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# *Argyreia nervosa* (Burm. f.): Receptor profiling of lysergic acid amide and other potential psychedelic LSD-like compounds by computational and binding assay approaches

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ABSTRACT

*Ethnopharmacological relevance:* The *convolvulacea Argyreia nervosa* (Burm. f.) is well known as an important medical plant in the traditional Ayurvedic system of medicine and it is used in numerous diseases (*e.g.* nervousness, bronchitis, tuberculosis, arthritis, and diabetes). Additionally, in the Indian state of Assam and in other regions *Argyreia nervosa* is part of the traditional tribal medicine (*e.g.* the Santali people, the Lodhas, and others). In the western hemisphere, *Argyreia nervosa* has been brought in attention as so called "legal high". In this context, the seeds are used as source of the psychoactive ergotalkaloid lysergic acid amide (LSA), which is considered as the main active ingredient.
*Aim of the study:* As the chemical structure of LSA is very similar to that of lysergic acid diethylamide (LSD), the seeds of *Argyreia nervosa* (Burm. f.) are often considered as natural substitute of LSD. In the present study, LSA and LSD have been compared concerning their potential pharmacological profiles based on the receptor binding affinities since our recent human study with four volunteers on p.o. application of *Argyreia nervosa* seeds has led to some ambiguous effects.
*Material and methods:* In an initial step computer-aided *in silico* prediction models on receptor binding were employed to screen for serotonin, norepinephrine, dopamine, muscarine, and histamine receptor subtypes as potential targets for LSA. In addition, this screening was extended to accompany ergotalkaloids of *Argyreia nervosa* (Burm. f.). In a verification step, selected LSA screening results were confirmed by *in vitro* binding assays with some extensions to LSD.
*Results:* In the *in silico* model LSA exhibited the highest affinity with a $pK_i$ of about 8.0 at $\alpha_{1A}$, and $\alpha_{1B}$. Clear affinity with $pK_i>7$ was predicted for 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$, 5-$HT_6$, 5-$HT_7$, and $D_2$. From these receptors the 5-$HT_{1D}$ subtype exhibited the highest $pK_i$ with 7.98 in the prediction model. From the other ergotalkaloids, agroclavine and festuclavine also seemed to be highly affine to the 5-$HT_{1D}$-receptor with $pK_i>8$. In general, the ergotalkaloids of *Argyreia nervosa* seem to prefer serotonin and dopamine receptors ($pK_i>7$). However, with exception of ergometrine/ergometrinine only for 5-$HT_{3A}$, and histamine $H_2$ and $H_4$ no affinities were predicted.

Compared to LSD, LSA exhibited lower binding affinities in the *in vitro* binding assays for all tested receptor subtypes. However, with a $pK_i$ of 7.99, 7.56, and 7.21 a clear affinity for 5-$HT_{1A}$, 5-$HT_2$, and $\alpha_2$ could be demonstrated. For DA receptor subtypes and the $\alpha_1$-receptor the $pK_i$ ranged from 6.05 to 6.85.
*Conclusion:* Since the psychedelic activity of LSA in the recent human study was weak and although LSA from *Argyreia nervosa* is often considered as natural exchange for LSD, LSA should not be regarded as LSD-like psychedelic drug. However, vegetative side effects and psychotropic effects may be triggered by serotonin or dopamine receptor subtypes.



## 1. Introduction

The *convolvulacea Argyreia nervosa* (Burm. f.) (synonyms: *Argyreia speciosa* Sweet, *Convolvulus speciosus*), also known as

*Abbreviations:* LSA, lysergic acide amide; LSD, lysergic acid diethylamide; 5-HT, serotonin; DA, dopamine; H, histamine; M, muscarine; FDP, feature-pair distribution; MOE, Molecular Operating Environment; IDW, inverse distance weighting.

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Hawaiian baby woodrose, is an important medical plant in traditional Indian medicine (Ashutosh et al., 2011; Jain and Dam, 1979) and the Ayurvedic system (Joseph et al., 2011) and it is used for therapy of numerous diseases (*e.g.* nervousness, bronchitis, tuberculosis, arthritis, and diabetes) (Galani et al., 2010; Warrier et al., 1996). Furthermore, in the Indian state of Assam and in other regions *Argyreia nervosa* is part of the traditional tribal medicine (*e.g.* the Santali people, the Lodhas, and others) (Joseph et al., 2011). For medical treatment the whole plant is employed as well as different parts of the plant (leaves, roots, seeds, and fruits) (Ashutosh et al., 2011; Joseph et al., 2011). In the western hemisphere, *Argyreia nervosa* is not part of the traditional phytomedicine, but rather it has been brought into attention since recent case reports have increased reporting the abuse of so-called "biogenic drugs" or "legal highs" by adolescents (Al-Assmar, 1999; Björnstad et al., 2009; Borsutzky et al., 2002; Gertsch and Wood, 2003; Göpel et al., 2003). Here, the seeds of *Argyreia nervosa,* which are used for treatment of anorexia, diabetes and various skin diseases in the traditional Indian medicine (Ashutosh et al., 2011), are consumed as psychedelic drug. In this context they act as source for lysergic acid amide (LSA), which is considered as the main active ingredient and mainly responsible for the psychotropic effect (Heim et al., 1968; Hofmann and Cerletti, 1961). On the other hand, LSA may be also responsible for (side) effects of a therapeutic use of the *Argyreia nervosa* seeds, but however clinical data is rare. Nonetheless, the spiritual use of LSA containing herbal drugs from the *convolvulacea* family is well known and there are numerous studies dealing with LSA containing traditional herbal preparations (*e.g.* the Aztec drug Ololiuqui) (Carod-Artal, 2011; Heim et al., 1968; Hofmann and Cerletti, 1961; Kinross-Wright, 1958; Osmond, 1955).

Animated by various tutorials available via the internet, the consumers ingest dried or fresh *Argyreia nervosa* seeds directly or produce a simple aqueous or alcoholic extract (L7Vega91, 2012; Neurosoup, 2011; Rapedbyaclown666, 2011; Yay, 2009a, 2009b) hoping for a hallucinogenic trip, which, however, differs from the anticipated lysergic acid diethylamide (LSD)-like experience. After LSA ingestion an altered visual perception (*e.g.* of colors and textures) is as well reported as some kind of mood elevation, but sedation is more commonly described (Borsutzky et al., 2002; Isbell and Gorodetzky, 1966; Osmond, 1955). Furthermore states of anxiety can occur under controlled conditions ("bad setting") (Gertsch and Wood, 2003) and changes in thought processes or sense of time are frequent (Whelan et al., 1968). Vegetative side effects range from nausea, vertigo, hypertension tachycardia, and tachypnea to mydriasis (Göpel et al., 2003). However, only few older (Cohen, 1964; Flach, 1967; Heim et al., 1968; Whelan et al., 1968) and some more recent scientific reports (Al-Assmar, 1999; Borsutzky et al., 2002; Gertsch and Wood, 2003; Göpel et al., 2003; Klinke et al., 2010; Kremer et al., 2012; Legriel et al., 2008) have been presented on the effects of human consumption of LSA or LSA containing seeds, but the mechanism of action remains unclear. In one *in vitro* experiment LSA showed vasoconstrictor activity at bovine lateral saphenous vein and dorsal metatarsal artery (Oliver et al., 1993), but although LSA is a well-known substance only little is known about receptor interaction. LSA binds at the dopamine $D_2$ receptor (Larson et al., 1999) and may act as partial agonist or antagonist at adrenergic ($\alpha_1$, $\alpha_2$) and serotonergic (5-HT) receptors (Oliver et al., 1993). Here, LSA may have a predilection for the 5-$HT_2$ receptor subtype (Oliver et al., 1993).

To elucidate the pharmacological effects of LSA containing "legal highs" more deeply, an ethic committee approved small human study employing seeds of *Argyreia nervosa* as source of LSA (Kremer et al., 2012) was performed. Unfortunately, the study had to be canceled because of severe side effects, which ranged from cardiovascular effects to a psychosis like state. To explain the observed effects and to investigate possible pharmacological mechanisms in a first step, a computer-aided prediction model was employed to estimate the affinity of LSA at different biogenic amine G-protein-coupled receptors (GPCRs), *e.g.*, serotonin (5-HT), norepinephrine (α, β), dopamine (DA), muscarine (M) and histamine (H) receptor subtypes. Based on the data of the prediction model, an *in vitro* receptor binding study with pure LSA was performed on a selection of potential targets, *e.g.*, 5-HT, α, and DA receptors. In addition, the results were compared to the receptor binding affinities of LSD, which were determined in the same assay systems. Although a large range of receptor models have been taken for the characterization, not all assays could be performed to match the virtual screening data.

As *Argyreia nervosa* contains further ergotalkaloids, which were reported to be present in lower concentrations by other researchers (Fig. 1), the computer-aided prediction experiment was additionally extended to these ergotalkaloids. As most of these compounds could not be purchased in a sufficient quality or in sufficient amounts, the prediction experiment could not be confirmed by our *in vitro* receptor binding experiments and thus, the *in silico* data obtained can be taken as orientation for follow-up experiments.

## 2. Material and methods

LSA stock solution (1 mg/mL in methanol, purity 99.2%) was purchased from THC Pharm (Frankfurt/Main, Germany) and LSD solution (100 μg/mL in acetonitrile) was obtained from LGC Standards (Wesel, Germany).

The *in silico* prediction model has been implemented in close analogy to the publication by Vidal and Mestres (Vidal and Mestres, 2010). Essentially, the ChemblDB database (Gaulton et al., 2012) was used to retrieve a dataset of ligands with reported $K_i$ values for 10 serotonine receptor (5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$, 5-$HT_{1E}$, 5-$HT_{2A}$, 5-$HT_{2C}$, 5-$HT_{3A}$, 5-$HT_{5A}$, 5-$HT_6$, and 5-$HT_7$), seven adrenoreceptor ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$, $\beta_1$, and $\beta_2$ ), five dopamine receptor ($D_1$, $D_2$, $D_3$, $D_4$, and $D_5$), five muscarinic receptor ($M_1$, $M_2$, $M_3$, $M_4$, and $M_5$), and four histamine receptor subtypes ($H_1$, $H_2$, $H_3$, and $H_4$). The raw data was curated by removing duplicates and compounds with $K_i$ values <5500 nM. For all compounds, the topological pharmacophore-based CATS descriptor (Schneider et al., 1999) was calculated using the MOE software suite (MOE: Molecular Operating Environment V2010.11, Chemical Computing Group Inc., Montreal, Canada). The feature-pair distribution (FDP) similarity as defined by Vidal et al. (Vidal et al., 2011) was used to calculate the similarity between two molecular compounds. The predicted $K_i$ values were retrieved by inverse distance weighting (IDW) interpolation as described by Rusu et al. (Rusu et al., 2008). The IDW interpolation weights the contribution of a data point to the interpolated value higher if the data points are close to each other. It is important to ensure that only very similar reference molecules are used for prediction of $K_i$ values therefore a cut-off similarity value was calculated as 95% quantile of the overall pairwise similarity distribution (0.6139). In case that no reference molecules with higher similarity than the cutoff are available the prediction of $K_i$ is outside of the applicability domain of this method.

Receptor binding studies have been performed using standard methods as radioligand displacement assays on membrane preparations as described previously (Bollinger et al., 2010; Hübner et al., 2000; Tomasch et al., 2012).

## 3. Results

In Table 1 the computer predicted p$K_i$ values of LSA and other related ergotalkaloides from *Argyreia nervosa* are summarized

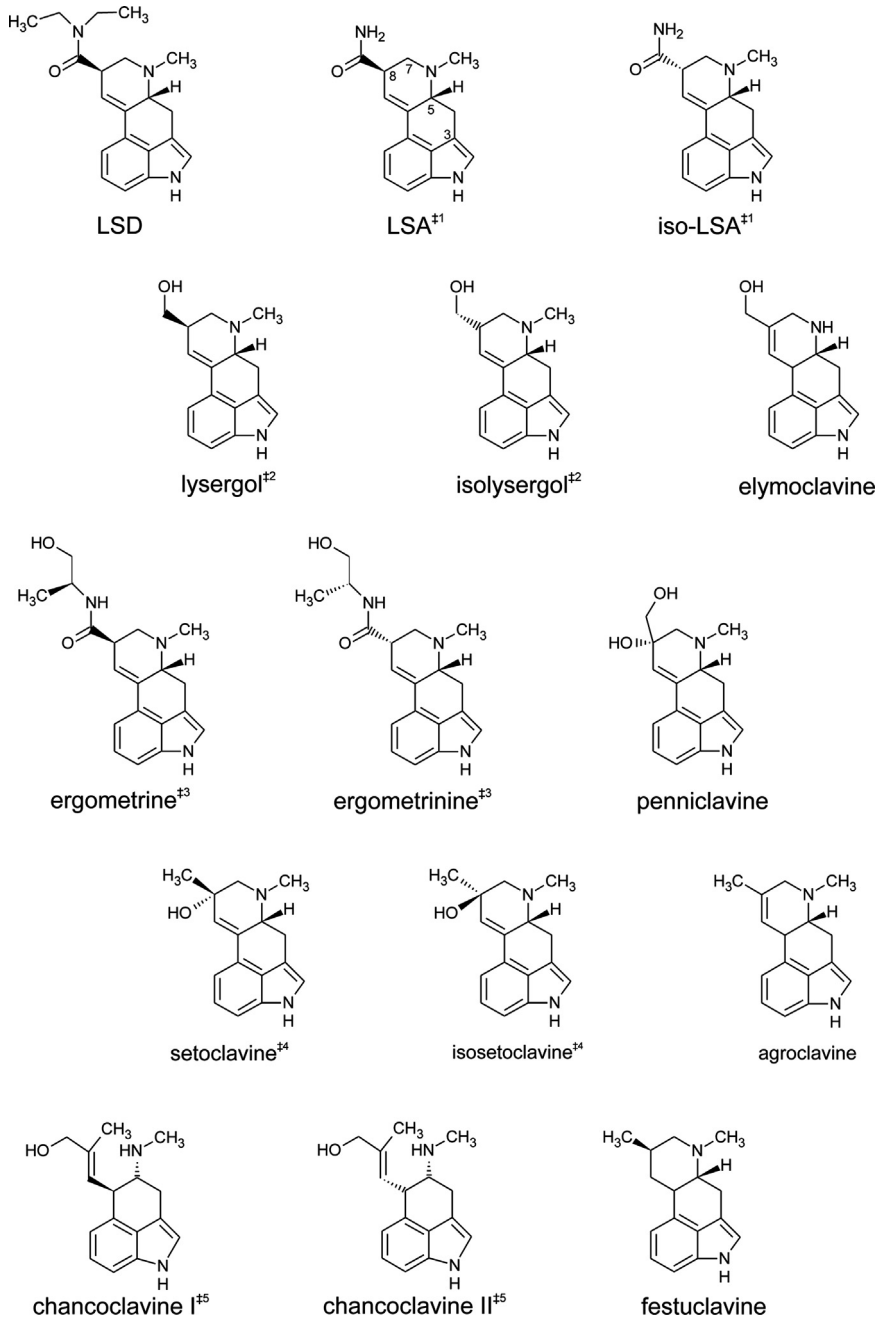


**Fig. 1.** Structures of the synthetic substance LSD and ergotalkaloids from *Argyreia nervosa* as reported by Chao and Der Marderosian (1973), Hylin and Watson (1965) and Eich (2007). Stereoisomeres are labeled (‡).

together with p$K_i$ values of LSD (literature data available from ChEMBL database (European Bioinformatics Institute, 2012). As the stereo-conformation was not taken into account by the computer model, the predicted p$K_i$ of each compound is also valid for stereoisomers (*e.g.* LSA/iso-LSA; c.f. Fig. 1). In the *in silico* model LSA showed the highest affinity with a p$K_i$ of about 8.0 at $\alpha_{1A}$, and $\alpha_{1B}$, and a clear affinity (p$K_i$>7) at 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_6$, 5-HT$_7$, and $D_2$ was predicted. From the serotonin receptors the 5-HT$_{1D}$ subtype showed the highest affinity with a p$K_i$ of 7.98 in the prediction model. From the other ergotalkaloids, agroclavine and festuclavine also seemed to be highly affine to the 5-HT$_{1D}$-receptor. They exhibited high p$K_i$ (p$K_i$>8) in the prediction model, which may be related to methyl-group on position 8.

In general, the ergotalkaloids of *Argyreia nervosa* seem to prefer serotonin and dopamine receptors as most of the ergotalkaloids exhibited p$K_i$>7 for these receptors. However, ergotalkaloids seem to interact with a broad range of receptors, and – with exception of ergometrine/ergometrinine – only for 5-HT$_{3A}$, and histamine $H_2$ and $H_4$ no affinities were predicted. Nevertheless, further research beyond a computer aided analysis is necessary to elucidate the exact pharmacological activity of each substance in more detail.

Table 2 gives an overview on the p$K_i$ values of LSA and LSD, which were determined in different *in vitro* membrane competition or displacement assays. Compared to LSD, LSA exhibited lower binding affinities in the *in vitro* binding assays for all tested receptor subtypes. However, with a p$K_i$ of 7.99, 7.56, and 7.21 a clear affinity in the nanomolar concentration range at 5-HT$_{1A}$, 5-HT$_2$, and $\alpha_2$ could be demonstrated. For DA receptor subtypes and the $\alpha_1$-receptor the p$K_i$ ranged from 6.05 to 6.85.

**Table 1**
p$K_i$ Values of LSA and other ergotalkaloides from *Argyreia nervosa* as determined with the computer aided prediction model,those of LSD were taken from ChEMBL database. (European Bioinformatics Institute, 2012).

| Receptor | LSA Iso–LSA | Agroclavine | Chancoclavine I, chanoclavine II | Elymoclavine | Festuclavine | Ergometrine, ergometrinine | Setoclavine, isosetoclavine | Penniclavine | Lysergol, isolysergol | LSD |
|---|---|---|---|---|---|---|---|---|---|---|
| 5-$HT_{1A}$ | 7.49 | 7.73 | 7.53 | 7.84 | 7.73 | 7.29 | 7.55 | 7.51 | 7.36 | 8.96 |
| 5-$HT_{1B}$ | 7.45 | 6.76 | 7.09 | 7.21 | 6.76 | 6.80 | 6.65 | 5.19 | 6.27 | |
| 5-$HT_{1D}$ | 7.98 | 8.14 | 7.40 | 7.31 | 8.14 | 7.29 | 6.99 | <5 | 7.99 | 8.41 |
| 5-$HT_{1E}$ | 6.47 | 6.20 | 5.78 | <5 | 6.20 | 6.40 | <5 | <5 | 6.47 | 7.03 |
| 5-$HT_{2A}$ | 6.24 | 7.61 | 7.74 | 7.76 | 7.61 | 7.21 | 7.42 | 7.10 | 6.46 | 8.48 |
| 5-$HT_{2C}$ | 6.07 | 6.79 | 6.71 | 7.57 | 6.79 | 6.60 | 7.12 | 6.77 | 6.32 | |
| 5-$HT_{3A}$ | 6.89 | <5 | <5 | <5 | <5 | 7.34 | <5 | <5 | <5 | |
| 5-$HT_{5A}$ | 6.87 | 6.56 | 6.10 | <5 | 6.56 | 6.97 | <5 | 6.02 | 6.97 | 8.05 |
| 5-$HT_6$ | 7.11 | 6.66 | 5.87 | 5.87 | 6.66 | 6.43 | 6.41 | 6.24 | 7.19 | 8.16 |
| 5-$HT_7$ | 7.45 | 6.52 | 7.23 | 7.23 | 6.52 | 7.76 | 6.74 | 6.89 | 7.46 | 8.18 |
| $D_1$ | 5.56 | 6.33 | 7.21 | 6.78 | 6.33 | 5.81 | 6.63 | 6.34 | 5.63 | |
| $D_2$ | 7.38 | 6.61 | 6.00 | 7.25 | 6.61 | 6.70 | 7.06 | 6.59 | 7.03 | 6.92 |
| $D_3$ | 6.63 | 7.31 | 7.38 | 7.14 | 7.31 | 7.28 | 6.99 | 6.96 | 6.61 | 7.57 |
| $D_4$ | 6.16 | 6.52 | 5.63 | 5.71 | 6.16 | 6.82 | 6.62 | 6.76 | 6.16 | 7.25 |
| $D_5$ | 5.95 | 7.02 | 7.51 | <5 | 5.95 | 6.37 | 6.50 | 6.31 | 6.14 | 6.47 |
| $\alpha_{1A}$ | 8.04 | 6.95 | 6.97 | <5 | 6.95 | 7.63 | 6.50 | 6.40 | 7.07 | |
| $\alpha_{1B}$ | 8.00 | 6.94 | 6.54 | <5 | 6.94 | 7.31 | 6.50 | 6.34 | 7.07 | |
| $\alpha_{2A}$ | 5.36 | <5 | 6.13 | <5 | <5 | 7.26 | 5.46 | 7.02 | 6.68 | |
| $\alpha_{2B}$ | 6.41 | <5 | 6.40 | <5 | <5 | 7.02 | 5.67 | 7.33 | 5.67 | |
| $\alpha_{2C}$ | 6.00 | <5 | 6.24 | <5 | <5 | 7.61 | 5.65 | 7.27 | 7.31 | |
| $\beta_1$ | 6.46 | 6.45 | <5 | <5 | 6.46 | 7.06 | 7.29 | 7.14 | 6.93 | 6.85 |
| $\beta_2$ | 5.50 | 5.49 | 6.12 | <5 | 5.55 | 7.37 | 5.91 | 7.79 | 6.16 | 6.13 |
| $M_1$ | <5 | <5 | 7.35 | <5 | <5 | 7.15 | 5.93 | 6.84 | 6.66 | |
| $M_2$ | <5 | <5 | 5.61 | <5 | <5 | 6.01 | 5.55 | 6.31 | 5.27 | |
| $M_3$ | <5 | <5 | 7.20 | <5 | <5 | 7.52 | 5.58 | 7.12 | 5.27 | |
| $M_4$ | <5 | <5 | <5 | <5 | <5 | 6.31 | 5.46 | <5 | 5.27 | |
| $M_5$ | <5 | <5 | <5 | <5 | <5 | 5.35 | 5.46 | <5 | 5.27 | |
| $H_1$ | 6.03 | 5.76 | 6.91 | 5.85 | 5.76 | 5.73 | 7.68 | 7.57 | 5.89 | 5.81 |
| $H_2$ | <5 | <5 | <5 | <5 | <5 | 5.56 | <5 | <5 | <5 | |
| $H_3$ | 6.95 | 7.03 | <5 | <5 | 7.03 | 7.16 | <5 | <5 | 7.38 | |
| $H_4$ | <5 | <5 | <5 | <5 | <5 | 6.86 | <5 | <5 | <5 | |

**Table 2**
p$K_i$ Values of LSA and LSD as determined with *in vitro* receptor binding assays.

| Receptor | p$K_i$ LSA *in vitro* assay | p$K_i$ LSD *in vitro* assay |
|---|---|---|
| 5-$HT_{1A}$(*) | 7.99 | 8.61 |
| 5-$HT_2$ | 7.56 | 9.06 |
| $D_1$(*) | 6.08 | 7.06 |
| $D_{2long}$ | 6.05 | 6.81 |
| $D_{2short}$ | 6.84 | 7.60 |
| $D_3$ | 6.36 | 7.19 |
| $D_{4.4}$ | 6.85 | 7.52 |
| $\alpha_1$ | 6.04 | 7.22 |
| $\alpha_2$ | 7.21 | 8.99 |

(*) porcine receptor.

## 4. Discussion

Although the chemical structure of LSA and LSD are of the same origin and share some structural features, the pharmacological effects of LSA differed in our human trial experiment (Kremer et al., 2012) from the reported effects of LSD. In this study no psychedelic effects like altered visual perception or mood elevation could be observed. Instead, – in accordance with (Göpel et al., 2003) – vegetative side effects with hypertension, nausea, vomiting, tremor and weakness were seen, and one participant exhibited a psychosis like state, which may represent a kind of "psychedelic bad trip". The virtual screening already suggests a different receptor binding profile of LSA or accompanying ergotalkaloids in comparison to that of LSD (c.f. Table 1). The predicted binding affinities of LSA or accompanying ergotalkaloids are in almost all cases lower than those reported for LSD (2012). Consequently, it can be speculated, that LSA or the other ergotalkaloids from *Argyreia nervosa* show weaker psychedelic activity than LSD and it could be doubted, that LSA or accompanying ergotalkaloids are a natural kind of LSD. However, in the prediction model LSA and some accompanying ergotalkaloids preferred serotonergic, dopaminergic, and adrenergic receptors, but not muscarinergic or histaminergic receptors. Consequently, it can be speculated, that the pharmacological effects of LSA or *Argyreia nervosa* material are mediated by serotonergic, dopaminergic or adrenergic systems. Muscarinergic or histaminergic receptors seem to play no dominant role in the way of action of LSA or *Argyreia nervosa*, respectively. With the discussion of the data it should be taken into account, that with this study we have not investigated efficacy, bioavailability, distribution, metabolism or other important factors for effects *in vivo*.

With the binding assays on selected serotonergic, dopaminergic and adrenergic receptor subtypes both, LSA and LSD, share similar receptor preferences, but LSA exhibited lower affinities in all cases. The differences in binding properties are between 1.8 and 0.7 log units depending on the target. However, both, LSA and LSD, showed clear affinities to 5-$HT_{1A}$ and 5-$HT_2$. The interpretation of these findings remains difficult, as the role of the 5-$HT_{1A}$ and 5-$HT_2$ receptors during psychotic or psychedelic processes have not yet been fully understood. Nevertheless, 5-$HT_{1A}$ as targets for LSA or accompanying ergotalkaloids of *Argyreia nervosa* must be given special attention, as the 5-$HT_{1A}$ receptor is involved in the pathomechanism of psychiatric disorders and thus a target for antipsychotic, anxiolytic and antidepressant drugs (Batool et al., 2010; Baumgarten and Grozdanovic, 1995; Ohno, 2011). Furthermore, the 5-$HT_{1A}$ receptor is located with a high density in the limbic system, the amygdala, the hippocampus, and in the *Nuclei raphe* (Baumgarten and Grozdanovic, 1995), and, *inter alia*, it contributes to the coordination of the core temperature (Hillegaart, 1991; Millan et al., 1993) and antinociception (Baumgarten and Grozdanovic, 1995). The 5-$HT_2$ receptor is

among others involved in the blood pressure regulation (Egashira et al., 1995) and the clear affinity of LSA and other ergotalkaloids from *Argyreia nervosa* to this receptor subtype (c.f. Tables 1 and 2) could be in part responsible for the hypertension observed in the human study. However, in the CNS the 5-$HT_2$ receptor is located in the neocortex, the brainstem nuclei and the amygdala, and it is considered as the main target for LSD and thus also responsible for hallucinogenic trips (Aghajanian and Marek, 1999; Benneyworth et al., 2005; Nichols, 2004). Like 5-$HT_{1A}$ it is also a target for antipsychotic drugs (Angelis, 2002). Compared to LSA, the markedly higher affinity of LSD for 5-$HT_2$ may be responsible for the more distinctive psychedelic activity of LSD, and may explain the weak psychedelic activity of LSA in the human study (Kremer et al., 2012). But further research is necessary to determine the efficacy of LSA at the 5-$HT_2$ receptor and thus to clarify the interaction of LSA and 5-$HT_2$ more deeply.

For LSA a significant affinity to 5-$HT_{1D}$ with a p$K_i$ of nearly 8 was predicted in the computer model, but devoid of a 5-$HT_{1D}$ assay could not be confirmed by an *in vitro* test. However, the 5-$HT_{1D}$ receptor is *inter alia* located in the CNS in the colliculus superior, the putamen, the gyrus dentatus and the neocortex (Baumgarten and Grozdanovic, 1995). It is involved in the release control of the neurotransmitters 5-HT, Ach, NA, and glutamate as well as in the activation of motor reactions or the control of pro-inflammatory peptides (Baumgarten and Grozdanovic, 1995). Clinically, it is a target for anti-migraine drugs (Baumgarten and Grozdanovic, 1995; Diener et al., 2001; Gomez-Mancilla et al., 2001). However, – at the present state of knowledge – no reports of a psychedelic activity linked to 5-$HT_{1D}$ are available and thus it remains unclear, whether 5-$HT_{1D}$ contributes to the observed psychotic effect in the human study.

For $\alpha_{1A}$ and $\alpha_{1B}$ p$K_i$ values for LSA of >8 were predicted in the computer model. As $\alpha_1$ is one of the most important receptors for blood pressure regulation (Kincaid-Smith, 1989; Sica, 2005), these results might explain the observed hypertension in the human study. On the other hand, these findings could not be confirmed in the *in vitro* assay, where LSA showed only a low p$K_i$ for $\alpha_1$. Therefore, at present it may be doubted, that $\alpha_1$ receptor activation is involved in the development of hypertension after LSA ingestion, *e.g.*, in the form of *Argyreia nervosa* preparations. However, in the *in vitro* assay LSA showed a relative high affinity to adrenergic $\alpha_2$ receptors, which also contribute to the blood pressure regulation, and again, further research will be necessary to elucidate the effect of LSA on this receptor more deeply.

LSA and its accompanying alkaloids exhibited moderate affinities to $D_2$ and $D_3$ receptors in the *in silico* and in the *in vitro* assays. This may explain the vomiting occurred in the human experiment, as $D_2$ and $D_3$ receptors – together with 5-$HT_{1A}$, 5-$HT_{2A/2C}$, 5-$HT_3$ and 5-$HT_4$ receptors – are considered as mainly responsible for nausea and emesis (Herrstedt, 1998). However, the affinity of LSA – compared to that of LSD – to all investigated dopamine receptors is weaker. But both, LSA and LSD, seem to have a predilection for the $D_2$ and $D_4$ receptors. It can therefore be speculated, that the dopaminergic system may be involved in the observed psychosis like state in one participant as well as in the observed tremor in all participants in the human study, as $D_2$ contributes to extrapyramidal syndromes and – together with $D_4$ – is involved in psychotic disorders (Casey, 1994; Martelle and Nader, 2008; Nikam and Awasthi, 2008; Nord and Farde, 2011; Strange, 2001). But again, more information about the efficacy of LSA on the dopamine receptors will be necessary to evaluate the effects of LSA on the dopaminergic system.

For the other examined receptors the p$K_i$ values have been determined *in silico* with the computer aided prediction model. The predictive power of the model is adequate if the *in silico* predicted p$K_i$ is in a range of one order of magnitude from the measured one. This validation holds true for the majority of predictions (data not shown), implicating that the model might be useful to get a first impression of the profile of the compound of interest. In this regard, the measurement of the p$K_i$ values for some of the receptors *in vitro* can be considered as a prospective evaluation for the quality of the *in silico* model. The underestimated binding affinities of LSA *in silico* at the 5-$HT_2$ and $\alpha_2$ receptors as well as the overestimated value for the $\alpha_1$ receptor (c.f. Tables 1 and 2) indicate that the prediction model is far from perfect and does not replace careful *in vitro* evaluation. Accordingly, the results of the *in silico* screening must be interpreted with caution. Thus, definitive statements on possible interactions of LSA with these receptors, which are solely based on virtual screening, would be speculative. Furthermore, the binding affinities of LSD could not be predicted due to the limited applicability domain of the models which should also be taken into account. Nevertheless, with aid of the computer based prediction model, possible targets for LSA could be identified or could be excluded. Consequently, as no affinity was predicted for M, $H_2$ or $H_4$, an interaction of LSA with these receptors seems improbable.

## 5. Conclusion

Although LSA from *Argyreia nervosa* is often considered as a natural kind of LSD, in our studies the pharmacological effects as well as the receptor binding profiles are quite different. Thus, LSA cannot be regarded as LSD-like psychedelic drug. Nevertheless the affinities at numerous biogenic amine receptor subtypes may be responsible for psychotic effects, which may be triggered mainly by 5-HT or DA receptor subtypes, but the vegetative side effects are more dominant. Nevertheless, the study exhibits a broad spectrum of possible molecular targets for the ergotalkaloids of *Argyreia nervosa*, but additional research is necessary to clarify the mechanisms of action in more detail.

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