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Systematic Mining of Generally Recognized as Safe (GRAS) Flavor Chemicals for Bioactive Compounds

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Supporting Information

ABSTRACT: Bioactive food compounds can be both therapeutically and nutritionally relevant. Screening strategies are widely employed to identify bioactive compounds from edible plants. Flavor additives contained in the so-called FEMA GRAS (generally recognized as safe) list of approved flavoring ingredients is an additional source of potentially bioactive compounds. This work used the principles of molecular similarity to identify compounds with potential mood-modulating properties. The ability of certain GRAS molecules to inhibit histone deacetylase-1 (HDAC1), proposed as an important player in mood modulation, was assayed. Two GRAS chemicals were identified as HDAC1 inhibitors in the micromolar range, results similar to what was observed for the structurally related mood prescription drug valproic acid. Additional studies on bioavailability, toxicity at higher concentrations, and off-target effects are warranted. The methodology described in this work could be employed to identify potentially bioactive flavor chemicals present in the FEMA GRAS list.

KEYWORDS: *bioactive food components, chemoinformatics, databases, foodinformatics, GRAS, histone deacetylase-1 (HDAC1), natural products, small molecules, structural similarity*

INTRODUCTION

Food constituents that promote health benefits by means other than straightforward nutrition are considered bioactive compounds. These extra-nutritional constituents are either naturally occurring in food or may be formed during processing. A prime example is the group of flavonoids, known for their antioxidant properties. The systematic analysis of edible plants to identify bioactive compounds is an extremely active area of research, as witnessed, for example, by the growing number of manuscripts published in this *Journal* under the heading "Bioactive Constituents and Functions". Recent examples include antioxidants, antidiabetics, and antimutagenics.[1−6] The different screening types and associated challenges (some sharing methodologies with those used in the pharmaceutical industry) are described in detail elsewhere.[7] Similar to pharmaceutical screening campaigns, systematic studies of compounds identified in food have been reported,[8] including use of Web-based servers[9−11] for the collection, organization, retrieval, and mining of data. A recent paper by Scalbert et al.[12] summarizes databases containing phytochemicals, with emphasis on the missing content in current data collections and recommendations for future development of databases. These studies and services highlight the need, feasibility, and growing interest in implementing data mining and chemoinformatic approaches to discovering bioactive food constituents. Chemoinformatic approaches have been widely employed in pharmaceutical screening, but they have by no means been restricted to that domain.[13] For example, visualization of the chemical space has been employed to analyze food databases.[13−15]

Unlike the screening techniques focused on identifying bioactive compounds through extraction, evaluation, and identification cycles, there are methods based on the structural comparison of known bioactive molecules to chemical libraries. Such comparisons employ the general notion that similar compounds have similar activity.[16]

In recent years, we reported[14] a chemoinformatic characterization of the flavor descriptors of substances (individual compounds only) contained in the Flavor and Extract Manufacturers Association (FEMA) generally recognized as safe (GRAS)[17−19] list of approved flavoring materials. Food ingredients designated GRAS are widely used in foods and beverages designed for human consumption. Beyond the organoleptic properties of GRAS compounds, these materials can be analyzed to identify potential bioactive components. For example, we previously reported potential mood-related properties for several GRAS chemicals.[20] To further characterize this data set, we performed a comprehensive and systematic study based on the comparison of various molecular properties, rings, atom counts, and structural fingerprints.[15] It was found that the lipophilicity profile of the GRAS database, a key property to predict human bioavailability, is similar to that of approved drugs and that GRAS chemicals overlap a broad region of the chemical space occupied by drugs.[15]

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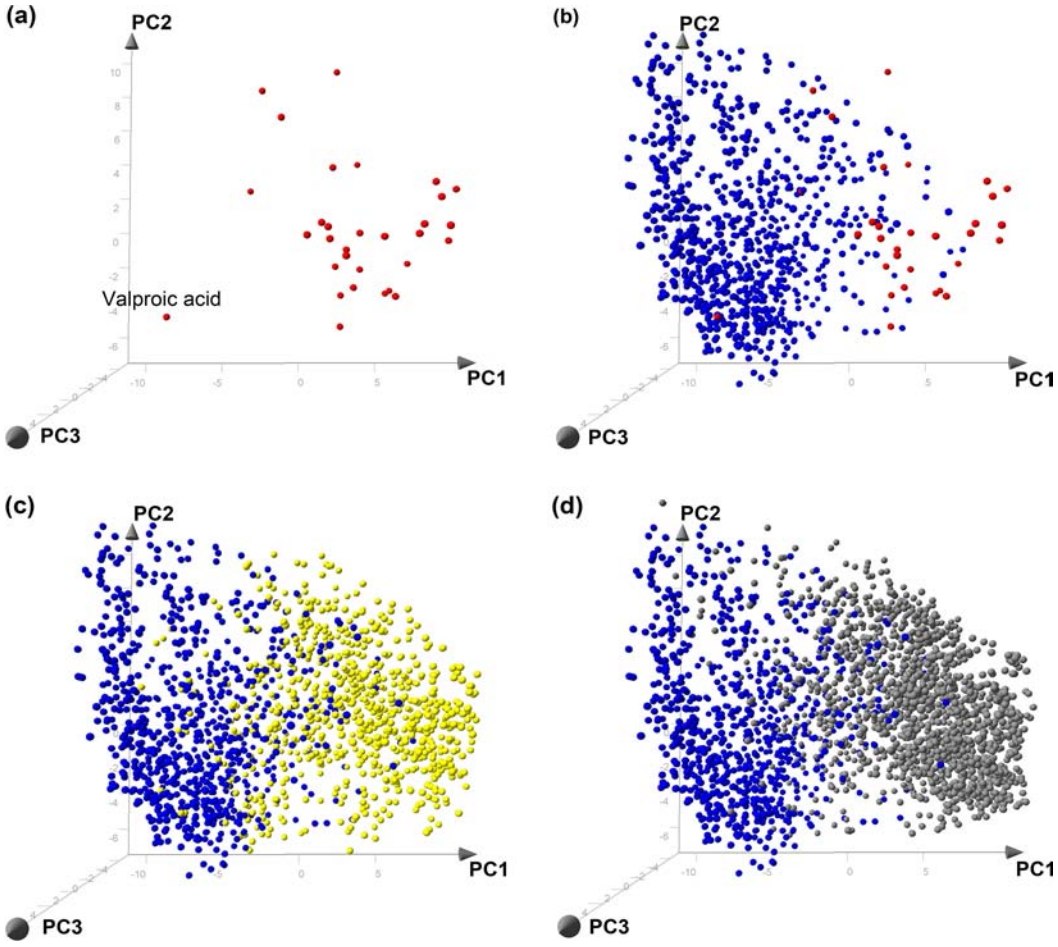


Figure 1. Visual representation of the structural space based on MACCS keys: (a) approved antidepressants (red); (b) approved antidepressants and GRAS chemicals (blue); (c) GRAS chemicals and DrugBank (yellow); (d) GRAS chemicals and ChEMBL antidepressant compounds (gray). The first three PCs account for 71.56% of the variance.

Valproic acid, a branched-chain carboxylic acid, is a drug approved for the treatment of disorders of the central nervous system (CNS) such as major depression, bipolar disorder, and epilepsy.[21] It binds and inhibits GABA transaminase and is also an HDAC inhibitor. Valproic acid is a well-known "promiscuous compound" (a primary example of polypharmacology). It is important to emphasize that there are different aspects related to polypharmacology that can lead to desirable or undesirable effects.[22] Moreover, in many instances, polypharmacology can be conceived of as a "normal" scenario, for example, in odor perception.[23] Knowledge of polypharmacology can also be used as a means not only for repurposing drugs but also in the repositioning of chemical compounds initially designed by man, or found to occur in nature, for other purposes.[24]

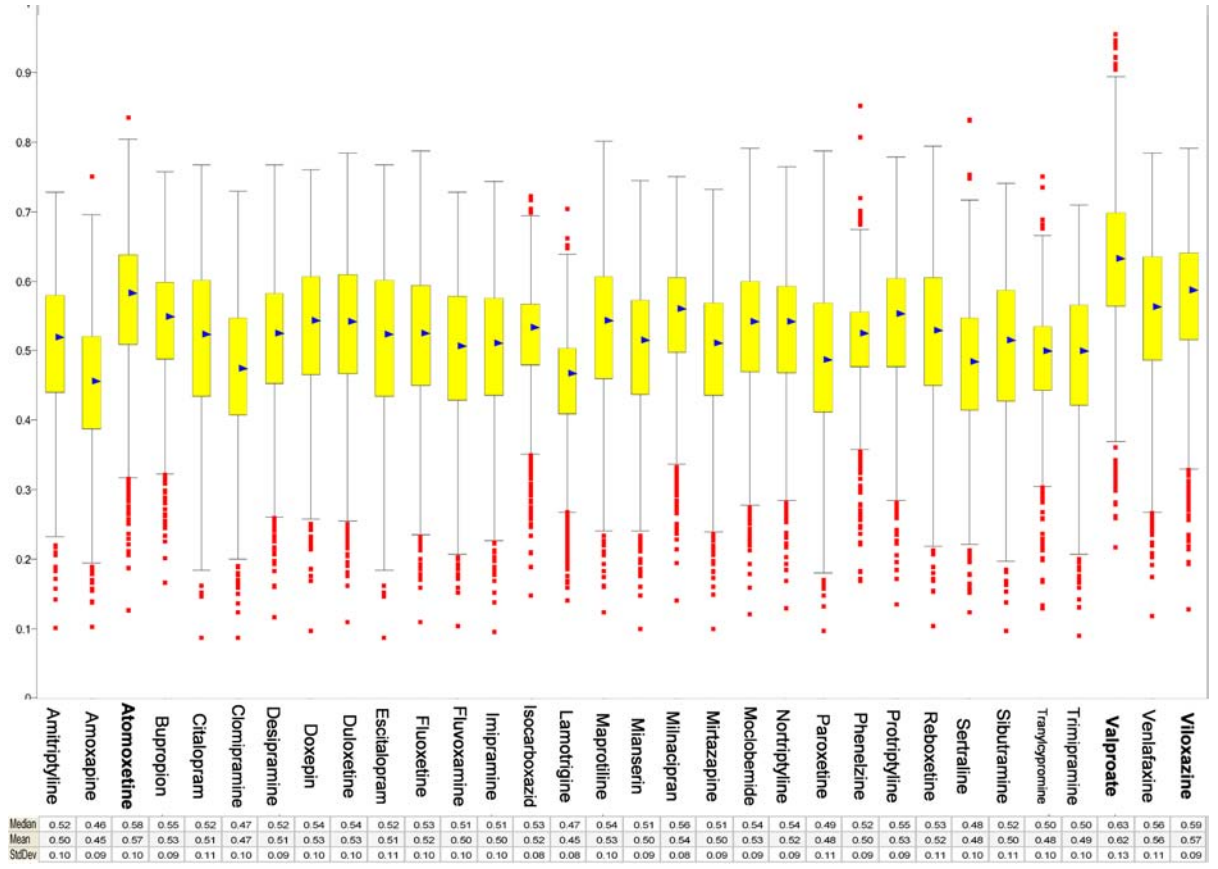
Valproic acid interacts with histone deacetylase-1 (HDAC1), with IC_{50} = 400 μM.[25] Remarkably, the HDAC inhibitory properties, as well as the teratogenic effects of valproic acid, mimic those of trichostatin A (TSA). On the basis of these observations, it has been proposed that the inhibition of HDAC could be associated with the efficacy of valproic acid in the treatment of biopolar disorder.[25]

As part of our continuing effort to identify the potential health-related benefits of GRAS chemicals, in this work we focused on the comparison of GRAS flavoring substances with 32 approved antidepressant drugs and performed an experimental evaluation with HDAC1.

METHODS

Computational. The following properties were computed with the program Molecular Operating Environment (MOE):[26] molecular weight (MW), number of rotatable bonds (RB) (the bonds were considered rotatable if they satisfied the criteria of bond order of 1, not a ring, and at least two heavy neighbors), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), topological polar surface area (TPSA), and octanol/water partition coefficient (SlogP). In MOE TPSA is calculated using group contributions to approximate the polar surface area from the connection table information only. The parametrization is that of Ertl et al.[27] Comparison with Clark's calculation of PSA[28] results showed almost no difference between the two approaches. The faster calculation of TPSA makes it particularly valuable for large data sets. SlogP is an atomic contribution model[29] that calculates the logP for a given structure. To obtain a visual representation of the property space,[30] a principal component analysis (PCA) was carried out in Spotfire 9.1.1[31] considering the above-mentioned physicochemical properties after normalization. LogBBB and QPlogHERG reported in Table 4 were computed with QikProp.[32]

Structural similarity between 4600 GRAS flavoring substances (discrete chemical entities only; list expanded to include all possible stereoisomers) and 32 approved antidepressant drugs (Figure 2) was evaluated by employing the widely used Molecular Access System (MACCS) keys (166 bits) as implemented in MOE. The molecular similarity was computed using the Tanimoto (T) coefficient with the equation $T = C/(A + B - C)$, where A and B are the numbers of features in the fingerprint representation of molecules A and B, respectively, and C is the number of common features in A and B. The selection of compounds was performed following four major steps: (1)



	Amitriptyline	Amoxapine	Atomoxetine	Bupropion	Citalopram	Clomipramine	Desipramine	Doxepin	Duloxetine	Escitalopram	Fluoxetine	Fluvoxamine	Imipramine	Isocarboxazid	Lamotrigine	Maprotiline	Mianserin	Milnacipran	Mirtazapine	Moclobemide	Nortriptyline	Paroxetine	Phenelzine	Protriptyline	Reboxetine	Sertraline	Sibutramine	Tranylcypromine	Trimipramine	Valproate	Venlafaxine	Viloxazine
Median	0.52	0.46	0.58	0.55	0.52	0.47	0.52	0.54	0.54	0.52	0.53	0.51	0.51	0.53	0.47	0.54	0.51	0.56	0.51	0.54	0.54	0.49	0.52	0.55	0.53	0.48	0.52	0.50	0.50	0.63	0.56	0.59
Mean	0.50	0.45	0.57	0.53	0.51	0.47	0.51	0.53	0.53	0.51	0.52	0.50	0.50	0.52	0.45	0.53	0.50	0.54	0.50	0.53	0.52	0.48	0.50	0.53	0.52	0.48	0.50	0.48	0.49	0.62	0.56	0.57
StdDev	0.10	0.09	0.10	0.09	0.11	0.10	0.09	0.10	0.10	0.11	0.10	0.10	0.10	0.08	0.08	0.10	0.09	0.08	0.09	0.09	0.09	0.11	0.09	0.09	0.11	0.10	0.11	0.10	0.10	0.13	0.11	0.09

Figure 2. Box and whisker plots of the distribution similarity values for each approved antidepressant versus FEMA GRAS chemicals. Statistics are shown at bottom. Boxes are delimited by first (Q1) and third (Q3) quartiles; gray lines delimit highest and lowest nonoutlier values, whereas red dots represent outliers. Nonoutlier values are defined as Q3 ± 1.5 IQR, where IQR represents the interquartile range (Q1−Q3). Overall, valproate is the most similar drug to all GRAS compounds, followed by atomoxetine and viloxazine.

For each of the 32 antidepressant molecules, the Tanimoto similarity to each compound in the expanded GRAS data set was calculated using the precomputed MACCS keys. (2) For each compound in the GRAS set, the maximum of the 32 Tanimoto similarities was computed. (3) The GRAS compounds were sorted by maximum similarity. (4) The compounds with the largest similarities were selected; these GRAS flavoring substances represent the "nearest neighbors" of the approved antidepressants.

Experimental. All reagents were purchased from Fisher Scientific and were of at least 95% purity. The initial assessment of HDAC inhibition consisted of a single-dose duplicate screening at 500 μM in an in vitro assay. HDAC1 control compound, trichostatin A (TSA), was tested in a 10-dose IC_{50} mode with 3-fold serial dilution, starting at 10 μM. The substrate, a fluorogenic moiety bound to specific p53 fragment, residues 379−392 (Arg-His-Lys-Lys(Ac)), which comprises an ε-acetylated lysine side chain, was incubated with HDAC1. Upon deacetylation of the substrate, the fluorophore was released, giving rise to fluorescence emission; the latter was measured by a fluorometer. IC_{50} values were then determined for six selected compounds, and for valproic acid, in duplicate in a 10-dose IC_{50} manner with 3-fold serial dilution starting at 500 μM. IC_{50} values were extracted by curve-fitting the dose/response slopes. Biological evaluation was performed by Reaction Biology Corp. (Malvern, PA, USA).

RESULTS AND DISCUSSION

The methodologies here employed are described in detail elsewhere;[16] this section covers from global comparisons (chemical space) to specific food related comparisons (organoleptic properties). We started with broad comparisons using and visualizing the chemical space. Then we compare GRAS chemicals to approved antidepressants, to identify structurally related compounds. The identified GRAS molecules were then experimentally evaluated, and we closed with the analysis of physicochemical and organoleptic properties of the relevant compounds.

Except for the experimental section, in each section we made use of chemical information tools. These and additional methodologies (not employed here) could be used for other purposes, for example, to explore the chemical space of food additives, for the assessment of structural diversity between food databases, for the exploration of physicochemical properties based on structural properties.

Thus, this study represents an example of the application of a limited number of chemoinformatic tools but aims to introduce specific concepts and methods to inspire other applications.

Structural Space Coverage. A variety of compounds have been evaluated for antidepressant activity. Compounds with antidepressant activity reported in the literature can be obtained from the ChEMBL database.[33] With the aim of visually assessing the structural proximity of the approved antidepressants to GRAS chemicals, we built the structural space of these data sets based on MACCS keys; for reference purposes, compounds reported in the ChEMBL database and marketed drugs from DrugBank were also included. The resultant structural space is shown in Figure 1. This structural space shows that valproic acid is separated from the rest of the approved antidepressants (red). It also shows that the collection of GRAS chemicals (blue) covers an area of the

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chemical space toward valproic acid and with a degree of overlap with certain other approved antidepressants. Not surprisingly, investigation of new compounds with antidepressant activity, represented by the ChEMBL data set (gray), covers the denser area of known drugs (yellow) and approved antidepressants (red). Thus, the chemical space covered by GRAS chemicals expands toward an unexplored region of chemical space representing current antidepressant drugs.

Similarity of GRAS Chemicals to Approved Antidepressant Drugs: Compound Selection. Figure 2 summarizes the distribution of similarity values for each approved antidepressant drug (cf. the FEMA GRAS flavoring substances). Overall, valproic acid was the antidepressant most similar to GRAS compounds, followed by atomoxetine and maprotiline (see chemical structures in Figure 3). The top 10

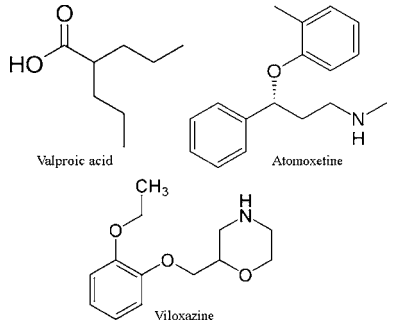

Figure 3. Chemical structures of the three approved antidepressant drugs most similar to GRAS chemicals.

GRAS flavoring substances identified as being most similar to the antidepressants (and all turn out to be most similar to valproic acid) are included in Figure 4, along with valproic acid. Not surprisingly, the compounds with highest similarity to valproic acid are carboxylic acids. The corresponding MACCS keys/Tanimoto similarity values are also shown.

To begin exploring the potential biological activity of the GRAS flavoring substances, we decided to test in vitro the HDAC1 inhibitory activity of selected compounds with high structural similarity to valproic acid.

Experimental Evaluation of Selected Compounds' Inhibition of HDAC1. First, 10 GRAS flavoring substances and valproic acid (see Figure 4) were tested for HDAC1 inhibition at a single dose (500 μM; see Methods for details). Table 1 summarizes the results obtained. In this exploratory screening, the average activity of HDAC1 relative to DMSO in the presence of valproic acid was 73%. Interestingly, in the presence of compound **1**, HDAC1 activity was 37%. For compounds **2**, **3**, **4**, **7**, and **10**, HDAC1 activity ranged from 62.8 to 88.8%. As a next step, compounds **1**− **4**, **7**, **10** and valproic acid (**11**) were tested in dose−response manner using the same protocol employed for the single-dose assays. Results are shown in Table 1. The initial testing concentration chosen was 500 μM, on the basis of valproic acid's IC_{50} value of 400 μM and the desire to find compounds exhibiting HDAC1 inhibition in the same range or lower than that observed for valproic acid. In addition, higher concentrations were avoided to decrease the potential for artifacts occurring in the enzymatic assay, such as aggregation.[34] Under these experimental conditions it was possible to evaluate the inhibition of HDAC1 in the presence of compounds **1** and **4**, having IC_{50} = 0.366 and 0.786 mM, respectively. None of the other compounds inhibited HDAC1 close to 50% at the concentrations evaluated; therefore, the extrapolated IC_{50} values represent only estimates; raw data are summarized in Table S1 of the Supporting Information. The estimated IC_{50} values for these compounds were in the range of 1−2 mM. Thus, among the compounds evaluated, **1** and **4** showed the most significant HDAC1 inhibition, at least equipotent to valproic acid.

Physicochemical and Organoleptic Properties of GRAS Compounds Identified with HDAC1 Inhibitory Activity. Chemoinformatic analyses can be used to analyze and correlate information. As an example, in this section we present the description of the physicochemical and organoleptic properties making use of our previous analysis of the GRAS database. The organoleptic properties, potential uses, usage levels, and occurrence for GRAS chemicals that yielded the best HDAC1 inhibition (**1** and **4**) are summarized in Table 2. The commercially available Leffingwell & Associates (LF) database, marketed as Flavor-Base Pro 2010, was used as the source of flavor data.[35] Being carboxylic acids, both give rise to fatty and dairy notes. Interestingly, depending on the source of the information, different odor notes have been described. Both chemicals are used as flavoring agents, and both occur in nature. Nonanoic acid (**1**) is present in a variety of fruits as well as in dairy products and also in lamb and coffee. *trans*-2-Decenoic acid (**4**) (herein named 2-decenoic acid) has been reported in fewer foods, for example, black tea, wort, and pork fat. The wide variety of odor notes attributed to nonanoic acid is reflected in the several uses reported, from dairy to tobacco notes. We reported previously[14] the frequency count of flavor descriptors occurring in the FEMA GRAS list, as catalogued in Leffingwell's database.[35] Table 3 lists frequently used descriptors, as well as those reported specifically for nonanoic acid and/or 2-decenoic acid, along with frequency counts and associated descriptors. The latter, highlighted in bold, correspond to those highly associated (in terms of co-occurrence) with the reference descriptor. Some combinations are expected, for instance, fatty acid/oily; some others are less intuitive, for instance, fatty acid/citrus. Full associations of descriptors can be found elsewhere.[14]

There are several physicochemical and pharmacokinetic properties that are associated with drug-likeness, bioavailability and toxicity. There are also particular properties that are common among CNS drugs. The parameters listed in Table 4 are associated with these desirable pharmacokinetic and ADMET (adsorption, distribution, metabolism, excretion, and toxicity) properties. The predicted values for the four compounds that showed best HDAC1 inhibition are summarized. Lipinski's so-called "Rule of Five",[36] which is associated with drug-likeness, states that the absorption or permeation of a molecule is more likely when the molecular weight is under 500, the value of logP is lower than 5, and the molecule has at most 5 H-donor and 10 H-acceptor atoms.

An important property of active drugs for the CNS is their ability to cross the blood−brain barrier (BBB). BBB penetration is associated with a drug molecule's lipophilicity and polar surface area. The average logP value (a measure of lipophilicity) for CNS drugs is ~2.50.[37] For CNS drugs, PSA values less than 60 Å^2 have been reported.[38] Additionally, QikProp[32] provides a model to predict a brain−blood partition coefficient. It should be noted that this model was developed for orally administered drugs (rather than specifically for CNS drugs), and the recommended range goes from −3.0 to 1.2.

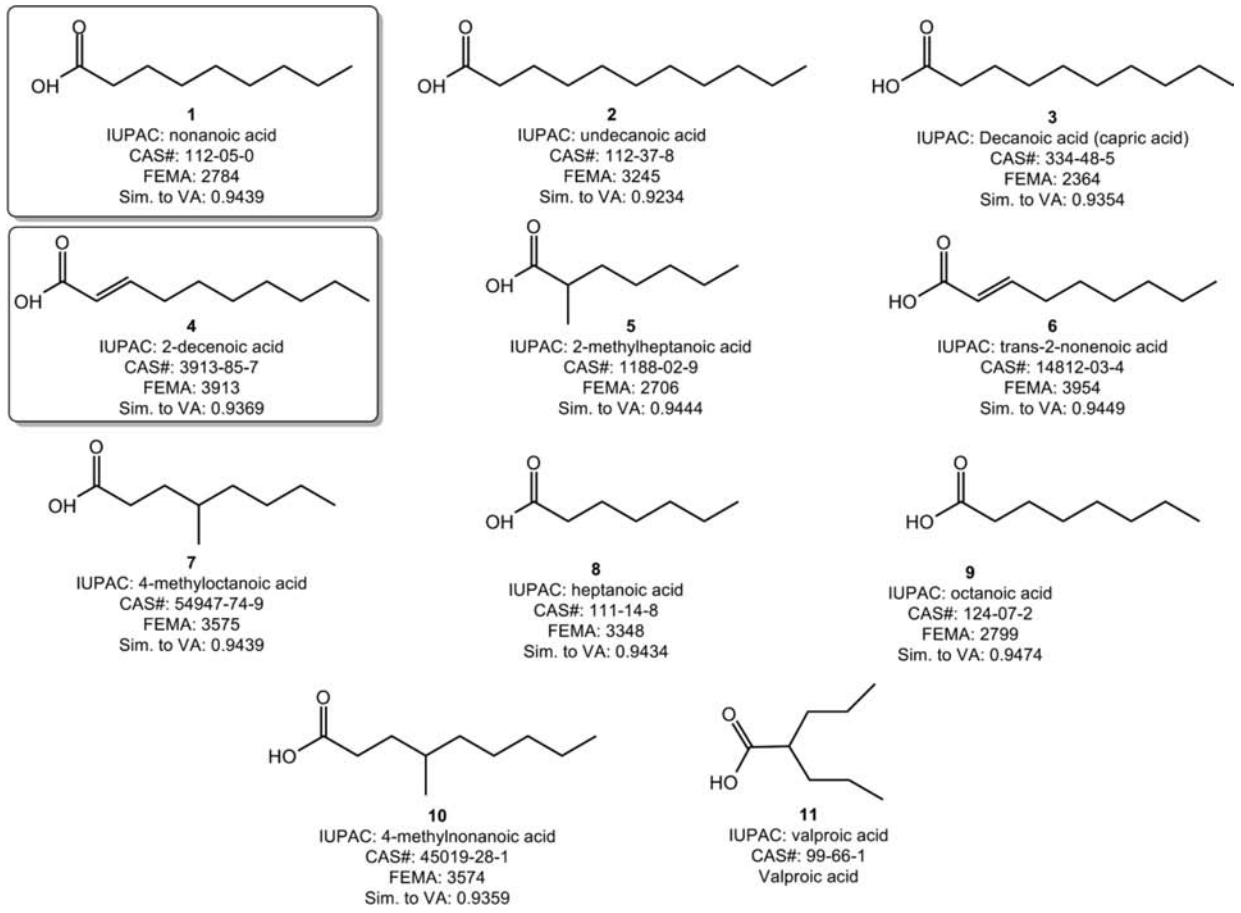

Figure 4. Chemical structures of GRAS compounds similar to valproic acid (labeled compound **11**).

Table 1. Average Percent Activity for HDAC1 Relative to DMSO with the 11 Compounds Tested

compd	% activity relative to DMSO	IC_{50} (mM)
1	**37.0**	**0.37**
2	75.8	≈1
3	88.8	≈1
4	**77.3**	**0.79**
5	91.4	
6	109.1	
7	86.5	≈2
8	103.8	
9	96.2	
10	62.8	≈2
11[a]	73.2	≈1

[a]Valproic acid.

Finally, the predicted IC_{50} value for blockage of HERG K+ channels[35] is listed in Table 4, named QPlogHERG; values below −5 are of concern. This is a challenging property for many compounds in drug development. However, the HERG values for the GRAS chemicals listed in Table 4 are predicted to be safe.

In terms of the predicted physicochemical properties, summarized in Table 4, these GRAS chemicals as a group have comparable logP values, as well as TPSA values, and they also fulfill Lipinski's Rule of Five and bioavailability guidelines for drug-likeness.

Pharmacophore for HDAC1 Inhibitors. The 18 human HDACs known to date can be classified into four classes. Selectivity toward the different isoforms is the subject of current experimental[39] and computational[40] studies aimed at understanding the function of the different HDAC isoforms as well as making better use of them as drug targets.

HDAC1 belongs to class I. Many inhibitors of HDAC1 interact with a zinc cation and have a hydrophobic cap group and an aliphatic linker; this general pharmacophore is depicted in Figure 5. A review of inhibitors falling into this accepted pharmacophore can be found elsewhere.[41] A variety of compound classes such as short-chain fatty acids, hydramic acids, benzamides, ketones, and cyclic peptides with a pendant functional group satisfy this pharmacophore, and accordingly exhibit HDAC inhibitory properties.

Both GRAS carboxylic acids (**1** and **4**) identified in this work as HDAC1 inhibitors have structures consistent with this pharmacophore. Therefore, it is expected that nonanoic acid and 2-decenoic acid inhibit HDAC1 via the accepted mechanism involving interactions with the zinc cation.

In summary, to identify potential bioactivity among the food flavoring components that comprise the so-called FEMA GRAS list, we conducted computational searching for compounds with structures similar to those of approved antidepressant drugs. In addition, we show relevant organoleptic and physicochemical properties of selected GRAS compounds making use of chemoinformatic analyses. Valproic acid was the antidepressant most similar to GRAS compounds. Guided by the proposal that the inhibition of HDAC1 could be

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Table 2. Organoleptic and Safety Properties of the Two GRAS Chemicals More Relevant in This Study[a]

name	nonanoic acid	2-decenoic acid
FEMA no.	2784	3913
flavor description	mild, fatty, dairy cheese, nut-like odor; fatty-waxy cheese and nutty taste	fruity and slightly oily waxy; fruity, sweet, and peach-like taste
occurrence	blue cheese, butter, cocoa, coffee, cooked lamb/mutton, geranium, grape, grape brandy, orris, raspberry, rose, strawberry, tea, Virginia tobacco	black tea, wort, pork fat
uses	used in dairy flavors (butter, cream, cheese), cooked meat flavors (pork, beef, mutton), chocolate, tea, fruit (guava, mango, papaya, berry) and wine/brandy flavors; in tobacco it is reported to give a fatty-waxy taste, which makes it useful in Virgina tobacco flavors	possible uses include Fruital flavors as well as dairy flavors
use levels	normal use levels in finished consumer product: 0.1−13 ppm; Council of Europe limits, foods (20 ppm); beverages (2 ppm)	no safety concern at current levels of intake when used as a flavoring agent
functional class	flavoring agent	flavoring agent

[a]Information obtained from Leffingwell and Associates.[28]

Table 3. Flavor Descriptors Described for Nonanoic Acid and 2-Decenoic Acid[a]

descriptor	frequency usage	odor descriptors commonly used in combination with reference descriptor[b]
fatty acid	168	**oily**, waxy, citrus, dairy, cheesy, nutty, green general, fruity, meaty, roasty
dairy	56	**cheesy**, waxy, fatty acid
cheesy	111	dairy, sour, fatty acid
nut	153	**roasted**, **earthy**, musty general, cocoa, fatty acid, caramel
waxy	107	**fatty** acid, dairy, green general, fruity, citrus, tropical, melon
fruity	665	**sweet**, **green general**, **floral**, tropical, apple, herbal
oily	74	**fatty acid**, waxy, citrus
sweet	463	**fruity**, **floral**, rose, balsam, spicy, caramel, herbal, woody

[a]Corresponding descriptor frequency usage in the Leffingwell database and their associated descriptors (see ref 7). [b]Frequent combinations were obtained from clustering analysis of the flavor similarity matrix of the descriptors in the Leffingwell database. Details are described elsewhere.[7] Ordered by stronger associations; notorious associations are highlighted in bold.

associated with the efficacy of valproic acid in the treatment of bipolar disorder, we screened the GRAS compounds most similar to valproic acid for HDAC1 inhibition. Two GRAS chemicals, namely, nonanoic acid and 2-decenoic acid, inhibited HDAC1 with potency comparable to that of valproic acid. The results of this work exemplify the feasibility of exploring the FEMA GRAS flavoring list as a potential source of biologically active molecules. It needs to be stressed that GRAS compounds are not expected to exhibit strong enzymatic inhibitory effects at the concentrations typically employed in flavor formulations designed for use in foods and beverages. However, as shown here, GRAS chemicals are able to bind to important therapeutic targets. Exploration of other biological effects of GRAS chemicals, their bioavailability, and an estimation of toxicity at higher concentrations (as noted above) will demonstrate the value of GRAS compounds as a potential source of new, already

Figure 5. Common pharmacophore for class I and II HDAC inhibitors.

in human use, bioactive compounds. A major perspective of this work, already in progress, is to perform in vivo behavioral studies. Additionally, the toxicology and bioavailability of nonanoic acid and 2-decenoic acid are both warranted. If confirmed as viable actives (active, bioavailable, and nontoxic), these molecules represent potential candidates for additional investigation as potential mood-enhancing chemicals. Testing will also include in vitro assays with other enzymes, for example, those that are also targeted by valproic acid. Additional research avenues for these compounds include investigations as combination therapies for cancer treatment and for HIV infection. This study shows how similarity searching followed by experimental evaluation can be used for rapid identification of GRAS chemicals with possible biological activity, with potential application for promoting health and wellness. This work also represents a step further in the growing field of foodinformatics; this field relates to the use of chemical information methods in food chemistry.[24]

■ ASSOCIATED CONTENT

Supporting Information

HDAC profiling. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 4. Physicochemical and Pharmacokinetic Properties of the Two GRAS Chemicals More Relevant in This Study[a]

	CAS Registry No.	MW	SlogP	donors	acceptors	TPSA	logBBB	QPlogHERG
1	112-05-0	158.24	3.05	1	2	37.30	−0.849	−2.005
4	3913-85-7	170.25	3.25	1	2	37.30	−0.931	−2.327

[a]MW, molecular weight; donor, hydrogen bond donor count; acceptor, hydrogen bond acceptor count; TPSA, topological polar surface area.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MACCS, molecular access system; MOE, molecular operating environment; GRAS, generally recognized as safe; FEMA, Flavor and Extract Manufacturers Association; VA, valproic acid; HDAC, histone deacetylase; HERG, human *ether-à-go-go*-related gene; CNS, central nervous system; TSA, trichostatin A

■ REFERENCES

(1) Cichewicz, R. H.; Nair, M. G. Isolation and characterization of stelladerol, a new antioxidant naphthalene glycoside, and other antioxidant glycosides from edible daylily (*Hemerocallis*) flowers. *J. Agric. Food Chem.* **2001**, *50*, 87−91.

(2) Eggen, T.; Lillo, C. Antidiabetic II drug metformin in plants: uptake and translocation to edible parts of cereals, oily seeds, beans, tomato, squash, carrots, and potatoes. *J. Agric. Food Chem.* **2012**, *60*, 6929−6935.

(3) Faudale, M.; Viladomat, F.; Bastida, J.; Poli, F.; Codina, C. Antioxidant activity and phenolic composition of wild, edible, and medicinal fennel from different Mediterranean countries. *J. Agric. Food Chem.* **2008**, *56*, 1912−1920.

(4) Nakahara, K.; Trakoontivakorn, G.; Alzoreky, N. S.; Ono, H.; Onishi-Kameyama, M.; Yoshida, M. Antimutagenicity of some edible thai plants, and a bioactive carbazole alkaloid, mahanine, isolated from *Micromelum minutum*. *J. Agric. Food Chem.* **2002**, *50*, 4796−4802.

(5) Tesoriere, L.; Fazzari, M.; Allegra, M.; Livrea, M. A. Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of Sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. *J. Agric. Food Chem.* **2005**, *53*, 7851−7855.

(6) Wang, S.; Bao, L.; Zhao, F.; Wang, Q.; Li, S.; Ren, J.; Li, L.; Wen, H.; Guo, L.; Liu, H. Isolation, identification, and bioactivity of monoterpenoids and sesquiterpenoids from the mycelia of edible mushroom *Pleurotus cornucopiae*. *J. Agric. Food Chem.* **2013**, *61*, 5122−5129.

(7) Schwager, J.; Mohajeri, M. H.; Fowler, A.; Weber, P. Challenges in discovering bioactives for the food industry. *Curr. Opin. Biotechnol.* **2008**, *19*, 66−72.

(8) Sprous, D. G.; Salemme, F. R. A comparison of the chemical properties of drugs and FEMA/FDA notified GRAS chemical compounds used in the food industry. *Food Chem. Toxicol.* **2007**, *45*, 1419−1427.

(9) Minkiewicz, P.; Dziuba, J.; Iwaniak, A.; Dziuba, M.; Darewicz, M. BIOPEP database and other programs for processing bioactive peptide sequence. *J. AOAC Int.* **2008**, *91*, 965−980.

(10) Dziuba, J.; Minkiewicz, P.; Nalecz, D.; Iwaniak, A. Database of biologically active peptide sequences. *Nahrung* **1999**, *43*, 190−195.

(11) Watanabe, S.; Zhuo, X. G.; Kimira, M. Food safety and epidemiology: new database of functional food factors. *BioFactors* **2004**, *22*, 213−219.

(12) Scalbert, A.; Andres-Lacueva, C.; Arita, M.; Kroon, P.; Manach, C.; Urpi-Sarda, M.; Wishart, D. Databases on food phytochemicals and their health-promoting effects. *J. Agric. Food Chem.* **2011**, *59*, 4331−4348.

(13) Martinez-Mayorga, K.; Medina-Franco, J. L. In *Chemoinformatics − Applications in Food Chemistry in Advances in Food and Nutrition Research*; Taylor, S. L., Ed.; Elsevier: Amsterdam, The Netherlands, 2009; Vol. 58, pp 33−56.

(14) Martínez-Mayorga, K.; Peppard, T. L.; Yongye, A. B.; Santos, R.; Giulianotti, M.; Medina-Franco, J. L. Characterization of a comprehensive flavor database. *J. Chemom.* **2011**, *25*, 550−560.

(15) Medina-Franco, J. L.; Martínez-Mayorga, K.; Peppard, T. L.; Del Rio, A. Chemoinformatic analysis of GRAS (generally recognized as safe) flavor chemicals and natural products. *PLoS ONE* **2012**, *7*, e50798.

(16) Johnson, M. A.; Maggiora, G. M. *Concepts and Applications of Molecular Similarity*; Wiley: New York, 1990.

(17) Burdock, G. The GRAS process. *Food Technol. (Chicago, IL, U.S.)* **2003**, *57*, 17.

(18) Burdock, G. A.; Carabin, I. G. Generally recognized as safe (GRAS): history and description. *Toxicol. Lett.* **2004**, *150*, 3−18.

(19) Burdock, G. A.; Carabin, I. G.; Griffiths, J. C. The importance of GRAS to the functional food and nutraceutical industries. *Toxicol.* **2006**, *221*, 17−27.

(20) López-Vallejo, F.; Peppard, T. L.; Medina-Franco, J. L.; Martínez-Mayorga, K. Computational methods for the discovery of mood disorder therapies. *Expert Opin. Drug Discov.* **2011**, *6*, 1227−1245.

(21) Boyadjieva, N.; Varadinova, M. Epigenetics of psychoactive drugs. *J. Pharm. Pharmacol.* **2012**, *64*, 1349−1358.

(22) Merino, A.; Bronowska, A. K.; Jackson, D. B.; Cahill, D. J. Drug profiling: knowing where it hits. *Drug Discovery Today* **2010**, *15*, 749−756.

(23) Malnic, B.; Hirono, J.; Sato, T.; Buck, L. B. Combinatorial receptor codes for odors. *Cell* **1999**, *96*, 713−723.

(24) Martinez-Mayorga, K.; Medina-Franco, J. L. FoodInformatics: applications of chemical information to food chemistry. Presented at the *245th ACS National Meeting of the American Chemical Society*, New Orleans, LA; American Chemical Society: Washington, DC, 2013.

(25) Phiel, C. J.; Zhang, F.; Huang, E. Y.; Guenther, M. G.; Lazar, M. A.; Klein, P. S. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.* **2001**, *276*, 36734−36741.

(26) Molecular Operating Environment (MOE), version 2012; Chemical Computing Group: Montreal, Canada.

(27) Ertl, P.; Rohde, B.; Selzer, P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J. Med. Chem.* **2000**, *43*, 3714−3717.

(28) Clark, D. E. Prediction of intestinal absorption and blood-brain barrier penetration by computational methods. *Comb. Chem. High Throughput Screening* **2001**, *4*, 477−496.

(29) Wildman, S. A.; Crippen, G. M. Prediction of physicochemical parameters by atomic contributions. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868−873.

(30) Medina-Franco, J. L.; Martinez-Mayorga, K.; Giulianotti, M. A.; Houghten, R. A.; Pinilla, C. Visualization of the chemical space in drug discovery. *Curr. Comput.-Aided Drug Des.* **2008**, *4*, 323−334.

(31) Spotfire version 9.1.1; TIBCO Software, Inc., Somerville, MA.

(32) QikProp 3.1, Schrodinger, LLC, New York, 2008.

(33) Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P. ChEMBL: a large-scale bioactivity database for drug discovery*Nucleic Acids Res.* **2011**.

(34) Jadhav, A.; Ferreira, R. S.; Klumpp, C.; Mott, B. T.; Austin, C. P.; Inglese, J.; Thomas, C. J.; Maloney, D. J.; Shoichet, B. K.; Simeonov, A. Quantitative analyses of aggregation, autofluorescence, and reactivity artifacts in a screen for inhibitors of a thiol protease. *J. Med. Chem.* **2009**, *53*, 37−51.

(35) Vanderberg, J. I.; Perry, M. D.; Perrin, M. J.; Mann, S. A.; Ke, Y.; Hill, A. P. hERG K(+) channels: structure, function, and clinical significance. *Physiol. Rev.* **2012**, *92*, 1393−1478.

(36) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliver. Rev* **1997**, *23*, 3−25.
(37) Pajouhesh, H.; Lenz, G. R. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx* **2005**, *2*, 541−553.
(38) Kelder, J.; Grootenhuis, P. J.; Bayada, D.; Delbressine, L. C.; Ploemen, J.-P. Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. *Pharm. Res.* **1999**, *16*, 1514−1519.
(39) Blackwell, L.; Norris, J.; Suto, C. M.; Janzen, W. P. The use of diversity profiling to characterize chemical modulators of the histone deacetylases. *Life Sci.* **2008**, *82*, 1050−1058.
(40) Silvestri, L.; Ballante, F.; Mai, A.; Marshall, G. R.; Ragno, R. Histone deacetylase inhibitors: structure-based modeling and isoform-selectivity prediction. *J. Chem. Inf. Model.* **2012**, *52*, 2215−2235.
(41) Mai, A.; Massa, S.; Rotili, D.; Cerbara, I.; Valente, S.; Pezzi, R.; Simeoni, S.; Ragno, R. Histone deacetylation in epigenetics: an attractive target for anticancer therapy. *Med. Res. Rev.* **2005**, *25*, 261−309.