Major Metabolites of Zolpidem: Expeditious Synthesis and Mass Spectra

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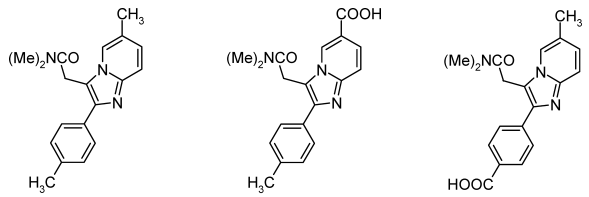
An expeditious route to the two major metabolites of Zolpidem—and readily applicable to the synthesis of the drug—was established *via* a cyclization reaction between a 2-aminopyridine and a suitable α-bromoacetophenone. The structures of the target compounds were confirmed from a 2D ^{1}H–^{15}N NMR correlation. Their mass spectra contribute to a reliable toxicological identification of the drug in the case of drug-facilitated crimes.

Key words Zolpidem; metabolite; regioselective cyclization; drug-facilitated crime; mass spectra

Zolpidem (Ambien®, Stilnox®) is a short-action non-benzodiazepine hypnotic drug which binds with a high affinity as an allosteric modulator to α_1 subunit-containing $GABA_A$ receptors.[1)] The α_1-$GABA_A$-preferring property of this imidazo[1,2-*a*]pyridine, which may be turned into an α_3 partial agonism for imidazo[1,2-*a*]pyrimidines,[2)] has been documented as being responsible for some behavioural effects consistent with sedative properties such as suppression of locomotor activity and procumbent posture in animal models,[3)] whereas stimulation of α_2- and α_3-$GABA_A$ subunits resulted in little or no sedation.[4)]

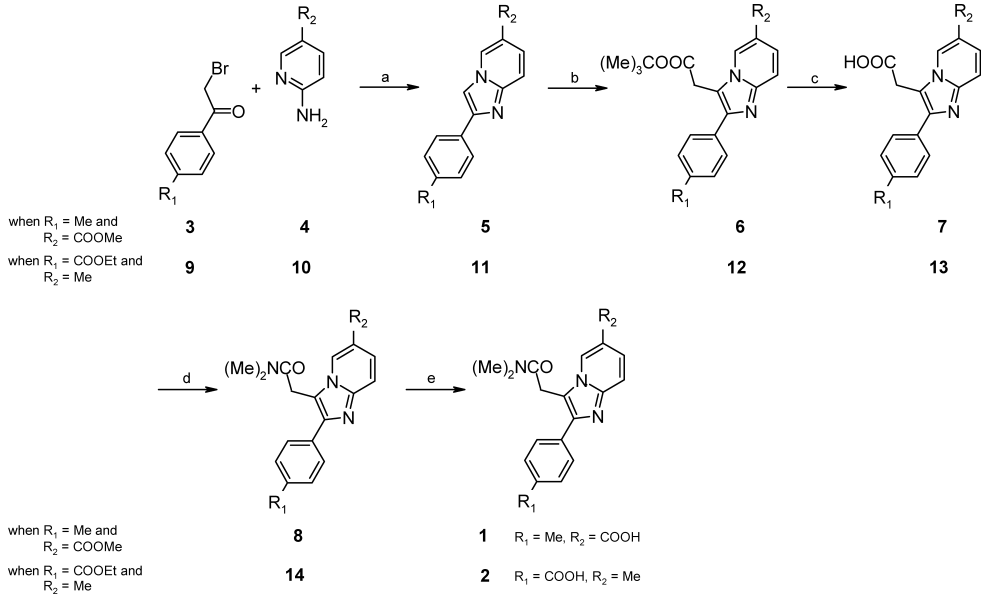
Moreover, reports dealing with "drug-facilitated crimes" (robbery, mugging, sexual assault) related to benzodiazepines and benzodiazepine-like hypnotics[5)] are tending to increase because of their short half-life, their rapid excretion and/or relatively long delay between the intake and urinary sampling. If classical techniques (GC/MS, RIA, CE, HPLC, DPV) have been used to identify and quantify the presence of Zolpidem in body fluids, an approach by LC-MS/MS has also been developed for hair analysis.[6,7)] Nevertheless, identification of metabolites is also of interest since convenient analysis could lead to specific quantifications and to unequivocally conclusive elements as to the origin of poisoning.

The aim of this work was to conclusively define an expeditious route to synthesize the two well-known major metabolites of Zolpidem[8)] (Chart 1), so as to characterize its criminal administration through its HPLC-MS parameters.



Zolpidem **1** **2**

Chart 1. Zolpidem Metabolites



Reagents and Conditions: (a) EtOH, reflux; (b) $N_2CH_2COOC(Me)_3$, Cu, toluene, reflux; (c) 50% TFA, CH_2Cl_2, rt; (d) HBTU, HOBt, DIEA, $(Me)_2NH$, rt; (e) 0.1 N LiOH, THF, rt.

Chart 2

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Results and Discussion

A synthetic route to the carboxylic metabolites **1** and **2** of Zolpidem was previously reported[9] and was obtained through a scheme inspired from the preparation of the ^{14}C-labelled drug.[10] As it is rather long, we decided to establish a shorter access to these molecules *via* two analogous pathways involving easily accessible reagents. The chosen procedure (Chart 2) reduced by half the reactional sequence and led to the creation of the carboxylic and tertiary amide functions of **1** and **2** *via* their corresponding cross-protected esters.

Following the classical procedure for the preparation of imidazo[1,2-*a*]pyridines,[11] the cyclization of α-bromo-4-methylacetophenone **3** with methyl 2-aminopyridine-5-carboxylate **4** gave the imidazopyridine **5**. Substitution on C-3 by a *tert*-butyl acetate group was completed with *tert*-butyl diazoacetate/Cu in toluene at reflux.[12,13] By chimioselective acidolysis of the *tert*-butyl ester of **6** (50% TFA/CH_2Cl_2)[14] carboxylic acid **7** was obtained, and this, in turn, reacted with dimethylamine (peptidic coupling conditions: HBTU, HOBt, DIEA) to give tertiary amide **8**. Target carboxylic acid **1** was obtained by saponification of compound **8** with 0.1 N LiOH/THF.[15] An analogous pathway using α-bromo-4-(carboethoxy)acetophenone **9** and 2-amino-5-methylpyridine **10** as starting materials readily gave imidazopyridine **11**. Substitution on C-3 by *N*,*N*-dimethylaminoacetamide (compound **14**) and final saponification into compound **2** were obtained as previously described for imidazopyridine **1**.

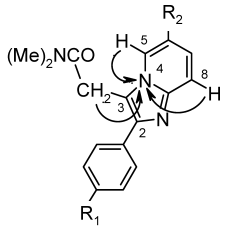
The structural identification of the regioisomer formed during the cyclization step (2-arylimidazo[1,2-*a*]pyridines **5** and **11**, respectively) was performed on final compounds **1** and **2**. The 2D ^1H–^{15}N NMR correlation (Chart 3), optimized on long range couplings (HMBC, $^2J_{N-H}$ and/or $^3J_{N-H}$), displayed cross peaks between N-4 at 203 ppm and protons H_5, H_8 and exocyclic CH_2, excluding the formation of the 3-aryl isomers: the presence of a withdrawing group (carboxylic ester) on β-carbon of pyridine did not evidently modify the nucleophilic character of ring nitrogen with regard to a bromomethylketone group.

In conclusion, a flexible and rapid method to prepare the two main carboxyl-containing metabolites of Zolpidem has been developed and may be applied to the synthesis of the drug. Their mass spectra (Chart 4) provide a specific clue as to their identification in biological fluids, particularly in cases of drug-facilitated crimes: both compounds **1** and **2** gave rise to peaks at m/z=266 and 265 corresponding to the loss of the fragment $(Me)_2NCO$ (m/z=72) and of the fragment $(Me)_2NCO+H^{\bullet}$ (m/z=73) from the quasimolecular ion (MH^+=338). Characteristic peaks of each molecule can be observed, such as fragmentation at m/z=92—indicative of a CH_2 substituent on pyridine—for metabolite **2**.

Moreover, as reliable concentrations could be achieved for standard solutions of **1** and **2**, their quantitative analysis is now available to estimate the intake of Zolpidem and provides a valuable tool in forensic toxicology since this drug leads to anterograde amnesia.[5]

Experimental

Melting points were determined with a Büchi 535 capillary melting point apparatus. Analytical thin-layer chromatography was performed on precoated Kieselgel 60F_{254} plates (Merck); the spots were located by UV (254, 366 nm); *Rf* values are given for guidance. Silica gel 60 230—400 mesh or 5—40 μm purchased from Merck were used for column or preparative thick



1 R_1 = Me; R_2 = COOH
2 R_1 = COOH; R_2 = Me

Chart 3. Selected HMBC Correlations for **1** and **2** (Solvent: DMSO-d_6)

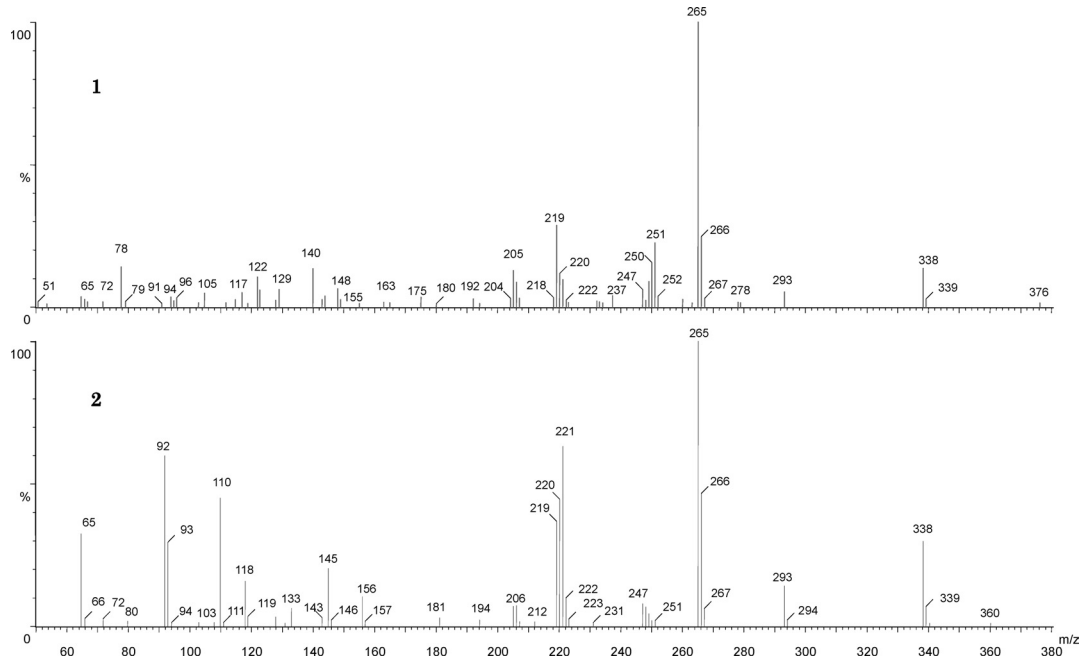


Chart 4. Mass Spectra of **1** and **2**

layer chromatography. The structures of all compounds were supported by IR (neat, FT-Bruker Vector 22 instrument) and by NMR on a Bruker AVANCE 300 spectrometer operating at 300.13, 75 and 31.42 MHz for ^{1}H, ^{13}C (HSQC and HMBC) and ^{15}N, respectively, with a 5 mm broad band inverse gradient probe. All chemical shifts were referenced with TMS and NH_4Cl signal at $\delta=0$ ppm for ^{1}H, ^{13}C and ^{15}N, respectively; the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. APCI$^+$ mass spectra were obtained on an LC-MS system Thermo Electron Surveyor MSQ. HPLC-ESI-MS analyses were performed on a Waters 2695 HPLC apparatus interfaced with a Waters ZQ quadrupole MS spectrometer: spectra were recorded in full scan mode between 50 and 450 *m*/*z* with positive electrospray at 30 V. Commercially available reagents and solvents were used throughout without further purification.

Methyl 2-(4-Methylphenyl)imidazo[1,2-*a*]pyridine-6-carboxylate (5) Bromomethylketone (**3**) (98%, 2 g, 9.3 mmol) was added while stirring to a hot solution of ester (**4**) (97%, 1.44 g, 9.3 mmol) in ethanol (40 ml). After refluxing for 24 h, the solution was cooled and the solid that precipitated was filtered and washed with ethanol (1.86 g, 75%). *Rf*=0.64 (ethyl acetate–cyclohexane, 3/2). IR cm^{-1}: 1720. ^{1}H-NMR (DMSO-d_6) δ: 2.39 (3H, s), 3.96 (3H, s), 7.41 (2H, d), 7.85 (2H, d), 8.17 (1H, d), 8.20 (1H, d), 8.80 (1H, s), 9.52 (1H, s). LC-MS (APCI$^+$) *m*/*z*: 267 (MH$^+$).

Methyl 3-*tert*-Butoxycarbonylmethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-6-carboxylate (6) Commercial *tert*-butyl diazoacetate (0.5 ml, 3.57 mmol) was added *via* a syringe to a suspension of ester (**5**) (931 mg, 3.5 mmol) in 30 ml of dry toluene, this was followed by small portions of copper powder (total amount: 250 mg). After adding another portion of *tert*-butyl diazoacetate (0.5 ml, 3.57 mmol) and of copper powder (total amount: 250 mg), the mixture was heated at reflux for 18 h. The mixture was cooled down to room temperature and the solvent was evaporated under reduced pressure. Ethyl acetate was added to the residue, the suspension was filtered on Celite and the filtrate was concentrated and used as such in the next reaction (480 mg, 36%): *Rf*=0.63 (ethyl acetate–cyclohexane, 3/2). LC-MS (APCI$^+$) *m*/*z*: 381 (MH$^+$).

Methyl 3-Carboxymethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-6-carboxylate (7) A solution of *tert*-butyl ester (**6**) (480 mg, 1.26 mmol) and trifluoroacetic acid (10 ml) in dichloromethane (10 ml) was stirred for 12 h at room temperature and concentrated without heating. The aqueous phase, resulting from the addition of a 10% potassium carbonate solution (50 ml), was washed with ethyl acetate and acidified (pH 6) with 6 N HCl. Further extractions with ethyl acetate and chloroform gave after evaporation a white solid which was washed with ethyl acetate and recrystallized (240 mg, 59%): mp 240—241 °C (ethyl acetate). *Rf*=0.05 (ethyl acetate–cyclohexane, 3/2). IR cm^{-1}: 1712, 1636. ^{1}H-NMR (DMSO-d_6) δ: 2.37 (3H, s), 3.89 (3H, s), 4.15 (2H, s), 7.31 (2H, d), 7.67 (4H, m), 9.02 (1H, s).

Methyl 3-Dimethylcarbamoylmethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-6-carboxylate (8) *O*-(1*H*-Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (969 mg, 2.56 mmol), 1-hydroxybenzotriazole (115 mg, 0.85 mmol), and *N*,*N*-ethyldiisopropylamine (0.6 ml, 3.47 mmol) were added to a suspension of carboxylic acid (**7**) (550 mg, 1.7 mmol) in dry dichloromethane (20 ml). The solution was stirred for 30 min in a dry atmosphere before dimethylamine (33%, 0.54 ml, 3.5 mmol) was added. After a further addition of dichloromethane (30 ml), the solution was washed with 0.5 N HCl, with a saturated aqueous NaCl solution, then with an aqueous 10% sodium bicarbonate solution. The organic phase was dried over $MgSO_4$ before the solvent was evaporated to give an oil which was chromatographed on silica gel (dichloromethane–methanol, 9/1) to give amide (**8**) (500 mg, 84%). *Rf*=0.41 (ethyl acetate–cyclohexane, 4/1). IR cm^{-1}: 1718, 1618. ^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, s), 2.97 (3H, s), 3.09 (3H, s), 4.04 (3H, s), 4.27 (2H, s), 7.35 (2H, d), 7.52 (2H, d), 8.18 (1H, d), 8.28 (1H, d), 8.93 (1H, s). LC-MS (APCI$^+$) *m*/*z*: 352 (MH$^+$).

3-Dimethylcarbamoylmethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-6-carboxylic Acid (1) 0.1 N Aqueous lithium hydroxide (28 ml, 2.8 mmol) was added dropwise to a stirred solution of amide (**8**) (500 mg, 1.42 mmol) in tetrahydrofuran (28 ml). After 18 h, the solvent was evaporated and the aqueous phase was successively washed with chloroform, acidified (pH 5) with 1 N HCl and extracted with 1-butanol. After removal of the organic solvent under reduced pressure, the resulting solid was washed with ethyl acetate and purified by thick layer chromatography (dichloromethane–methanol, 4/1): 72 mg, 15%. *Rf*=0.13 (dichloromethane–methanol, 9/1). ^{1}H-NMR (CD_3OD) δ: 2.46 (3H, s), 3.06 (3H, s), 3.24 (3H, s), 4.35 (2H, s), 7.35 (1H, d), 7.55 (1H, d), 7.85 (2H, m), 8.65 (1H, s). HPLC-ESI-MS *m*/*z*: 338 (M+H$^+$).

α-Bromo-4-(carboethoxy)acetophenone (9) Bromine (0.27 ml, 5.2 mmol) was added dropwise to a stirred solution of ethyl 4-acetylbenzoate (98%, 1 g, 5.2 mmol) in acetic acid (15 ml) in a nitrogen atmosphere. The solution was stirred at room temperature for 18 h, then poured into water (200 ml) and the precipitate was successively washed with water and cyclohexane. The solid was recrystallized (0.9 g, 64%): mp 75—76 °C (hexane) (lit.[16] 71 °C). *Rf*=0.70 (ethyl acetate–cyclohexane, 7/3). IR cm^{-1}: 1706. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, t), 4.43 (2H, q), 4.48 (2H, s), 8.04 (2H, d), 8.11 (2H, d).

Ethyl 4-[(6-Methyl)imidazo[1,2-*a*]pyridin-2-yl]benzoate (11) 2-Amino-5-methylpyridine (95%, 1.5 g, 13.2 mmol) was added to a stirred solution of bromomethylketone (**9**) (3 g, 11 mmol) in ethanol (40 ml) in a dry atmosphere. After 24 h, the precipitate was filtered, washed with ethanol and recrystallized (2.7 g, 87%): mp 229—230 °C (ethanol). *Rf*=0.47 (ethyl acetate–cyclohexane, 3/2). IR cm^{-1}: 1708. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t), 2.42 (3H, s), 4.35 (2H, q), 7.78 (1H, d), 7.81 (1H, d), 8.15 (4H, m), 8.71 (1H, s), 8.87 (1H, s). LC-MS (APCI$^+$) *m*/*z*: 281 (MH$^+$).

Ethyl 4-[(3-*tert*-Butoxycarbonylmethyl-6-methyl)imidazo[1,2-*a*]-pyridin-2-yl]benzoate (12) Commercial *tert*-butyl diazoacetate (1 ml, 7.03 mmol) was added in small amounts to a boiling solution of ester (**11**) (1 g, 3.55 mmol) in 30 ml of dry toluene, followed by small portions of copper powder (total amount: 500 mg). When the additions were completed, boiling was continued for a further 3 h. The mixture was cooled down to room temperature, the suspension was filtered on Celite and the filtrate was concentrated under reduced pressure. Purification was carried out by column chromatography on silica gel (ethyl acetate–cyclohexane, 1/1) and gave 600 mg (43%) of *tert*-butyl ester (**12**) which was immediately used in the next reaction. LC-MS (APCI$^+$) *m*/*z*: 395 (MH$^+$).

Ethyl 4-[(3-Carboxymethyl-6-methyl)imidazo[1,2-*a*]pyridin-2-yl]benzoate (13) A solution of *tert*-butyl ester (**12**) (650 mg, 1.65 mmol) and trifluoroacetic acid (5 ml) in dichloromethane (5 ml) was stirred for 1 h at room temperature and concentrated without heating. The aqueous phase resulting from adding a 10% potassium carbonate solution (50 ml) was washed twice with diethyl ether and acidified (pH 6) with 6 N HCl. The resulting precipitate was filtered, then washed with diethyl ether and recrystallized (200 mg, 36%): mp 177—178 °C (acetonitrile). *Rf*=0.05 (ethyl acetate). IR cm^{-1}: 1719, 1701, 1661. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t), 2.46 (3H, s), 4.30 (2H, s), 4.38 (2H, q), 7.83 (4H, m), 8.18 (2H, d), 8.78 (1H, s), 13.20 (1H, m).

Ethyl 4-[(3-Dimethylcarbamoylmethyl-6-methyl)imidazo[1,2-*a*]-pyridin-2-yl]benzoate (14) Amide (**14**) was prepared from carboxylic acid (**13**) (200 mg, 0.59 mmol), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (335 mg, 0.88 mmol), 1-hydroxybenzotriazole (40 mg, 0.29 mmol), *N*,*N*-ethyldiisopropylamine (152 mg, 1.18 mmol) and dimethylamine (33%, 0.18 ml, 1.18 mmol) in a similar manner to that described for the synthesis of **8**: 163 mg, 76%. *Rf*=0.58 (ethyl acetate–cyclohexane, 4/1). IR cm^{-1}: 1711, 1610. ^{1}H-NMR (CD_3OD) δ: 1.39 (3H, t), 2.41 (3H, s), 3.04 (3H, s), 3.22 (3H, s), 4.27 (2H, s), 4.42 (2H, q), 7.31 (1H, d), 7.54 (1H, d), 7.74 (2H, d), 8.08 (1H, s), 8.14 (2H, d). LC-MS (APCI$^+$) *m*/*z*: 366 (MH$^+$).

4-[(3-Dimethylcarbamoylmethyl-6-methyl)imidazo[1,2-*a*]pyridin-2-yl]benzoic Acid (2) An aqueous solution of 0.1 N lithium hydroxide (14 ml) was added dropwise to an ice-cold stirred solution of amide **14** (250 mg, 0.68 mmol) in tetrahydrofuran (14 ml). After 24 h, the solution was diluted with 10% aqueous potassium carbonate (20 ml), washed with ethyl acetate, then acidified (pH 5) with 1 N HCl to give a solid which was washed with ethyl acetate and chromatographed on silica gel (CH_2Cl_2–CH_3OH, 9/1). An analytical sample was recrystallized (149 mg, 65%): mp >250 °C (2-propanol). *Rf*=0.44 (dichloromethane–methanol, 9/1). IR cm^{-1}: 1655. ^{1}H-NMR (CD_3OD) δ: 2.40 (3H, s), 3.04 (3H, s), 3.20 (3H, s), 4.12 (2H, s), 7.26 (1H, d), 7.51 (1H, d), 7.63 (2H, d), 8.06 (1H, s), 8.07 (2H, d). ^{13}C-NMR (DMSO-d_6) δ: 17.8, 28.9, 35.3, 37.0, 115.6, 115.9, 120.6, 122.4, 126.5, 127.0, 129.2, 135.0, 139.5, 142.6, 142.9, 168.1, 169.0. HPLC-ESI-MS *m*/*z*: 338 (M+H$^+$).

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