ARTICLE IN PRESS

Journal of Ethnopharmacology ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Review

Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides

Jente Boonen [a], Antoon Bronselaer [b], Joachim Nielandt [b], Lieselotte Veryser [a], Guy De Tré [b], Bart De Spiegeleer [a,*]

[a] Drug Quality and Registration (DruQuaR) Group, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[b] Department of Telecommunications and Information Processing, Faculty of Engineering and Architecture, Ghent University, Sint-Pietersnieuwstraat 41, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form
21 May 2012
Accepted 22 May 2012

Keywords:
N-alkylamides (NAAs)
Plant taxonomy
Bioactivity
Alkamid database
Chemical structure classification

ABSTRACT

Ethnopharmacological relevance: *N*-Alkylamides (NAAs) are a promising group of bioactive compounds, which are anticipated to act as important lead compounds for plant protection and biocidal products, functional food, cosmeceuticals and drugs in the next decennia. These molecules, currently found in more than 25 plant families and with a wide structural diversity, exert a variety of biological–pharmacological effects and are of high ethnopharmacological importance. However, information is scattered in literature, with different, often unstandardized, pharmacological methodologies being used. Therefore, a comprehensive NAA database (acronym: Alkamid) was constructed to collect the available structural and functional NAA data, linked to their occurrence in plants (family, tribe, species, genus).

Materials and methods: For loading information in the database, literature data was gathered over the period 1950–2010, by using several search engines. In order to represent the collected information about NAAs, the plants in which they occur and the functionalities for which they have been examined, a relational database is constructed and implemented on a MySQL back-end.

Results: The database is supported by describing the NAA plant-, functional- and chemical-space. The chemical space includes a NAA classification, according to their fatty acid and amine structures.

Conclusions: The Alkamid database (publicly available on the website http://alkamid.ugent.be/) is not only a central information point, but can also function as a useful tool to prioritize the NAA choice in the evaluation of their functionality, to perform data mining leading to quantitative structure–property relationships (QSPRs), functionality comparisons, clustering, plant biochemistry and taxonomic evaluations.

© 2012 Elsevier Ireland Ltd. All rights reserved.

UNCORRECTED PROOF

Contents

* Corresponding author. Tel.: +32 9264 8100; fax: +32 9264 8193.
E-mail address: Bart.DeSpiegeleer@UGent.be (B. De Spiegeleer).

0378-8741/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2012.05.038

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

1. Introduction

In the last two decades, the biomedical interest in *N*-alkylamides (NAAs) has increased enormously. These plant-derived amides mostly contain a poly-unsaturated aliphatic fatty acid chain and a shorter substituent at the amine side. Both might include cyclic systems and/or heteromolecules (nitrogen, sulfur, oxygen) (Fig. 1). At the core is the amide bond, which resembles the peptide link –C(=O)NH– as observed in polypeptides and proteins. Due to its resonance characteristics, amide bonds are planar and relatively stable, possess partial double bond characters and are at the origin of its large dipole moment.

NAAs are widely present in the whole biological kingdom. The pharmaceutically important ergot alkaloids, which comply with our definition of NAAs, are produced by fungi of different genera (*e.g. Claviceps*, *Penicillium* and *Aspergillus*) (Wallwey and Li, 2011). 9*Z*-octadecenamide was identified in the lichen *Stereocaulon alpinum* as bioactive NAA (Ingolfsdottir et al., 1997). Ceramides, fatty acid linked sphingosines, are major lipid components in *Pseudomonas*-like Gram (−) bacteria and important physiological constituents in eukaryotic cell membranes (Minamino et al., 2003). In human and other mammalian skin, ceramides play a key role against transepidermal water loss and harmful environmental influences (Raith et al., 2004). However, most importantly, NAAs as novel drug leads are found as secondary metabolites in the plant kingdom.

Due to these secondary metabolites, several plants have been used traditionally for organoleptic, as well as medical purposes, like toothache, gum, skin and gastric diseases, sexual dysfunctions and viral infections (Barnes et al., 2005; Boonen et al., 2010; Sharma et al., in press; Wang et al., 2007; Wu et al., 2004; Yang, 2008). These different uses reflect the wide variety of ethnopharmacological viewpoints: NAA containing plants are used in numerous Traditional medicine systems (TMS) all over the world. Some typical usages are exemplified in Table 1, where beside the TMS and originating area, the local plant name and indication are depicted. Moreover, from the work of different research groups focusing on plants containing NAAs, it became clear that these physiologically active molecules possess a broad functional spectrum via multiple mechanisms of action and targets. NAAs are thus becoming a new meta-group of drugs (like oligo-peptides, -saccharides and -nucleotides), interfering with different pathophysiologies. Hundreds of publications report the identification and functionality of NAAs, found in more than twenty different plant families. These studies are mostly fragmented, from different chemical, biopharmaceutical or chemotaxonomic fields, and with a strong ethnopharmacological view point. Up till now however, no global data-base overview of botanical NAAs is available. Seen this multi-disciplinarity, one identical molecule for example has historically received several names based upon their origin (α-sanshool, echinacein, neoherculin) (Crombie, 1955). Therefore, we present here a structured overview of plant-occurring NAAs with the acronym "Alkamid", an online accessible chemical and functional database (http://alkamid.ugent.be). We will describe the occurrence of NAAs in the different plant families, including their possible biosynthetic pathways and intrinsic roles (plant space), as well as their main functionalities outside the plant (functionality space) and their chemistry (chemical space).

2. Material and methods

For loading information in the database, literature data was gathered by using the search engines Web of Knowledge, PubMed, Espacenet and Google. 'Alkamide', 'alkylamide' and 'amide', each separately, as well as 'plant' and 'activity', using the Boolean operation "AND", covering the period 1950–2010.

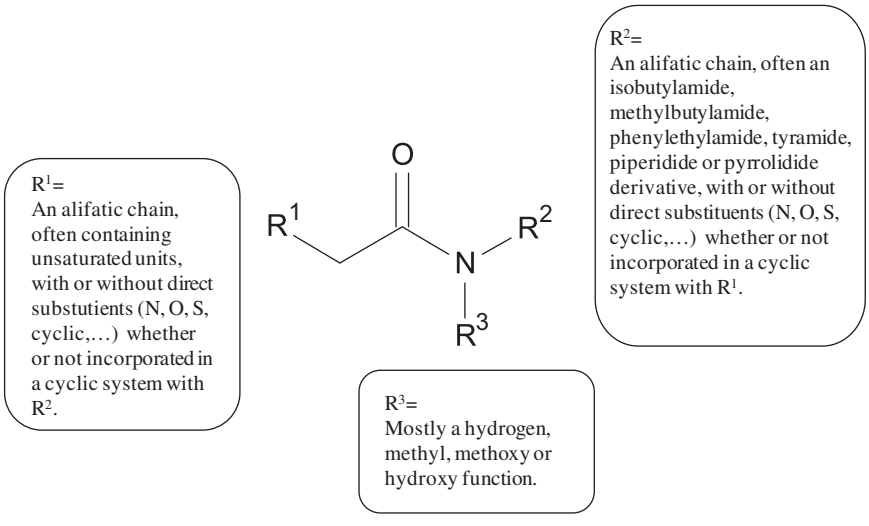


Fig. 1. Structural properties of NAAs.

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Table 1
Typical ethnopharmacological uses of NAA containing plants.

Traditional medicine system	Originating area	Family	Scientific accepted name	Local name	Indication	Reference
Chinese	East Asian	Aristolochiaceae	*Asarum heterotropoides* F.Schmidt	*Xi-xin*	Pain, cough, allergy	Zhang et al. (2005)
Ayurveda	South and Southeast Asian	Asteraceae	*Spilanthes acemella* (L.) L.	–	Sexual deficiencies	Sharma et al. (2011)
Unani		Asteraceae	*Anacyclus pyrethrum* (L.) Lag.	*Aaqarqarhaa*	Toothache, rheumatic and neuralgic affections, rhinitis, epilepsy	Khare (2004)
Siddha		Zygophyllaceae	*Tribulus terrestris* L.	*Nerujil*	Arthritis	Wilson et al. (2007)
		Asteraceae	*Anacyclus pyrethrum* (L.) Lag.	*Akkara karam*	Joint pain	
		Euphorbiaceae	*Ricinus communis* L.	*Amanakku*	Joint pain, swelling	
Roman	Mediterranean and Near Eastern (Italy, Tunesia)	Asteraceae	*Helianthus annuus* L.	*Ain el, Girasole*	Sunstroke, hypertension, hyperglycemia	Leporatti and Ghedira (2009)
		Euphorbiaceae	*Ricinus communis* L.	*Kharwaa, Ricino*	Bronchitis, headache, fever, rheumatic pain, crude skin, pus	
		Poaceae	*Zea mays* L.	*Ktania, Mais, Granturco*	Constipation, gonorrhoae, bronchitis	
		Solanaceae	*Capsicum annuum* L.	*Felfel, Peperoncino*	Otitis, headache, rheumatism, alopecia, hemorrhoids, hypertension	
Yoruba	African	Poaceae	*Zea mays* L.	*Ewe okporokporo*	Malaria	Ene et al. (2010)
		Solanaceae	*Nicotiana tabacum* L.	*Ewe taba*	Convulsions, stimulant	
Mayan	American	Solanaceae	*Capsicum annuum* L.	*Maax iik*	Hard swelling	Caamal-Fuentes et al. (2011)
Tacana		Euphorbiaceae	*Ricinus communis* L.	*Tahua dhahua*	Wounds, pimples, swelling, cough	Bourdy et al. (2000)
Brazilian		Asteraceae	*Achillea millefolium* L.	*Novalgina*	Fever, headache, pain, stomach complaints, bad cold	Di Stasi et al. (2002)
		Asteraceae	*Spilanthes acmella* (L.) L.	–	Multi-resistant bacterial infection	Machado et al. (2003)

UNCORRECTED PROOF

In methodologies where an increase or decrease towards a placebo sample was observed, the result (“value”) was standardized as the percentage relative to placebo NAA and was calculated as follows:

$$\% = \frac{Ctr - x}{Ctr} \times 100$$

where *Ctr* is the value for the placebo sample; *x* the value for the investigated NAA.

In order to represent the collected information about NAAs, the plants in which they occur and the functionalities for which they have been examined, a relational database is constructed (Codd, 1970). The database design is shown schematically in Fig. 4. In this visual representation, each rectangular block describes the structure of a table. In the header of the block, the table name is given. Below this table name, the names of the columns are listed. The first column name(s) listed, *i.e.* the column name(s) above the dotted line, constitute(s) the primary key of the table, that is, a unique identifier for rows in the table. As an example, consider the block in the left bottom. This block describes a table called ‘Molecule’, which has nine columns. Each row in this table is uniquely identified by its moleculeID. One-to-many links are shown by means of arrows between blocks.

The conceptual idea of the database is as follows. Information about NAAs regarding their chemical structure is stored in the table ‘Molecule’. Information about plants is stored in a normalized way in four tables: ‘PlantSpecies’, ‘PlantGenus’, ‘PlantTribe’ and ‘PlantFamily’. The links between these tables grasp the hierarchical structure that is used to categorize plants. In the ‘PlantSpecies’ table, information is stored about the ethnopharmacological systems (*e.g.* Ayurveda medicine) in which NAAs are used. The observed occurrences of NAAs in plants are stored in the table ‘In Species’. Hereby, the review or publication in which the observation was made is also stored. Observed functionalities are stored in the table ‘Functionality’. Several aspects such as the used method (‘methodName’) to measure the functionality are stored in separate tables in order to maximize consistency in the database. For each reported measurement concerning a particular functional behavior, the measured value is stored in the column ‘Measurement’. In the case where the measured value indicates a change, column ‘Measurement Change’ indicates whether the measured value is an increase or a decrease. Column ‘Measurement Operation’ encodes whether the measured value is an exact number, an upper bound or a lower bound.

Functionalities observed by performing measurements directly on a(n) (group of) NAA(s) are linked to this (group of) NAA(s) through the table ‘Molecule Functionality’. Functionalities observed by performing measurements on whole plant extracts are linked to these plants through the table ‘Plant Functionality’. For each tested and observed functional behavior, the review or publication is mentioned in which this behavior is reported.

The Alkamid database has been implemented on a MySQL backend and is publicly available on the website http://alkamid.ugent.be. The website is implemented by using the content management system Drupal.

3. Results and discussion

3.1. Alkamid database

The Alkamid database is a resource of plant occurring NAAs. Ethnopharmacological and biofunctional data of specific NAAs can be searched for, together with their physicochemical properties

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

and plant origin. Based upon the input-question of the user (name, structural formula, plant origin, activity, literature), this search page will give all available NAAs in a structured manner. In order to make the online Alkamid database easily searchable, it provides the most significant chemical identifiers of chemical structures (*i.e.* chemical name, IUPAC name, trivial name, SMILES string and structural formula). In addition, some physicochemical properties are included and a structured overview of NAA functionalities is provided. Because the extraction and collection of such results are labor intensive, the online database provides facilities for users to communicate their results and/or knowledge to the database administrators.

Besides the functional part of the database, the link between NAAs and the plants in which they occur, is given. This way, starting from a plant (specified by family, genus, tribe or species), it is easy to obtain an overview of the NAAs occurring in that plant and by extension also an overview of all functionalities reported for these NAAs.

In order to facilitate the use of the database, a simple (keyword based) search interface is provided that allows to search by NAA, by plant, by functionality and by article specifications (author, title, year).

3.2. Plant space

3.2.1. Plant families

Up till now, NAAs are found in 26 different plant families comprising more than 100 plant species. Table 2 illustrates these plant families, their corresponding tribes, genera, some of the NAA containing plant species and the NAAs found herein (numbers can be found in Table S1 in the supplementary material, which correspond to the molecule identities in the database). The species names are in accordance with the international taxonomic database "The plant list" (www.theplantlist.org). Species names

Table 2
Plant origin of NAAs.

Acanthaceae
+ Hydrangeae
• Aphelandra
- *squarrosa* Nees **(277,278)**
Amaranthaceae
+ Gomphreneae
• Gomphrena
- *globosa* L. **(266)**
Aristolochiaceae
+ Asareae
• Asarum
- *forbesii* Maxim. **(4,77,286)**
- *heterotropoides* F.Schmidt **(3,4,80)**
Asteraceae
+ Anthemideae
• Achillea
- *ageratifolia* (Sibth. & Sm.) Benth. & Hook.f. **(106,107,108,109,110,111,112,113,114,115, 116,117,118)**
- *asiatica* Serg. **(80,90,97,98)**
- *aspleniifolia* Vent. **(33,80,83,90,91,93,94,95,97,98,105)**
- *biebersteinii* Afan. **(141)**
- *collina* (Becker ex Rchb.f.) Heimerl **(33,80,83,90,91,93,94,95,97,98,105)**
- *crithmifolia* Waldst. & Kit. **(90,98)**
- *falcata* L. **(80,91,96,97,98,140)**
- *grandifolia* Friv. **(141,150)**
- *lanulosa* Nutt. [nom. illeg.] **(80,97,98)**
- *latiloba* Ledeb. ex Nordm. **(33,80,91,93,97,98,100,104)**
- *ligustica* All. **(139)**
- *lycaonica* Boiss. & Heldr. **(146,147,148,149)**
- *macrophylla L. (Achillea)* **(80,91)**
- *millefolium* L. **(33,78,80,83,90,91,93,94,95,96,97,98, 99,100,101,102,103,104,105)**
- *nana* L. **(55,78,103,133,134,135,136,137,138)**
- *pannonica* Scheele [nom. illeg.] **(80,90,94,95)**
- *ptarmica* L. **(132)**
- *setacea* Waldst. & Kit. **(80,90,94,95)**
- *spinulifolia* Fenzl ex Boiss. **(80,91,132,145,151)**
- *tomentosa* L. **(117,144)**
- *wilhelmsii* K.Koch [nom. illeg.] **(119,120,121,122,123,124,125,126, 127,128,129,130,131)**
• Anacyclus
- *monanthos* (L.) Thell. **(88)**
- *pyrethrum* (L.) Lag. **(19,66,78,79,80,81,82,83,84,85, 86,87,103,104,279)**
• Artemisia
- *dracunculus* L. **(80,152,153)**
• Chrysanthemum
- *frutescens* L. [nom. illeg.] **(33,86,154,155,156,157)**
• Leucocyclus
- *formosus* Boiss. [nom. illeg.] **(66,80,86,133,134,142,143)**
• Otanthus
- *maritimus* **(86,133,134,312,313,314)**
+ Heliantheae
• Spilanthes
- *acmella* (L.) L. **(1,2,3,4,5,6,7,8,9,10,11,12,14,15)**
- *alba* [nom. illeg.] **(4,15,22,23,24,25,26,27,56,59)**
- *callimorpha* A.H. Moore **(3,4,9,14,16,17,19,20)**
- *ciliata Kunth* [nom. illeg.] **(1,2,4,6,10,11,12,14,21,28,29,30,1,31,32,33,34,35,36, 37,38,39,40,41,42,43,44,45,46,47,57,62)**
- *oppositifolia* var. *oppositifolia* (Lam.) R.K. Jansen **(1,2,4)**
- *radicans* Schrad. ex DC. [nom. illeg.] **(1,2,12,28,31,33,38,39,40,48,49,50,51)**
+ Senecioneae
• Senecio
- *erechthithoides* F.Muell. [nom. illeg.] **(262)**
Brassicaceae
+ Brassiceae
• Arabidopsis
- *thaliana* (L.) Heynh. **(267,272)**
• Brassica
- *oleracea* L. **(267)**
+ Lepidieae
• Lepidium
- *meyenii* Walp. **(249,250)**
Bromeliaceae
+ Ananaseae
• Ananas
- *comosus* (L.) Merr. **(265)**
Caryophyllaceae
+ Diantheae
• Dianthus
- *caryophyllus* L. **(268)**
Convolvulaceae
+ Ipomoeeae
• Ipomoea
- *aquatica* Forssk. **(247)**
- *nil* (L.) Roth **(252,342)**
- *obscura* (L.) Ker Gawl. **(248)**
- *quinquefolia* [nom. illeg.] **(247)**
Ephedraceae
+ Ephedreae
• Ephedra
- *aphylla* Forssk. **(278)**
Euphorbiaceae
+ Acalypheae
• Acalypha
- *indica* L. *(Acalypha)* **(257)**
• Ricinus
- *communis* L. *(Ricinus)* **(272)**
Extraction artefacts **(300,301)**
Fabaceae
+ Aeschynomeneae
• Arachis
- *hypogaea*L. **(272)**

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Table 2 (*continued*)

- • Echinacea
  - - *angustifolia* DC. **(3,4,6,62,63,69,74,75,76,162,286)**
  - - *pallida* (Nutt.) Nutt. **(63)**
  - - *purpurea* (L.) Moench **(3,4,6,7,9,56,57,58,59,60,61,62,63,65,66,67,68,69,70,71,72,73,75,105,162,286)**
- • Helianthus
  - - *annuus* L. **(264)**
- • Heliopsis
  - - *buphthalmoides* (Jacq.) Dunal **(52,53,54,55)**
  - - *helianthoides* (L.) Sweet **(53)**
  - - *longipes* (A.Gray) S.F.Blake **(1,2)**
- • Salmea
  - - *scandens* (L.) DC. **(3,4,12,14,22,286)**

Liliaceae
- + Lilieae
  - • Lilium
    - - *sp. (Lilium)* **(265)**

Malvaceae
- + Gossypieae
  - • Gossypium
    - - *hirsutum* L.**(272)**
- + Hibisceae
  - • Abelmoschus
    - - *esculentus* (L.) Moench **(272)**

Meliaceae
- + Aglaieae
  - • Aglaia
    - - *tenuicaulis* Hiern **(258,259,260)**

Menispermacea
- + Menispermeae
  - • Cissampelos
    - - *glaberrima* A.St.-Hil. **(80,243)**

Not-plant
(**270**)

Piperaceae
- + Pipereae
  - • Piper
    - - *aduncum* L**(283)**
    - - *amalago* L.**(274,280)**
    - - *arboreum* Aubl. **(287,288,289,290)**
    - - *demeraranum* (Miq.) C.DC. **(281)**
    - - *longum* L. **(80,234,237,238,239,240,241,242,243,273)**
    - - *nigrum* L. **(80,86,229,230,231,232,233,234,235,236,310)**
    - - *retrofractum* Vahl **(66,80,86,133,240,241,244,245, 261)**
    - - *sarmentosum* Roxb. **(66,80,86,243,244,245,246)**
    - - *sylvaticum* Roxb. **(293)**
    - - *tuberculatum* Jacq. **(292,293,294,295)**

Poaceae
- + Andropogoneae
  - • Zea
    - - *mays* L. **(272)**
- + Bambuseae
  - • Phyllostachys
    - - *bambusoides* Siebold & Zucc. **(269,308)**
- + Paniceae
  - • Pennisetum
    - - *americanum* (L.) Leeke [nom. illeg.] **(263)**

Rhamnaceae
- + Phyliceae
  - • Phylica
    - - *pubescens* Phylica **(269)**

Rosaceae
- + Pyreae
  - • Pyrus
    - - *communis* L. *(Pyrus)* **(264)**
- + Rubeae
  - • Rubus
    - - *idaeus* L. **(264)**

Rutaceae
- + Amyrideae
  - • Amyris
    - - *balsamifera* L. **(197)**
- + Phaseoleae
  - • Glycine
    - - *max* (L.) Merr. **(251,272)**
- + Vicieae
  - • Pisum
    - - *savitum* L. **(272)**
  - • Vicia
    - - *faba* L. **(264)**

Hippocastanaceae
- + Aesculeae
  - • Aesculus
    - - *hippocastanum* L. **(264)**

Lauraceae
- + Perseeae
  - • Persea
    - - *gratissima* [nom. illeg.] **(263)**
- + Clauseneae
  - • Clausena
    - - *indica* (Dalzell) Oliv. *(Clausena)* **(224,225,226,227,228)**
    - - *lansium* (Lour.) Skeels **(51)**
  - • Glycosmis
    - - *chlorosperma* (Blume) Spreng. **(254,255,345)**
    - - *calcicola* B.C.Stone [nom. illeg.] **(343)**
    - - *parviflora* (Sims) Little **(344)**
    - - *cyanocarpa* (Blume) Spreng. **(256,299,302,303,305,306)**
    - - *mauritiana* (Lam.) Tanaka **(296,297,298,304,307)**
- + Pilocarpeae
  - • Pilocarpus
    - - *trachyllophus* Holmes [nom. illeg.] **(80)**
- + Zanthoxyleae
  - • Zanthoxylum
    - - *ailanthoides* var. ailanthoides **(161,164,184,185)**
    - - *armatum* DC. **(188,189)**
    - - *beecheyanum* K. Koch [nom. illeg.] **(162,206,207,208,209,210,211,212,213, 214,215,217)**
    - - *bungeanum* Maxim. **(158,159,160,161,164,168,169,170, 172,173,174)**
    - - *gilletii* (De Wild.) P.G.Waterman **(80)**
    - - *heitzii* (Aubrév. & Pellegr.) P.G.Waterman [nom. illeg.] **(186,187,188)**
    - - *fagara* (L.) Sarg. **(193,194,195,196)**
    - - *integrifolium* (Merr.) Merr. **(134,161,164,165,166,167,168,169,170)**
    - - *lemairie* [nom. illeg.] **(86,191,192,199,238,242)**
    - - *macrophylla* [nom. illeg.] *(Zanthoxylum)* **(188,238,243,311)**
    - - *piperitum* (L.) DC. [nom. illeg.] **(158,159,161,162,163,164,172,175,176, 177,178,179,180,181,182,183,315)**
    - - *planispinum* Siebold & Zucc. [nom. illeg.] **(158,162)**
    - - *rubescens* Planch. ex Hook. **(191,198,199,200,201,202,203, 204,309)**
    - - *schinifolium* Siebold & Zucc. **(158,159,161,168,170,172)**
    - - *setulosum* P. Wilson **(276)**
    - - *simulans* Hance **(212,213,214,218,219,220,221,222, 223)**
    - - *thomense* A.Chev. ex Waterman [nom. illeg.] **(199)**

Salicaceae
- + Saliceae
  - • Salix
    - - *sp. (Salix)* **(263)**

Scrophulariaceae
- + Antirrhineae
  - • Chaenorhinum
    - - *minus* (L.) Lange **(278)**
  - • Schweinfurthia
    - - *papilionacea* Boiss. [nom. illeg.] **(278)**

Solanaceae
- + Capsiceae
  - • Capsicum
    - - *annuum* L. **(253,264,266,269,271,317)**
    - - *pubescens* Ruiz & Pav. **(319,320,321)**
- + Nicotianeae
  - • Nicotiana
    - - *tabacum* L. **(263)**
- + Solaneae
  - • Solanum
    - - *lycopersicum* Lam. **(263,264,272)**

Synthetic
(64,284,291,316,318,322,323,324,325,326,327,328,329,330,331,332, 333,334,335,336,337,338,339,340,341)

Zygophyllaceae
- + Tribuleae
  - • Tribulus
    - - *terrestris* L. **(266,285)**

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

which are not generally accepted are indicated with [nom. illeg.]. In Fig. 2, some representative structures are presented, while the numbers between brackets refer to the molecule identities in accordance to the NAAs in the database and Table S1.

3.2.1.1. Asteraceae family. Some typical chemical properties can be assigned to individual families. Beside the alkene (double bond) fatty acid patterns (*e.g.* **1**), which are found in most NAA containing plant families, alkyne (triple) chains (*e.g.* **25**) are only present in Asteraceae (Greger, 1984). Christensen et al. reviewed the occurrence of acetylenes and related compounds (including NAAs) in three different tribes of the Asteraceae family (Asteracea, Heliantheae and Anthemideae) (Christensen, 1992; Christensen and Lam, 1991a,b). Additionally, NAAs were found in the Asteraceae–Senecioneae tribe (Ndom et al., 2010).

In the Asteracea tribe (Brachycome genus), only one NAA, dodeca-2*E*,4*E*,8*E*,11-tetraene acid isobutylamide (**273**) was found (Christensen and Lam, 1991a).

Greger first reported 21 different NAAs in the Heliantheae tribe (Greger, 1984). Anno 2011, more than 70 NAAs have already been identified herein. Their fatty acid moiety contains a C_4, C_6, C_8–C_{16} or C_{18} chain, while the amide residue can be an isobutylamide (IBA), 2-hydroxy isobutylamide (2-OH IBA), 2-methylbutylamide (2-MBA), saturated phenylethylamide (PEA) or unsaturated (1*E*/*Z*) phenylethylamide (styrylamides) (Christensen and Lam, 1991b). These styrylamides were only identified in *Spilanthes alba* (*e.g.* **25**) and can possess an epoxy-derivative in its acid chain (**27**) (Bohlmann et al., 1980). In addition, this epoxy group was identified with a PEA residue in *Spilanthes acmella*, *radicans* and *ciliata* and in *Salmea scandens* (**12**) (Bohlmann et al., 1985; Boonen et al., 2010; Martin and Becker, 1985; Rios-Chavez et al., 2003). Globally, C_8–C_{14} IBAs, 2-MBAs and PEAs are documented in the *Spilanthes* genus. The short chained C_8 NAAs are only identified in *Spilanthes radicans* and *Spilanthes ciliata*. Moreover, the isovalerate ester (in 10-hydroxyspilantholisovalerate (**29**) and 10-hydroxyspilanthol-3-methylacrylate (**30**)) has simply been demonstrated in *Spilanthes ciliata* (Martin and Becker, 1984). NAAs with fatty acid moieties, containing hydroxy- and dihydroxy groups, are found in *Spilanthes ciliata* and *Spilanthes callimorpha* (*e.g.* **16, 17**). Both are also reported in the Rutaceae (*Zanthoxylum piperitum*) (Hatano et al., 2004), while the hydroxyl-derivative only, has been detected in Asteraceae–Anthemideae (*Anacylcus monanthos*) (**88**) and Piperaceae family (*e.g. Piper nigrum*) (*e.g.* **231**) (2005; Siddiqui et al., 2003).

Spilanthol (or affinin) (**1**) is the best known NAA of several *Spilanthes* species, although this deca-2*E*,6*Z*,8*E*-trienoic acid IBA and its 2-MBA derivative (homospilanthol) (**2**) are also found in the *Heliopsis longipes* (Molina-Torres et al., 1996). In contrast, two other *Heliopsis* genera (*buphthalmoides* and *helianthoides*) contain C_{18} NAAs with the rarely occurring pentaene acids (**53**, **54**) (Bohlmann et al., 1983).

Echinaceae NAAs always possess a C_2 unsaturation in their acid chain and have a relatively longer chain acid moiety, starting from C_{11} up to C_{16}. The majority of NAAs in *Echinaceae* are IBAs and 2-MBAs, while no PEAs are present in this genus.

Deviating from all other NAAs in the Heliantheae tribe, the hydroxy cinnamamides (HCAAs) were found in *Helianthus annuus* (**264**) (Martintanguy et al., 1978).

The Anthemideae tribe possesses C_{10}–C_{18} acid moieties linked to various amine parts. Initial investigations indicated that C_{14} fatty acids are predominant in Anthemideae (Greger, 1984). However, not

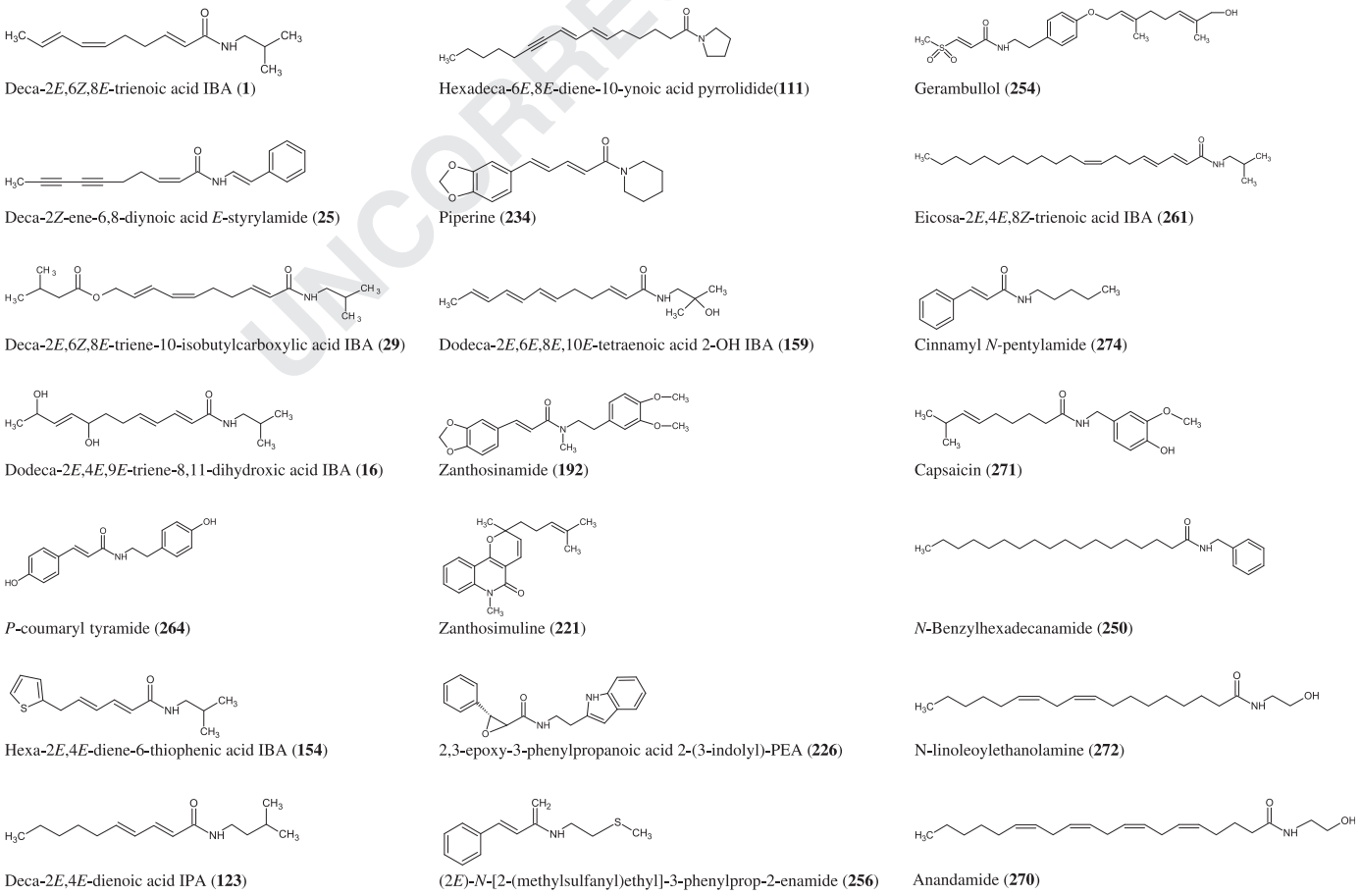


Fig. 2. Some representative NAA structures found in different plant families.

UNCORRECTED PROOF

only anacycline (**78**) and its derivatives (C_{14}), but also pellitorine-like homologs (C_{10}) are widely distributed in various genera of the Anthemideae (*e.g. Achillea*, *Anacyclus*, *Artemisia*, *Leucocyclus*, *Chamaemelum*, *Cladanthus*, *Argyranthemum* and *Matricaria*) (Christensen, 1992). Since then however, those fatty acid moieties are also found in the Heliantheae tribe and other families: Rutaceae, Piperaceae, Aristolochiaceae and Menispermacea (AndradeNeto et al., 1996; Burden and Crombie, 1969; Greger et al., 1981; Greger and Werner, 1990; Saadali et al., 2001; Weenen et al., 1990b; Yasuda et al., 1981a). Nevertheless, the thiophene fatty acid moiety is a typical characteristic of Anthemideae, which was identified in *e.g. Otanthus* and *Chrysanthemum* genera (*e.g.* **154**, **157**) (Bohlmann and Wegner, 1982; Bohlmann and Zdero, 1967; Bohlmann et al., 1974; Greger and Hofer, 1984). The Anthemideae also possesses typical NAA amine residues. In addition to the common IBAs, 4-hydroxy PEAs (tyramides) (*e.g.* **83**, **84**, **85**) occur, which are also found in several other families like Piperaceae and Rutaceae (Burden and Crombie, 1969; Greger et al., 1981; Kubo et al., 1984; Martintanguy et al., 1978; Matsuda et al., 2009; Stöhr et al., 1999). More recently, other tyramides, with an alkynic structure on the acid side, were identified in *Anacyclus pyrethrum* (Boonen et al., submitted for publication) (*e.g.* **279**), while from another Asteraceae tribe, Senecioneae, the fully saturated pentacosyl tyramide was identified (**262**) (Ndom et al., 2010). The biogenetic characteristic isopentylamides (IPAs) were only described in *Achillea* (*e.g.* **123**) (Greger and Hofer, 1987), while the *N*-methyl IBA (*e.g.* **79**) are found in the Anthemideae tribe (*Anacyclus pyrethrum*) (Jente et al., 1972) as well as in the Rutaceae family, encompassing Clauseneae tribe (Riemer et al., 1997) and Zanthoxyleae tribe (Adesina et al., 1997; Adesina and Reisch, 1989; Cheng et al., 2004). In Anthemideae, the amide part occurs particularly in cyclic systems (*i.e.* piperidide, piderideide, pyrrolide, pyrrolidide, 2,3-didehydropyrrolidide). Pyrrole amines, encompassing pyrrolidides (*e.g.* **111**), 2,3-didehydropyrrolidides (*e.g.* **144**) and pyrrolides (*e.g.* **110**), are mainly present in *Achillea* even though pyrrolidides have also been identified in the Convolvulaceae as well as in the Meliaceae family (Greger et al., 1981, 1983, 1984, 1987, 2008; Tofern et al., 1999). In the latter case, it concerns a bisamide structure (**259**) (Greger et al., 2008). The piperidide amides are typically found in Anthemideae tribe and Piperaceae family. Unlike the Anthemideae, the Piperaceae piperidides principally enclose a 3,4-(methylenedioxy)phenyl residue in their acid group (*e.g.* **234**). On the other hand, the 2,3-didehydroderivatives of piperidides (piperideides) (*e.g.* **93**) are only seen in the Anthemideae and more particularly in *Achillea* species (Greger et al., 1981, 1983, 1984; Greger and Werner, 1990; Wu et al., 2004). The 4-hydroxic piperideide (**140**) was reported exceptionally in *Achillea falcata* (Greger et al., 1983). Finally, the pyrrole and piperidide amides with completely saturated C_{18} fatty acid chains and their corresponding dehydro-derivatives were only reported in *Achillea lycaonica* (**146–149**) (Greger et al., 1982).

3.2.1.2. Rutaceae family. NAAs found in Rutaceae only possess alkenic structures in their acid part. The conventional straight chain NAAs consist mainly out of C_{12} and C_{14} acid moieties. The *Zanthoxylum* genus possesses the so-called sanshools. This name is derived from the Japanese term sanshō (*i.e.* Sichuan pepper, the outer pod of the fruit of various *Zanthoxylum* species), added with the suffix -ol, demonstrating the possible presence of an alcohol group at the amine side of these NAAs. Dependent on their double bond configuration, the dodeca-2,6,8,10-tetraenoic acid IBAs are known as α-, β-, and ε-sanshools (*e.g.* **158-160, 162, 163, 175, 176**) (Chen et al., 1999; Jang et al., 2008; Kashiwada et al., 1997; Yang, 2008; Yasuda et al., 1982), while the γ-sanshools are tetradeca-2,4,8,10,12-pentaenoic acid derivatives (*e.g.* **161, 164, 172, 174, 315**) (Chen et al., 1999; Iseli et al., 2007; Kashiwada et al., 1997; Xiong et al., 1997; Yang, 2008). The *Zanthoxylum* genus also contains the bungeanools (*e.g.* **168, 169, 170, 173**) which are typically tetradeca-2*E*,4*E*(,8,10)-di-, tri- or tetraenoic acid 2-hydroxy IBAs (Chen et al., 1999; Iseli et al., 2007; Xiong et al., 1997). Their derivatives, enclosing an oxo group in their fatty acid part and/or lacking the hydroxyl group at the IBA part, are the lanyuamides (*e.g.* **165, 166, 184, 185**) (Chen et al., 1999; Cheng et al., 2003). Next to C_{14} tetradeca-2*E*,4*Z*-dienoic acid IBA (**86**), the 2*E*,4*E*-dienoic acid IBA homologs with chain length C_8 (**243**), C_{10} (pellitorine) (**80**) and C_{20} (**242**), like in the Asteraceae family (*e.g.* **66, 99, 135, 142**) and Piperaceae family (*e.g.* **244, 245, 246**), are present. Pellitorine was not only identified in *Zanthoxylum*, but also in *Pilocarpus* (Kalia et al., 1999; Kubo et al., 1984; Yasuda et al., 1981a). Furthermore, NAAs from the Rutaceae family can be composed of a *2E*/*Z*-phenylethyl derivative as fatty acid, like (**187–192**, **199-204, 206, 207, 215, 217**). These NAAs are cinnamamides, which include an IBA (*e.g.* **188**), (di)methoxy phenylethyl (*e.g.* **200, 206**) or 3,4-methylenedioxy phenylethyl (*e.g.* **204**) amine moiety (Adesina et al., 1997; Adesina and Reisch, 1989).

Apart from the NAAs described above, the Rutaceae family includes compounds in which the entire amide function is part of one cyclic system, mainly 6-membered (*Zanthoxylum*) (*e.g.* **221**) (Chen et al., 1997; Cheng et al., 2004; de Moura et al., 2002), but occasionally 8-membered (*Clausena*) (*e.g.* **227, 228**) rings (Riemer et al., 1997). These NAAs frequently harbor three to five cyclic units. Moreover, in the *Clausena* genus, tryptamine derived NAAs were identified (*e.g.* **226**) (Riemer et al., 1997). Finally, sulfur-containing NAAs are described in the leaves of Rutaceae *Glycosmis* genera, which represent a typical chemical character of this genus (Chansriniyom et al., 2009; Cuong et al., 1999; Greger et al., 1992, 1993a,b, 1996; Hinterberger et al., 1994, 1998; Hofer et al., 1995, 2000; Rahmani et al., 2004, 2010). Both, in the fatty acid residue, as well as in the amine moiety, a sulfur atom can occur, mostly two carbon atoms away from the amide function (*e.g.* **256**) (Greger et al., 1992, 1993a,b, 1996; Hinterberger et al., 1998). The sulfur moieties, most probably derived from the amino acid cysteine, can additionally be oxidized to sulfones and sulfoxides or shortened by α-oxidation (*e.g.* **254**) (Chansriniyom et al., 2009; Greger et al., 1994, 1996; Hofer et al., 2000; Rahmani et al., 2010). Moreover, Meliaceae comprises sulfur containing amides (*e.g.* **258**), but also bisamides (*e.g.* **265**) and an amide alcohol (*e.g.* **260**), which all can be defined as cinnamamides (Greger et al., 2008).

3.2.1.3. Piperaceae family. The NAAs from Piperaceae were thoroughly reviewed by Strunz, (2000). Shortly, they have a straight chain acid moiety (from C_6 up to C_{26}) (*e.g.* **273**) with or without aromatic terminus, which is often a 3,4-methylenedioxy phenylethyl (*e.g.* **232–239**). In most cases, the acid chain has an even number of carbon atoms when there is no aromatic substituent. On the other hand, harboring an aromatic group, an uneven carbon number is present. The IBA, piperidide and pyrrolidide predominate as amine moiety. Exceptionally, a 2-MBA (*e.g.* **246**), a *n*-pentyl (*e.g.* **274**), isopentyl (*e.g.* **280**), 3,4-didehydro-2-pyrrolidone (*e.g.* **281**), 3,4-didehydro-2-piperidone (*e.g.* **293**) or 4-hydroxy,5-methoxyphenylethyl (*e.g.* **283**) amine moiety can occur. Like in Rutaceae, typical plant cinnamamide derivatives are also present in the Piperaceae family (*e.g.* **274**) (Achenbach et al., 1986).

3.2.1.4. Others. Capsaicinoids are a group of NAAs which are only synthesized in nature in chili pepper fruits (Solanaceae) (Aza-Gonzalez et al., 2011). These pungent components are distinguished from other NAAs by the presence of a vanillin amine moiety (*e.g.* **271**) (Aza-Gonzalez et al., 2011).

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

The Solanaceae and at least seventeen other plant families produce the metabolic important hydroxy cinnamamides (HCAAs) (Amaranthaceae, Aristolochiaceae, Asteracae, Brassicaceae, Bromeliaceae, Caryophyllaceae, Convolvulaceae, Fabaceae, Hippocastanaceae, Lauraceae, Liliacea, Poaceae, Rhamnaceae, Rosaceae, Rutaceae, Salicaceae, Zygophyllaceae) (Cheng et al., 2004; Li et al., 1998; Martintanguy et al., 1978; Tanaka et al., 2003; Tofern et al., 1999; Wu et al., 1994). These NAAs are composed of hydroxycinnamate derivatives as fatty acid part (*e.g.* *p*-coumaryl, di-*p*-coumaryl, caffeoyl, feruloyl, diferuloyl moieties), linked to an aromatic amine (*e.g.* serotonin, tyramine) or polyamine (*e.g.* putrescine, spermidine) part (Facchini et al., 2002; Han et al., 2002; Kang et al., 2010; King and Calhoun, 2010; Martintanguy et al., 1978; Negrel et al., 1996; Park et al., 2009; Parr et al., 2005; Turnock et al., 2001; Yoshihara et al., 1981) (*e.g.* **263-269**).

Next to HCAAs, the Brassicaceae species *Lepidium meyenii* ("Maca") contains benzylated or 3-methoxybenzylated amides which are not found in other plants (Wang et al., 2007). In these so called macamides, the C_{14}–C_{18} or C_{24} fatty acid residues predominate and can be fully saturated (*e.g.* **250–251**) (McCollom et al., 2005; Muhammad et al., 2002; Wang et al., 2007). Moreover, the Zygophyllaceae family contains more complex lignanamides (*e.g.* **285**) (Li et al., 1998), the Convolvulaceae family possesses macrolactam-type indole alkaloids (ipobscurines) (*e.g.* **248**) (Jenett-Siems et al., 2003) and the Euphorbiaceae family contains cyanogenic and non-cyanogenic pyridone derivatives (*e.g.* **257**) (Hungeling et al., 2009).

The *N*-acylethanolamides (NAEs) (*e.g.* **272**) occur in different plant families (Malvaceae, Poaceae, Brassicaceae, Fabaceae, Solanaceae and Euphorbiaceae). Their acid residue contains twelve, sixteen or eighteen carbon atoms, with maximally three double bonds (Chapman, 2004; Lopez-Bucio et al., 2006).

At last, macrobicyclic spermine alkaloids (*e.g.* aphelandrine (**277**)) are a rare class of naturally occurring polyamide conjugates present in the Brassicaceae, Acantahaceae, Scrophulariaceae and Ephedraceae family (Facchini et al., 2002; Nezbedová et al., 2001; Sagner et al., 1998).

Next to this extensive enumeration of NAAs occurring in higher plants, we have included some NAAs from the not-plant biological system in the database *e.g.* a mammalian signaling alkylamide, the NAE anandamide (**270**). This NAAs are classified as "not-plant" NAAs. Moreover, synthetic *e.g.* **64**, as well as semi-synthetic *e.g.* **300**, NAAs are included and referred to as "synthetic".

3.2.2. Biosynthesis

A brief overview of the biosynthetic pathways of the most important plant NAAs is summarized here. They all consist of a fatty acid moiety and an amine part which are combined via an amide linkage.

For NAEs, the fatty acid part is delivered by lauric (12:0), myristic (14:0), palmitic (16:0) or linoelaidic (18:2, *cis*,*cis*) acid (Chapman, 2004). Most straight chain fatty acids derive from non-aromatic acyl precursor, like oleic (18:1), linoleic (18:2) and linolenic (18:3) fatty acids (Greger and Hofer, 1987; Greger et al., 1983; Martin and Becker, 1985). Successive dehydrogenations and dehydrations, frequently accompanied by isomerization, lead to characteristic alkenyc and alkynic structures (Greger, 1984), while different oxidative processes contribute to chain shortening or epoxide structures (Christensen and Lam, 1991a; Greger, 1984; Greger et al., 1983, 1987; Martin and Becker, 1985). A well-known exception is *e.g.* capsaicin (**271**), where the acid part arises from isobutyryl CoA and three acetyl groups (Keipert, 2009). Aromatic fatty acid chains (*e.g.* piperine, **234**) are derived from the shikimic acid pathway (Strunz, 2000), while sulfur atoms are delivered by a cysteine unit (*e.g.* **255, 256**) (Greger et al., 1993a; Keipert, 2009). In HCAAs, the fatty acid part is delivered by hydroxy-cinnamic acids, like *p*-coumaric (*e.g.* **269**), ferulic (*e.g.* **266**), sinapinic (*e.g.* **267**) and caffeic acid (Handrick et al., 2010; Kang et al., 2010; Kang and Back, 2006).

The amine moiety of NAEs is provided by phosphatidylethanolamine. In other NAAs, amines often derive from decarboxylation of different biogenic amino acids. Valine, isoleucine, phenylalanine, tyrosine and leucin serve as precursors for the isobutyl-, methylbutyl-, phenylethyl-, 4-hydroxyphenylethyl- and isopentylamine, respectively (Greger, 1984; Keipert, 2009). After cyclisation and decarboxylation of lysine or cadaverine, the piperidine and piperideine amines arise. The similar biosynthesis of pyrrolidine rings from ornithine/putrescine was noted (Strunz, 2000). However, decarboxylation of a proline derivative also produces pyrrolidines (Strunz, 2000). Further dehydrogenation of pyrrolidines leads to pyrrolines and 2,3-didehydropyrrolidines (Greger, 1984). Decarboxylation of tyrosine, tryptophan and dihydroxyphenylalanine yields the amine moieties for the HCAAs (*e.g.* tyramine, tryptamine, serotonin) (Kang and Back, 2006).

The amide formation occurs via an enzyme-catalyzed reaction of the fatty acid part with the amino part. Synthesizing NAEs, NAPE synthase links free fatty acids with phosphatidylethanolamine molecules, resulting in *N*-acetylphosphatidylethanolamines. Next, NAEs are formed from *N*-acylphosphatidylethanolamine by phospholipase D, with a release of phosphatidic acid (Chapman, 2004). For most other NAAs, including the HCAAs and macrocyclic polyamides, specific transferases condense the CoA thioesters activated fatty acid with the amine part (Kang and Back, 2006; Martin and Becker, 1985; Nezbedová et al., 2001). For example, in the synthesis of HCAAs, the family of the BAHD-like acyltransferases is responsible for the transfer of het hydroxyl-cinnamoyl residues from CoA to the amine (Handrick et al., 2010).

3.2.3. Intrinsic role in the plant

Only for a few NAA subclasses, the botanical meaning was investigated. These studies focused mainly on the involvement of NAAs in growth and development processes and on their antimicrobial defense.

HCAAs are believed to act as controlling agents in several developmental processes like sexual organogenesis, cytomorphogenesis, floral induction and flower formation (Facchini et al., 2002; Kang and Back, 2006), while straight chain NAAs like spilanthol and derivatives were found to promote growth and alter root development in a concentration dependent manner (Ramirez-Chavez et al., 2004). NAEs on the contrary, inhibit the seedling root development, possibly due to their inhibitory effect on phospholipase D or their interaction with plant hormones (Kim et al., 2010b).

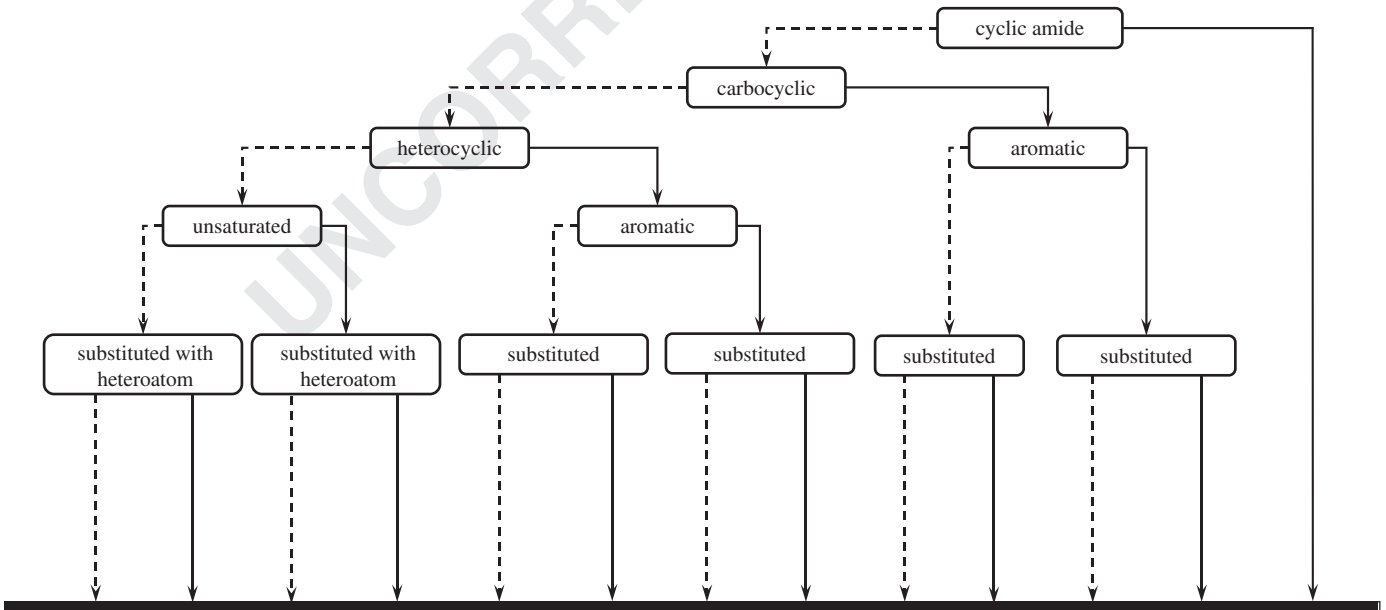
The defensive properties of capsaicinoids and HCAAs were documented (Ramirez-Chavez et al., 2004; Tewksbury et al., 2008). Because four nitrogen possessing macrocyclic amides consume quite some energy for their biosynthesis, their importance in the plant physiology and role against endophytic or pathogenic fungi was postulated (Werner et al., 1997). For NAEs, the mechanism of their protective role was explored (Kim et al., 2010b). NAE accumulation was assumed to play a role in pathogen defenses by two hypothetical mechanisms: (1) NAE accumulation could modulate the level of other lipids (*e.g.* phosphatidic acid) in response to pathogens or (2) NAEs might interfere with the quorum sensing mechanism (*i.e.* inter-bacterial communication to coordinate their activities) of bacteria. A binding protein for NAEs with properties similar to the NAE receptor of vertebrates (cannabinoid receptor) has also been identified in plant membranes. Therefore, it is believed that NAEs are endogenous signaling compounds in plant systems (Chapman, 2004; Kim et al., 2010b). Finally, macrocyclic polyamides are believed to play a role in the cell metal ion homeostasis (uptake, turnover and transport of metal ions) (Nezbedová et al., 2001).

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

3.3. Chemical space

From a chemical point of view, NAAs are clearly a heterogeneous class of structurally different molecules (see Fig. 1). Currently, there is no consistent formal classification system for the NAAs, and different subgroups are used by the various research groups *e.g.* *N*-isobutyl-, 2-methylbutyl-, isopentyl-, phenylethyl amides, sanshools, tyramides, capsaicinoids, HCAAs and NAEs (Kim et al., 2010b). Unlike the lipid classification system (LIPID MAPS), based on well-defined (bio)chemical pathways and hydrophobic/hydrophilic principles (Fahy et al., 2005, 2009), for the first time, a chemical division for NAAs is presented here. NAAs are constructed of a fatty acid part and an amino part. In Fig. 1 the R^1 group corresponds with the fatty acid part, while the amine part is presented by the R^2 group. Our proposed classification is build up from these two parts, which are characterized by a series of repeated methyl groups. For both parts, thirteen similar groups are defined. They can differ in length, degree, place and configuration of unsaturation. They can possess heteroatoms and carbocyclic and/or heterocyclic (whether or not aromatic and/or substituted) systems. The NAA structured classification name starts with "F" (indicative for fatty acids part) followed by the fatty acid category (from 1 to 13) and ends with "M" (indicative for the amino part) followed by the amino category (from 1 to 13). Combining the F part with the M part yields 25 chemical NAA classes. Fig. 3 presents the hierarchic scheme for classification of the NAAs. Number #1 stands for saturated chain NAAs which are not substituted with a heteroatom (sulfur, nitrogen, oxygen), while #2 points to the saturated substituted chains. Their length can differ from C_0 up to at least C_{16} for the fatty acid part. On contrary, a $ROCNH_2$ amide part does not meet the definition of *N*-"alkyl"amide and hence, minimally one methyl group must be present at the amino side. Unsaturated chains, not substituted and substituted with a heteroatom are assigned in group #3 and #4, respectively. All groups can be branched with methylene entities. Heterocyclic NAAs may include a sulfur, oxygen or nitrogen (whether or not incorporating the nitrogen of the amide structure). They can be non-aromatic, non-substituted (#5), non-aromatic, substituted (#6), aromatic, non-substituted (#7) or aromatic, substituted (#8). Substituted cyclic systems involve an additional group at the opposite site of the amide entity. Moreover, carbocyclic systems, only including carbons in their ring structure, are defined. Like heterocyclic systems, they may possess non-aromatic, non-substituted (#9), non-aromatic, substituted (#10), aromatic, non-substituted (#11) or aromatic, substituted (#12) units. At last, NAAs of which the entire amide group is included in a cyclic part are categorized in one group (#13). As for the cyclic amides, the fatty acid as well as the amine part is the same: only the F13M13 combination can be made. Examples for each category are presented in Fig. 3, while for each NAA the chemical classification name is given in the Alkamid database. Where our classification used a simple and short FxMy nomenclature, the LIPID MAPS assigns a twelve character LIPID ID to its lipids, containing a fixed database designation (LM) followed by a two letter category code (*e.g.* FA), a two digit class code (*e.g.* 03), a two digit subclass code (*e.g.* 02) and terminated by a unique four character identifier within subclass. The LIPID MAPS contains eight lipid categories, of which one, the fatty acid category, includes the fatty amide class. This class has four subclasses with some of them enclosing a limited number of specific NAAs. The first subclass, the primary amides, cannot be considered as NAAs. The second subclass is a large *N*-acyl amine group, containing – whether or not unsaturated – C_{16} up to C_{22} fatty acid parts combined with diversity of amine groups mostly consisting of a simple alkyl-group or a peptide derived entity. The quorum sensing fatty acyl homoserine lactones are defined as a small, third subclass. The fourth subclass contains the *N*-acyl ethanolamines (*e.g.* endocannabinoids). Unlike the LIPID MAPS



#	1	2	3	4	5	6	7	8	9	10	11	12	13
F	**213** (F1M12) **225** (F1M11)	**304** (F2M11) **307** (F2M11)	**20** (F3M11) **230** (F3M1)	**29** (F4M1) **148** (F4M5)	-	**12** (F6M11) **110** (F6M7)	**154** (F7M1)	**157** (F8M1)	**198** (F9M12)	-	**191** (F11M12) **260** (F11M2)	**264** (F12M12) **186** (F12M1)	
M	**2** (F3M1) **88** (F4M1)	**158** (F3M2) **256** (F11M2)	-	**258** (F11M4) **281** (F12M4)	**97** (F3M5) **247** (F1M5)	**140** (F3M6) **259** (F11M6)	**107** (F3M7) **110** (F6M7)	-	-	-	**249** (F4M11) **275** (F6M11)	**83** (F3M12) **189** (F12M12)	**193** (F13M13) **257** (F13M13)

Fig. 3. NAA classification scheme.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

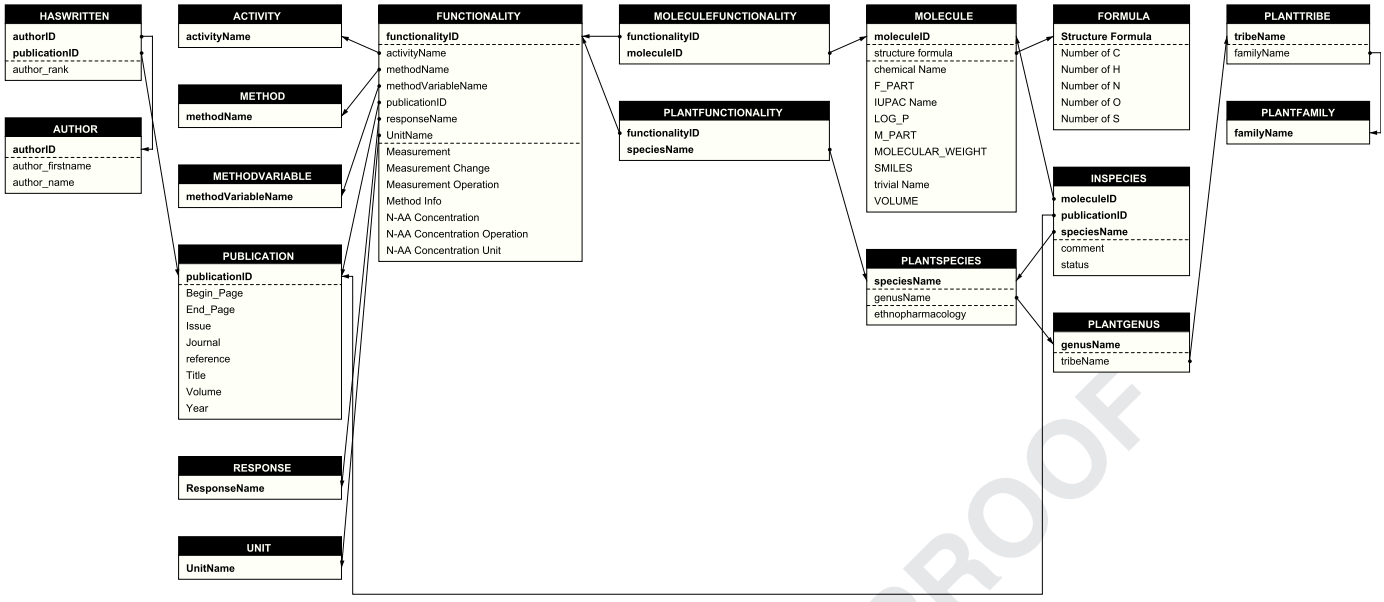


Fig. 4. Scheme of Alkamid database design.

classification, which serves a more global objective, our NAA classification only considers a chemical approach, dividing them in more categories ($n=25$) compared to the fatty amine LIPID MAPS classification ($n=4$). This increased classification allows *i.a.* fine tuning in SAR studies. This approach is interesting for future linking of NAAs to specific functionalities. The reason is the not fully comprehensive stage of database. It is very plausible that in future, some representatives will be assigned to these categories.

3.4. Functional space

In this section, the different bioactivities of NAAs are generally described. It is impossible to cite all papers exhaustively in this field and therefore the main emphasis is placed on reviews and recent investigations in the field. NAAs elicit multiple functional actions, which make them very promising in the development of novel drugs. However, whole plant extracts, sometimes even without a reasonable purification or characterization of NAAs, are frequently used in pharmacological assays. This approach can only yield pilot information, as compounds other than NAAs could contribute or even be the main responsible for the observed pharmacological effects. Only using well-characterized compounds, as mono- or combination preparation, unequivocal conclusions can be made on their pharmacological effects (Goel et al., 2002; Matthias et al., 2008). In this context, it should be mentioned that NAAs cannot automatically freely interact with target receptors as they can form micelles above their critical micelle concentration. For dodeca-2*E*,4*E*-dienoic acid IBA (66) in water ($\pm$0.05% BSA) for example, a CMC range of 200–300 nM was established (Raduner et al., 2007). This is the lowest CMC ever reported for a bioactive natural products. Seen the outcome of *in-vitro* as well as *in-vivo* experiments depends on the solubility and physicochemical behavior, it is recommended to investigate and incorporate the aggregating behavior of NAAs in the evaluation and conclusions of the pharmacological assays. Moreover, the adsorption behavior of NAAs towards surfaces, proteins and membranes is to be considered as well.

3.4.1. Antimicrobial and related activities

Numerous studies dealt with the antibacterial and antifungal, but also with the antiparasitic, molluscicidal and insecticidal activities of NAAs.

Antimicrobial activities of NAAs were evaluated by different test methods, *e.g.* dilution, disk diffusion and even TLC-hyphenated bioassays (Rahalison et al., 1994). In this so-called spray method, a spore suspension in glucose and salt solution was sprayed on TLC chromatograms and incubated 72 h in darkness at 25 °C. The inhibition zones against a dark background indicate the minimal inhibiting amount of the separated compound on TLC.

The antibacterial effect of NAAs was mainly studied by Molina-Torres et al. (1999, 2004, 2008). Minimal inhibitory concentrations (MIC) against several Gram (−) and Gram (+) bacteria are presented in Table 3. Different unsaturated C_{10} IBAs (*e.g* **1**) and capsaicin (**271**) were investigated against Gram (−) bacteria. The specific recognition of their chain length by β-hydroxydecanoyl thioester dehydratase (enzyme responsible for the introduction of a double bond in vital unsaturated fatty acids of *Escherichia coli*) is assumed to be responsible for the observed activity (Molina-Torres et al., 1999). In *Escherichia coli* and *Pseudomonas solanacearum*, a 2*E* unsaturation in the fatty acid chain is preferable for activity. An additional 6*Z*,8*E* unsaturation diminishes the activity. One study found that the investigated NAAs were inactive against Gram (−) *Erwinia carotovora* (Molina-Torres et al., 2008), while another study indicated high antibacterial activity of one of these NAAs against *Erwinia carotovora i.e.* deca-2*E*-enoic acid IBA (Molina-Torres et al., 2004). MIC values of the investigated NAAs for *Escherichia coli*, *Pseudomonas solanacearum* and *Erwinia carotovora* range between 5 and 300 μg/mL. The degree of unsaturation as well as the chain length of the acid moiety influence the growth inhibition of Gram (+) bacteria (*Bacillus subtilis*). For IBAs, a 2*E* unsaturation is favorable (MIC= 25 μg/mL), while full saturation (MIC= 75 μg/mL) and additional 6*Z*,8*E* unsaturation (MIC= 50 μg/mL) decrease the activity. C8 up to C12 NAA have a higher activity (MIC=75 μg/mL) than C6 (MIC=150 μg/mL). For PEA, on the contrary, 2*E* unsaturation in deca-2*E*-enoic acid IBA (MIC=150 μg/mL) has a negative contribution *versus* the fully saturated pattern (MIC=10 μg/mL). This latter C_{10} decanamide also has the optimal chain length compared to the activity of C_8 (MIC=15 μg/mL) > C_6 (MIC=75 μg/mL) > C_{12} (MIC=150 μg/mL) (Molina-Torres et al., 2008). Above, the amide moiety impacts the inhibitory effect, with generally higher activities of PEAs compared to IBAs (Molina-Torres et al., 2008). An invention relates to NAAs of D, L, L(−) and D(+)-carnitine possessing antibacterial activity. These NAA derivatives are prepared via a well-defined

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Table 3
MIC (μg/mL) on bacterial growth of NAAs (+ % inhibition).

NAA	Gram (−)		Gram (+)		Method	Reference
	Escherichia coli	*Pseudomonas solanacearum*	*Erwinia carotovora*	*Bacillus subtilis*		
Isobutylamides (IBA)						
Hexanoic acid (332)	-[na]	-[na]	/	150 (53%)	Diet	Molina-Torres et al. (2008)
Octanoic acid (333)	-[na]	-[na]	/	75 (96%)	Diet	Molina-Torres et al. (2008)
	-[na]	-[na]	-[na]	75 (60%)–150 (100%)	Diet	Molina-Torres et al. (2004)
Decanoic acid (334)	-[na]	-[na]	/	150 (0%)	Diet	Molina-Torres et al. (2008)
	5 (100%)	-[na]	5 (100%)	25 (15%)–50 (100%)	Diet	Molina-Torres et al. (2004)
Deca-2*E*-enoic acid (335)	-[na]	-[na]	/	150 (0%)	Diet	Molina-Torres et al. (2008)
	25 (90%)	50 (20%)–150 (100%)	-[na]	50 (20%)–150 (100%)	Dilution	Molina-Torres et al. (1999)
Deca-2*E*,6*Z*,8*E*-trienoic acid (1)	75 (100%)	-[na]	/	50 (20%)–150 (100%)	Diet	Molina-Torres et al. (2004)
Dodecanoic acid (336)	-[na]	-[na]	/	75 (98%)	Diet	Molina-Torres et al. (2008)
Vanillyl amide (3-methoxy,4-hydroxy-benzyl amide)						
8 Methyl-Nona-6*E*-enoic acid (271)	300 (0%)	300 (20%)	-[na]	25 (100%)	Dilution	Molina-Torres et al. (1999)
Phenylethylamides (PEA)						
Hexanoic acid (337)	-[na]	-[na]	/	75 (97%)	Diet	Molina-Torres et al. (2008)
Octanoic acid (338)	-[na]	-[na]	/	15 (92%)	Diet	Molina-Torres et al. (2008)
Decanoic acid (339)	-[na]	-[na]	/	10 (93%)	Diet	Molina-Torres et al. (2008)
Deca-2*E*-enoic acid (340)	-[na]	-[na]	/	150 (92%)	Diet	Molina-Torres et al. (2008)
Dodecanoic acid (341)	-[na]	-[na]	/	150 (0.9%)	Diet	Molina-Torres et al. (2008)

-[na]: not available.
/: no inhibitory effect up to a dosage of 150 μg/mL.

UNCORRECTED PROOF

process, whereafter the pharmaceutical and cosmetic compositions are made comprising an amount of at least one of the NAAs suitable for promoting an effective antibacterial action. Toxicological tests via the oral route and antibacterial and antidandruff activity were performed (Cavazza and Fiorentini, 1988, 1989). Administration of plants containing ingredients with pronounced antimicrobial activity (*e.g. Spilanthes mauritiana*) eliminates the resistance features of antibiotics. Prepared extracts can be used to treat different infectious diseases of the gastro-intestinal tract, infections of the eye, infections of wounds, mucosa and skin, etc. (Wabnitz and Angsorg, 1997).

Antifungal effects of NAA were discussed manifold (Table 4). Generally, a 2*E* unsaturation at the acid or the amine side is favorable for fungal growth inhibition. NAAs possessing a sulfur atom in their acid or in their amine part showed increased antifungal effects. From the NAAs with a sulfur atom in the acid chain, the methylthioethyl imides are highly toxic towards *Cladosporium herbarum*, especially penimide A (**302**) ($MIC_{penimide\ A} \sim 4$ μM). Methylthioethyl amides with a secondary amine part (like penangin (**300**) and isopenangin (**301**)) however, are methylthioethyl imide artifacts. These NAAs are formed during extraction and storage with methanol and have no antifungal activity (Pacher et al., 2010). The phenylethyl group at the amine side is thus essential for the activity. The methylthiocarbonic acid NAAs combined with a phenylethyl amine group (ninarins (**304–306**)) are also antifungal, however, more than 100 times less than penimide A. The all-*trans* dehydroninarin B was shown to be more effective than others due to different steric hindrance of the rotation about the amide C–N bond. Changing the methylthioethyl to the amine side and the phenylethyl group to the acid part gives moderate antifungal NAAs (illukumbins (**296–298**) and sinarhins (**256, 299**)) (MIC ~ 40–130 μM). The effect is more pronounced with a 2*E* unsaturation > a 2*Z* unsaturation > fully saturated methylthioethyl amine. A similar MIC effect is seen with the pyrrolidine NAAs possessing a benzo[1,3]dioxol acid group (*e.g.* **289**). Their related piperidines (*e.g.* **292**), dihydropyridones (*e.g.* **294**) and IBAs (*e.g.* **188**) are nearly ineffective (MIC in mM range). In straight chain acid IBAs, like spilanthol (**1**), the 2*E*,6*Z*,8*Z* unsaturation is needed for fungal inhibition. The 2*E*- or fully unsaturated spilanthol derivatives, pellitorine (**80**) and fagaramide (**188**) are ineffective against fungal growth (MIC in mM range). Sanshools also do not have any significant bacterial or fungal activity (Jang et al., 2008).

The antiparasitic activity of some NAAs was evaluated. The antiplasmodial activity of some NAAs is summarized in Table 5. From different studies, it was suggested that the α,β-unsaturated carbonyl function rather than the *N*-isobutyl substituent is the responsible active site (Penali et al., 2007; Sittie et al., 1998; Weenen et al., 1990a). Longer, straight chain acid moieties and substituted (rather than primary) amines seem advantageous. Besides the moderate antiplasmodial effect, a high antitrypanosomal activity of sulfonyl-containing NAAs was documented (**343**) (Astelbauer et al., 2010). The EC_{50} values against *Trypanosoma cruzi* of methyldambullin (**344**), methylgerambullin (**343**) and sakambullin (**345**) after 72 h exposure were 1.70, 1.23,

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

Table 4
MIC (μg/mL) and % inhibition between brackets of NAAs on fungal growth.

NAA	*Sclerotium rolfsii*	*Cladosporium sphaerospermum*	*Cladosporium cladosporioides*	*Cladosporium herbarum*	*A. take*	*Hypocrella bambusae*	*Shiraia bambusicola*	Method	Reference
Deca acid IBA (**3 3 4**)	/	-na	-na	-na	-na	-na	-na	Diet	Molina-Torres et al. (2004)
Deca-2*E*-ene acid IBA (**335**)	/	-na	-na	-na	-na	-na	-na	Diet	Molina-Torres et al. (2004)
Spilanthol (**1**)	50 (100%)	-na	-na	-na	-na	-na	-na	Diet	Molina-Torres et al. (2004)
Penta-2*E*,4*Z*-ene-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidide (**287**)	-na	1000	-na	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Arboreumine (**288**)	-na	500	-na	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Penta-2*E*-ene-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidide (**289**)	-na	10	10	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Penta-2*E*,4*E*-ene-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidide (**290**)	-na	10	10	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Penta-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidide (**291**)	-na	500	-na	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Pellitorine (**80**)	-na	-na	500	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
$\Delta^{\alpha\beta}$dihydropiperine (**292**)	-na	-na	500	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
Piplartine (**293**)	-na	-na	500	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)
	-na	10	500	-na	-na	-na	-na	Spray	Vasques-da-Silva et al. (2002)

UNCORRECTED PROOF

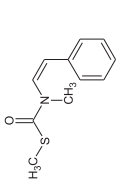
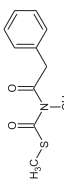
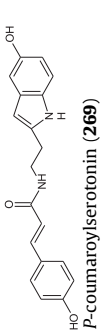
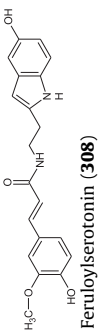
Dihydropiplartine (**294**)	_na	_na	500	_na	_na	_na	_na	Spray	Vasques-da-Silva et al. (2002)
Cis-piplartine (**295**)	_na	_na	1000	_na	_na	_na	_na	Spray	Vasques-da-Silva et al. (2002)
Fagaramine (**188**)	_na	_na	10.0	5.5 (50%)	_na	_na	_na	Spray, dilution	Greger et al. (1993b) and Greger et al. (1996)
Methylillukumbin A (**296**)	_na	_na	10.0	_na	_na	_na	_na	Spray	Greger et al. (1993b)
Methylillukumbin B (**297**)	_na	_na	10.0	_na	_na	_na	_na	Spray	Greger et al. (1993b)
Illukumbin B (**298**)	_na	_na	20.0	_na	_na	_na	_na	Spray	Greger et al. (1993b)
Sinharine (**256**)	_na	_na	30.0	17.6 (50%)	_na	_na	_na	Spray, dilution	Greger et al. (1993b and Greger et al. (1996)
Methylsinharin (**299**)	_na	_na	/	> 200 (50%)	_na	_na	_na	Spray	Greger et al. (1993b)
Penangin (**300**)	_na	_na	/	_na	_na	_na	_na	Spray	Greger et al. (1993b)
Isopenangin (**301**)	_na	_na	/	0.9	_na	_na	_na	Spray	Greger et al. (1993b)
Penimide A (**302**)	_na	_na	/	_na	_na	_na	_na	Spray	Greger et al. (1993b)
Penimide B (**303**)	_na	_na	_na	28.0 (50%)	_na	_na	_na	Dilution	Greger et al. (1996)
Niranin (**304**)	_na	_na	_na	100.0 (50%)	_na	_na	_na	Dilution	Greger et al. (1996)
Dehydroniranin A (**305**)	_na	_na	_na	8.4 (50%)	_na	_na	_na	Dilution	Greger et al. (1996)

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Table 4 (*continued*)

NAA	*Sclerotium rolfsii*	*Cladosporium sphaerospermum*	*Cladosporium cladosporioides*	*Cladosporium herbarum*	*A. take*	*Hypocrella bambusae*	*Shiraia bambusicola*	Method	Reference
 Dehydroniranin B (**306**)	_na	_na	_na	156.0 (50%)	_na	_na	_na	Dilution	Greger et al. (1996)
 Ritigalin (**307**)	_na	_na	_na	_na	100	> 100	> 100	Dilution	Tanaka et al. (2003)
 *P*-coumaroylserotonin (**269**)	_na	_na	_na	_na	> 100	> 100	> 100	Dilution	Tanaka et al. (2003)
 Feruloylserotonin (**308**)									

5.18 μM, respectively, making them potential drugs against the Chagas disease.

The insecticidal activity was demonstrated for NAAs of several plant families (Asteraceae, Menispermaceae, Rutaceae, Aristolochiaceae, Piperaceae) (see Table 6). Different methodological assays have been used for a wide range of insects and their larvae. Topical application of the test solution in an organic solvent (contact test) (Su and Horvat, 1981) and the disk chamber test according to Hamraoui and Regnault-Roger (Hamraoui and Regnault-Roger, 1997) are used for adult insects. In the diet and dilution test, on contrary, larvae are fed with an agar/water/dry insect food or brought in a liquid medium, both containing the test substance (Roth et al., 1998; Zhang et al., 1997). Dependent on the insect under investigation, different structural properties in the fatty acid and/or amine moiety of NAAs are essential.

Considering the fatty acid part, the 2*E* unsaturation, the configuration and place of other unsaturations play a key role in the toxicity of NAAs against insects (Crombie, 1955; Crombie and Denman, 1984; Jacobson, 1954). For example, undeca-2*E*,4*E*,10*Z*-triene-11-(benzo[1,3]dioxol-5-yl) acid IBA (pipercide) (**232**) and deca-2*E*,4*E*-dienoic acid IBA (pellitorine) (**80**) are more effective towards *Musca domestica* than their 2*E*,4*E*,10*Z*- and 2*E*,4*Z*-stereomer, respectively (Crombie, 1955; Crombie and Denman, 1984). Several insects are more susceptible to longer fatty acid chains NAAs and alkyne containing NAAs are preferable compared to purely alkenic NAAs (Clifford et al., 2002; Kubo et al., 1984; Ramsewak et al., 1999; Saadali et al., 2001). However, the purely alkenic dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide (**3, 4**) (LD_{100}=10 μg/mL) shows higher activity than the alkynic derivatives (*e.g.* **60**) (LD_{100}=100 μg/mL) against the yellow fever mosquito *Aedes aegyptii* (Clifford et al., 2002). Considering the amine moiety in NAAs, insect toxicity of IBAs against *Aedes aegyptii* and cowpea weevils (*Causus maculatus*) is superior to that of 2-MBAs and piperidides, respectively (Clifford et al., 2002; Su and Horvat, 1981). Nevertheless, compared to IBAs, piperidides demonstrate higher activity against *Cyclommatus scutellaris* ants (Christodoulopoulou et al., 2005). Strunz made some general assumptions for activity against the adzuki bean weevil, *Cryptocarya chinensis* (Strunz, 2000), which are in line with our above-mentioned determining structural properties for insect toxicity.

3.4.2. Tingling and related organoleptic effects

Application of NAAs might result in a tingling but also a burning sensation. The characteristic "tingling" activity of some NAAs, called "tingle compounds", has been established for decades (Humphries, 1979). This tingling effect can be described as producing a buzzy, numbing anesthetic, pungent, pin and needles effect. While some authors try to make a distinction between the denominations tingling, numbing and pungent, this sensation is one here classified as single bioactive effect, called "tingling". However, the tingling effect is totally different from the hot, spicy, pain associated, burning sensation *e.g.* after eating chilli peppers, which will be classified as "burning".

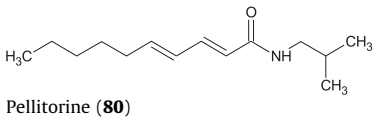
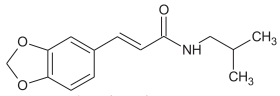
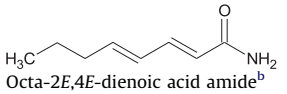
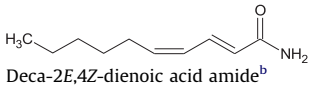
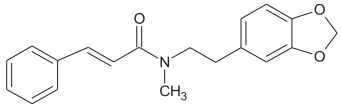
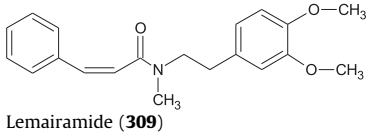
Several *in-vivo* as well as *in-vitro* tests are applied to evaluate the sensation of NAAs. First, in *in-vivo* human sensory tests, the test solution is brought on the tongue, followed by rinsing the mouth with an aqueous solution, which minimizes desensitization. The sensation can be scored using a rating system (Correa et al., 2011; Iseli et al., 2007; Ley et al., 2004, 2005a,b, 2006) or the test solution can be compared to a placebo/reference solution possibly brought on the opposite side of tongue. Evaluation of the relative sensation (quality, intensity) using a relative numeric value is performed (magnitude estimation scaling (MES)) (Bryant and Mezine, 1999) (Castillo et al., 2007). The threshold test is another well-known sensory test in which the panelists indicate

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Table 5
Antimalarial activity of NAAs.

NAA	*Plasmodium falciparum*	IC_{50} (μM)	Method	Reference
	K_1[a]	24.1		
			[^3H]Hypoxanthine incorporation method	Weenen et al. (1990a)
Pellitorine (**80**)				
	K_1[a]	50.0		
			[^3H]Hypoxanthine incorporation method	Weenen et al. (1990a)
Fagaramine (**188**)				
	3D7[c]	192.8		
			Lymphocyte proliferation method	Sittie et al. (1998)
Octa-2*E*,4*E*-dienoic acid amide[b]	Dd2[a]	200.0		
	3D7[c]	87.1		
			Lymphocyte proliferation method	Sittie et al. (1998)
Deca-2*E*,4*Z*-dienoic acid amide[b]	Dd2[a]	91.3		
	3D7[c]	89.7		
			Isotopic method	Penali et al. (2007)
Zanthomamide (**199**)	FCM29[a]	101.1		
	3D7[c]	133.8		
			Isotopic method	Penali et al. (2007)
Lemairamide (**309**)	FCM29[a]	149.9		

[a] Chloroquine-resistant.
[b] These primary amides were not included in the database as they do not meet the definition of NAAs.
[c] Chloroquine-sensitive.

UNCORRECTED PROOF

whether or not they are able to detect a sensation at increasing test concentration (Castillo et al., 2007). Self-, de-, cross-sensitization, time intensity, thermal–tingle and tactile–tingle interactions also can be examined (Albin and Simons, 2010). The *in-vivo* drinking behavior test records the amount of water mice have drunk the day before and after the chemical test compound was added (Bautista et al., 2008; Lennertz et al., 2010). In the mice licking test, the number of licks after intradermal injection of test compound was counted and compared to vehicle-treated group (Koo et al., 2007). Second, *ex-vivo in-vitro* neural assays are described. In the skin–nerve experiment, several kinds of fibers are exposed to electrical-, mechanical- and chemical-evoked action potentials. Fibers sensitive to a chemical demonstrate more action potentials additionally to their baseline firing rate (Lennertz et al., 2010). At last, Ca^{2+}-imaging with TRPV and/or TRPA expressing HEK293 cells from mice/rats trigeminal ganglia or dorsal root ganglion neurons are cultured and loaded with a calcium indicator (*e.g.* Fluo-4 AM). Their response to test compounds is then measured with calcium flux imaging. The fluorescence ratio ($F_{test}/F_{0(baseline)}$) evaluates their sensitivity (Bautista et al., 2008; Bryant and Mezine, 1999; Koo et al., 2007; Lennertz et al., 2010; Sugai et al., 2005).

Table 7 gives an overview of tingling and burning NAAs. For tingling sensation, the 2*E* unsaturation in the fatty acid part of NAAs is preferable, but not necessary *e.g.* dihydrospilanthol (**21**) (Ley et al., 2004). Further unsaturation (alkenic and/or alkynic) generally leads to stronger effects in which the presence of a Z configuration is preferable (Bryant and Mezine, 2002; Galopin et al., 2004; Ley et al., 2004, Ley et al., 2005a,b; Sugai et al., 2005). The unnatural 2*E*,4*Z* pellitorine *cis*-isomer (**316**) has a profound tingling effect compared to the natural *all-trans* pellitorine (Ley et al., 2004). The chain length of the fatty acid part seems to influence the tingling sensation negatively, *e.g.* deca-2*E*,4*E*-dienoic acid IBA (**80**) (rating value 5) is more potent than undeca-2*E*,4*E*-dienoic acid IBA (**143**) (rating value 3) (Ley et al., 2005a,b). Also, the amine part plays a role in the tingling effect. Determining the threshold value of sanshools, it was found that 2-OH IBAs have a lower tingling effect than the IBAs. It is assumed that the lower log P, and hence, the lower membrane permeability of 2-OH IBAs are the reasons for their lower effect (Sugai et al., 2005). In decreasing order, the influence of the amine part is as follows: IBA > piperidine > ethanolamine and 2-ethylhexylamine > pyyrolidine > 3-methyl butylamine > butylamine and MBA (Ley et al., 2005a,b). Finally, it is interesting to notice that in similar compounds, containing no amide, like the undeca-7*Z*,9*E*-dienoic acid 2-ketol ester, acmellonate also has tingling effects, although to a lesser extent than (homo)spilanthol (**1, 2**) (Ley et al., 2006).

Capsaicinoids are responsible for the burning effect of chilli peppers. It was stated that this effect was associated with the presence of an amide bond linking an acyl chain with a vanillyl ring (Castillo et al., 2007). Capsaicin (**271**) and dihydrocapsaicin have been reported as most potent burning, followed by capsaicin's 8-acyl derivative (**324**) > vanillylnonamide (**318**) > norhydrocapsaicin (**319**) > homocapsaicin (**320**) > homodihydrocapsaicin (**321**) > capsaicin's 10-acyl derivative (**325**) (Table 7). In the fatty acid NAA side, a pronounced burning sensation is found between C_8–C_{11}. C_6 and C_{12} derivatives of capsaicin show a very low effect. Beyond this range of fatty acid chain length, no effect has been observed anymore. The amine part also influences the burning effect: benzyl- or 3-methoxybenzylamines give inactive compounds (Castillo et al., 2007).

Although it was originally thought that the target side for the tingling and burning effect was the same, namely the transient

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

UNCORRECTED PROOF

Table 6
Insecticidal activity of NAAs.

NAA	*Caususmaculatus* LD_{50} (μg)	*Pectinophora gossypiella* ED_{50} (ppm)	*Heliothis virescens* ED_{50} (ppm)	*Helicoverpa zea* ED_{50} (ppm)	*Spodoptera frugiperda* ED_{50} (ppm)	*Culex pipiens* LD_{100} (ppm)	*Xyleborusglabratus* LD_{100} (ppm)	Reference
Trideca-2*E*,4*E*,10*E*-triene-13-(benzo[1,3]dioxol-5-yl) acid IBA (**310**)	0.3[a], 1.4[b]	-[na]	-[na]	-[na]	-[na]	-[na]	-[na]	Su and Horvat (1981)
Pipercide (**232**)	0.8[a], 3.9[b]	-[na]	-[na]	-[na]	-[na]	-[na]	-[na]	Su and Horvat (1981)
Piperine (**234**)	> 100[a], [b]	-[na]	-[na]	-[na]	-[na]	-[na]	-[na]	Su and Horvat (1981)
Fagaramine (**188**)	-[na]	440	350	510	530	15	200	Kubo et al. (1984)
Piperlongumine (**238**)	-[na]	430	370	-[na]	500	10	> 200	Kubo et al. (1984)
Octa-2*E*,4*E*-dienoic acid IBA (**243**)	-[na]	70	600	600	280	15	200	Kubo et al. (1984)
4,5-Dihydropiperlongumine (**311**)	-[na]	800	-[na]	-[na]	1700	-[na]	-[na]	Kubo et al. (1984)
Pellitorine (**80**)	2.2[a], 6.7[b]	15	270	210	230	5	> 200	Kubo et al. (1984) and Su and Horvat (1981)

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

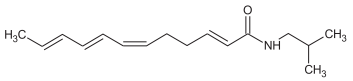
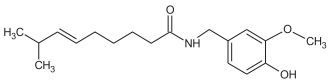
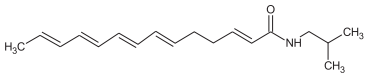
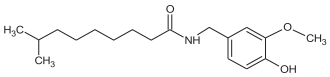
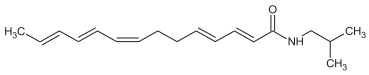
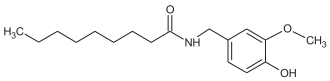
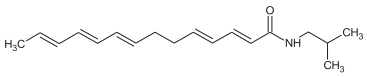
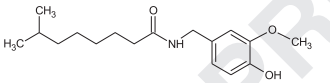
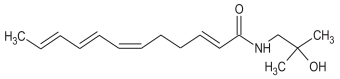
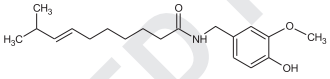
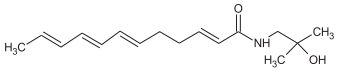
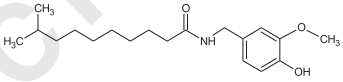
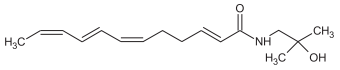
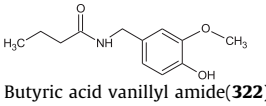
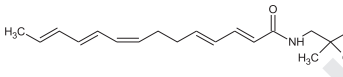
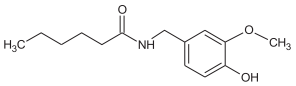
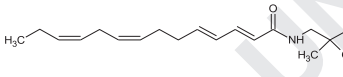
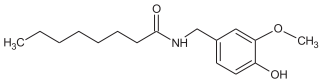
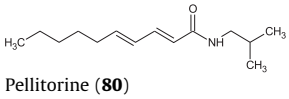
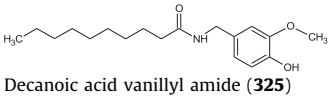
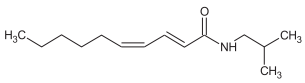
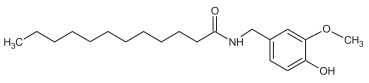
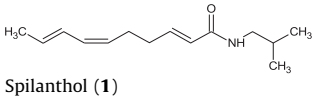
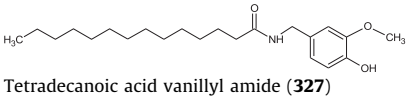
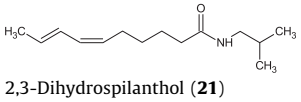
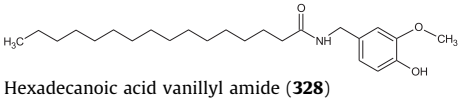
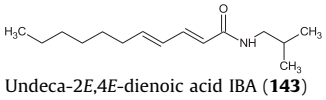
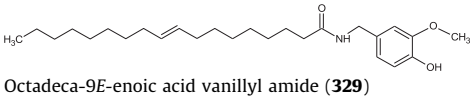
UNCORRECTED PROOF

NAA	*Aedes aegyptii*	*Sitophilusoryzae*	*Rhyzopertha dominia*	*Cyclommatus scutellaris*	Reference
	LD_x (μg/mL)	LD_x (μg/mL)	LD_x (μg/mL)	LD_{50} (μg)	
Spilanthol (**1**)	6.5 (LD_{50})	-[na]	-[na]	-[na]	Ramsewak et al. (1999)
Undeca-2*E*,7*Z*,9*E*-trienoic acid IBA (**10**)	6.5 (LD_{50})	-[na]	-[na]	-[na]	Ramsewak et al. (1999)
Undeca-2*E*-ene-8,10-diynoic acid IBA (**6**)	6.5 (LD_{30})	-[na]	-[na]	-[na]	Ramsewak et al. (1999)
Neopellitorine A (**153**)	-[na]	200 (LD_{100})	200 (LD_{100})	-[na]	Saadali et al. (2001)
Neopellitorine B (**153**)	-[na]	200 (LD_{80})	200 (LD_{50})	-[na]	Saadali et al. (2001)
Tetradeca-2*E*,4*E*(,8*Z*(,11*Z*))-di/tri/tetraenoic acid piperidide (**312, 313, 314**)	-[na]	-[na]	-[na]	80	Christodoulopoulou et al. (2005)
Tetradeca-2*E*,4*E*(,8*Z*(11*Z*))-di/tri/tetraenoic acid IBA (**86, 173, 134**)	-[na]	-[na]	-[na]	80	Christodoulopoulou et al. (2005)
Pellitorine (**80**)	-[na]	200 (LD_{80})	200 (LD_{50})	-[na]	Saadali et al. (2001)

[a] Male insects.
[b] Female insects.

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

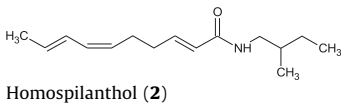
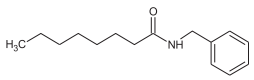
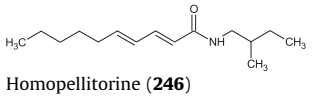
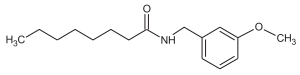
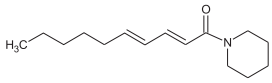
Table 7
Overview of investigated NAA with tingling and burning effects.

Tingling compound	Amount	Rating (%)	Reference	Burning compounds	Amount	Rating (%)[a]	Reference
 α-Sanshool (**162**)	65 μg	-[na]	Sugai et al. (2005)[b]	 Capsaicin (**271**)	0.43 μg	-[na]	Sugai et al. (2005)[b]
					1.2 ppm	100	Castillo et al. (2007)
 β-Sanshool (**163**)	70 μg	-[na]	Sugai et al. (2005)[b]	 Dihydrocapsaicin (**317**)	-[na]	100	Castillo et al. (2007)
 γ-Sanshool (**164**) **Q19**	45 μg	-[na]	Sugai et al. (2005)[b] and Yasuda et al. (1981b)	 Vanillylnonamide (**318**)	-[na]	56	Castillo et al. (2007)
 γ-Isosanshool (**315**)	45 μg	-[na]	Sugai et al. (2005)[b]	 Norhydrocapsaicin (**319**)	-[na]	56	Castillo et al. (2007)
 Hydroxy-α-sanshool (**158**)	50 μg	-[na]	Bryant and Mezine (1999)	 Homocapsaicin (**320**)	-[na]	53	Castillo et al. (2007)
	190 μg		Sugai et al. (2005)[b]				
 Hydroxy-β-sanshool (**159**)	> 100 μg	-[(3)]	Bryant and Mezine (1999)	 Homodihydrocapcaisin (**321**)	-[na]	53	Castillo et al. (2007)
	390 μg	-[na]	Sugai et al. (2005)[b]				
 Hydroxy-ε-sanshool (**160**)	50 μg	-[na]	Bryant and Mezine (1999)	 Butyric acid vanillyl amide(**322**)	28 ppm	/	Castillo et al. (2007)
 Hydroxy-γ-sanshool (**161**)	-[na]	-[na]	Yasuda et al. (1981b)	 Hexanoic acid vanillyl amide (**323**)	28 ppm	2	Castillo et al. (2007)
 Bungeanool (**170**)	-[na]	-[na]	Hiserodt et al. (2004)	 Octanoic acid vanillyl amide (**324**)	1.2 ppm	66	Castillo et al. (2007)
 Pellitorine (**80**)	10 ppm	56	Ley et al. (2004) and Ley et al. (2005a,b)	 Decanoic acid vanillyl amide (**325**)	1.2 ppm	35	Castillo et al. (2007)
 *Cis*-pellitorin (**316**)	< 10 ppm	56	Ley et al. (2004) and Ley et al. (2005a,b)	 Dodecanoic acid vanillyl amide (**326**)	28 ppm	3	Castillo et al. (2007)
 Spilanthol (**1**)	30 ppm	89	Ley et al. (2005a,b)	 Tetradecanoic acid vanillyl amide (**327**)	28 ppm	/	Castillo et al. (2007)
 2,3-Dihydrospilanthol (**21**)	10 ppm	22	Ley et al. (2005a,b)	 Hexadecanoic acid vanillyl amide (**328**)	28 ppm	/	Castillo et al. (2007)
 Undeca-2*E*,4*E*-dienoic acid IBA (**143**)	10 ppm	33	Ley et al. (2005a,b)	 Octadeca-9*E*-enoic acid vanillyl amide (**329**)	28 ppm	/	Castillo et al. (2007)
	10 ppm	56			28 ppm	/	

UNCORRECTED PROOF

ARTICLE IN PRESS

Table 7 (*continued*)

Tingling compound	Amount	Rating (%)	Reference	Burning compounds	Amount	Rating (%)[a]	Reference
 Homospilanthol (**2**)			Ley et al. (2005a,b)	 Octanoic adic phenylmethylamide (**330**)			Castillo et al. (2007)
 Homopellitorine (**246**)	10 ppm	33	Ley et al. (2005a,b)	 Octanoic adic 3-methoxy phenylmethylamide (**331**)	28 ppm	/	Castillo et al. (2007)
 Achilleamide (**97**)	10 ppm	56	Ley et al. (2005a,b)				

-[na]: not available.
/: no tingling or burning sensation.
[c]Inactive/tasteless.
[a] Relative to reference capsaicin.
[b] Mean threshold value.

receptor potential (TRP) channel (Kalil-Gaspar et al., 2007; Koo et al., 2007), it was recently found that their mechanism of action is different (Albin and Simons, 2010; Viana, 2011). Where the burning effect is evoked by activation of the mechano- and heat-sensitive ion channel TRPV1 (transient receptor potential cation channel subfamily V member 1) (the so called capsaicin/vanilloid receptor) (Viana, 2011), the tingling effect is obtained by excitation of light-touch TrkC (tyrosine kinase) mechanoreceptors by inhibition of the pH- and anesthetic-sensitive two-pore potassium channels in sensory (trigeminal) neurons (KCNK 18) as well as in keratinocytes (KCNK 3, 9) (Bautista et al., 2008; Lennertz et al., 2010). Recently, it was found that hydroxy-α-sanshool excites the D-hair fibers, a distinct subset of ultrasensitive light-touch receptors in the skin and targets novel populations of Aβ and C fiber nerve afferents (Lennertz et al., 2010).

Above, NAAs are found to have remarkably strong umami tasting properties (Winkel, et al., 2008). Umami-taste can be described as salty and brothy and has been recognized as the fifth basic taste sensation next to sweet, salt, bitter and sour. The umami taste is associated with a G-protein-couple receptor, but the question which receptor protein is responsible, and if there are more proteins important, remains unclear at this moment (Winkel, et al., 2008). Several patents claim the taste and flavor enhancing effect of food, drinks and drugs by NAAs, which have sweet, salt or umami taste and flavor enhancement quality. *N*-substituted unsaturated alkylamide containing C_{2-9} linear or branched alkyl, alkenyl, dienealkyl, or phenyl fatty acid chain combined with a C_{4-13} linear or branched alkyl, alkenyl, alkyldienyl, acyclic or monocyclic amine substituent are claimed to enhance or change the sense (*i.e.* sweetness, sourness, saltiness, bitterness, and umami) of food, drinks, drugs (Dewis et al., 2006). The umami flavoring potency of the synthetic geranylamine derivatives have also been provided (Kaouas et al., 2010). Symrise GMBH and Co patented the use of an alkamide and/or a mixture comprising two or more different alkamides, like trans-pellitorine (**80**), cis-pellitorine (**316**), spilanthol (**1**), homospilanthol (**2**), α–sanshool (**162**), hydroxyl-α-sanshool (**158**), hydroxyl-γ-sanshool (**161**), hydroxyl-γ-isosanshool (**172**), γ-sanshool (**164**), bungeanool (**170**), isobungeanool (**168**), for changing, masking or decreasing the unpleasant flavor effect in unpleasant tasting material (Langer et al., 2009). This company also patented the use of NAAs in order to produce a feeling of warmth upon consumption and/or to intensify/mimic the taste of ethanol. The effect can be established by a NAA having a fatty acid moiety consisting of a C7 up to C14 chain combined with a C1 up to C5 chain on the amine side, or a mixture of two or more of these NAAs. The fatty acid part is preferably a C8–12 chain, while the optimal amine moiety is an isobutyl or *N*-methylbutyl (Ley et al., 2005a,b). In a Japanese patent, organoleptic, but no pharmacological effects of spilanthol and the essential oil of *Spilanthes oleracea* are also demonstrated (Yoshida and Uematsu, 1985; Yoshida and Yamagishi, 1986; Sugano and Yoshida, 1987).

3.4.3. Anti-inflammatory and immunomodulatory effects

Anti-inflammatory activity and the closely associated immunomodulatory effects can be examined *in-vitro* via several targets or biomarkers from different related pathways. First, the arachidonic acid (AA) pathway can be explored in immune-related assays: the enzymes cyclooxygenase 1 and 2 (COX 1 and COX 2) and 5-lipoxygenase (5-LO) convert AA via intermediates into measureable pro-inflammatory prostaglandins, thromboxanes and leukotrienes. The RNA and/or protein expression of the interfering enzymes can be investigated *e.g.* by means of PCR or their activity might be established by measuring substrate/product levels or the consumption of oxidizing or reducing substrates (*e.g.* di-oxygen). Second, the level of nitric oxide and cytokines, produced by a variety of inflammation involved cells like macrophages can be measured, mostly by specific ELISA assays. Cytokines, produced by T helper (TH) cells, can be proinflammatory (TNF-α, interleukine (IL)-1, IL-2, IL-8, IL-12) or anti-inflammatory (IL-4, IL-5, IL-10 and IL-13). TH cells can be divided in TH1 and TH2 cells. This differentiation occurs in the presence of a mitogenic T cell receptor (TCR) stimulus and is driven by the microenvironmental cytokine concentration (Biedermann et al., 2004). After activation of native T helper cells, TH0 cells are developed. If IL-12 or interferon (IF)-α dominates the microenvironment together with the TCR signal, TH cells differentiate in TH1 cells. These cells tend to produce the proinflammatory cytokines upon stimulation. The TH1 type cytokines are involved in the immunity against intracellular infections and carry out autoimmune effects if directed against autoantigens. A mitogenic stimulus in the presence of IL-4 polarizes TH0 cells to TH2 cells, producing anti-inflammatory cytokines upon activation. TH2 type cytokines are associated with promotion of IgE, generally mediating antibody responses. They are also involved in allergy. Where excess of TH1 leads to an uncontrolled tissue damage, TH2 excess counters the TH1 mediated antimicrobial action. Therefore, a balance in TH1

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

and TH2 responses is essential for an appropriate immune function (Berger, 2000). The granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF) and tissue inhibition-1 of metallopeptidase (TIMP-1) are also immunomodulatory cytokines. Moreover, the inactivation of NF-κB, resulting in a reduction of pro-inflammatory mediator (*e.g.* IL-1, IL-8, TNF-α) production, can be measured as well. Finally, it is suggested that many immune associated signal transduction pathways of NAAs are related to the cannabinoid receptors, a family of GPC receptors (Gertsch, 2008; Raduner et al., 2006; Woelkart et al., 2005). Receptor binding of cannabinoids can be examined by radioligand displacement assays after which the binding inhibition Ki can be obtained or measuring the intracellular Ca^{2+} concentration. The endogenous cannabinoid, an unsaturated fatty acid ethanolamide, anandamide (**270**) is often included in this receptor binding studies.

Especially for *Echinaceae* NAAs, the anti-inflammatory and immuno-modulatory properties have been well investigated, confirmed and patented (Woelkart and Bauer, 2007; Esanu, 1981). However, also the, immune effects of *Anacyclus pyrethrum* and anti-inflammatory effects of *Spilanthes*, *Heliopsis*, *Piper* and *Achillea* species are established (Hernandez et al., 2009; Mullerjakic et al., 1994; Rimbau et al., 1996; Stöhr et al., 1999; Wu et al., 2008).

Different targets for the anti-inflammatory properties have been identified. Spilanthol inactivates NF-κB, shows significant topical anti-inflammatory effects in the mouse ear edema test and is the only NAA that has a demonstrated influence on the transcription and translation of the COX enzymes (Hernandez et al., 2009; Wu et al., 2008). All other investigated NAAs have no influence on transcriptional nor translational level of COX (Hinz et al., 2007), but their enzyme activity decreases in the presence of low concentrations of NAAs containing multiple alkyne groups in their fatty acid chain (Clifford et al., 2002). At higher concentrations *i.e.* from 12.5 μg/mL, also NAAs containing double bonds in their acid chain have an inhibitory effect on the COX pro-inflammatory PGE2 product (23% up to 90% inhibition) (Cech et al., 2010; Hinz et al., 2007; Lalone et al., 2007; Mullerjakic et al., 1994). In the fatty acid part of the NAA, the 2*E* configuration shows a higher inhibitory effect than the 2*Z* configuration, (*e.g.* **6**) with 46% inhibition towards (**77**) with 23% inhibition. Three double bonds are superior to two double bonds, (*e.g.* **90**) with 37% inhibition *versus* (**98**) with 27% inhibition, while a thiophene containing NAA (*e.g.* **154**) is favorable for potent PGE2 inhibition. The amine moiety of the NAA also plays a role in the following decreasing activity: 2-MBA, IBA, piperid(e)ide, tyramide and isopentylamide. A pyrrolide moiety is destructive for activity. Only half of these investigated NAAs are also inhibitors of the 5-HPETE level produced by 5-LO (Mullerjakic et al., 1994). Pronounced 5-HPETE inhibition was only seen if the IBA possessed four multiple bonds or a thiophene in their acid chain, or if the IBA is replaced by a pyrrolide, a 2,3-didehydro piperidine or tyramide moiety.

The pro-inflammatory TNF-α levels in chemically induced human blood or other immune cell lines are diminished by the endogenous anadamide (**270**) and/or different C_{11} to C_{12} IBAs containing a C_2 unsaturation in their fatty acid chain (**3, 6, 56 66**). The production of the other pro-inflammatory (IL-1β, IL-2, IL-6, IL-8 IL-12) and the anti-inflammatory (IL-10) mediators is suppressed or enhanced, depending on the nature of chemical stimulation and the structural properties of the NAA (Cech et al., 2010; Matthias et al., 2007; Raduner et al., 2006; Stevenson et al., 2005; Wu et al., 2008).

Although different receptor binding studies confirmed the affinity of NAAs to the CB1 and even more to the CB2 receptor, their anti-inflammatory effects are not exclusively mediated by CB receptor interaction (Gertsch et al., 2006a). Moreover, NAAs are rather potential, unexplored therapeutics in the CB system (Gertsch et al., 2006a; Woelkart et al., 2008). Interaction with CB1 affects the central nerve system, while agonists of peripheral CB2 can be effective as therapeutic for chronic inflammation conditions like arthritis and chronic liver inflammation, for autoimmune diseases, chronic pain relief, osteoporosis, cancer therapeutic and for neurodegradation (Alzheimer's disease). Several C_{11} to C_{14} 2*E* unsaturated IBAs and 2-MBAs showed affinities to the CB2 receptor (Ki_{CB2} values < 20 μM) (Matovic et al., 2007; Raduner et al., 2006; Woelkart et al., 2005). On contrast, the synthetic *all-trans* tetradeca-2*E*,4*E*,8*E*,10*E*-tetraenoic acid IBA (**284**) shows no CB2 affinity, while the naturally occurring 2*E*,4*E*,8*Z*,10*Z* isomer shows very high affinity ($Ki_{CB2} = 57$ nM). Above, using an immobilized CB1/CB2 open tubular column for fast screening, shows that a plant extract (*Zanthoxylum* sp.) rich in NAAs has high affinity at the CB1/CB2 receptors (Moaddel et al., 2001) Depending on their fatty acid moiety, some synthetic *N*-benzyl NAAs also show CB2 receptor affinity. Despite their structural similarity with anandamide, NAEs show no affinity for CB receptors (Gertsch et al., 2006a,b), possibly due to the lack of the 2*E* unsaturation at the fatty acid moiety. Indeed, for CB interaction, the 2*E* double bond in NAAs is necessary (Gertsch, 2008). The 4*E* double bond is not essential for CB1 receptor binding, but increases – depending on the fatty acid chain – the affinity of CB2. Moreover, for CB2 receptor interaction, the fatty acid alkyl chain needs to be longer than ten carbon atoms, and IBA or dimethylbutyl amine moiety seem to favor this CB2 interaction. On the molecular level, the Tyr190 aromatic ring of the CB receptor exhibits a H-bond interaction and π–π interactions with the NAA: the hydrophilic pocket of the CB receptor stabilizes the amide function and is surrounded by the residues Asp189 and Tyr190 (Gertsch et al., 2006a).

It is noteworthy that the synergistic action of NAAs has been demonstrated in numerous studies involving different anti-inflammatory pathways (PGE2/5-HPETE/cytokine levels, NF-κB activity, CB2 activation) (Chicca et al., 2009; Lalone et al., 2007; Matthias et al., 2008, 2007; Mullerjakic et al., 1994; Woelkart et al., 2006). This again indicates the importance of the knowledge of phytochemical components present in an investigated and/or used extract. In this context, it is interesting that no individual NAAs but only NAA mixtures decreased the LPS-stimulated NO production (Kim et al., 2010b; Stevenson et al., 2005).

3.4.4. Others

That NAAs are a very promising group of therapeutics becomes clear from the high amount of bioactivities they have been associated with. Although at the moment, a limited amount of drugs and cosmeceuticals containing one or more NAAs as main bioactive compound are in clinical trials or on the market. Capsaisin is already commercially available as dermal formulation in pain relief and local inflammatory diseases (*e.g.* Stilene®, Rado-Salil®) and is being investigated in several clinical trials for several indications which have been reviewed manifold (Bode and Dong, 2011; Derry et al., 2009; Qureshi et al., 2008; Reyes-Escogido et al., 2011; Wallace and Pappagallo, 2011). Various *Echinacea* formulations (*e.g.* Echinacin®, Echinaforce®) are on the market to enhance the immune system and local applications of *Spilanthes acmella* or its main bioactive spilanthol (**1**) (*e.g.* Buccaldol®, (Indolphar®) for benign mouth diseases and fungi) are on the market, we believe that NAAs will gain even more ethnopharmacological interest in the next years. The link with the exponential growing medicinal peptides is very close and traditional as well as current knowledge of the pharmacological effects of NAAs makes them optimal lead compounds in the development of highly potent new drugs. A brief description of some important ethnomedicinal applications is given below. NAAs can exhibit

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

analgesic effect through central GABA release *e.g.* spilanthol (**1**) (Rios et al., 2007), by interfering with voltage-gated sodium channels (Gertsch et al., 2008) or via desensitization of the TRPV1 receptor. TRPV1 agonists initially stimulate sensory neurons and release of substance P, followed by a long-lasting refractory period, during which the previously excited neurons are no longer responsive to a broad range of stimuli (Bode and Dong, 2011; Luo et al., 2011; Pal et al., 2009). Therefore, prolonged ingestion or topical application of capsaicinoids can be successful in managing painful conditions such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy, postherpetic, neuralgia, postmastectomy pain syndrome, cluster headache, reflex sympathetic dystrophy and gastro-intestinal related problems like reflux and functional dyspepsia (Holzer, 2011; Philip and Thakur, 2011). On contrast, depending on the type of NAA, acute ingestion of NAAs induce gastric mobility via binding to the TRPV1 receptor (capsaicinoids), indirectly by releasing acethylcholine and other neurotransmitters or by acting directly on the smooth muscles (sanshools) (Hashimoto et al., 2001; Holzer, 2011).

Gastro-protective effects against aspirin-induced erosions (also via TRPV1 interaction) and hepato-protective properties (by inhibiting D-GalN/TNF-α-induced death of hepatocytes) can also be assigned to NAAs. Several piperidines and IBAs having a C_3 to C_{15} fatty acid moieties containing a benzyl or benzo[1,3]dioxol-5-yl terminus show hepato-protective effects (μM range). A 1,9-decadiene structure between the benzene ring and amine part, however, enhances the activity (Matsuda et al., 2009).

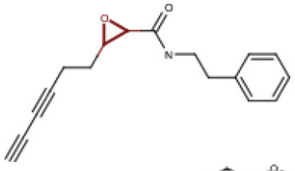
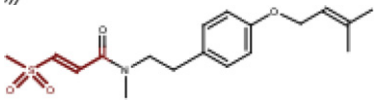
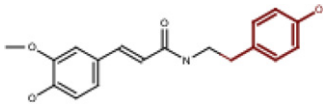
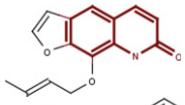
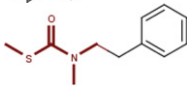
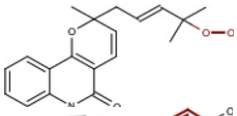
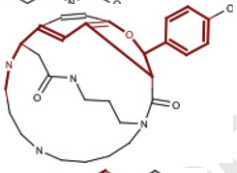
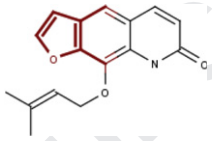
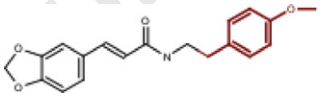
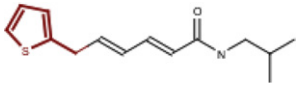
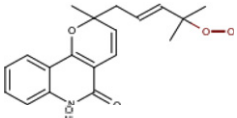
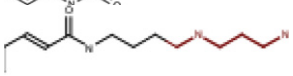
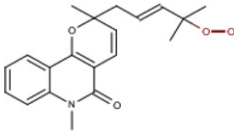
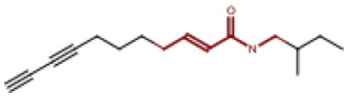
A recent study showed that hexadeca-2*E*,9*Z*,12*Z*,14*E*-tetraenoic acid IBA (**68**) has the potential to manage insulin resistance and type-2 diabetes by activation of the peroxisome proliferator activated receptor gamma (PPARγ) without stimulation of adipocyte differentiation (Christensen et al., 2009). NAAs with a C_{16} to C_{20} fatty acid chain length have the highest PPARγ affinity. NAAs with the amide part in one cyclic system (**208**, **212, 214, 218, 220**) or with an ethylphenyl fatty acid and amine moiety (**206, 207**) showed total inhibition of platelet aggregation induced by arachidonic acid, collagen and PAF at 100 μg/mL (Chen et al., 1997; Cheng et al., 2004). *N-cis*-ferulyltyramide (**217**) is even more potent, with a total anti-platelet activity at 10 μg/mL. However, none of the investigated NAAs show activity against thrombin induced aggregation. Finally, *in-vitro* as well as *in-vivo* studies indicate that NAAs are moderate (IC50∼μM range) anti-cancer therapeutics. Pellitorine (**80**) and capsaicin (**271**) are cytotoxic against breast cancer cell lines (Ee et al., 2010; Luo et al., 2011). While pellitorine also shows inhibitory properties against leukemia cell lines, capsaicinoids induce the apoptosis of prostate cancer cell lines and autography in colon cancer cell lines. 1,3-benzodioxol-acid IBAs like fagaramide (**188**) and derivatives (**187, 188**), however, possess no cytotoxicity against prostate tumor cell lines (Mbaze et al., 2009). (2*E*/*Z*)-*N*-(4-amino-2,3-dihydroxybutyl)-3-(4-hydroxyphenyl)prop-2-enamide (pharnilatin A (**252**) and B (**342**)) show *in-vitro* cytotoxicity against skin melanoma and lung, ovary and colon carcinoma (Kim et al., 2010a). Moreover, a sulfone-NAA (**343**) has anticancer activity against CEM-SS and KU812F (leukemia cells), HT29 (colon cancer) and UACC-62 (melanoma), and was found to be non-toxic to peripheral blood mononuclear cells (Astelbauer et al., 2010). *In-vivo* oral ingestion or direct injection of capsaicinoids in mice reduces the size of breast tumors with 50% and inhibits the pre-neoplastic development of breast lesions with 80% (Luo et al., 2011).

Very often not purified plant material is used in ethnopharmaceutical assays. In this case, it is likely that endogenous plant compounds, other than NAAs are responsible for the observed effect. Although in these studies the contribution of NAAs to the pharmaceutical effect is questioned, the biofunctionality of some NAA containing plant extracts has been described. The antioxidant effects of *Anacyclus pyrethrum* and *Spilanthes acmella* have been demonstrated (Kalim et al., 2010; Wongsawatkul et al., 2008). Synthetic N-(1-oxo-1,3-dihydro-2-benzofuran-5-yl)alkylamide derivatives, preferably containing a fluorine or chlorine atom in their fatty acid part are claimed to preserve the antioxidant and strengthening effects of the skin (Boulle, 2008). The hydroalcoholic and chloroform extract of *Anacyclus pyrethrum* are protective against seizures and therefore has been shown to be a potential anticonsulvant (Pahuja et al., 2010; Zaidi et al., 2009). Moreover, different types of *Echinaceae pallida*, *Echinaceae purpurea*, *Echinaceae angustifolia*, *Echinaceae vegetalis and Echinaceae atribactilus* preparations showed anti-herpetic properties (Squires Meryl, 2001; Schneider et al., 2010). One group of antimicrobial compounds herein are isobutylamides dosed from 0.003% to 0.009%, m/m. The improved wound healing effects of *Echinacea pallida* may be attributable to their bioavailable NAAs (Zhai et al., 2009). Maca (*Lepidium meyenii*, Brassicacae), *Anacyclus pyrethrum* and *Spilanthes acmella* has been shown to improve the fertility and sexual performance, possibly by their NAA content (Sharma et al., in press; Sharma et al., 2009; Wang et al., 2007). The diuretic activity in male rats of a *Spilanthes acmella* water extract was established (Ratnasooriya et al., 2004). The MTT assay reveals that spilanthol (**1**) (40 μg/mL) only exerts a slight decrease of in cell viability (10%) *in-vitro* in RAW 264.7 cell lines and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (**3**) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (**66**) (both 10 μg/mL) do not influence the cell viability of Jurkat cells in a XTT experiment at all. Although no profound cytotoxicy for the investigated NAAs was demonstrated, *in-silico* experiments by Derek Nexus 2.0 (Lhasa Limited) indicate specific toxicological endpoints for some functional groups *i.e.* toxicophores, in NAAs (Table 8). Derek is an expert system for the assessment of toxicity of chemicals that predicts whether a chemical is toxic in humans, other mammals and bacteria. It consists of a chemical rulebase of descriptions of toxicophores called "structural alerts", which correlate with specific toxicological endpoints, like carcinogenicity, genotoxicity, hepatotoxicity, irritation of the skin/gastrointestinal tract/eye/respiratory tract, nephrotoxicity, neurotoxicity, teratogenicity, occupational asthma, etc. For each endpoint where at least one structural alert is found, the outcome of the rules results in a level of likelihood (certain, probable, plausible, equivocal, doubted, improbable, impossible, open, contradicted) for every endpoint, giving a qualitative indication for the toxicity of a chemical in endpoints. Plausible and equivocal likelihood, indicating the weight of evidence supports the proposition and representing an equal weight of evidence for and against the proposition, respectively, were found for several toxicophores found in NAAs. Nineteen toxicophores with a plausible risk and eight with a equivocal risk are present in our set of NAAs. The alerts with a plausible likelihood are considered important. The highest awareness goes to a minor part (∼4%) of the reported NAAs harboring toxicophores with a plausible cancer related toxicity. Epoxides possess a plausible carcinogenicity in mammal, chromosome damage *in-vitro* in mammal and mutagenicity *in-vitro* in bacterium, while α,β-unsaturated sulphones, alkylphenols and hydroperoxide have chromosome damage *in-vitro* in mammal and quinolones, thiocarbamates and hydroperoxide have mutagenicity *in-vitro* in mammal and/or bacteria. Besides, two NAAs harboring a HERG Pharmacophore II found to result plausibly in a HERG channel inhibition *in-vitro* in mammal with potential to develop the long QT syndrome, a fatal heart disorder. Other awareness goes to the furan, para-alkylphenol or derivative, thiophene or organic peroxide, in total covering 10% of the reported NAAs. This toxicophores plausibly result in hepatotoxicity in mammal. Less critical, but important knowledge in drug development are the eye, respiratory tract and or skin irritation of diamines and alkyl hydroperoxides, only attribution for 1% of the NAAs. At last, several toxicophores present in NAAs (epoxides, α,β-unsaturated sulphones, α,β-unsaturated amides or precursors, phenols or precursors, α,β-unsaturated ketones or precursors, catechols

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

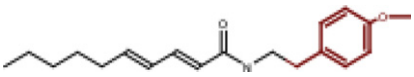
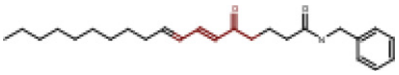
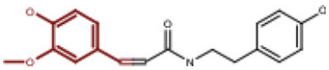
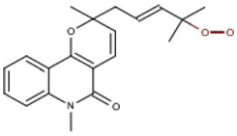
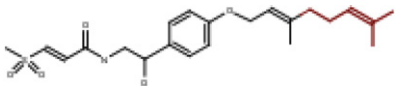
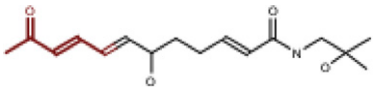
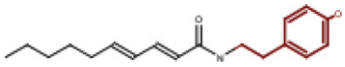
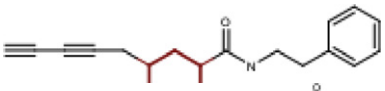
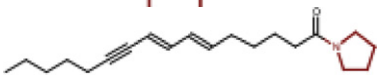
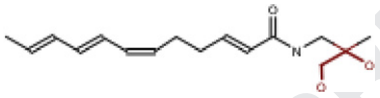
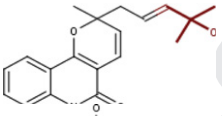
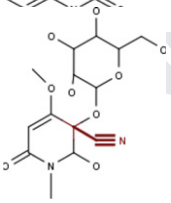
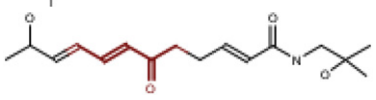
UNCORRECTED PROOF

Table 8
In-silico toxicity data from Derek Nexus 2.0 (Lhasa Limited).

Toxicophore	Structural group	Endpoint	Level of likelihood	NAA examples (IDs—to database)
Epoxide		Carcinogenicity in mammal Chromosome damage *in vitro* in mammal Developmental toxicity in mammal Mutagenicity *in vitro* in bacterium Irritation of the eye/skin in mammal Skin sensitisation in mammal	Plausible	12, 27, 226, 275,
α,β-Unsaturated sulphone		Chromosome damage *in vitro* in mammal Skin sensitisation in mammal	Plausible Plausible	254, 255, 343, 344, 345
Alkylphenol		Chromosome damage *in vitro* in mammal	Plausible	217, 264, 266
Quinoline		Mutagenicity *in vitro* in bacterium Mutagenicity *in vivo* in mammal	Plausible	193
Thiocarbamate		Mutagenicity *in vitro* in bacterium	Plausible	304
Hydroperoxide		Mutagenicity *in vitro* in bacterium Chromosome damage *in vitro* in mammal	Plausible Equivocal	219
HERG Pharmacophore II		HERG channel inhibition *in vitro* in mammal	Plausible	277, 278
Furan		Hepatotoxicity in mammal	Plausible	193
para-Alkylphenol or derivative		Hepatotoxicity in mammal	Plausible	83, 99, 141, 192, 197, 200, 206, 253, 278, 281, 317-329
Thiophene		Hepatotoxicity in mammal Rapid prototypes: nephrotoxicity in mammal	Plausible Equivocal	154, 157
Organic peroxide		Hepatotoxicity in mammal Rapid prototypes: nephrotoxicity in mammal	Plausible Equivocal	219
Diamine		Irritation of the respiratory tract in mammal	Plausible	267, 268, 277, 278
Alkyl hydroperoxide		Irritation of the eye/respiratory tract/skin in mammal	Plausible	219
α,β-Unsaturated amide or precursor		Skin sensitisation in mammal	Plausible	1-11, 22-26, 47-106, 112-185, 199-207, 229-246, 254-256, 342-345

UNCORRECTED PROOF

Table 8 (*continued*)

Toxicophore	Structural group	Endpoint	Level of likelihood	NAA examples (IDs—to database)
Phenol or precursor		Skin sensitisation in mammal	Plausible	83, 84, 99, 189, 206, 215, 251, 217, 253, 262, 266, 278, 331
α,β-Unsaturated ketone or precursor		Skin sensitisation in mammal	Plausible	249
Catechol or precursor		Skin sensitisation in mammal	Plausible	217, 248, 253, 266, 268, 271, 308, 317-329
Allyl hydroperoxide		Skin sensitisation in mammal	Plausible	219
Terpenoid		Skin sensitisation in mammal	Plausible	255, 343
α,β-Unsaturated ketone		Chromosome damage *in vitro* in mammal	Equivocal	178, 179, 249
para-Alkylphenol		Rapid prototypes: nephrotoxicity in mammal	Equivocal	83, 84, 217, 248, 253, 266, 262, 264
1,3-Dihydroxypropane or derivative		Rapid prototypes: hepatotoxicity in mammal Rapid prototypes: nephrotoxicity in mammal	Equivocal	42
Pyrrolidine or derivative		Rapid prototypes: hepatotoxicity in mammal	Equivocal	111–115, 135–138, 148, 247, 290
1,2-Ethyleneglycol or derivative		Rapid prototypes: nephrotoxicity in mammal	Equivocal	175–176, 180–181, 252, 342
Tertiary alcohol or ether		Rapid prototypes: hepatotoxicity in mammal Rapid prototypes: nephrotoxicity in mammal	Equivocal	218
Aliphatic nitrile		Rapid prototypes: hepatotoxicity in mammal	Equivocal	257
α,β-Unsaturated ketone or precursor		Skin sensitisation in mammal	Equivocal	178, 179

or precursors, allyl hydroperoxides and terpenoids), covering more than 70% of the NAAs in the database, plausibly result in skin sensitisation in mammal.

1.4.5. PK/PD interactions

Recently, pharmacokinetic and pharmacodynamics interaction between NAA containing plants and conventional drugs have been investigated. Pharmacokinetic interactions of these species mainly are assigned to absorption and metabolism phenomena. *In-vitro* and *in-vivo* P-glycoprotein (P-gp) transport studies suggest no significant clinical interaction risk between NAA containing plants, like *Echinacea* spp., and drugs being P-gp substrates (Gurley et al., 2008; Hansen and Nilsen, 2008, 2009). NAAs are metabolized (dealkylation, epoxydation, hydroxylation, and carboxylation) by the cytochroom (CYP) P450 system (Matthias et al., 2005; Spelman, 2009; Toselli et al., 2010). Besides the alteration of their own activity after metabolism (Cech et al., 2006), NAA containing plants (like *Echinacea* spp., *Heliopsis* spp., *Piper* spp., *Capsicum* spp.) and isolated NAAs with a terminal alkyne group inhibit P450 enzymes (Matthias et al., 2005; Pandit et al., 2012; Rodeiro et al., 2009; Subehan et al., 2006; Usia et al., 2006) and hence can also influence the metabolism of other drugs. Nevertheless, *in-vitro* as well as *in-vivo* studies showed this herb–drug interaction is clinically not significant (Toselli et al., 2009). *In-vitro* studies are indicative for the additive and synergetic effects between NAAs themselves or between NAAs and other phytochemicals present in the natural mixtures (Mbeunkui

CORRECTED PROOF

ARTICLE IN PRESS

et al., 2011), endogeneous compounds (Chicca et al., 2009) or other conventional drugs (Hohmann et al., 2011). For this phenomena, multiple mechanisms are suggested (Chicca et al., 2009). Dependent on their structure, isolated NAAs also exert inverse or partial agonistic effects (Acosta-Madrid et al., 2009). Overall it can be concluded that it is unlikely that NAA containing plants pose serious health treats in patients combining it with conventional drugs.

4. Conclusions

NAAs are secondary metabolites present in more than 25 plant families. These versatile molecules elicit multiple biofunctional actions, which make them very promising lead compounds in the development of novel drugs and bioactives. Although fragmental studies have reported over 300 plant NAAs, a structured overview of this new "meta-group" of compounds is highly warranted. NAAs were described here according to their plant origin, their functionality and chemical characteristics. The related Alkamid database presents the botanical occurrence (plant family, tribe, genus and species) of NAAs, their 3D structure, chemical classification name and physico-chemical properties. Moreover, functionality results and a NAA structured clustering are given.

We want to debate the frequent use of unpurified plant material which impairs unambiguous activity results. Hence, at this stage, explicit QSAR studies are mostly hampered due to lack of unequivocal chemical-functional data. Therefore, isolation and/or synthesis of pure NAAs, or at least highly purified plant extracts are one of the future ways to perform standardized *in-vitro* as well as *in-vivo* pharmacological tests and consequently, to open up this class of promising bioactive compounds. Furthermore, as NAA containing plants are frequently used in traditional medicine, development of standardized production procedures and quality attributes is recommended, which will strengthen *in-vitro* and *in-vivo* activity, toxicity and interaction experiments. In this way, consistent efficacy and safety of these traditionally used medicinal plants is guaranteed.

Acknowledgment

This research was funded by a Ph.D. Grant of "Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen)" (No. 91257).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2012.05.038.

References

Achenbach, H., Fietz, W., Worth, J., Waibel, R., Portecop, J., 1986. Constituents of tropical medicinal plants.19. GC MS investigations of the constituents of *Piper amalago*—30 new amides of the piperine-type. Planta Medica, 12–18.

Acosta-Madrid, I.I., Castaneda-Hernandez, G., Cilia-Lopez, V.G., Carino-Cortes, R., Perez-Hernandez, N., Fernandez-Martinez, E., Ortiz, M.I., 2009. Interaction between *Heliopsis longipes* extract and diclofenac on the thermal hyperalgesia test. Phytomedicine 16, 336–341.

Adesina, S.K., Olugbade, T.A., Akinwusi, D.D., Bergenthal, D., 1997. Extractives from *Zanthoxylum lemairie* root and stem. Pharmazie 52, 720–724.

Adesina, S.K., Reisch, J., 1989. Amides from *Zanthoxylum rubescens*. Phytochemistry 28, 839–842.

Albin, K.C., Simons, C.T., 2010. Psychophysical evaluation of a sanshool derivative (Alkylamide) and the elucidation of mechanisms subserving tingle. PLoS One, 5.

AndradeNeto, M., Mendes, P.H., Silveira, E.R., 1996. An imidazole alkaloid and other constituents from *Pilocarpus trachyllophus*. Phytochemistry 42, 885–887.

Astelbauer, F., Obwaller, A., Raninger, A., Brem, B., Greger, H., Duchene, M., Wernsdorfer, W., Walochnik, J., 2010. High antitrypanosomal activity of plant-derived sulphur-containing amides. International Journal of Antimicrobial Agents 36, 570–572.

Aza-Gonzalez, C., Nunez-Palenius, H.G., Ochoa-Alejo, N., 2011. Molecular biology of capsaicinoid biosynthesis in chili pepper (Capsicum spp.). Plant Cell Reports 30, 695–706.

Barnes, J., Anderson, L.A., Gibbons, S., Phillipson, J.D., 2005. Echinacea species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): a review of their chemistry, pharmacology and clinical properties. Journal of Pharmacy and Pharmacology 57, 929–954.

Bautista, D.M., Sigal, Y.M., Milstein, A.D., Garrison, J.L., Zorn, J.A., Tsuruda, P.R., Nicoll, R.A., Julius, D., 2008. Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. Nature Neuroscience 11, 772–779.

Berger, A., 2000. Science commentary: Th1 and Th2 responses: what are they? British Medical Journal 321, p. 424.

Biedermann, T., Rocken, M., Carballido, J.M., 2004. TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin. Journal of Investigative Dermatology Symposium Proceedings 9, 5–14.

Bode, A.M., Dong, Z., 2011. The two faces of capsaicin. Cancer Research 71, 2809–2814.

Bohlmann, F., Gerke, T., Ahmed, M., King, R.M., Robinson, H., 1983. Polyacetylenic compounds.269. New N-isobutylamides from Heliopsis species. Liebigs Annalen Der Chemie, 1202–1206.

Bohlmann, F., Hartono, L., Jakupovic, J., 1985. Highly unsaturated amides from *Salmea scandens*. Phytochemistry 24, 595–596.

Bohlmann, F., Wegner, P., 1982. Naturally occurring terpene derivatives.410. Entbeyer-15-ene derivatives from *Nidorella anomala*. Phytochemistry 21, 1175–1177.

Bohlmann, F., Zdero, C., 1967. Polyacetylenverbindugen.119. Uber zwei neue isobutylamdies aus *Chrisanthemum frutescens* L. Chemische Berichte-Recueil 100, 104.

Bohlmann, F., Zdero, C., Suwita, A., 1974. Polyacetylenic compounds. 225. Further amides from the tribe Anthemideae. Chemische Berichte-Recueil 107, 1038–1043.

Bohlmann, F., Ziesche, J., Robinson, H., King, R.M., 1980. Polyacetylenic compounds.256. New amides from *Spilanthes alba*. Phytochemistry 19, 1535–1537.

Boonen, J., Baert, B., Roche, N., Burvenich, C., De Spiegeleer, B., 2010. Transdermal behaviour of the N-alkylamide spilanthol (affinin) from *Spilanthes acmella* (Compositae) extracts. Journal of Ethnopharmacology 127, 77–84.

Boonen, J., Sharma, V., Dixit, V.K., De Spiegeleer, B. LC–MS N-alkylamide profiling of an ethanolic *Anacyclus pyrethrum* root extract. Planta Medica, submitted for publication. Q6

Bryant, B., Mezine, I., 2002. Pungency and tingling: sensations and mechanisms of trigeminal chemical sensitivity. In: Given, P.P.D. (Ed.), Chemistry of Taste: Mechanisms, Behaviors, and Mimics, 825; 2002, pp. 202–212.

Bryant, B.P., Mezine, I., 1999. Alkylamides that produce tingling paresthesia activate tactile and thermal trigeminal neurons. Brain Research 842, 452–460.

Burden, R.S., Crombie, L., 1969. Amides of vegetable origin.12. A new series of alka-2,4-dienoic tyramide amides from *Anacyclus pyrethrum* DC (Compositae). Journal of the Chemical Society C-Organic, 2477.

Castillo, E., Torres-Gavilan, A., Severiano, P., Arturo, N., Lopez-Munguia, A., 2007. Lipase-catalyzed synthesis of pungent capsaicin analogues. Food Chemistry 100, 1202–1208.

Cavazza, P., Fiorentini, G., 1989. N-Alkylamides of D,L and L(-)-Carnitine Having Antibacterial Activity, Process for their Preparation and Pharmaceutical and Cosmetic Compositions Containing Same. EP19880830495 19881121. Avantgarde Spa, Italy.

Cavazza, P. and Fiorentini G., 1988. N-Alkylamides of d (+)-Carnitine Having Antibacterial Activity, Process for Their Preparation and Pharmaceutical and Cosmetic Compositions Containing Same. Avantgarde Spa. US19890410001 19890919. Italy.

Cech, N.B., Tutor, K., Doty, B.A., Spelman, K., Sasagawa, M., Raner, G.M., Wenner, C.A., 2006. Liver enzyme-mediated oxidation of *Echinacea purpurea* alkylamides: production of novel metabolites and changes in immunomodulatory activity. Planta Medica 72, 1372–1377.

Cech, N.B., Kandhi, V., Davis, J.M., Hamilton, A., Eads, D., Laster, S.M., 2010. Echinacea and its alkylamides: Effects on the influenza A-induced secretion of cytokines, chemokines, and PGE(2) from RAW 264.7 macrophage-like cells. International Immunopharmacology 10, 1268–1278.

Chansriniyom, C., Ruangrungsi, N., Lipipun, V., Kumamoto, T., Ishikawa, T., 2009. Isolation of acridone alkaloids and N-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives from *Glycosmis parva* and their anti-herpes simplex virus activity. Chemical and Pharmaceutical Bulletin 57, 1246–1250.

Chapman, K.D., 2004. Occurrence, metabolism, and prospective functions of N-acylethanolamines in plants. Progress in Lipid Research 43, 302–327.

Chen, I.S., Chen, T.L., Lin, W.Y., Tsai, I.L., Chen, Y.C., 1999. Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*. Phytochemistry 52, 357–360.

Chen, I.S., Tsai, I.W., Teng, C.M., Chen, J.J., Chang, Y.L., Ko, F.N., Lu, M.C., Pezzuto, J.M., 1997. Pyranoquinoline alkaloids from *Zanthoxylum simulans*. Phytochemistry 46, 525–529.

Cheng, M.J., Tsai, I.L., Chen, I.S., 2003. Chemical constituents from the root bark of Formosan *Zanthoxylum ailanthoides*. Journal of the Chinese Chemical Society 50, 1241–1246.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Cheng, M.J., Wu, C.C., Tsai, A.L., Chen, I.S., 2004. Chemical and antiplatelet constituents from the stem of *Zanthoxylum beecheyanum*. Journal of the Chinese Chemical Society 51, 1065–1072.

Chicca, A., Raduner, S., Pellati, F., Strompen, T., Altmann, K.-H., Schoop, R., Gertsch, J., 2009. Synergistic immunomopharmacological effects of N-alkylamides in *Echinacea purpurea* herbal extracts. International Immunopharmacology. 9, 850–858.

Christensen, K.B., Petersen, R.K., Petersen, S., Kristiansen, K., Christensen, L.P., 2009. Activation of PPAR gamma by metabolites from the flowers of purple coneflower (*Echinacea purpurea*). Journal of Natural Products 72, 933–937.

Christensen, L.P., 1992. Acetylenes and related compounds in Asteraceae (=Compositae).4. Acetylenes and related compounds in Anthemideae. Phytochemistry 31, 7–49.

Christensen, L.P., Lam, J., 1991a. Acetylenes and related compounds in Asteraceae (=Compositae).3. Acetylenes and related compounds in Astereae. Phytochemistry 30, 2453–2476.

Christensen, L.P., Lam, J., 1991b. Acetylenes and related compounds in Heliantheae. Phytochemistry 30, 11–49.

Christodoulopoulou, L., Tsoukatou, M., Tziveleka, L.A., Vagias, C., Petrakis, P.V., Roussis, V., 2005. Piperidinyl amides with insecticidal activity from the maritime plant *Otanthus maritimus*. Journal of Agricultural and Food Chemistry 53, 1435–1439.

Clifford, L.J., Nair, M.G., Rana, J., Dewitt, D.L., 2002. Bioactivity of alkamides isolated from *Echinacea purpurea* (L.) Moench. Phytomedicine 9, 249–253.

Codd, E.F., 1970. A relational model of data for large shared data banks. Communications of the ACM 13, 377.

Correa, E., Sterner, O., Echeverri, F., 2011. Prenylhydroxybenzoic acid compounds with pungent activity from *Piper arieianum* (CDC) leaves. Phytochemistry 72, 238–241.

Crombie, L., 1955. Amides of vegetable origin.4. the nature of pellitorine and anacyclin. Journal of the Chemical Society, 999–1006.

Crombie, L., Denman, R., 1984. Insecticidal amides—synthesis of natural 2(E),4(E),10(E)-pipercide, its 2(E),4(E),10(Z)-stereomer, and related isobutylamides. Tetrahedron Letters 25, 4267–4270.

Cuong, N.M., Taylor, W.C., Sung, T.V., 1999. Glypetelotine, a sulphur-containing indole alkaloid from *Glycosmis petelotii*. Phytochemistry 52, 1711–1714.

de Moura, N.F., Morel, A.F., Dessoy, E.C., Zanatta, N., Burger, M.M., Ahlert, N., Porto, G.P., Baldisserotto, B., 2002. Alkaloids, amides and antispasmodic activity of *Zanthoxylum hyemale*. Planta Medica 68, 534–538.

Derry, S., Lloyd, R., Moore, R.A., McQuay, H.J., 2009. Topical capsaicin for chronic neuropathic pain in adults. Cochrane Database of Systematic Reviews, 4.

Ee, G.C.L., Lim, C.M., Rahmani, M., Shaari, K., Bong, C.F.J., 2010. Pellitorine, a potential anti-cancer lead compound against HL60 and MCT-7 cell lines and microbial transformation of piperine from *Piper Nigrum*. Molecules 15, 2398–2404.

Esanu, A., 1981. Phenoxy Alkylamides. US19790050106 19790619. Chimique, Soc d etudes prod chimique, US.

Facchini, P.J., Hagel, J., Zulak, K.G., 2002. Hydroxycinnamic acid amide metabolism: physiology and biochemistry. Canadian Journal of Botany–Revue Canadienne De Botanique 80, 577–589.

Fahy, E., Subramaniam, S., Brown, H.A., Glass, C.K., Merrill, A.H., Murphy, R.C., Raetz, C.R.H., Russell, D.W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M.S., White, S.H., Witztum, J.L., Dennis, E.A., 2005. A comprehensive classification system for lipids. Journal of Lipid Research 46, 839–861.

Fahy, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R.H., Shimizu, T., Spener, F., van Meer, G., Wakelam, M.J.O., Dennis, E.A., 2009. Update of the LIPID MAPS comprehensive classification system for lipids. Journal of Lipid Research 50, S9–S14.

Galopin, C.C., Furrer, S.M., Goeke, A., 2004. Pungent and tingling compounds in Asian cuisine. In: Hofmann, T.H.C.T.P.W. (Ed.), Challenges in Taste Chemistry and Biology, 867; 2004, pp. 139–152.

Gertsch, J., 2008. Immunmodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system. Planta Medica 74, 638–650.

Gertsch, J., Raduner, S., Altmann, K.-H., 2006a. Review—new natural noncannabinoid ligands for cannabinoid type-2 (CB2) receptors. Journal of Receptors and Signal Transduction 26, 709–730.

Gertsch, J., Raduner, S., Feyen, F., Altmann, K.H., 2006b. Exploration of natural alkylamides and synthetic analogs as source for new ligands for the cannabinoid type-2 receptor. Planta Medica 72, 970–971.

Gertsch, J., Raduner, S., Tytgat, J., Peigneur, S., Quednow, B., Bilkei-Gorzo, A., Zimmer, A., Altmann, K.H., 2008. Analgesic and neuropsychological effects of Echinacea N-alkylamides. Planta Medica 74, 1014.

Goel, V., Chang, C., Slama, J.V., Barton, R., Bauer, R., Gahler, R., Basu, T.K., 2002. Echinacea stimulates macrophage function in the lung and spleen of normal rats. Journal of Nutritional Biochemistry 13, 487–492.

Greger, H., 1984. Alkamides—structural relationships, distribution and biological activity. Planta Medica 50, 366–375.

Greger, H., Grenz, M., Bohlmann, F., 1981. Polyacetylenic compounds.260. Amides from Achillea species and *Leucocyclus formosus*. Phytochemistry 20, 2579–2581.

Greger, H., Grenz, M., Bohlmann, F., 1982. Polyacetylenic compounds.261. Piperidides and other amides from Achillea species. Phytochemistry 21, 1071–1074.

Greger, H., Hadacek, F., Hofer, O., Wurz, G., Zechner, G., 1993a. Different types of sulfur-containing amides from Glycosmis CF chlorosperma. Phytochemistry 32, 933–936.

Greger, H., Hofer, M., Teichmann, K., Schinnerl, J., Pannell, C.M., Vajrodaya, S., Hofer, O., 2008. Amide-esters from *Aglaia tenuicaulis*—first representatives of a class of compounds structurally related to bisamides and flavaglines. Phytochemistry 69, 928–938.

Greger, H., Hofer, O., 1984. On the pungent principle of *Matricaria pubescens*. Phytochemistry 23, 1173–1174.

Greger, H., Hofer, O., 1987. Highly unsaturated isopentyl amides from *Achillea wilhelmsii*. Journal of Natural Products 50, 1100–1107.

Greger, H., Hofer, O., Kahlig, H., Wurz, G., 1992. Sulfur- containing cinnamides with antifungal activity from *Glycosmis cyanocarpa*. Tetrahedron 48, 1209–1218.

Greger, H., Hofer, O., Zechner, G., Hadacek, F., Wurz, G., 1994. Sulfones derived from methylthiopropenoiec acid amides from *Glycosmis angustifolia*. Phytochemistry 37, 1305–1310.

Greger, H., Werner, A., 1990. Comparative HPLC analyses of alkamides within the *Achillea millefolium* group. Planta Medica 56, 482–486.

Greger, H., Zdero, C., Bohlmann, F., 1983. Polyacetylenic compounds.268. Further unsaturated amides from Achillea species. Liebigs Annalen Der Chemie, 1194–1201.

Greger, H., Zdero, C., Bohlmann, F., 1984. Pyrrolidine and piperidine amides from Achillea. Phytochemistry 23, 1503–1505.

Greger, H., Zdero, C., Bohlmann, F., 1987. Pyrrole amides from *Achillea ageratifolia*. Phytochemistry 26, 2289–2291.

Greger, H., Zechner, G., Hofer, O., Hadacek, F., Wurz, G., 1993b. Sulfur-containing amides from glycosmis species with different antifungal activity. Phytochemistry 34, 175–179.

Greger, H., Zechner, G., Hofer, O., Vajrodaya, S., 1996. Bioactive amides from Glycosmis species. Journal of Natural Products 59, 1163–1168.

Gurley, B.J., Swain, A., Williams, D.K., Barone, G., Battu, S.K., 2008. Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: comparative effects of St. John's wort, Echinacea, clarithromycin, and rifampin on digoxiin pharmacokinetics. Molecular Nutrition and Food Research 52, 772–779.

Hamraoui, A., Regnault-Roger, C., 1997. Monoterpenoids insecticidal effect comparison on two phytophagic insects, *Ceratitis capitata* and *Rhopalosiphum padi*. Acta Botanica Gallica 144, 413–417.

Han, S.H., Lee, H.H., Lee, I.S., Moon, Y.H., Woo, E.R., 2002. A new phenolic amide from Lycium chinense Miller. Archives of Pharmacal Research 25, 433–437.

Handrick, V., Vogt, T., Frolov, A., 2010. Profiling of hydroxycinnamic acid amides in *Arabidopsis thaliana* pollen by tandem mass spectrometry. Analytical and Bioanalytical Chemistry 398, 2789–2801.

Hansen, T.S., Nilsen, O.G., 2009. *Echinacea purpurea* and P-glycoprotein drug transport in Caco-2 cells. Phytotherapy Research 23, 86–91.

Hashimoto, K., Satoh, K., Kase, Y., Ishige, A., Kubo, M., Sasaki, H., Nishikawa, S., Kurosawa, S., Yalabi, K., Nakamura, T., 2001. Modulatory effect of aliphatic acid amides from *Zanthoxylum piperitum* on isolated gastrointestinal tract. Planta Medica 67, 179–181.

Hatano, T., Inada, K., Ogawa, T., Ito, H., Yoshida, T., 2004. Aliphatic acid amides of the fruits of *Zanthoxylum piperitum*. Phytochemistry 65, 2599–2604.

Hellum, B.H., Nilsen, O.G., 2008. *In vitro* inhibition of CYP3A4 metabolism and P-glycoprotein-mediated transport by trade herbal products. Basic and Clinical Pharmacology and Toxicology 102, 466–475.

Hernandez, I., Marquez, L., Martinez, I., Dieguez, R., Delporte, C., Prieto, S., Molina-Torres, J., Garrido, G., 2009. Anti-inflammatory effects of ethanolic extract and alkamides-derived from *Heliopsis longipes* roots. Journal of Ethnopharmacology 124, 649–652.

Hinterberger, S., Hofer, O., Greger, H., 1994. Synthesis and corrected structures of sulfur-containing amides from Glycosmis species—sinharines, penimides, and illukumbins. Tetrahedron 50, 6279–6286.

Hinterberger, S., Hofer, O., Greger, H., 1998. Synthesis of amides from Glycosmis species: methylthiopropenoic acid, methylsulfonylpropenoic acid, thiocarbamic acid S-methyl ester, and senecioic acid amides. Tetrahedron 54, 487–496.

Hinz, B., Woelkart, K., Bauer, R., 2007. Alkamides from Echinacea inhibit cyclooxygenase-2 activity in human neuroglioma cells. Biochemical and Biophysical Research Communications 360, 441–446.

Hiserodt, R.D., Pope, B.M., Cossette, M., Dewis, M.L., 2004. Proposed mechanisms for the fragmentation of doubly allylic alkenamides (tingle compounds) by low energy collisional activation in a triple quadrupole mass spectrometer. Journal of the American Society for Mass Spectrometry 15, 1462–1470.

Hofer, O., Greger, H., Lukaseder, B., Vajrodaya, S., Bacher, M., 2000. Prenylated sulfonyl amides from Glycosmis species. Phytochemistry 54, 207–213.

Hofer, O., Zechner, G., Vajrodaya, S., Lutz, G., Greger, H., 1995. New anthranilic and methylsulfonylpropenoic acid amides from Thai Glycosmis species. Liebigs Annalen, 1789–1794.

Hohmann, J., Redei, D., Forgo, P., Szabo, P., Freund, T.F., Haller, J., Bojnik, E., Benyhe, S., 2011. Alkamides and a neolignan from *Echinacea purpurea* roots and the interaction of alkamides with G-protein-coupled cannabinoid receptors. Phytochemistry 72, 1848–1853.

Holzer, P., 2011. Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. Pharmacology and Therapeutics 131, 142–170.

Humphries, C.J., 1979. A revision of the genus Anacylcus (Compositae: Anthemideae). Bulletin of the British Museum—Natural History: Botany 7, 83–142.

Hungeling, M., Lechtenberg, M., Fronczek, F.R., Nahrstedt, A., 2009. Cyanogenic and non-cyanogenic pyridone glucosides from *Acalypha indica* (Euphorbiaceae). Phytochemistry 70, 270–277.

Ingolfsdottir, K., Gissurarson, S.R., Nenninger, A., Neszmelyi, A., Wiedemann, B., Wagner, H., 1997. Biologically active alkamide from the lichen *Stereocaulon alpinum*. Phytomedicine 4, 331–334.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Iseli, V., Potterat, O., Hagmann, L., Egli, J., Hamburger, M., 2007. Characterization of the pungent principles and the essential oil of *Zanthoxylum schinifolium* pericarp. Pharmazie 62, 396–400.

Jacobson, M., 1954. Constituents of Heliopsis species. 3. Cis–trans isomerism of affinin. Journal of American chemistry Society 76, 4606–4608.

Jang, K.H., Chang, Y.H., Kim, D.-D., Oh, K.-B., Oh, U., Shin, J., 2008. New polyunsaturated fatty acid amides isolated from the seeds of *Zanthoxylum piperitum*. Archives of Pharmacal Research 31, 569–572.

Jenett-Siems, K., Weigl, R., Kaloga, M., Schulz, J., Eich, E., 2003. Ipobscurines C and D: macrolactam-type indole alkaloids from the seeds of *Ipomoea obscura*. Phytochemistry 62, 1257–1263.

Jente, R., Bohlmann, F., Bonnet, P.H., 1972. Polyacetylenic compounds.206. Constituents of *anacyclus pyrethrum* DC. Chemische Berichte-Recueil 105, 1694.

Kalia, N.K., Singh, B., Sood, R.P., 1999. A new amide from *Zanthoxylum armatum*. Journal of Natural Products 62, 311–312.

Kalil-Gaspar, P., Marcuzzo, S., Rigon, P., Molina, C.G., Achaval, M., 2007. Capsaicin-induced avoidance behavior in the terrestrial *Gastropoda Megalobulimus abbreviatus*: evidence for TRPV-1 signaling and opioid modulation in response to chemical noxious stimuli. Comparative Biochemistry and Physiology A—Molecular and Integrative Physiology 148, 286–291.

Kalim, M.D., Bhattacharyya, D., Banerjee, A., Chattopadhyay, S., 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Complementary and Alternative Medicine, 10.

Kang, K., Lee, K., Ishihara, A., Park, S., Kim, Y.S., Back, K., 2010. Induced synthesis of caffeoylserotonin in pepper fruits upon infection by the anthracnose fungus, *Colletotrichum gloeosporioides*. Scientia Horticulturae 124, 290–293.

Kang, S., Back, K., 2006. Enriched production of N-hydroxycinnamic acid amides and biogenic amines in pepper (*Capsicum annuum*) flowers. Scientia Horticulturae 108, 337–341.

Kaouas, A., Renes, H., Winke, C., 2010. Geranylamine derivatives as flavouring agents. Givaudan, Switzerland. (13/202.194).

Kashiwada, Y., Ito, C., Katagiri, H., Mase, I., Komatsu, K., Namba, T., Ikeshiro, Y., 1997. Amides of the fruit of Zanthoxylum spp. Phytochemistry 44, 1125–1127.

Keipert, R., 2009. Acmella ciliata (H.B.K.) Cassini Phytochemische und enzymatische Untersuchungen, galenisch Präformulierungen. Biologie, Chemie, Pharmazie, vol. Doktors der Naturwissenschaften. Freien Universitat Berlin, Berlin.

Kim, K.H., Choi, S.U., Son, M.W., Lee, K.R., 2010a. Two new phenolic amides from the seeds of pharbitis nil. Chemical and Pharmaceutical Bulletin 58, 1532–1535.

Kim, S.-C., Chapman, K.D., Blancaflor, E.B., 2010b. Fatty acid amide lipid mediators in plants. Plant Science 178, 411–419.

King, R.R., Calhoun, L.A., 2010. A feruloyltyramine trimer isolated from potato common scab lesions. Phytochemistry 71, 2187–2189.

Koo, J.Y., Jang, Y., Cho, H., Lee, C.-H., Jang, K.H., Chang, Y.H., Shin, J., Oh, U., 2007. Hydroxy-alpha-sanshool activates TRPV1 and TRPA1 in sensory neurons. European Journal of Neuroscience 26, 1139–1147.

Kubo, I., Matsumoto, T., Klocke, J.A., Kamikawa, T., 1984. Molluscicidal and insecticidal activities of isobutylamides isolated from *Fagara macrophylla*. Experientia 40, 340–341.

Lalone, C.A., Hammer, K.D.P., Wu, L., Bae, J., Leyva, N., Liu, Y., Solco, K.S., Kraus, G.A., Murphy, P.A., Wurtele, E.S., Kim, O.-K., Seo, K.I.I., Widrlechner, M.P., Birt, D.F., 2007. Echinacea species and alkamides inhibit prostaglandin E-2 production in RAW264.7 mouse macrophage cells. Journal of Agricultural and Food Chemistry 55, 7314–7322.

Langer, K., Ley, J., Reinders, G., Kindel, G., Krammer, G., 2009. Use of alkamides for masking an unpleasant flavour. EP20080168519 20081106. Symrise GMBH & Co., Germany.

Lennertz, R.C., Tsunozaki, M., Bautista, D.M., Stucky, C.L., 2010. Physiological basis of tingling paresthesia evoked by hydroxy-alpha-sanshool. Journal of Neuroscience 30, 4353–4361.

Ley, J.P., Blings, M., Krammer, G., Reinders, G., Schmidt, C.O., Bertram, H.J., 2006. Isolation and synthesis of acmellonate, a new unsaturated long chain 2-ketol ester from *Spilanthes acmella*. Natural Product Research 20, 798–804.

Ley, J.P., Kindel, G., Krammer, G., 2005a. Use of alkene carboxylic acid n alkylamides as flavouring agents. WO2004EP52683 20041028. Symrise GMBH & Co., Germany.

Ley, J.P., Hilmer, J.M., Weber, B., Krammer, G., Gatfield, I.L., Bertram, H.J., 2004. Stereoselective enzymatic synthesis of cis-pellitorine, a taste active alkamide naturally occurring in tarragon. European Journal of Organic Chemistry, 5135–5140.

Ley, J.P., Krammer, G., Reinders, G., Bertram, H.J., 2005b. Structure activity relationships of trigeminal effects for artificial and naturally occuring alkamides related to spilanthol. In: Proceedings of the 11th Weurnann Flavour Research Symposium. Symrise, Roskilde, Denmark.

Li, J.X., Shi, Q., Xiong, Q.B., Prasain, J.K., Tezuka, Y., Hareyama, T., Wang, Z.T., Tanaka, K., Namba, T., Kadota, S., 1998. Tribulusamide A and B, new hepatoprotective lignanamides from the fruits of *Tribulus terrestris*: indications of cytoprotective activity in murine hepatocyte culture. Planta Medica 64, 628–631.

Lopez-Bucio, J., Acevedo-Hernandez, G., Ramirez-Chavez, E., Molina-Torres, J., Herrera-Estrella, L., 2006. Novel signals for plant development. Current Opinion in Plant Biology 9, 523–529.

Luo, X.-J., Peng, J., Li, Y.-J., 2011. Recent advances in the study on capsaicinoids and capsinoids. European Journal of Pharmacology 650, 1–7.

Martin, R., Becker, H., 1984. Spilanthol related amides from *Acmella ciliata*. Phytochemistry 23, 1781–1783.

Martin, R., Becker, H., 1985. Amides and other constituents from *Acmella ciliata*. Phytochemistry 24, 2295–2300.

Martintanguy, J., Cabanne, F., Perdrizet, E., Martin, C., 1978. Distribution of hydroxycinnamic acid amides from flowering plants. Phytochemistry 17, 1927–1928.

Matovic, N., Matthias, A., Gertsch, J., Raduner, S., Bone, K.M., Lehmann, R.P., DeVoss, J.J., 2007. Stereoselective synthesis, natural occurrence and CB2 receptor binding affinities of alkylamides from herbal medicines such as Echinacea sp. Organic and Biomolecular Chemistry 5, 169–174.

Matsuda, H., Ninomiya, K., Morikawa, T., Yasuda, D., Yamaguchi, I., Yoshikawa, M., 2009. Hepatoprotective amide constituents from the fruit of *Piper chaba*: structural requirements, mode of action, and new amides. Bioorganic and Medicinal Chemistry. 17, 7313–7323.

Matthias, A., Gillam, E.M.J., Penman, K.G., Matovic, N.J., Bone, K.M., De Vos, J.J., Lehmann, R.P., 2005. Cytochrome P450 enzyme-mediated degradation of *Echinacea alkylamides* in human liver microsomes. Chemico-Biological Interactions 155, 62–70.

Matthias, A., Banbury, L., Bone, K.M., Leach, D.N., Lehmann, R.P., 2008. Echinacea alkylamides modulate induced immune responses in T-cells. Fitoterapia 79, 53–58.

Matthias, A., Banbury, L., Stevenson, L.M., Bone, K.M., Leach, D.N., Lehmann, R.P., 2007. Alkylamides from Echinacea modulate induced immune responses in macrophages. Immunological Investigations 36, 117–130.

Mbaze, L.M.a., Lado, J.A., Wansi, J.D., Shiao, T.C., Chiozem, D.D., Mesaik, M.A., Choudhary, M.I., Lacaille-Dubois, M.-A., Wandji, J., Roy, R., Sewald, N., 2009. Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark of *Fagara heitzii* (Rutaceae). Phytochemistry 70, 1442–1447.

Mbeunkui, F., Grace, M.H., Lategan, C., Smith, P.J., Raskin, I., Lila, M.A., 2011. Isolation and identification of antiplasmodial N-alkylamides from Spilanthes acmella flowers using centrifugal partition chromatography and ESI-IT-TOF-MS. Journal of Chromatography B—Analytical Technologies in the Biomedical and Life Sciences 879, 1886–1892.

McCollom, M.M., Villinski, J.R., McPhail, K.L., Craker, L.E., Gafner, S., 2005. Analysis of macamides in samples of Maca (*Lepidium meyenii*) by HPLC-UV-MS/MS. Phytochemical Analysis 16, 463–469.

Minamino, M., Sakaguchi, I., Naka, T., Ikeda, N., Kato, Y., Tomiyasu, I., Yano, I., Kobayashi, K., 2003. Bacterial ceramides and sphingophospholipids induce apoptosis of human leukaemic cells. Microbiology 149, 2071–2081.

Molina-Torres, J., Garcia-Chavez, A., Ramirez-Chavez, E., 1999. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. Journal of Ethnopharmacology 64, 241–248.

Molina-Torres, J., Salazar-Cabrera, C.J., Armenta-Salinas, C., Ramirez-Chavez, E., 2004. Fungistatic and bacteriostatic activities of alkamides from *Heliopsis longipes* roots: affinin and reduced amides. Journal of Agricultural and Food Chemistry 52, 4700–4704.

Molina-Torres, J., Santos-Escobar, F., González-Arnao, M., Ramírez-Chávez, E., Sánchez Pavón, E., 2008. Síntesis Enzimática de Alcamidas en Sistemas No Acuosos y Ensayo de su Bioactividad *In Vitro*. Latin American Journal of Pharmacy 27, 852–858.

Molina-Torres, J., SalgadoGarciglia, R., RamirezChavez, E., DelRio, R.E., 1996. Purely olefinic alkamides in *Heliopsis longipes* and *Acmella* (Spilanthes) *oppositifolia*. Biochemical Systematics and Ecology. 24, 43–47.

Muhammad, I., Zhao, J.P., Dunbar, D.C., Khan, I.A., 2002. Constituents of *Lepidium meyenii* 'maca'. Phytochemistry 59, 105–110.

Mullerjakic, B., Breu, W., Probstle, A., Redl, K., Greger, H., Bauer, R., 1994. *In-vitro* inhibition of cyclooxygenase and 5-lipoxygenase by alkamides from echinacea and achillea species. Planta Medica 60, 37–40.

Ndom, J.C., Mbafor, J.T., Meva'a, L.M., Kakam, Z., Phanuel, A.S., Ndongo, E., Harwood, L.M., Mpondo, T.N., 2010. New alkamide and ent-kaurane diterpenoid derivatives from *Senecio erechtitoides* (Asteraceae). Phytochemistry Letters 3, 201–206.

Negrel, J., Pollet, B., Lapierre, C., 1996. Ether-linked ferulic acid amides in natural and wound periderms of potato tuber. Phytochemistry 43, 1195–1199.

Nezbedová, L., Drandarov, K., Deschamps, N., Werner, C., Hesse, M., 2001. Biogenesis and function of macrocyclic spermine alkaloids. Arkivoc, 154–164.

Pacher, T., Raninger, A., Lorbeer, E., Brecker, L., But, P.P.-H., Greger, H., 2010. Alcoholysis of naturally occurring imides: misleading interpretation of antifungal activities. Journal of Natural Products 73, 1389–1393.

Pahuja, M., Mehla, J., Reeta, K.H., Joshi, S., Gupta, Y.K., 2010. Protective effect of *Anacyclus pyrethrum* against seizures and seizure-induced cognitive impairment in rats. European Neuropsychopharmacology 20, S246–S247.

Pal, M., Angaru, S., Kodimuthali, A., Dhingra, N., 2009. Vanilloid receptor antagonists: emerging class of novel anti-inflammatory agents for pain management. Current Pharmaceutical Design 15, 1008–1026.

Pandit, S., Mukherjee, P.K., Mukherjee, K., Gajbhiye, R., Venkatesh, M., Ponnusankar, S., Bhadra, S., 2012. Cytochrome P450 inhibitory potential of selected Indian spices—possible food drug interaction. Food Research International 45, 69–74.

Park, S., Kang, K., Lee, K., Choi, D., Kim, Y.-S., Back, K., 2009. Induction of serotonin biosynthesis is uncoupled from the coordinated induction of tryptophan biosynthesis in pepper fruits (*Capsicum annuum*) upon pathogen infection. Planta 230, 1197–1206.

Parr, A.J., Mellon, F.A., Colquhoun, I.J., Davies, H.V., 2005. Dihydrocaffeoyl polyamines (kukoamine and allies) in potato (*Solanum tuberosum*) tubers detected during metabolite profiling. Journal of Agricultural and Food Chemistry 53, 5461–5466.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Penali, L., Mulholland, D.A., Tano, K.D., Cheplogoi, P.K., Randrianarivelojosia, M., 2007. Low antiplasmodial activity of alkaloids and amides from the stem bark of *Zanthoxylum rubescens* (Rutaceae). Parasite-Journal De La Societe Francaise De Parasitologie 14, 161–164.

Philip, A., Thakur, R., 2011. Post herpetic neuralgia. Journal of Palliative Medicine 14, 765–773.

Qureshi, A.A., Qureshi, A.A., Omer, S., Sanghai, D.B., Setty, S.R., Bhajipale, N.S., 2008. Capsaicin: preclinical and clinical studies. Plant Archives 8, 7–11.

Raduner, S., Majewska, A., Chen, J.Z., Xie, X.Q., Hamon, J., Faller, B., Altmann, K.H., Gertsch, J., 2006. Alkylamides from Echinacea are a new class of cannabinomimetics—cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. Journal of Biological Chemistry 281, 14192–14206.

Raduner, S., Bisson, W., Abagyan, R., Altmann, K.-H., Gertsch, J., 2007. Self-assembling cannabinomimetics: supramolecular structures of N-alkyl amides. Journal of Natural Products 70, 1010–1015.

Rahalison, L., Hamburger, M., Monod, M., Frenk, E., Hostettmann, K., 1994. Antifungal tests in phytochemical investigations—comparison of bioautographic methods using phytopathogenic and human pathogenic fungi. Planta Medica 60, 41–44.

Rahmani, M., Leng, K.W., Ismail, H.B.M., Hin, T.Y.Y., Sukari, M.A., Ali, A.M., Kulip, J., 2004. A new flavonoid and sulphur-containing amides from *Glycosmis chlorosperma*. Natural Product Research 18, 85–88.

Rahmani, M., Serang, R.M., Hashim, N.M., Sukari, M.A., Ee, G.C.L., Ali, A.M., Ismail, H.B.M., 2010. Alkaloids and sulphur-containing amides from *Glycosmis citrifolia* and *Glycosmis elongata*. Sains Malaysiana. 39, 445–451.

Raith, K., Farwanah, H., Wartewig, S., Neubert, R.H.H., 2004. Progress in the analysis of *Stratum corneum* ceramides. European Journal of Lipid Science and Technology 106, 561–571.

Ramirez-Chavez, E., Lopez-Bucio, J., Herrera-Estrella, L., Molina-Torres, J., 2004. Alkamides isolated from plants promote growth and alter root development in arabidopsis. Plant Physiology 134, 1058–1068.

Ramsewak, R.S., Erickson, A.J., Nair, M.G., 1999. Bioactive N-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 51, 729–732.

Ratnasooriya, W.D., Pieris, K.P.P., Samaratunga, U., Jayakody, J., 2004. Diuretic activity of *Spilanthes acmella* flowers in rats. Journal of Ethnopharmacology 91, 317–320.

Reyes-Escogido, M.D., Gonzalez-Mondragon, E.G., Vazquez-Tzompantzi, E., 2011. Chemical and pharmacological aspects of capsaicin. Molecules 16, 1253–1270.

Riemer, B., Hofer, O., Greger, H., 1997. Tryptamine derived amides from *Clausena indica*. Phytochemistry 45, 337–341.

Rimbau, V., Risco, E., Canigueral, S., Iglesias, J., 1996. Antiinflammatory activity of some extracts from plants used in the traditional medicine of North-African countries. Phytotherapy Research 10, 421–423.

Rios-Chavez, P., Ramirez-Chavez, E., Armenta-Salinas, C., Molina-Torres, J., 2003. Acmella radicans var. radicans: *in vitro* culture establishment and alkamide content. *In Vitro* Cellular and Developmental Biology—Plant 39, 37–41.

Rios, M.Y., Aguilar-Guadarrama, A.B., del Carmen Gutierrez, M., 2007. Analgesic activity of affinin, an alkamide from *Heliopsis longipes* (Compositae). Journal of Ethnopharmacology 110, 364–367.

Rodeiro, I., Donato, M.T., Jimenez, N., Garrido, G., Molina-Torres, J., Menendez, R., Castell, J.V., Gomez-Lechon, M.J., 2009. Inhibition of human P450 enzymes by natural extracts used in traditional medicine. Phytotherapy Research 23, 279–282.

Roth, G.N., Chandra, A., Nair, M.G., 1998. Novel bioactivities of *Curcuma longa* constituents. Journal of Natural Products 61, 542–545.

Saadali, B., Boriky, D., Blaghen, M., Vanhaelen, M., Talbi, M., 2001. Alkamides from *Artemisia dracunculus*. Phytochemistry 58, 1083–1086.

Sagner, S., Shen, Z.W., Deus-Neumann, B., Zenk, M.H., 1998. The biosynthesis of lunarine in seeds of *Lunaria annua*. Phytochemistry 47, 375–387.

Schneider, S., Reichling, J., Stintzing, F.C., Messerschmidt, S., Meyer, U., Schnitzler, P., 2010. Anti-herpetic properties of hydroalcoholic extracts and pressed juice from *Echinacea pallida*. Planta Medica 76, 265–272.

Sharma, V., Boonen, J., Chauhan, N.S., Thakur, M., De Spiegeleer, B., Dixit, V.K. Spilanthes acmella ethanolic flower extract: LC–MS alkylamide profiling and its effects on sexual behavior in male rats. Phytomedicine, in press. **Q7**

Sharma, V., Thakur, M., Chauhan, N.S., Dixit, V.K., 2009. Evaluation of the anabolic, aphrodisiac and reproductive activity of *Anacyclus Pyrethrum* DC in male rats. Pharmaceutical Biology 77, 97–110.

Siddiqui, B.S., Gulzar, T., Begum, S., Rasheed, M., Saftar, F.A., Afshan, F., 2003. Two new insecticidal amides and a new alcoholic amide from *Piper nigrum* LINN. Helvetica Chimica Acta 86, 2760–2767.

Sittie, A.A., Lemmich, E., Olsen, C.E., Hviid, L., Christensen, S.B., 1998. Alkamides from *Phyllanthus fraternus*. Planta Medica 64, 192–193.

Spelman, K., 2009. The extraction, stability, metabolism and bioactivity of the alkylamides in Echinacea spp.. Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, p. 210.

Squires, Meryl, 2001. Antimicrobial Treatment for Herpes Simplex Virus and Other Infectious Diseases. EA19980000893 19970312. Squires Meryl, US.

Stevenson, L.M., Matthias, A., Banbury, L., Penman, K.G., Bone, K.M., Leach, D., Lehmann, R.P., 2005. Modulation of macrophage immune responses by Echinacea. Molecules 10, 1279–1285.

Stöhr, J.R., Xiao, P.G., Bauer, R., 1999. Isobutylamides and a new methylbutylamide from *Piper sarmentosum*. Planta Medica 65, 175–177. **Q11**

Strunz, G.M., 2000. Unsaturated amides from Piper species (Piperaceae). Studies in Natural Products Chemistry 24, 683–738.

Su, H.C.F., Horvat, R., 1981. Isolation, identification, and insecticidal properties of *piper-nigrum* amides. Journal of Agricultural and Food Chemistry 29, 115–118.

Subehan, Usia, Kadota, T., Tezuka, Y., S., 2006. Alkamides from *Piper nigrum* L. and their inhibitory activity against human liver microsomal cytochrome P450 2D6 (CYP2D6). Natural Product Communications 1, 1–7.

Sugai, E., Morimitsu, Y., Iwasaki, Y., Morita, A., Watanabe, T., Kubota, K., 2005. Pungent qualities of sanshool-related compounds evaluated by a sensory test and activation of rat TRPV1. Bioscience Biotechnology and Biochemistry 69, 1951–1957.

Sugano, H., Yoshida, F., 1987. Composition for oral cavity application. L. Corp., Japan (JP62198611). **Q12**

Tanaka, E., Tanaka, C., Mori, N., Kuwahara, Y., Tsuda, M., 2003. Phenylpropanoid amides of serotonin accumulate in witches' broom diseased bamboo. Phytochemistry 64, 965–969.

Tewksbury, J.J., Reagan, K.M., Machnicki, N.J., Carlo, T.A., Haak, D.C., Calderon Penaloza, A.L., Levey, D.J., 2008. Evolutionary ecology of pungency in wild chilies. Proceedings of the National Academy of Sciences of the United States of America 105, 11808–11811.

Tofern, B., Mann, P., Kaloga, M., Jenett-Siems, K., Witte, L., Eich, E., 1999. Aliphatic pyrrolidine amides from two tropical convolvulaceous species. Phytochemistry 52, 1437–1441.

Toselli, F., Matthias, A., Bone, K.M., Gillam, E.M.J., Lehmann, R.P., 2010. Metabolism of the Major Echinacea Alkylamide N-isobutyldodeca-2E,4E,8Z,10Z-tetraenamide by Human Recombinant Cytochrome P450 Enzymes and Human Liver Microsomes. Phytotherapy Research 24, 1195–1201.

Toselli, F., Matthias, A., Gillam, E.M.J., 2009. Echinacea metabolism and drug interactions: the case for standardization of a complementary medicine. Life Sciences 85, 97–106.

Turnock, J., Cowan, S., Watson, A., Bartholomew, B., Bright, C., Latif, Z., Sarker, S.D., Nash, R.J., 2001. N-trans-feruloyltyramine from two species of the Solanaceae. Biochemical Systematics and Ecology 29, 209–211.

Usia, T., Iwata, H., Hiratsuka, A., Watabe, T., Kadota, S., Tezuka, Y., 2006. CYP3A4 and CYP2D6 inhibitory activities of Indonesian medicinal plants. Phytomedicine 13, 67–73.

Vasques-da-Silva, R., Navickiene, H.M.D., Kato, M.J., Bolzania, V.D.S., Meda, C.I., Young, M.C.M., Furlan, M., 2002. Antifungal amides from *Piper arboreum* and *Piper tuberculatum*. Phytochemistry 59, 521–527.

Viana, F., 2011. Chemosensory properties of the trigeminal system. ACS Chemical Neuroscience 2, 38–50.

Wabnitz, R., Angsorg, R., 1997. Agent for treating infections. Asche AG., Germany (DE19951026206 19950718). **Q13**

Wallace, M., Pappagallo, M., 2011. Qutenza (R): a capsaicin 8% patch for the management of postherpetic neuralgia 11, 15–27. **Q14**

Wallwey, C., Li, S.-M., 2011. Ergot alkaloids: structure diversity, biosynthetic gene clusters and functional proof of biosynthetic genes. Natural Product Reports 28, 496–510.

Wang, Y., Wang, Y., McNeil, B., Harvey, L.M., 2007. Maca: an Andean crop with multi-pharmacological functions. Food Research International 40, 783–792.

Weenen, H., Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo, L.S., Kilimali, V., Wijnberg, J., 1990a. Antimalarial activity of Tanzanian plants.2. Anitmalarial compounds containing an alpha,beta-unsaturated carbonyl moiety from Tanzanian medicinal plants. Planta Medica 56, 371–373.

Weenen, H., Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo, L.S., Kilimali, V., Wijnberg, J., 1990b. Antimalarial activity of Tanzanian plants.2. Antimalarial compounds containing an alpha,beta-unsaturated carbonyl moiety from Tanzanian medical plants. Planta Medica 56, 371–373.

Werner, C., Petrini, O., Hesse, M., 1997. Degradation of the polyamine alkaloid aphelandrine by endophytic fungi isolated from *Aphelandra tetragona*. FEMS Microbiology Letters 155, 147–153.

Winkel, C., de Klerk, A., Visser, J., de Rijke, E., Bakker, J., Koening, T., Renes, H., 2008. New developments in Umami (enhancing) molecules. Chemistry and Biodiversity 5, 1195–1203.

Woelkart, K., Bauer, R., 2007. The role of alkamides as an active principle of Echinacea. Planta Medica 73, 615–623.

Woelkart, K., Bauer, R., Hinz, B., 2006. Alkamides from echinacea angustifolia roots inhibit cyclooxygenase-2-dependent prostaglandin synthesis in human neuroglioma cells. Planta Medica 72, 1062.

Woelkart, K., Salo-Ahen, O.M.H., Bauer, R., 2008. CB receptor ligands from plants. Current Topics in Medicinal Chemistry 8, 173–186.

Woelkart, K., Xu, W., Pei, Y., Makriyannis, A., Picone, R.P., Bauer, R., 2005. The endocannabinoid system as a target for alkamides from *Echinacea angustifolia* roots. Planta Medica 71, 701–705.

Wongsawatkul, O., Prachayasittikul, S., Isarankura-Na-Ayudhya, C., Satayavivad, J., Ruchirawat, S., Prachayasittikul, V., 2008. Vasorelaxant and antioxidant activities of *Spilanthes acmella* Murr. International Journal of Molecular Sciences 9, 2724–2744.

Wu, L.-c., Fan, N.-c., Lin, M.-h., Chu, I.-r., Huang, S.-j., Hu, C.-Y., Han, S.-y., 2008. Anti-inflammatory effect of spilanthol from Spilanthes acmella on murine macrophage by down-regulating LPS-induced inflammatory mediators. Journal of Agricultural and Food Chemistry 56, 2341–2349.

Wu, S.H., Sun, C.R., Pei, S.F., Lu, Y.B., Pan, Y.J., 2004. Preparative isolation and purification of amides from the fruits of *Piper longum* L. by upright counter-current chromatography and reversed-phase liquid chromatography. Journal of Chromatography A 1040, 193–204.

Wu, T.S., Ou, L.F., Teng, C.M., 1994. Aristolochic acids, aristolactam alkaloids and amides from *Aristolochia kankauensis*. Phytochemistry 36, 1063–1068.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038

ARTICLE IN PRESS

Xiong, Q.B., Shi, D.W., Yamamoto, H., Mizuno, M., 1997. Alkylamides from pericarps of *Zanthoxylum bungeanum*. Phytochemistry 46, 1123–1126.

Yang, X., 2008. Aroma constituents and alkylamides of red and green huajiao (*Zanthoxylum bungeanum* and *Zanthoxylum schinifolium*). Journal of Agricultural and Food Chemistry 56, 1689–1696.

Yasuda, I., Takeya, K., Itokawa, H., 1981a. Structures of amides from *Asiasarum hetrotropoides* Maek var mandshuricum Maek. Chemical and Pharmaceutical Bulletin 29, 564–566.

Yasuda, I., Takeya, K., Itokawa, H., 1981b. Two new pungent principles isolated from the pericarps of *Zanthoxylum ailanthoides*. Chemical and Pharmaceutical Bulletin 29, 1791–1793.

Yasuda, I., Takeya, K., Itokawa, H., 1982. Distribution of unsaturated aliphatic acid amides in Japanese Zanthoxylum species. Phytochemistry 21, 1295–1298.

Yoshida, F., Yamagishi, K., 1986. Composition for oral purpose. L. Corp, Japan (JP61155315).

Yoshida, F., Uematsu, M., 1985. Composition for oral cavity. L. Corp., Japan (JP60075424).

Yoshihara, T., Yamaguchi, K., Takamatsu, S., Sakamura, S., 1981. Phenolic compounds from the roots of Solanaceae plants.2. A new lignan amide, grossamide, from Bell pepper (*Capsicum annuum* var. *grossum*). Agricultural and Biological Chemistry 45, 2593–2598.

Zaidi, S.M.A., Pathan, S.A., Jain, G.K., Ahmad, F.J., Jamil, S., Singh, S., Khar, R.K., 2009. Anticonvulsant and neuropharmacological studies of *Anacyclus pyrethrum* root extract. Neuroscience Research 65, S250.

Zhai, Z., Haney, D.M., Wu, L., Solco, A.K., Murphy, P.A., Wurtele, E.S., Kohut, M.L., Cunnick, J.E., 2009. Alcohol extract of *Echinacea pallida* reverses stress-delayed wound healing in mice. Phytomedicine 16, 669–678.

Zhang, D., Nair, M.G., Murry, M., Zhang, Z.Z., 1997. Insecticidal activity of indanomycin. The Journal of Antibiotics 50, 617–620.

UNCORRECTED PROOF

Please cite this article as: Boonen, J., et al., Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. Journal of Ethnopharmacology (2012), http://dx.doi.org/10.1016/j.jep.2012.05.038