Full Paper

Synthesis and Characterization of Hydroxylated Mesocarb Metabolites for Doping Control

Mikko Vahermo[1], Tina Suominen[2], Antti Leinonen[2], and Jari Yli-Kauhaluoma[1]

[1] Faculty of Pharmacy, Division of Pharmaceutical Chemistry, University of Helsinki, Helsinki, Finland
[2] United Laboratories Ltd., Doping Control Laboratory, Helsinki, Finland

The synthesis and method of analysis of hydroxylated mesocarb metabolites are described. Six potential hydroxylated mesocarb metabolites were prepared, characterized, and compared with the mesocarb metabolites synthesized enzymatically *in vitro* using human liver proteins and also compared with metabolites extracted from human urine after oral administration of mesocarb. *p*-Hydroxymesocarb was the most prevalent metabolite (conjugated and non-conjugated) observed. With respect to doping analysis, synthesis of *p*-hydroxymesocarb, the main urinary metabolite of mesocarb, and its availability as a reference material is important.

Keywords: Doping analysis / Mesocarb / Metabolite / Sydnone imine

Received: August 6, 2008; accepted: December 12, 2008

DOI 10.1002/ardp.200800144

Introduction

Mesocarb (Sydnocarb), 3-(1-methyl-2-phenylethyl)-5-[[(phenylamino)carbonyl]amino]-1,2,3-oxadiazolium (Fig. 1) is a psychostimulant that is used in Russia as a drug for treating psychiatric disorders such as schizophrenia and depression [1]. The pharmacological profile of mesocarb is similar to other stimulants but evidence suggests that it has a different mode of action [2]. When compared to amphetamines, the effects build up more gradually and last longer. Mesocarb does not cause euphoria or locomotor excitation, and it has weak addictive liability [3].

Mesocarb was added to the list of banned compounds in sports by the Medical Commission of the International Olympic Committee in 1991 [4]. Several analytical methods for detecting mesocarb or its metabolites have been developed [5 – 8]. However, method development has been difficult, because, until now, no pure metabolites have been available [5] as reference substances, and researchers have been dependent on the metabolites extracted in minute quantities from either rat [6, 9, 10] or human urine [8]. Several anti-doping laboratories have studied the metabolism of mesocarb in man [8, 11 – 16]. Phase I metabolism of mesocarb introduces one or more hydroxy groups to the phenylpropyl, phenylcarbamoyl moiety or to both. The sulphate conjugate of *p*-hydroxymesocarb **6a** is likely to be the main phase II metabolite, and a small amount of β-D-glucuronide conjugate has also been detected [5]. In addition, Appolonova *et al*. have found several mono-, di-, and trihydroxylated mesocarb metabolites [8]. However, the precise regiochemistry of these metabolites has remained unknown.

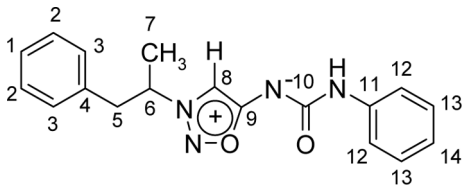
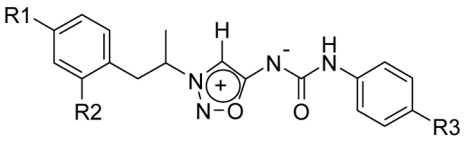


Figure 1. Structure of mesocarb.

In this paper, we describe a convenient route to synthesize six potential mesocarb metabolites **6a – f** (Fig. 2). Synthesis methods of mesocarb have been published [17 – 19], but to our knowledge no attempts to synthesize its hydroxylated metabolites have been reported in the liter-

Correspondence: Jari Yli-Kauhaluoma, Faculty of Pharmacy, Division of Pharmaceutical Chemistry, P. O. Box 56 (Viikinkaari 5 E), FI-00014 University of Helsinki, Finland.
E-mail: Jari.Yli-Kauhaluoma@helsinki.fi
Fax: +358 9 191 59556

Abbreviation: multiple reaction monitoring (MRM)



6a: R1 = H, R2 = H, R3 = OH
6b: R1 = OH, R2 = H, R3 = H
6c: R1 = H, R2 = OH, R3 = H
6d: R1 = OH, R2 = OH, R3 = OH
6e: R1 = H, R2 = OH, R3 = OH
6f: R1 = OH, R2 = H, R3 = OH

Figure 2. Synthesized mesocarb metabolites.

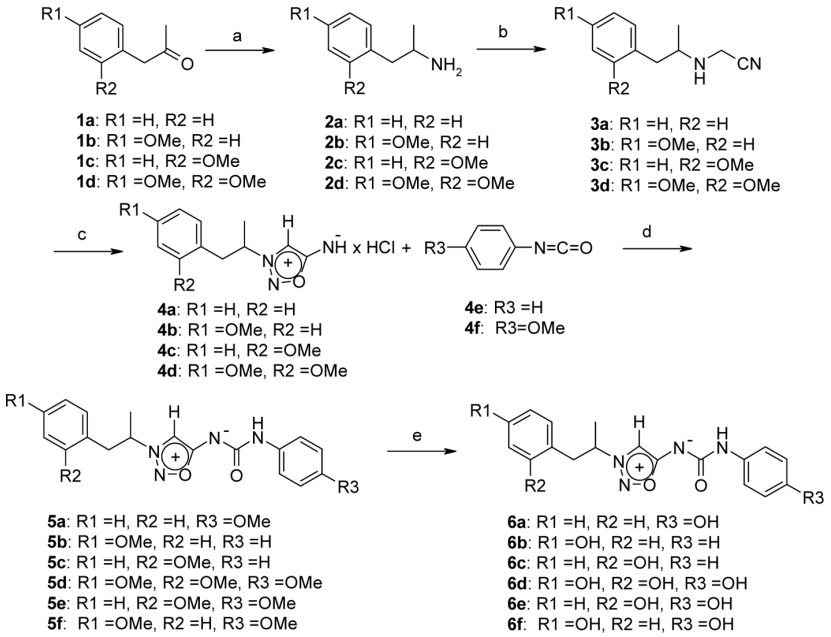
ature. The synthesized metabolites were characterized by means of ^{1}H-NMR, ^{13}C-NMR, FTIR spectrometry, and liquid chromatography – mass spectrometry (LC-MS). As a part of the characterization protocol, the synthesized metabolites were compared to those purified from urine samples collected after oral administration of mesocarb, and to those synthesized enzymatically *in vitro* using human liver proteins.

Results and discussion

Synthetic chemistry

Six hydroxylated mesocarb metabolites were selected for the synthesis. We found out that the most straightforward approach to prepare metabolites with correct regiochemistries was to use appropriately substituted starting materials; methoxyphenylacetones **1b–d** and *p*-methoxyphenylisocyanate **4f**, where the methoxy groups are in the correct positions. Additionally, the demethylation reaction could be carried out as a last step of the synthesis route (Scheme 1).

Phenylacetones **1a–d** were converted to the corresponding amphetamines **2a–d** via reductive amination using ammonium formate as a source for hydrogen and nitrogen [20]. The amphetamines **2a–d** were treated with KCN and formaldehyde at pH 2-3 to yield alkyl acetonitriles **3a–d** [17]. These alkyl acetonitriles were subsequently nitrosated with $NaNO_2$ in dilute HCl [21]. The ring closure to the sydnone imines was achieved by refluxing *N*-nitroso-α-aminoacetonitriles in concentrated HCl. The obtained crude oily products were dissolved in ethanol and precipitated with diethyl ether to give the corresponding hydrochloride salts and were used without further purification. Sydnone imine hydrochlorides **4a–d** were then reacted with phenylisocyanates **4e–f** in anhydrous pyridine to yield the corresponding methoxy-substituted mesocarbs **5a–f**. Demethylation of the phenolic methyl ether was successfully carried out with an aluminium chloride-ethanethiol system in dichloromethane [22]. Other demethylation reagents, such as BBr_3 [23] and thiophenol [24] were also tried but neither of them worked as well as the $AlCl_3$-EtSH system.



1a: R1 =H, R2 =H
1b: R1 =OMe, R2 =H
1c: R1 =H, R2 =OMe
1d: R1 =OMe, R2 =OMe

2a: R1 =H, R2 =H
2b: R1 =OMe, R2 =H
2c: R1 =H, R2 =OMe
2d: R1 =OMe, R2 =OMe

3a: R1 =H, R2 =H
3b: R1 =OMe, R2 =H
3c: R1 =H, R2 =OMe
3d: R1 =OMe, R2 =OMe

4a: R1 =H, R2 =H
4b: R1 =OMe, R2 =H
4c: R1 =H, R2 =OMe
4d: R1 =OMe, R2 =OMe

4e: R3 =H
4f: R3=OMe

5a: R1 =H, R2 =H, R3 =OMe
5b: R1 =OMe, R2 =H, R3 =H
5c: R1 =H, R2 =OMe, R3 =H
5d: R1 =OMe, R2 =OMe, R3 =OMe
5e: R1 =H, R2 =OMe, R3 =OMe
5f: R1 =OMe, R2 =H, R3 =OMe

6a: R1 =H, R2 =H, R3 =OH
6b: R1 =OH, R2 =H, R3 =H
6c: R1 =H, R2 =OH, R3 =H
6d: R1 =OH, R2 =OH, R3 =OH
6e: R1 =H, R2 =OH, R3 =OH
6f: R1 =OH, R2 =H, R3 =OH

Reagents and conditions: (a) Pd/C, $HCOONH_4$, MeOH, H_2O, r.t., overnight, 56–95%; (b) KCN, HCHO, H_2O, pH 2–3, 0°C → r.t., overnight, 80–91%; (c_i) $NaNO_2$, THF, 1 M aqueous HCl, 0°C → r.t., 3 h; (c_{ii}) conc. HCl, reflux, 40 min, 75–93%; (d) pyridine, r.t., overnight, 49–92%; (e) $AlCl_3$, EtSH, CH_2Cl_2, r.t., 1 h, 20–74%.

Scheme 1. Synthesis of the metabolites.

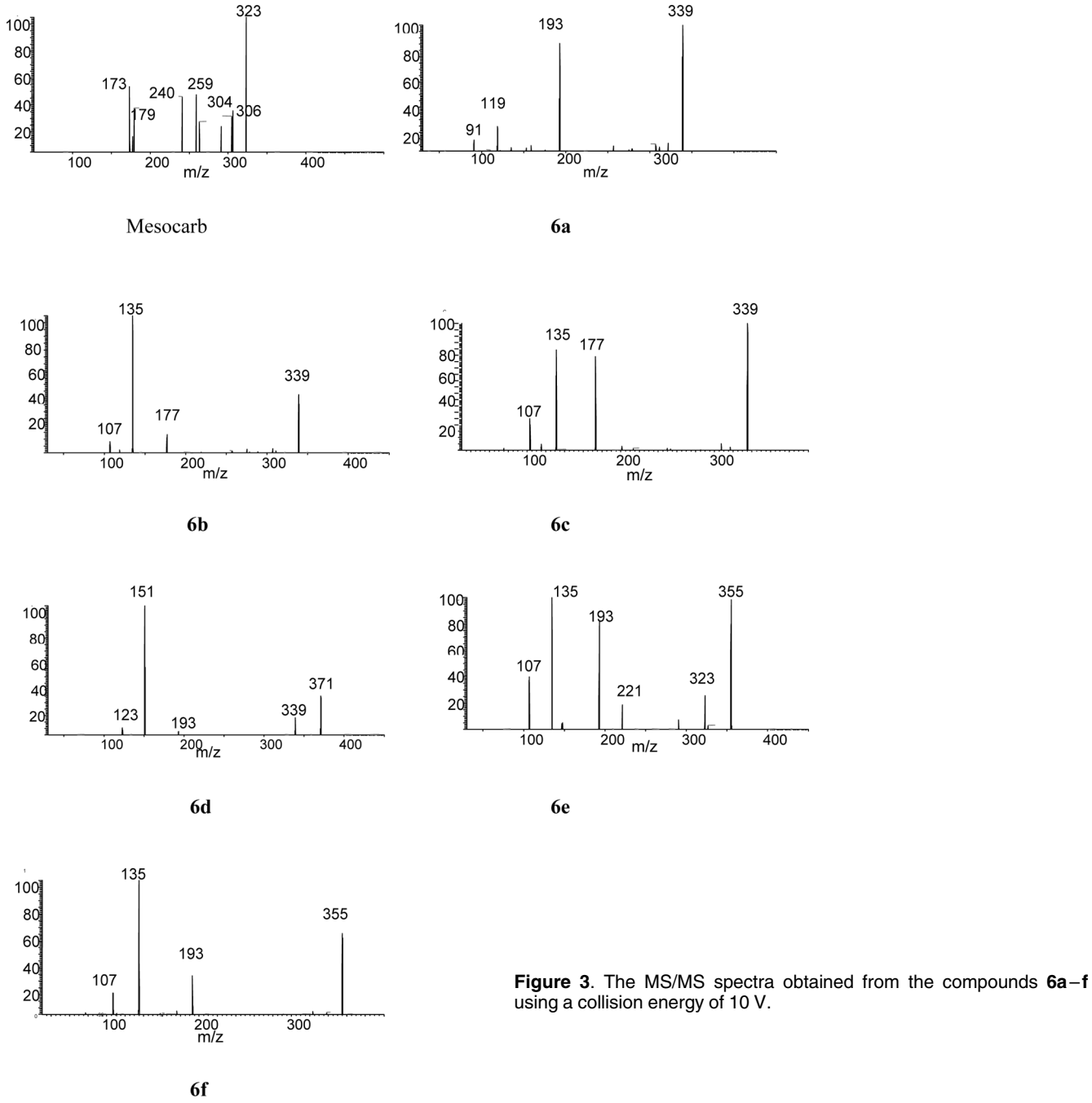


Figure 3. The MS/MS spectra obtained from the compounds **6a**–**f** using a collision energy of 10 V.

The purities of the final products **6a**–**f** were determined by LC/UV-VIS and were within the range of 94.9–99.6%. All the metabolites were structurally characterized by NMR spectroscopy. In addition to the ^{1}H and ^{13}C spectra, the structure of the main metabolite **6a** was confirmed by the HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation) spectra. ^{1}H signals were assigned from the HMBC and ^{1}H spectra, and the corresponding carbons from the HSQC spectrum (Table 1).

LC-MS analytics

The hydroxylated mesocarb metabolites were also analyzed using LC-MS and LC-MS/MS. $[M + H]^+$ was the dominating ion for all the metabolites, and was thus used as the precursor ion in the tandem mass spectrometric

Table 1. ^{1}H- and ^{13}C-NMR chemical shifts of **6a** in ppm (δ).

	δ		δ
H1 – 3	7.19 – 7.32 (5H)	C(1)	127.1
H5	3.28 (CH_2)	C(2)	128.5 (2C)
H6	5.10	C(3)	128.9 (2C)
H7	1.63 (CH_3)	C(4)	136.1
H8	8.14	C(5)	40.6
H12	7.39 (2H)	C(6)	62.5
H13	6.62 (2H)	C(7)	19.5
H(NH)	9.01	C(8)	102.1
H(OH)	8.92	C(9)	172.0
		C(10)	158.7
		C(11)	132.8
		C(12)	119.4 (2C)
		C(13)	114.9 (2C)
		C(14)	151.9

For numbering of the atoms, see Fig. 1.

Table 2. Compound-specific MRM parameters and retention times of the mesocarb metabolites.

Compound	Precursor ion $[M + H]^+$ (m/z)	Product ions (m/z)	Collision energy (V)	RT (min)
Mesocarb	323	119	24	6.05
		91	44	
6a	339	193	10	5.37
		91	48	
6b	339	107	40	5.49
		135	18	
6c	339	107	40	5.84
		135	18	
6d	371	123	36	4.64
		151	20	
6e	355	107	38	5.16
		135	20	
6f	355	107	38	4.81
		135	20	
17α-Methyltestosterone (internal standard)	303	109	36	6.61
		97	36	

experiments. The fragmentation of the compounds (Fig. 3) was efficient producing several specific ions, which is essential for the reliable verification of suspicious samples in doping control. The two most abundant and specific product ions were selected for multiple reaction monitoring (MRM). The optimized MRM parameters used are presented in Table 2. The performance of the LC-MS/MS method was examined for the identification of mesocarb metabolites in urine. Each metabolite could be detected at a concentration level of 50 ng/mL (n = 4) with signal-to-noise ratios higher than 150. Intra-day precision determined at concentration levels of 50 and 500 ng/mL (n = 4) varied between 2.7 and 10.4%. Four aliquots of drug-free urine were analyzed to check the selectivity of the assay. The biological background was low with no interfering peaks. The recovery of the extraction procedure was between 82 and 93% at a concentration level of 500 ng/mL (n = 4). Ion suppression of the sample matrix was studied using the approach of Dams *et al.* [25] and did not indicate any ion suppression at the retention time range where the analytes eluted.

Enzymatic synthesis

As a final part of the characterization, the chemical structures of the synthesized metabolites were compared both to the metabolites synthesized enzymatically *in vitro,* using human liver microsomal protein and fraction S9 of human liver protein, and to an authentic human urine sample, which was collected during 0 to 48 hours after *p.o*. administration of 5 mg of mesocarb (donated from the Antidoping Centre Moscow).

In-vitro enzymatic synthesis yielded only *p*-hydroxymesocarb **6a** (Fig. 2) which was also the dominating metabolite found in human urine collected after oral administration of mesocarb. Most of the *p*-hydroxymesocarb in urine was sulphated (76%), but also some glucuronidated (22%) and free (2%) metabolite was detected. Traces of the dihydroxylated metabolite **6f** (Fig. 2) were also found in urine. The results agree with the literature, where *p*-hydroxymesocarb has been considered to be the main metabolite. Appolonova *et al*. also detected several di- and trihydroxylated mesocarb metabolites in human urine [8, 26], but in this experiment those metabolites could not be detected. A possible explanation for this might be the administered single low dose and the individual differences in the metabolism of mesocarb.

Conclusion

We have developed a method of synthesis and analysis for different hydroxy-substituted mesocarb metabolites. The synthesized metabolites were compared to the metabolites obtained from *in-vitro* and *in-vivo* experiments. LC-MS/MS analysis of the metabolites from the experiments agreed with the literature that *p*-hydroxymesocarb is the main metabolite of mesocarb. Thus, with respect to doping analysis, synthesis of this metabolite and its availability as a reference material, is naturally of great importance.

The authors thank Mrs. Teija Moisander for expert technical assistance and the Antidoping Centre Moscow for an authentic human urine mesocarb sample. The project was funded by the World Anti-Doping Agency WADA (Grant 05D4JY/2005).

The authors have declared no conflict of interest.

Experimental

General

The reagents and materials for synthetic purposes were obtained from commercial suppliers and were used without further purification. Pyridine and dichloromethane were distilled and dried prior to use. Reactions were monitored by thin-layer chromatography on silica gel 60-F_{256} plates acquired from Merck (Darmstadt, Germany). Column chromatography was carried out manually by using silica gel 60 (230 – 400 mesh) from E. Merck or by Biotage SP1 purification system (Charlottesville, VA, USA) using Flash 25+M silica cartridges. FTIR spectra were measured on a Bruker Vertex 70 FT-IR spectrometer (Bruker, Ettlingen, Germany) with KBr technique. ^{1}H-NMR and ^{13}C-NMR were recorded on Varian Mercury Plus 300 MHz spectrometer (Varian, Palo Alto, CA., USA) using DMSO-d_6 as a solvent. Chemical shifts are reported relative to TMS. *J* values are given in Hz. In order to observe ^{1}H-^{13}C correlation between the mesoionic proton 8 and carbon 8 in the HSQC spectrum, the value of one bond coupling constant had to be altered from the default setting of 140 to 180 MHz. Purity of the synthesized metabolites was checked using an HP1100 LC/UV-VIS system (Hewlett-Packard, Waldbronn, Germany) with a Zorbax Eclipse XDB-C18 (2.1 × 50 mm, 3.5 µm; Agilent, Germany) column using the same chromatographic conditions, which were used in LC-MS/MS detection of the metabolites.

Chemistry

1-Methyl-2-phenylethylamine ***2a***

The substance was synthesized under the permission by the National Agency for Medicines (3/2005, Helsinki, Finland). Ammonium formate (49 g, 780 mmol) was dissolved in a mixture of water (18 mL) and MeOH (160 mL). Phenylacetone (10 g, 75 mmol) and palladium (2.4 g, 3.0 mmol) on activated charcoal (10 w/w%) were added. The resulting mixture was stirred overnight at room temperature. The reaction mixture was filtered through a pad of Celite to remove the palladium catalyst. The Celite was washed with MeOH, the filtrate was evaporated and concentrated HCl (14 mL) was added to the residue. This mixture was extracted with Et_2O (2 × 15 mL). The aqueous phase was made alkaline with solid KOH, and the resulting amphetamine base separated. It was removed, and the aqueous phase was extracted with Et_2O (15 mL). The ethereal phase was combined with the amphetamine layer and the solvent was evaporated. Amphetamine was dissolved in MeOH and filtered through a pad of silica. Silica was washed with methanol. Evaporation of the solvent afforded a pale orange oil.

Yield: 9.5 g (95%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.25 (m, 5H), 3.09 (sextet, *J* = 6.6 Hz, 1H), 2.66 (dd, *J* = 6.6, 13.2 Hz, 1H), 2.52 (dd, *J* = 6.9, 13.2 Hz, 1H), 0.97 (d, *J* = 6.3 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 139.2, 129.2, 128.2, 126.0, 48.2, 44.7.0, 21.8; FTIR (KBr, cm^{-1}): 3365, 3281, 3027, 1947, 1604.

2-(4-Methoxyphenyl)-1-methylethylamine ***2b***

A pale orange oil. Yield: 1.67 g (55%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.08 (td, *J* = 3.0, 8.4 Hz, 2H), 6.83 (td, *J* = 3.3, 8.4 Hz, 2H), 3.71 (s, 3H), 2.93 (sextet, *J* = 6.3 Hz, 1H), 2.60 (d, *J* = 7.2 Hz, 2H), 0.92 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 157.5, 131.9, 130.0, 113.5, 54.9, 48.4, 45.3, 23.2; FTIR (KBr, cm^{-1}): 3359, 3271, 3030, 2059, 1612.

2-(2-Methoxyphenyl)-1-methylethylamine ***2c***

A pale orange oil. Yield: 1.32 g (65%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.17 (td, *J* = 1.8, *J* = 8.4 Hz, 1H), 7.09 (dd, *J* = 1.5, 7.2 Hz, 1H), 6.93 (dd, *J* = 0.6, 7.2 Hz, 1H), 6.85 (td, *J* = 0.9, 7.2 Hz, 1H), 3.75 (s, 3H), 3.17 (s, 2H), 3.02 (sextet, *J* = 6.6 Hz, 1H), 2.51 (d, *J* = 6.6 Hz, 1H), 0.92 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 157.3, 130.7, 128.0, 127.2, 120.2, 110.6, 55.2, 48.6, 46.7, 23.5; FTIR (KBr, cm^{-1}): 3400 – 2800 (br), 2055, 1600.

2-(2,4-Dimethoxyphenyl)-1-methylethylamine ***2d***

A pale orange oil. Yield: 1.1 g (56%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.01 (d, *J* = 8.4 Hz, 1H), 6.52 (d, *J* = 2.7 Hz, 1H), 6.45 (dd, *J* = 2.4, 8.4 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.07 (sextet, *J* = 6.3 Hz, 1H), 2.58 (dd, *J* = 6.6, 12.6 Hz, 1H), 2.49 (dd, *J* = 7.2, 12.9 Hz, 1H), 0.96 (d, *J* = 6.3 Hz, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 159.2, 158.1, 131.1, 119.0, 104.4, 98.4, 55.3, 55.1, 46.8, 38.2, 21.5; FTIR (KBr, cm^{-1}): 3359, 2836, 2065, 1612.

(1-Methyl-2-phenylethylamino)acetonitrile ***3a***

2a (2.50 g, 14.6 mmol) was suspended in water (35 mL). The pH of the solution was adjusted to 2 – 3 with concentrated HCl. The reaction mixture was cooled with an ice bath, and formaldehyde (1.10 mL, 14.6 mmol) and potassium cyanide (0.95 g, 14.6 mmol) were added. After stirring the reaction mixture overnight at room temperature, it was extracted with Et_2O (4 × 15 mL). The combined extracts were dried with Na_2SO_4 and evaporated to give light yellow oil.

Yield: 2.12 g (83%). R_f = 0.79 (EtOAc / MeOH 4 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.25 (m, 5H), 3.54 (s, 2H), 3.17 (m, 1H), 2.67 (m, 2H), 1.11 (d, *J* = 6.3 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 138.4, 129.4, 128.9, 126.8, 117.8, 53.1, 43.5, 35.0, 19.6; FTIR (KBr, cm^{-1}): 3333, 3063, 2234, 1952, 1603.

[2-(4-Methoxyphenyl)-1-methylethylamino]acetonitrile ***3b***

A colorless oil. Yield: 570 mg (86%). R_f = 0.39 (EtOAc / Hex 1 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.08 (td, *J* = 3.0, 8.4 Hz, 2H), 6.83 (td, *J* = 2.7, 9.0 Hz, 2H), 3.72 (s, 3H), 3.65 (d, *J* = 3.9 Hz, 2H), 2.86 (sextet, *J* = 5.7 Hz, 1H), 2.68 (dd, *J* = 5.7, 13.5 Hz, 1H), 2.39 (bs, 1H), 2.38 (dd, *J* = 7.2, 13.2 Hz, 1H), 0.90 (d, 3H, 6.3 Hz); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 157.5, 131.9, 130.0, 113.5, 54.9, 48.4, 45.3, 23.2; FTIR (KBr, cm^{-1}): 3332, 3061, 2234, 2058, 1614.

[2-(2-Methoxyphenyl)-1-methylethylamino]acetonitrile ***3c***

A colorless oil. Yield: 660 mg (91%). R_f = 0.30 (EtOAc / Hex 1 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.20 (td, *J* = 1.8, 7.8 Hz, 1H), 7.10 (dd, *J* = 1.8, 7.2 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 6.86 (td, *J* = 0.9, 7.2 Hz, 1H), 3.77 (s, 3H), 3.64 (bd, *J* = 5.1 Hz, 2H), 2.95 (m, sextet, *J* = 6.6 Hz, 1H), 2.77 (dd, *J* = 5.1, 12.9 Hz, 1H), 2.40 (bs, 1H), 2.36 (dd, *J* = 8.1, 12.9 Hz, 1H), 0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 157.3, 130.8, 127.5, 127.1, 120.1, 119.3, 110.7, 55.2, 51.7, 36.7, 34.4, 19.3; FTIR (KBr, cm^{-1}): 3333, 3065, 2234, 2051, 1601.

[2-(2,4-Dimethoxyphenyl)-1-methylethylamino]acetonitrile ***3d***

A pale yellow oil. Yield: 590 mg (91%). R_f = 0.31 (EtOAc / Hex 1 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.99 (d, *J* = 8.4 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.44 (dd, *J* = 2.1, 8.4 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 2.88 (sextet, *J* = 6.6 Hz, 1H), 2.67 (dd, *J* = 5.1, 13.2 Hz, 1H), 2.34 (bs, 1H), 2.29 (dd, *J* = 7.5, 13.2 Hz, 1H), 0.88 (d, *J* = 6.3 Hz, 3H); ^{13}C-NMR

(75 MHz, DMSO-d_6) δ: 159.1, 158.1, 131.1, 119.3, 119.2, 104.3, 98.3, 55.3, 55.1, 51.8, 36.1, 34.4, 19.2; FTIR (KBr, cm^{-1}): 3333, 3078, 2234, 2060, 1614.

3-(1-Methyl-2-phenylethyl)sydnone imine ***4a***

3a (2.12 g, 12.2 mmol) was dissolved in THF (11 mL). The solution was cooled to 0°C and 1 M HCl (8.6 mL) was added. $NaNO_2$ (0.84 g, 12.2 mmol) was dissolved in water (3 mL) and added dropwise over 20 min. The reaction mixture was stirred at room temperature for 3 hours after which it was evaporated to dryness. The residue was suspended to concentrated HCl (13 mL) and refluxed for 40 min. The reaction mixture was evaporated to dryness, and the residue was dissolved in a small amount of ethanol and cooled. Et_2O was added. The mixture was stirred vigorously on an ice bath for 20 min after which the off-white sydnone imine hydrochloride was filtered.

Yield: 760 mg (68%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.96 (s, 2H), 8.30 (s, 1H), 7.27 (m, 5H), 5.32 (sextet, J = 6.9 Hz, 1H), 3.31 (d, J = 7.2 Hz, 2H), 1.63 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 169.0, 135.6, 129.2, 129.0, 128.6, 127.2, 101.2, 63.8, 40.5, 18.9; FTIR (KBr, cm^{-1}): 3373, 3091, 1684.

3-[1-Methyl-2-(4-methoxyphenyl)ethyl]sydnone imine ***4b***

An off-white solid. Yield: 558 mg (93%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.95 (s, 2H), 8.27 (s, 1H), 7.13 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.25 (sextet, J = 6.9 Hz, 1H), 3.71 (s, 3H), 3.23 (d, J = 7.8 Hz, 2H), 1.61 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 168.9, 158.3, 130.1, 127.3, 114.0, 101.1, 64.0, 55.0, 39.8, 18.8; FTIR (KBr, cm^{-1}): 3334, 3151, 1697.

3-[1-Methyl-2-(2-methoxyphenyl)ethyl]sydnone imine ***4c***

A light brown solid. Yield: 1.3 g (85%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.91 (s, 2H), 8.2 (s, 1H), 7.25 (td, J = 1.8, 8.4 Hz, 1H), 7.11 (dd, J = 1.8, 7.2 Hz, 1H), 6.97 (dd, J = 0.6, 8.4 Hz, 1H), 6.87 (td, J = 0.9, 7.2 Hz, 1H), 5.20 (sextet, J = 6.9 Hz, 1H), 3.73 (s, 3H), 3.26 (d, J = 6.9 Hz, 2H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 168.9, 157.3, 130.8, 129.0, 123.1, 120.4, 110.8, 101.2, 62.6, 55.3, 36.0, 18.7; FTIR (KBr, cm^{-1}): 3345, 3039, 1677.

3-[1-Methyl-2-(2,4-dimethoxyphenyl)ethyl]sydnone imine ***4d***

4d failed to precipitate from EtOH / Et_2O. Water was evaporated and the residue dried *in vacuo* before the next reaction step.

Yield: 521 mg (75%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.73 (s, 2H), 8.14 (s, 1H), 7.01 (d, J = 7.8 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 6.45 (dd, J = 2.4, 8.1 Hz, 1H), 5.13 (sextet, J = 7.5 Hz, 1H), 3.73 (s, 3H), 3.71 (s, 3H), 3.16 (d, J = 6.9 Hz, 2H), 1.62 (d, J = 7.2 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 168.9, 160.1, 158.3, 131.3, 115.1, 104.8, 101.1, 98.3, 62.9, 55.4, 55.1, 35.6, 18.6; FTIR (KBr, cm^{-1}): 3335, 2999, 1670.

3-(1-Methyl-2-phenylethyl)-5-[[(4-methoxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5a***

4a (1.11 g, 4.61 mmol) was dissolved in anhydrous pyridine (25 mL) under argon atmosphere. The solution was cooled on an ice bath and 4-methoxyphenyl isocyanate (1.20 mL, 9.22 mmol) was added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched by pouring the mixture to ice water. The aqueous phase was extracted with EtOAc (4 × 15 mL). The extracts were combined and washed with aqueous $CuSO_4$ solution and water. The organic phase was then dried with Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography using EtOAc / toluene (2 : 1) as an eluent to give a yellow solid.

Yield: 1.4 g (92%). R_f = 0.50 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.14 (s, 1H), 8.17 (s, 1H), 7.53 (d, J = 8.7 Hz, 2H), 7.26 (m, 5H), 6.80 (d, J = 9 Hz, 2H), 5.11 (sextet, J = 6.6 Hz, 1H), 3.69 (s, 3H), 3.28 (m, 2H), 1.64 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 158.7, 153.9, 136.1, 134.3, 128.9, 128.5, 127.0, 119.1, 113.6, 102.2, 62.5, 55.1, 40.5, 19.4; FTIR (KBr, cm^{-1}): 3259, 3032, 1635, 1601.

3-(1-Methyl-2-(4-methoxyphenyl)ethyl)-5-[[(phenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5b***

A pale yellow solid. Yield: 482 mg (59%). R_f = 0.59 (EtOAc / Hex 3 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.29 (s, 1H), 8.20 (s, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.20 (t, J = 7.5 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.86 (m, 3H), 5.06 (sextet, J = 6.6 Hz, 1H), 3.70 (s, 3H), 3.22 (m, 2H), 1.62 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.3, 158.9, 158.2, 141.1, 129.9, 128.4, 127.8, 121.1, 117.8, 113.9, 102.3, 62.8, 54.9, 39.9, 19.3; FTIR (KBr, cm^{-1}): 3228, 3035, 1638, 1593.

3-(1-Methyl-2-(2-methoxyphenyl)ethyl)-5-[[(phenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5c***

A yellow solid. Yield: 369 mg (57%). R_f = 0.45 (EtOAc / Hex 3 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.27 (s, 1H), 8.13 (s, 1H), 7.62 (d, J = 7.8 Hz, 2H), 7.22 (m, 3H), 7.08 (dd, J = 1.8, 7.8 Hz, 1H), 6.97 (d, J = 1.2, 8.4 Hz, 1H), 6.86 (m, 2H), 5.06 (sextet, J = 6.9 Hz, 1H), 3.74 (s, 3H), 3.24 (d, J = 7.5 Hz, 2H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.3, 158.9, 157.3, 141.1, 130.5, 128.8, 128.4, 121.9, 120.3, 117.8, 110.8, 102.3, 61.2, 55.3, 36.1, 19.2; FTIR (KBr, cm^{-1}): 3231, 3023, 1639, 1586.

3-(1-Methyl-2-(2,4-dimethoxyphenyl)ethyl)-5-[[(4-methoxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5d***

A yellow solid. Yield: 350 mg (49%). R_f = 0.47 (EtOAc / toluene 1 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.13 (s, 1H), 8.08 (s, 1H), 7.53 (d, J = 9.3 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 8.7 Hz, 2H), 6.52 (d, J = 2.1 Hz, 1H), 6.42 (dd, J = 2.1, 8.7 Hz, 1H), 4.98 (sextet, J = 6.6 Hz, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.69 (s, 3H), 3.15 (d, J = 7.5 Hz, 2H), 1.61 (d, J = 6.3 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 159.9, 158.8, 158.3, 153.8, 134.4, 131.0, 119.1, 115.7, 113.6, 104.6, 102.0, 98.3, 61.3, 55.4, 55.1, 35.6, 19.2; FTIR (KBr, cm^{-1}): 3228, 3001, 1637, 1589.

3-(1-Methyl-2-(2-methoxyphenyl)ethyl)-5-[[(4-methoxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5e***

A pale yellow solid. Yield: 337 mg (49%). R_f = 0.47 (EtOAc / Hex 3 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.13 (s, 1H), 8.09 (s, 1H), 7.53 (d, J = 9 Hz, 2H), 7.23 (td, J = 1.8, 8.4 Hz,1H), 7.07 (dd, J = 1.5, 7.5 Hz, 1H), 6.96 (d, J = 7.5 Hz, 1H), 6.82 (m, 3H), 5.05 (sextet, J = 6.9 Hz, 1H), 3.75 (s, 3H), 3.69 (s, 3H), 3.23 (d, J = 6.9 Hz, 2H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 158.8, 157.3, 153.9, 134.3, 130.5, 128.8, 123.6, 120.3, 119.1, 113.6,

110.8, 102.1, 61.1, 55.3, 55.1, 36.1, 19.3; FTIR (KBr, cm^{-1}): 3242, 3002, 1643, 1594.

3-(1-Methyl-2-(4-methoxyphenyl)ethyl)-5-[[(4-methoxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***5f***

A yellow solid. Yield: 468 mg (59%). R_f = 0.16 (EtOAc / Hex 2 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.14 (s, 1H), 8.16 (s, 1H), 7.53 (d, J = 9.0 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 6.80 (d, J = 8.7Hz, 2H), 5.04 (sextet, J = 6.6 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.21 (m, 2H), 1.62 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 158.7, 158.2, 153.9, 134.3, 129.9, 127.8, 119.1, 113.9, 113.6, 102.1, 62.8, 55.1, 54.9, 19.3; FTIR (KBr, cm^{-1}): 3225, 3029, 1637, 1592.

3-(1-Methyl-2-phenylethyl)-5-[[(4-hydroxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6a***

5a (0.515 g, 1.45 mmol) was dissolved in dry dichloromethane (5 mL). Ethanethiol (5 mL) was added. The solution was cooled to 0°C and aluminium chloride (0.66 g, 4.95 mmol) was added. The reaction mixture was stirred at room temperature for 1 hour. The reaction was quenched by adding water (10 mL). The product precipitated, and the resulting mixture was stirred for 5 min, after which the supernatant was decanted, concentrated, and extracted with dichloromethane. The organic layer was combined with the precipitated product and evaporated to dryness. The crude product was purified by silica gel column chromatography, and subsequently recrystallized from ethanol to give light orange crystals.

Yield: 290 mg (59%). LC-UV/VIS purity 99.3%; R_f = 0.35 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.01 (s, 1H), 8.92 (s, 1H), 8.14 (s, 1H), 7.39 (d, J = 8.7 Hz, 2H), 7.25 (m, 5H), 6.62 (d, J = 9 Hz, 2H), 5.1 (sextet, J = 6.6 Hz, 1H), 3.28 (m, 2H), 1.63 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.0, 158.7, 151.9, 136.1, 132.8, 128.9, 128.5, 127.1, 119.4, 114.9, 102.1, 62.5, 40.6, 19.5; FTIR (KBr, cm^{-1}): 3155, 1601, 1542, 1433, 1312.

3-[1-Methyl-2-(4-hydroxyphenyl)ethyl]-5-[[(phenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6b***

A pale yellow solid. Yield: 595 mg (58%). LC-UV/VIS purity 96.6%; R_f = 0.54 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.31 (s, 1H), 9.28 (s, 1H), 8.17 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.20 (t, J = 8.1 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 6.88 (t, J = 7.2 Hz, 1H), 6.66 (d, J = 8.4 Hz, 2H), 5.01 (sextet, J = 6.6 Hz, 1H), 3.15 (m, 2H), 1.61 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.3, 158.9, 156.3, 141.1, 129.9, 128.4, 126.0, 121.1, 117.8, 115.3, 102.3, 62.9, 39.9, 19.2; FTIR (KBr, cm^{-1}): 3265, 3184, 1639, 1592, 1517, 1313.

3-[1-Methyl-2-(2-hydroxyphenyl)ethyl]-5-[[(phenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6c***

A yellow solid. Yield: 190 mg (57%). LC-UV/VIS purity 98.9%; R_f = 0.63 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.62 (s, 1H), 9.27 (s, 1H), 8.14 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 8.1 Hz, 1H), 7.06 (td, J = 1.8, 7.5 Hz, 1H), 6.97 (dd, J = 1.2, 7.5 Hz, 1H), 6.88 (t, J = 7.8 Hz, 1H), 6.8 (d, J = 7.8 Hz, 1H), 6.68 (td, J = 0.9, 7.2 Hz, 1H), 5.1 (sextet, J = 7.2 Hz, 1H), 3.21 (d, J = 7.2 Hz, 2H), 1.65 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.4, 158.9, 155.5, 141.1, 130.6, 128.4, 121.9, 121.1, 118.9, 117.8, 115.0, 102.2, 61.1, 36.2, 19.3; FTIR (KBr, cm^{-1}): 3228, 3186, 1638, 1593, 1313.

3-[1-Methyl-2-(2,4-dihydroxyphenyl)ethyl]-5-[[(4-hydroxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6d***

A yellow solid. Yield: 204 mg (20%). LC-UV/VIS purity 94.9%; R_f = 0.20 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.42 (s, 1H), 9.13 (s, H), 8.98 (s,1H), 8.91 (s, 1H), 8.03 (s, 1H), 7.39 (d, J = 9 Hz, 2H), 6.71 (d, J = 8.4 Hz, 1H), 6.61 (d, J = 8.7 Hz, 2H), 6.27 (d, J = 2.1 Hz, 1H), 6.09 (dd, J = 2.4, 8.4 Hz, 1H), 4.97 (sextet, J = 7.5 Hz, 1H), 3.06 (d, J = 7.2 Hz, 2H), 1.60 (d, J = 7.2 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 158.7, 157.5, 156.3, 151.8, 132.8, 130.9, 119.4, 114.9, 112.4, 106.2, 102.4, 101.8, 61.3, 35.8, 19.2; FTIR (KBr, cm^{-1}): 3314 (br), 3191, 1620, 1513, 1309.

3-[1-Methyl-2-(2-hydroxyphenyl)ethyl]-5-[[(4-hydroxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6e***

A yellow solid. Yield: 290 mg (65%). LC-UV/VIS purity 99.3%; R_f = 0.40 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.61 (s, 1H), 8.99 (s, 1H), 8.93 (s, 1H), 8.07 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 7.05 (td, J = 1.5, 9.3 Hz, 1H), 6.97 (dd, J = 1.5, 7.2 Hz, 1H), 6.80 (d, J = 7.2 Hz, 1H), 6.63 (m, 3H), 5.07 (sextet, J = 7.2 Hz, 1H), 3.19 (d, J = 7.2 Hz, 2H), 1.63 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.1, 158.8, 155.5, 151.9, 132.8, 130.6, 128.5, 122.0, 119.5, 119.0, 115.1, 114.9, 101.9, 61.0, 36.3, 19.4; FTIR (KBr, cm^{-1}): 3221, 1627, 1514, 1310.

3-[1-Methyl-2-(4-hydroxyphenyl)ethyl]-5-[[(4-hydroxyphenylamino)carbonyl]amino]-1,2,3-oxadiazolium ***6f***

A yellow solid. Yield: 275 mg (74%). LC-UV/VIS purity 99.6%; R_f = 0.30 (EtOAc / Hex 4 : 1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.31 (s, 1H), 9.01 (bs, 1H), 8.91 (s, 1H), 8.10 (s, 1H), 7.39 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 6.62 (d, J = 9 Hz, 2H), 4.98 (sextet, J = 6.9 Hz, 1H), 3.14 (m, 2H), 1.60 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 172.0, 158.7, 156.3, 151.8, 132.8, 129.8, 126.0, 119.4, 115.3, 114.9, 102.0, 62.8, 40.0, 19.3; FTIR (KBr, cm^{-1}): 3185, 1637, 1600, 1514.

In-vitro **enzymatic synthesis of mesocarb metabolites**

In-vitro synthesis of the metabolites was based on the procedure described by Keski-Hynnilä *et al*. [27]. The reaction mixture for enzyme-assisted synthesis of phase I metabolites of mesocarb consisted of 50 μM of mesocarb in methanol, 5 mM NADPH in buffer, human liver microsomal protein (0.5 mg/mL) and fraction S9 of human liver protein (0.5 mg/mL). 50 mM phosphate solution (pH 7.4, 5 mM $MgCl_2$) was used as an incubation buffer. The total reaction volume was 100 μL.

The phase I reaction was started by addition of NADPH to the reaction mixture. The reaction was carried out at 37°C in Eppendorf tubes in a dry bath. The incubation was stopped after 4 hours by addition of ice-cold 4 M perchloric acid. After centrifugation, the supernatant was moved to a glass vial and its pH was adjusted to 6–7 with 1 M NaOH. The aqueous phase was extracted with ethyl acetate (4 × 0.5 mL), and the combined EtOAc extracts were evaporated. The analytical recovery of the used extraction procedure was 92–95% depending on the

metabolite. The mesocarb metabolites were thermally stable during incubation at 37°C (data not shown).

LC-MS/MS detection of metabolites

The LC-MS/MS-experiments were performed on a ThermoFinnigan Surveyor LC from ThermoFinnigan, and a TSQuantum triple quadrupole mass spectrometer, also from ThermoFinnigan (Germany).

The examination of the analytes' mass spectrometric properties was conducted using an electrospray ionization (ESI) in the positive ion mode. The MS- and MS/MS-spectra were measured in the scan range m/z 50 – 500 and two-ion transitions were chosen per each compound for MRM. Argon was used as the collision gas at a pressure of 1.5 mTorr. Nitrogen was used both as sheath and auxiliary gas (49 and 5 arbitrary units). The spray voltage was 4000 V and the capillary temperature 270°C.

The chromatographic separation was obtained at ambient temperature on a Zorbax 300SB-CN (2.1 × 150 mm, 5 μm) column from Agilent, equipped with a guard column of the same packing material (2.1 × 12.5 mm). The mobile phase consisted of A: 2.5 mM ammonium acetate with 0.1% acetic acid, pH 4, and B: methanol. A linear gradient was run with a flow rate of 200 μL/min from 40% B to 100% B in 6 min, followed by an equilibration step of 2 min.

Urine sample pre-treatment

17α-Methyltestosterone was used as an internal standard (500 ng/mL). A 3 mL-aliquot of urine was applied to a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), which was previously conditioned with 2.5 mL of MeOH and 5 mL of water. The sample was first rinsed with 5 mL of water, and then eluted with 3 mL of MeOH. The eluate was divided into three parts, which were evaporated to dryness and dissolved in 1 mL of an appropriate buffer for the isolation of the free and conjugated metabolites. Enzymatic hydrolysis of the β-D-glucuronide conjugates was performed in a 0.1 M phosphate buffer (pH 7) with 25 μL of β-glucuronidase from *Escherichia coli* K12 (Boehringer Mannheim, Mannheim, Germany) at 50°C for 1 h. Sulphate conjugates were enzymatically hydrolyzed in 1 mL of 0.1 M acetate buffer (pH 5) containing 5 μM saccharic acid lactone with 10 μL of arylsulfatase from *Helix pomatia* (Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 12 h. For isolation of the free metabolites no hydrolyzing enzyme was added. All three aliquots were finally extracted twice with 3 mL of ethyl acetate at pH 8 on a vortex-mixer for 30 s. After centrifugation, the organic phase was separated, evaporated to dryness under a nitrogen stream at 40°C, and finally dissolved in 100 μL of the initial LC mobile phase prior to injection to the LC-MS/MS. The analytical performance of the method was evaluated with respect to detection capability, precision, specificity, extraction recovery, and ion suppression of the sample matrix.

References

[1] E. Anderzhanova, K. S. Rayevsky, P. Saransaari, E. Riitamaa, S. S. Oja, *Eur. J. Pharmacol.* **2001**, *428*, 87 – 95.

[2] R. R. Gainetdinov, T. D. Sotnikova, T. V. Grekhova, K. S. Rayevsky, *Eur. J. Pharmacol.* **1997**, *340*, 53 – 58.

[3] M. D. Mashkokovsky, R. A. Altshuler, G. Ya. Avrutsky, Yu. A. Aleksandrovich, R. A. Smulevich, *Korsakov J. Neurol. Psychiatr.* **1971**, *71*, 1704 – 1709.

[4] Medical Commission, International Olympic Committee, *International Olympic Charter Against Doping in Sport*, IOC, Lausanne, **1990**, updated December 1991.

[5] R. Ventura, T. Nadal, P. Alcalde, J. Segura, *J. Chromatogr.* **1993**, *647*, 203 – 210.

[6] H. Pyo, S. J. Park, J. Park, J. K. Yoo, B. Yoon, *J. Chromatogr. B* **1996**, *687*, 261 – 269.

[7] J. Lee, H. Jeung, K. Kim, D. Lho, *J. Mass Spectrom.* **1999**, *34*, 1079 – 1086.

[8] S. A. Appolonova, A. V. Shpak, V. A. Semenov, *J. Chromatogr. B*, **2004**, *800*, 281 – 289.

[9] M. Polgár, L. Vereczkey, L. Szporny, G. Czira, *et al.*, *Xenobiotica* **1979**, *9*, 511 – 520.

[10] J. Tamás, M. Polgár, G. Czira, L. Vereczkey, *Recent Dev. Mass Spectrum. Biochem. Med.* **1979**, *2*, 43 – 52.

[11] Y. Shumin, Z. Changjiu, C. Kairong in *Proceedings of the 10th Cologne workshop on Dope Analysis Cologne*, Germany, June **1992** (Eds.: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth), Sport und Buch Strauss, Edition Sport, Cologne, **1993**, pp. 199 – 212.

[12] R. Ventura, T. Nadal, M. J. Pretel, A. Solans, *et al.*, *in Proceedings of the 10th Cologne workshop on Dope Analysis Cologne*, Germany, June **1992**, (Eds.: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth), Sport und Buch Strauss, Edition Sport, Cologne, **1993**, pp. 231 – 248.

[13] A. Breidbach, G. Sigmund, M. Donike in *Proceedings of the 12th Cologne workshop on Dope Analysis Cologne*, Germany, April 1994, (Eds.: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke), Sport und Buch Strauss, Edition Sport, Cologne, **1995**, pp. 301 – 304.

[14] D. Thieme, J. Grobe, R. Lang, R. K. Mueller in *Proceedings of the 12th Cologne workshop on Dope Analysis Cologne*, Germany, April **1994**, (Eds.: M. Donike , H. Geyer, A. Gotzmann, U. Mareck-Engelke), Sport und Buch Strauss, Edition Sport, Cologne, **1995**, pp. 275 – 284.

[15] Y. Shumin, Z. Shaotang, Y. Zeyi in *Proceedings of the 14th Cologne workshop on Dope Analysis Cologne, Germany*, March **1996**, (Eds.: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke), Sport und Buch Strauss, Edition Sport, Cologne, **1997**, pp. 377 – 385.

[16] L. M. P. Damascenco, R. M. A. Bento, L. N. F. L. Gomes, R. de La Torre, F. R. Aquino Neto in *Proceedings of the 19th Cologne workshop on Dope Analysis Cologne*, Germany, March **2001**, (Eds.: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke), Sport und Buch Strauss, Edition Sport, Cologne, **2002**, pp. 253 – 257.

[17] Z. A. Olovyanishnikova, V. A. Parshin, V. V. Ogorodnikova, M. D. Maskovskii, V. G. Yashunskii, *Khimiko-Farmasevticheskii Zhurnal* **1987**, *21*, 1305 – 1309.

[18] Z. A. Olovyanishnikova, T. M. Ivanova, V. E. Sviridova, V. G. Yashunskii, *Khimiya Geterotsiklicheskikh Soedinenii* **1978**, 170 – 175.

[19] V. G. Yashunskii, V. V. Ogorodnikova, L. E. Kholodov, *Khimiya Geterotsiklicheskikh Soedinenii* **1980**, 1244 – 1247.

[20] J. Gonzalez-Sabin, V. Gotor, F. Rebolledo, *Tetrahedron Asymmetry* **2002**, *13*, 1315 – 1320.

[21] S. R. Hitchcock, G. P. Nora, C. Hedberg, D. M. Casper, *et al.*, *Tetrahedron* **2000**, *56*, 8799 – 8807.

[22] M. Node, K. Nishide, K. Fuji, E. Fujita, *J. Org. Chem.* **1980**, *45*, 4275 – 4277.

[23] J. F. W. McOmie, D. E. West, *Org. Synth.* **1969**, *49*, 50.

[24] A. K. Chakraborti, L. Sharma, M. K. Nayak, *J. Org. Chem.* **2002**, *67*, 6406 – 6414.

[25] R. Dams, M. A. Huestis, W. E. Lambert, C. M. Murphy, *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1290 – 1294.

[26] A. V. Shpak, S. A. Appolonova, V. A. Semenov, *J. Chromatogr. Sci.* **2005**, *43*, 11 – 21.

[27] H. Keski-Hynnilä, M. Kurkela, E. Elovaara, L. Antonio, *et al.*, *Anal. Chem*. **2002**, *74*, 3449 – 3457.

