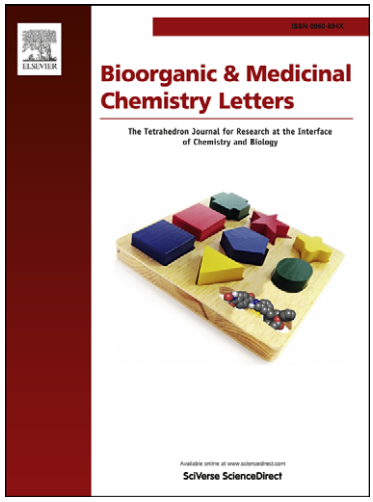

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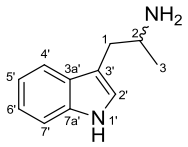

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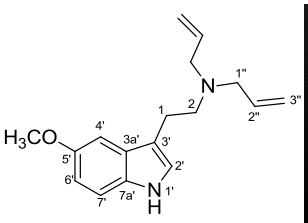

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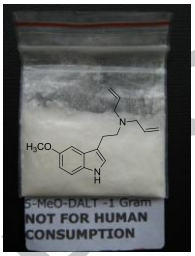

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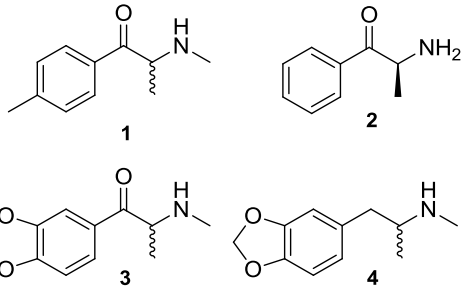
ABSTRACT

Novel Psychoactive Drugs (NPD) can be sold without restriction and are often synthetic analogues of controlled drugs. The tryptamines are an important class of NPD as they bind to the various serotonin (5-HT) receptor subtypes and cause psychosis and hallucinations that can lead to injury or death through misadventure. Here we report on the structure elucidation and receptor binding profiles of two widely marketed tryptamine-derived NPDs, namely alpha-methyl-tryptamine and 5-methoxy-*N*,*N*-diallyl-tryptamine.

Novel Psychoactive Drugs (NPD) have attracted considerable global attention over the last 4 years.[1] These materials are either biomass of plants or fungi, their extracts or single chemical entity drugs that are structurally close to an existing psychoactive compound that is, typically, a controlled substance.[2]

NPD can be based on an existing chemical scaffold; for example one of the first prominent NPD was the synthetic beta-keto-amphetamine mephedrone (**1**),[3] an analogue of the natural product cathinone (**2**) from the East African tree *Catha edulis* (Khat).[4] Analogues of mephedrone such as methylone (**3**) show striking similarity with the drug of abuse MDMA (Ecstasy, **4**), which in part explains why NPS continue to be popular even after the introduction of control measures.

NPD can be categorized according to chemical class such as the amphetamine group (e.g. camfetamine (**5**)),[5] the many



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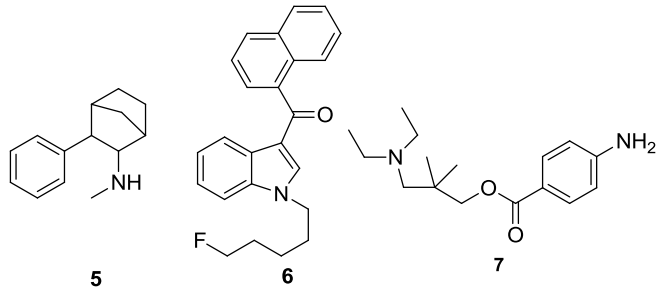

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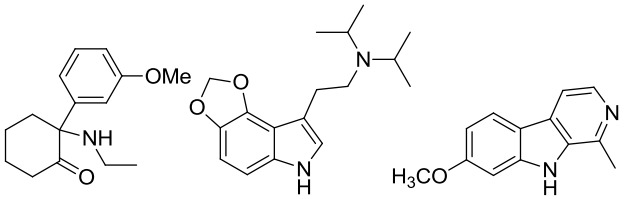

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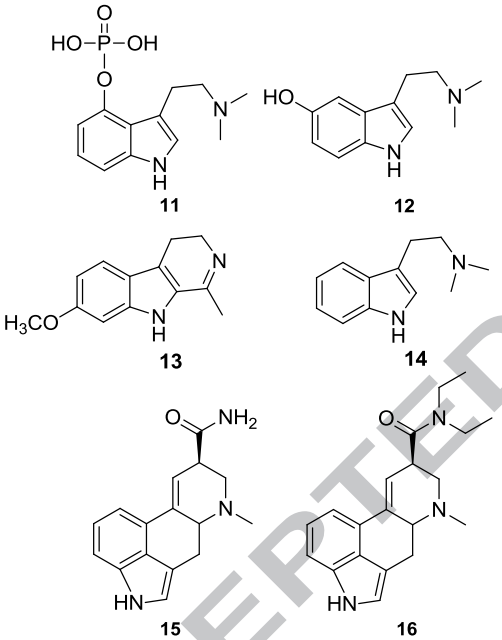
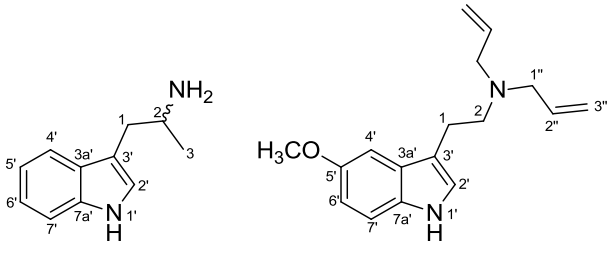
classes of cannabinoid receptor agonists of which AM-2201 (**6**) is a member,[6] synthetic cocaine derivatives such as dimethocaine (**7**),[7] ketamine analogues (e.g. methoxetamine, (**8**))[8] and tryptamines which can include synthetic compounds (e.g. 4,5-methylenedioxy-*N*,*N*-diisopropyltryptamine (**9**))[9] and natural products such as harmine (**10**).[10]

Nature is exceptionally adept at producing psychoactive compounds based on the tryptamine nucleus[2] and these products mimic the mammalian endogenous neurotransmitter serotonin (5-hydroxy-tryptamine; 5-HT), exerting their effects by binding to and activating various 5-HT receptor subtypes - in particular 5-HT_{2A} serotonin receptors. Examples include psilocybin (**11**)[11] from "magic mushrooms" of the genus *Psilocybe*, amphibian-derived metabolites related to bufotenin (**12**),[12] and a plethora of plant natural products which are either simple tryptamines such as the Ayahuasca components, harmine (**10**), harmaline (**13**) and *N*,*N*-dimethyl-tryptamine (**14**, DMT)[13-14] and the more complex natural products from *Ipomoea* sp. such as ergine (**15**)[10] to which LSD[15] (**16**) is closely related.



11 **12** **13** **14** **15** **16**

Natural tryptamines also serve as templates for simple synthetic NPD. However, there is often a paucity of their spectral data and even less in-depth evaluation of their *in vitro* pharmacological activities. This lack of data can make assessing the potential harms associated with these materials exceptionally difficult. We recently acquired two emerging NPD, namely alpha-methyl-tryptamine (AMT, α-MT) (**17**) and 5-methoxy-*N*,*N*-diallyl-tryptamine (5-MeO-DALT) (**18**) and here report their full spectroscopic analysis and pharmacological profile. These materials are being widely promoted on NPD webshops for their psychoactive effects but, in most jurisdictions, are not controlled drugs.



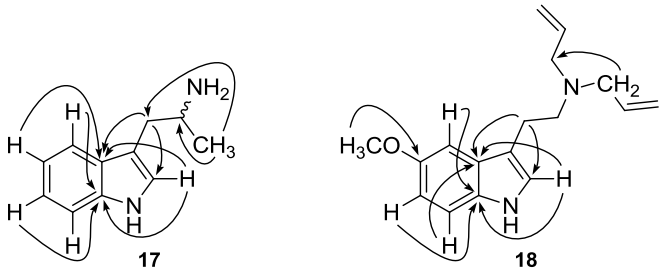
17 **18**

The identity and quality of materials purchased from NPD webshops is unpredictable and so must be verified before being used in studies.[16-17] This is particularly important where closely related materials are also being marketed as NPD. For example, 5-(2-aminopropyl)indole ("5-IT") is a positional isomer of alpha-methyl-tryptamine (AMT), which is also being sold as an NPD and which has been linked to a number of fatalities.[18] A recent study reported the use of various analytical techniques including 1D-NMR, GC-EI/CI ion trap MS, U/HPLC-DAD and HPLC-MS to differentiate AMT and 5-IT.[19] However, the full spectroscopic data and neuropharmacological studies were not reported. Previous ^{1}H NMR data has only been reported at 80 MHz with no assignment of the complete resonances [20] and the ^{13}C data is only available for the 1,2-dideuterated derivative.[21]

A sample of alpha-methyl-tryptamine (**17**, 1 g) was acquired from an internet site and a portion of this (15 mg) was subjected to full structure elucidation.

The ESIMS in the positive mode gave an $[M+H]^+$ peak at 175 which was consistent with a molecular formula of $C_{11}H_{14}N_2$. This was confirmed by high-resolution data (observed 175.1232 calculated for 175.1235) supporting the identity of the NPD as alpha-methyl-tryptamine (1-(indol-3-yl)-2-aminopropane). **17** was subjected to ^{1}H, ^{13}C, DEPT135, COSY, HMQC, HMBC and NOESY experiments and all spectra indicated a pure product that was essentially free of starting materials (supporting information). The ^{1}H NMR spectrum (Table 1; supporting information) revealed the presence of a deshielded NH hydrogen (δ_H = 10.78, bs), four aromatic resonances which were in a 1,2,3,4-substitution pattern, and an olefinic hydrogen (δ_H 7.11 d, J = 2 Hz) completing the ^{1}H resonances associated with the indole moiety.

A separate spin system was also present and comprised a methyl doublet (δ_H 0.98) which in the COSY spectrum was coupled to a nitrogen-bearing methine (δ_H 3.07, δ_C 47.4), which in turn coupled to a methylene functionality (δ_H 2.62) giving a CH_3-CH(NH_2)-CH_2- moiety, again supporting the identity of **17** as alpha-methyl-tryptamine. Full unambiguous assignment of all ^{1}H and ^{13}C resonances was achieved by careful inspection of the HMQC and HMBC spectra (supporting information). HMBC correlations (Figure 1) were observed for the methyl doublet hydrogens (CH_3-3) to the methine carbon to which it was directly attached (C-2, δ_C 47.4) and to the methylene of the COSY spin system (C-1).



17 **18**

Figure 1. Key HMBC correlations for compounds **17** and **18**.

The hydrogens associated with this methylene coupled to C-2' and C-3' (to which CH_2-1 was directly attached) and to a ring junction quaternary carbon (C-3a'). The olefinic hydrogen also coupled to C-3a' and to the other ring junction quaternary carbon (C-7a'). Further HMBC correlations to this ring junction carbon included ^3J couplings from H-4' and H-6' to C-7a' verifying its position. The downfield resonance of this carbon at δ_C 136.2 was

as a result of bearing the indole nitrogen. The remaining ^{13}C resonances were assigned on the basis of HMQC analysis (Table 1 supporting information). In addition to the HMBC considerations above, inspection of the NOESY spectrum supported placement of the CH_3-CH(NH_2)-CH_2- moiety at C-3' of the indole nucleus by through-space correlations between H-4' and H_2-1, H-2' and H-2 and between CH_3-3 and H-2' and H-4' (Figure 2).

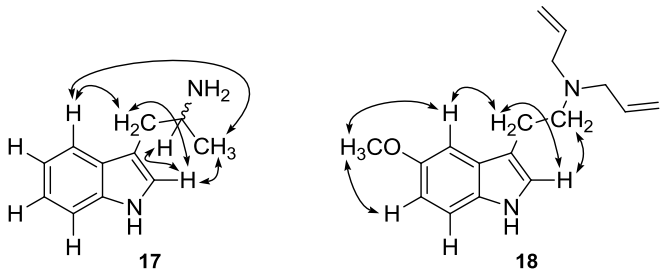


Figure 2. Key NOESY correlations for compounds **17** and **18**.

On the above evidence compound **17** was therefore assigned as 1-(indol-3-yl)-2-aminopropane (alpha-methyl-tryptamine). Elemental analysis of **17** gave the composition as 75.88 % C, 8.67% H and 15.92% N, correlating closely with the theoretical result of 75.82% C, 8.10% H and 16.08% N, indicating that NPD **17** was sold as the free base form of alpha-methyl-tryptamine. An alpha D measurement of 0 further suggested that compound **17** was racemic.

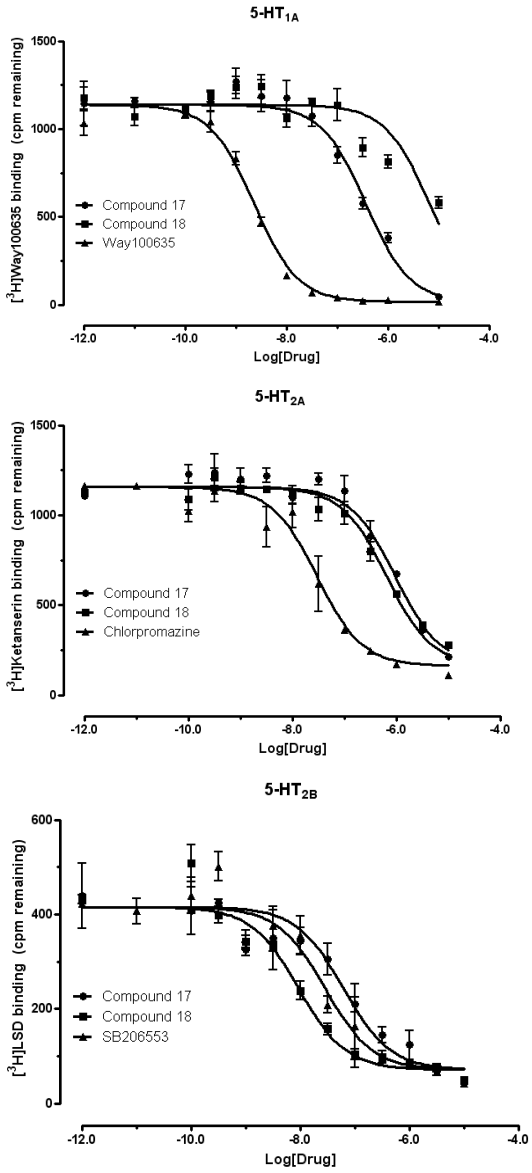
Compound **18** was also purchased from an internet website as a white amorphous solid. High-resolution ESIMS in the positive mode gave an $[M+H]^+$ peak at 271.1819 (theoretical calculated 271.1810), being consistent with a molecular formula of $C_{17}H_{22}N_2O$ supporting the identity of **18** as 5-MeO-*N*,*N*-diallyl-tryptamine (1-(5-methoxy-indol-3-yl)-*N*,*N*-diallyl-ethylamine). Surprisingly there is a lack of literature spectral data on 5-methoxy-*N*,*N*-diallyl-tryptamine (5-MeO-DALT) with only ^1H NMR reported[22] and the deuterated derivative has been synthesised and characterized by Brandt and co-workers.[23]

18 was subjected to an identical structure elucidation process as compound **17** and the NMR data again showed a compound which was apparently pure with resonances readily attributable to the target tryptamine (supporting information). The ^1H NMR data (Table 2; supporting information) revealed the presence of a highly deshielded nitrogen-bearing hydrogen (δ_H 10.56) with a 1,2,4-aromatic hydrogen substitution pattern and an olefinic hydrogen (δ_H 7.05, H-2') of the indole nucleus.

Further resonances included two co-incidental olefinic hydrogens (H-2'', 2H, m), four hydrogens of two co-incidental olefinic methylene groups (δ_H 5.13 and 5.25, 2H each, C-3''), and two deshielded co-incident methylene doublets (δ_H 3.16, C-1''). These resonances were coupled in the COSY spectrum to allow their assignment as two equivalent allyl substituents (-CH_2-CH=CH_2). The deshielded nature of the C-1'' methylene groups (δ_H 3.16, δ_C 56.2) indicated that they were attached to nitrogen. The remaining resonances were evident for two mutually coupled methylene groups (CH_2-1 and CH_2-2) of the tryptamine side chain, and a methoxyl group (δ_H 3.75).

Inspection of the HMQC and HMBC spectra (Figure 1) again allowed full unambiguous assignment of all ^1H and ^{13}C resonances (Table 2; supporting information). The hydrogens of CH_2-1 showed ^3J correlations to C-2' and C-3a' fixing the position of the tryptamine (ethylamine) side chain at C-3'. Correlations from H-2' to C3a' and C-7a', again allowed assignment of the ring-junction quaternary carbons of the indole nucleus. H-4' and H-6' exhibited ^3J correlations to C-7a', and H-7' coupled to C-3a' again supporting the assignments of C-3a' and C-7a'. HMQC spectra led to assignment of all hydrogen-bearing carbons. Positioning of the methoxyl at C-5' was achieved on the basis of its NOESY correlations with H-4' and H-6' (Figure 2), and a ^3J correlation to the carbon to which it was directly attached (δ_C 152.8). Further HMBC correlations supporting the proposed structure included correlations from the hydrogens of C-1'' to the C-1'' of the neighbouring allyl group (Figure 1) and from CH_2-1'' to C-2. Compound **18** was therefore elucidated as 1-(5-methoxy-indol-3-yl)-*N*,*N*-diallyl-ethylamine. Elemental analysis of **18** gave the composition as 75.25 % C, 8.51% H and 10.13% N which was very close to the theoretically predicted values of 75.52% C, 8.20% H and 10.36% N for the free base form.

Both compounds were assessed in a large battery of assays via the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (PDSP). We discovered that both compounds had high affinities mainly for a number of human, cloned 5-HT receptors (Figure 3 and Table 3 supporting information) as well as the 5-HT transporter (SERT), and σ1 receptors.



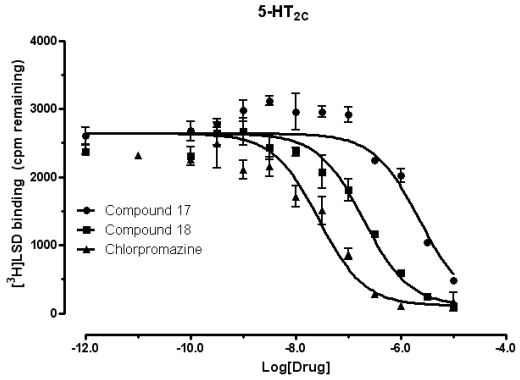
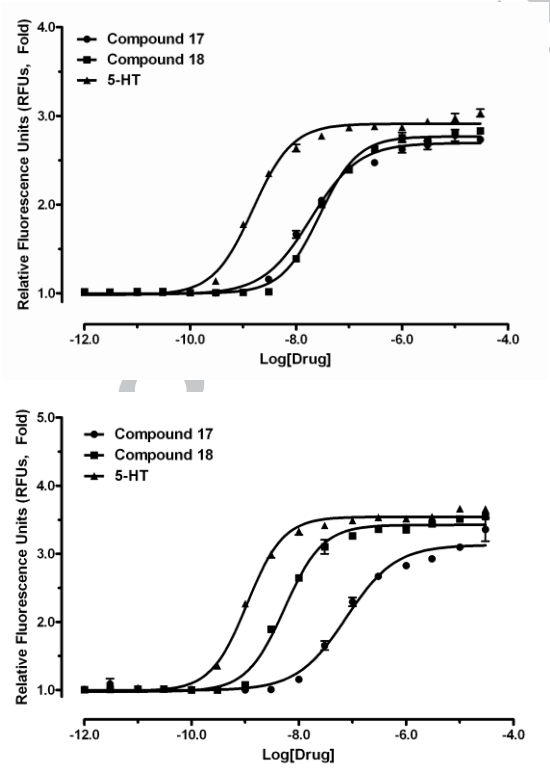


Figure 3. Radioligand competition binding isotherms for standards and **17** and **18** at heterologously expressed 5-hydroxytryptamine (5-HT) receptor subtypes. Membrane fractions of cells stably (HEK293) or transiently (HEK293T) expressing the indicated 5-HT receptor were incubated with radioligand (indicated on the y-axis) at a final concentration equal to its K_D in the presence of various concentrations of reference or test compounds.

The pKi (see Table 3, supporting information) was fit directly using an equation built into GraphPad Prism 5.0. Notably, **18** exhibited the greatest affinity for 5-HT_{2B} receptors.

Given the centrality of the 5-HT_{2A} receptor for mediating the psychoactive effects of many hallucinogens,[14] 5-HT_{2B} receptors for mediating cardiovascular side-effects of legal and illicit medications[24] and 5-HT_{2C} receptors for mediating anorectic actions of drugs, we examined the effects of **17** and **18** on 5-$HT_{2A/B/C}$ functional responses. We found that **17** and **18** had low nM potencies for activating 5-$HT_{2A/B/C}$ receptors (Figure 4).



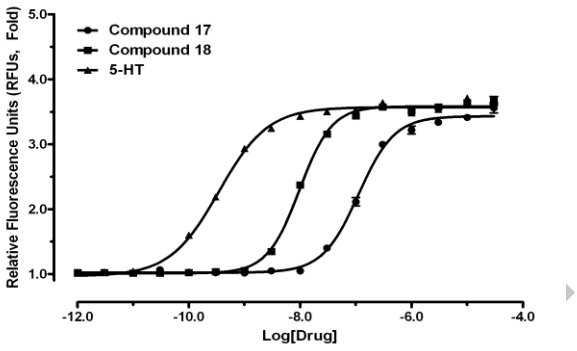


Figure 4. Agonist potencies for **17** and **18** at 5-HT_2-family receptors. Shown are representative concentration-response curves for 17 and 18 at human 5-HT_{2A} (Top), 5-HT_{2B} (Middle) and 5-HT_{2C} (Bottom)-expressing cell lines. The EC_{50} and Emax values are found in Table 5, supporting information. Notably, both compounds are potent, full agonists at human 5-HT_{2A} receptors.

We also found that **17** and **18** had greatly attenuated activity at various transporters (Table 4, supporting information). Given their high potencies and efficacies at 5-HT_{2A} receptors, these are likely to be essential for exerting their psychoactive effects in humans.

In this regard, it is well known that classical hallucinogens are potent 5-HT_{2A} agonists[25] and that their hallucinogenic actions are mediated via 5-HT_{2A} agonism.[14] This was highlighted by the tragic case of a 26-year-old man, who after consuming 5-MeO-DALT was killed after walking on to a motorway in the UK in 2010. The Coroner recorded a "narrative verdict of death from injuries sustained when he was in collision with a lorry while under the influence of 5-MeO-DALT".[26] Consumption of tryptamine containing plants such as Hawaiian Baby Woodrose can also result in death by misadventure, with a case of one fatality from jumping from a building where ergine was found to be present in the blood and urine.[27] According to users' experience, AMT is a long-lasting NPD producing psychedelic effects up to 14 hours depending on dosage (15-200 mg orally)[28] whilst 5-MeO-DALT is short acting with oral dosage ranges from 5-25 mg.[29]

It is the ability of these hallucinogenic NPD to cause such accidents that makes these materials potentially dangerous and their prevalence should monitored by organizations tasked with evaluating the harms of legal and illegal drugs of abuse.

Experimental Section

NPS **17** and **18** were dissolved in DMSO-d6 (500 µL) in a standard 5 mm NMR tube (Sigma Aldrich). NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer and HMBC spectra were optimized for couplings of 7 Hz.

Instrumental methods, UV-Vis and IR spectral data, optical rotation, elemental analysis and all NMR spectra are presented in the supporting information.

Details of the biological evaluation protocols are in the supporting information.

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References

1. Hillebrand, J.; Olszewski, D.; Sedefov, R. *Subst Use Misuse* **2010,** *45*, 330.
2. Gibbons, S. *Clin Toxicol (Phila)* **2012,** *50*, 15.
3. Gustavsson, D.; Escher, C. *Lakartidningen* **2009,** *106*, 2769.
4. Brenneisen, R.; Fisch, H. U.; Koelbing, U.; Geisshusler, S.; Kalix, P. *Br J Clin Pharmacol* **1990,** *30*, 825.
5. Kavanagh, P.; Angelov, D.; O'Brien, J.; Power, J. D.; McDermott, S. D.; Talbot, B.; Fox, J.; O'Donnell, C.; Christie, R. *Drug Test. Anal.*, Ahead of Print.
6. Nakajima, J. i.; Takahashi, M.; Nonaka, R.; Seto, T.; Suzuki, J.; Yoshida, M.; Kanai, C.; Hamano, T. *Forensic Toxicol.* **2011,** *29*, 132.
7. Woodward, J. J.; Compton, D. M.; Balster, R. L.; Martin, B. R. *Eur J Pharmacol* **1995,** *277*, 7.
8. Wood, D. M.; Davies, S.; Puchnarewicz, M.; Johnston, A.; Dargan, P. I. *Eur J Clin Pharmacol* **2011**.
9. Kline, T. B.; Benington, F.; Morin, R. D.; Beaton, J. M. *J. Med. Chem.* **1982,** *25*, 908.
10. Hylin, J. W.; Watson, D. P. *Science* **1965,** *148*, 499.
11. Halpern, J. H. *Pharmacology & Therapeutics* **2004,** *102*, 131.
12. Lyttle, T.; Goldstein, D.; Gartz, J. *J Psychoactive Drugs* **1996,** *28*, 267.
13. McKenna, D. J.; Towers, G. H. *J Psychoactive Drugs* **1984,** *16*, 347.
14. Keiser, M. J.; Setola, V.; Irwin, J. J.; Laggner, C.; Abbas, A. I.; Hufeisen, S. J.; Jensen, N. H.; Kuijer, M. B.; Matos, R. C.; Tran, T. B.; Whaley, R.; Glennon, R. A.; Hert, J.; Thomas, K. L.; Edwards, D. D.; Shoichet, B. K.; Roth, B. L. *Nature* **2009,** *462*, 175.
15. Hofmann, A. *J Psychedelic Drugs* **1979,** *11*, 53.
16. Davies, S.; Wood, D. M.; Smith, G.; Button, J.; Ramsey, J.; Archer, R.; Holt, D. W.; Dargan, P. I. *QJM* **2010,** *103*, 489.
17. Brandt, S. D.; Sumnall, H. R.; Measham, F.; Cole, J. *Drug Testing and Analysis* **2010,** *2*, 377.
18. Seetohul, L. N.; Maskell, P. D.; Paoli, G. D.; Pounder, D. J. *BMJ* **2012,** *345*.
19. Elliott, S. P.; Brandt, S. D.; Freeman, S.; Archer, R. P. *Drug Testing and Analysis* **2012**.
20. Nichols, D. E.; Lloyd, D. H.; Johnson, M. P.; Hoffman, A. J. *J Med Chem* **1988,** *31*, 1406.
21. Wang, Y.-Y.; Chen, C. *Journal of the Chinese Chemical Society* **2007,** *54*, 1363.
22. Zhao, H.-w.; Hong, Y.-y.; Liu, C.-c. *Huaxue Shiji* **2006,** *28*, 307.
23. Brandt, S. D.; Tirunarayanapuram, S. S.; Freeman, S.; Dempster, N.; Barker, S. A.; Daley, P. F.; Cozzi, N. V.; Martins, C. P. B. *J. Labelled Compd. Radiopharm.* **2008,** *51*, 423.
24. Roth, B. L. *New England Journal of Medicine* **2007,** *356*, 6.
25. Nichols, D. E. *Pharmacol Ther* **2004,** *101*, 131.
26. [cited 2012 1 February]; Available from: http://www.cambridge-news.co.uk/Home/Familys-vow-over-legal-high-drugs-danger.htm.
27. Klinke, H. B.; Muller, I. B.; Steffenrud, S.; Dahl-Sørensen, R. *Forensic Science International* **2010,** *197*, e1.
28. [cited 2013 20 February]; Available from: http://www.erowid.org/chemicals/amt/amt_dose.shtml.
29. [cited 2013 19 February]; Available from: http://www.erowid.org/chemicals/5meo_dalt/5meo_dalt_dose.shtml.