitor (no mode determined)	2003	[43]
	inhibitor (no mode determined)	2012	[36]
chrysin (**19**)	inhibitor (no mode determined)	2007	[6]
cinnamic acid (**20**)	inhibitor (no mode determined) (incl. IC_{50})	2009	[44]
	inhibitor (no mode determined) (incl. IC_{50})	2011	[45]
	non-competitive inhibitor (incl. IC_{50})	2016	[46]
isoferulic acid (**21**)	inhibitor (no mode determined)	2007	[42]
ferulic acid (**22**)	inhibitor (no mode determined)	2007	[42]
isoliquiritigenin (**23**)	competitive inhibitor (incl. IC_{50})	2003	[47]
	semi-competitive inhibitor (incl. IC_{50})	2004	[48]
	competitive inhibitor (incl. IC_{50})	2005	[49]
	inhibitor (no mode determined) (incl. IC_{50})	2012	[36]
	inhibitor (no mode determined) (incl. IC_{50})	2016	[50]
	inhibitor (no mode determined) (incl. IC_{50})	2018	[51]

butein (**24**)	competitive inhibitor (incl. IC_{50})	2004	[48]
	alternative substrate	2005	[52]
	inhibitor (no mode determined) (incl. IC_{50})	2017	[53]
2*R*,3*S*-catechin (**25**)	alternative substrate	2001	[54]

[a] no references to tyrosinase inhibition. [b] arbutin might act as prodrug of hydroquinone. [c] literature reviews

S8 – References

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