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Synthesis and identifications of potential metabolites as biomarkers of the synthetic cannabinoid AKB-48

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ABSTRACT

AKB-48 belongs to the family of synthetic cannabinoids. It has strong binding affinity to CB_1 receptor and is psychoactive. It is banned in many countries including USA, Japan, Germany, New Zealand, Singapore and China etc. But the difficulty in detecting the parent compound in urine samples highlights the importance of studies of its metabolites. Here we report the synthesis of 19 potential metabolites of AKB-48, among which, compounds **2**, **9**, **10**, **30** and **31**, together with the commercially available substance **5** were identified as metabolites of AKB-48 by comparison with one authentic human urine sample and human liver microsomal data. Compounds **10** and **30** could be of use as biomarkers in detecting AKB-48 in human urine samples.

1. Introduction

Synthetic cannabinoids have emerged and grown to become a common substitute to cannabis in recent years. The rate at which old synthetic cannabinoids are replaced on the market with new ones as they become classified as harmful substances puts great pressure on forensic laboratories and governments. Synthetic cannabinoids are not always detected in routine cannabinoid urine screening methods; however, their metabolites can be detected and are therefore important to enable identifications of synthetic cannabinoid intakes in analysis of urine samples. As a member of the synthetic cannabinoid family, AKB-48 (Fig. 1) (APINACA) has stronger binding affinity to CB_1 receptor compared to tetrahydrocannabinol,[1] and is used as an alternative to cannabis. It is banned in many countries including USA, Japan, Germany, New Zealand, Singapore and China etc.

AKB-48 was first reported by a Japanese group in 2012 as an ingredient in a synthetic cannabis smoking blend.[2] In 2013 the study of its metabolites was carried out by Gandhi et al. using human hepatocytes and high-resolution mass spectrometry.[3] Further investigation by Holm et al. found that CYP3A4 mediated the oxidative metabolism of AKB-48.[4] The oxidative metabolism happened mainly on the pentyl chain and the adamantyl moiety giving mono-, di- or tri-hydroxylated metabolites together with metabolites as ketones or carboxylic acids. Vikingsson et al. also put great effort in identification of AKB-48 metabolites using human liver microsomes and time of flight mass spectrometry.[5] Based on these research groups' findings more knowledge of AKB-48's metabolism is well described. However, the exact structure of the metabolites cannot be determined by LC-MS/MS alone. In a previous study we have synthesized and identified an interesting and important metabolite of AKB-48, with a secondary alcohol on the adamantane ring.[6] This is uncommon because metabolism of the adamantyl moiety normally leads to metabolites with a tertiary alcohol at the bridge-carbon as major.[7] Apart from the adamantyl moiety that is found in several synthetic cannabinoids, such as AB-001, SDB-001, STS-135; AKB-48 also contains a pentyl chain, which is an even more common moiety in other synthetic cannabinoids. There are some commercially available synthetic cannabinoid metabolites with a hydroxyl group at position 4 or 5 of the pentyl chain (Fig. 1), but unambiguous structure identification is lacking for several major metabolites.[5] To the best of our knowledge, there

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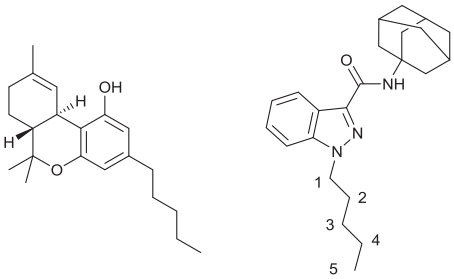


Fig. 1. Structures and activities of AKB-48 and tetrahydrocannabinol.

are not any synthesis and identification studies on the metabolic position of the pentyl chain of synthetic cannabinoids. To have a more complete picture of the metabolite profile of AKB-48, we continued our study of the synthesis and identifications of other metabolites of AKB-48. By comparison with an authentic urine sample we can identify its major metabolites, which could eventually lead us to discover better biomarkers for AKB-48, or even for other synthetic cannabinoids containing a pentyl chain or an adamantane moiety, in urine samples.

2. Results and discussion

According to our previous human liver microsome results[5] and the research by Gandhi et al.,[3] the oxidative metabolism of AKB-48 gave metabolites in the form of mono- or di-hydroxylations of the pentyl chain and/or of the adamantyl moiety. Therefore, the synthesis of potential metabolites of AKB-48 was focused on adding hydroxyl group(s) on its pentyl and/or adamantyl side chains. Furthermore, the synthetic compounds were used as references in the analysis of an authentic urine sample.

Compound **2** was synthesized using 3-indazole carboxylic acid **1** and 1-adamantanamine hydrochloride as starting materials with EDC as the coupling reagent in 48% yield. The yield was unfortunately low because of incomplete conversion.

Compounds **4** and **5** are commercially available. They can also be synthesized using hydroboration-oxidation, which was described below for the synthesis of compounds **19** and **20**. Alkylation of **2** with 5-bromopent-1-ene followed by dihydroxylation with OsO_4/NMO to give compound **6** (Scheme 1). Similar methods were applied to the synthesis of mono- or di-hydroxylations of the pentyl side chain at different position from compound **7** to give compounds **8**, **9** and **10**, and from **17** to give **18**, **19** and **20**, and from compound **21** to **22**, **23**, and **24**, which are shown in Scheme 2 and 4. The yield of compound **10** was low, and the purification of its mixture with compound **9** was difficult. It's probably easier to achieve **10** by reduction of compound **11** with $NaBH_4$ instead.

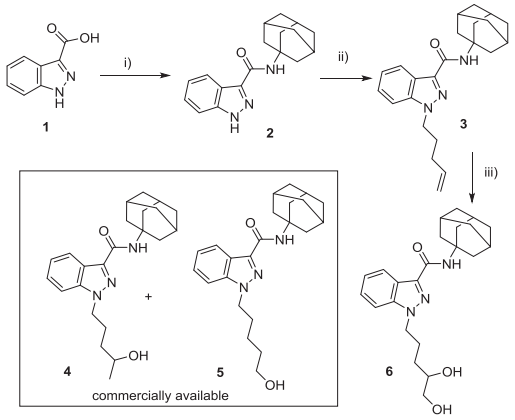
The ketones **11** and **12** were synthesized by microwave irradiation of the mixture of compound **2** or **15** and 1-chloro-3-pentanone in THF with t-BuOK as base at 120 °C for one hour (Scheme 3). Full conversion was not achieved, resulting in low yields, 24% and 28% respectively. Unfortunately, we were not able to find compound **11** or **12** in the authentic urine sample. Therefore, the optimization was not continued.

The adamantyl moiety of AKB-48 was prone to be oxidized by CYP3A4.[2,4] 1-adamantylamine **13** was oxidized instead with concentrated H_2SO_4/HNO_3 to give compound **14** containing a hydroxyl group.[8] With **14** as starting material other potential metabolites of AKB-48, compounds **12**, **15**, **16**, **18**–**20** and **22**–**24** were synthesized using similar methods as described above, showed in Scheme 4. When compound **15**, a similar structure to **2** was synthesized, another amide coupling reagent TBTU was used instead, and a good yield (81%) was achieved compared to that of EDC (27%) or DCC (31%). Hydroboration-oxidation of **17** gave a mixture of alcohols **19** and **20**, where the yield of **20** was very low (3%) because of anti-Markovnikov's rule and therefore there were also difficulties in the purification. Another synthetic route was developed to achieve **20** with better yield (49%, two steps) by first epoxidation of **17** with m-CPBA, followed by reduction of the resulting epoxide with $NaBH_4$ in *i*-PrOH at 60 °C for 3 h.

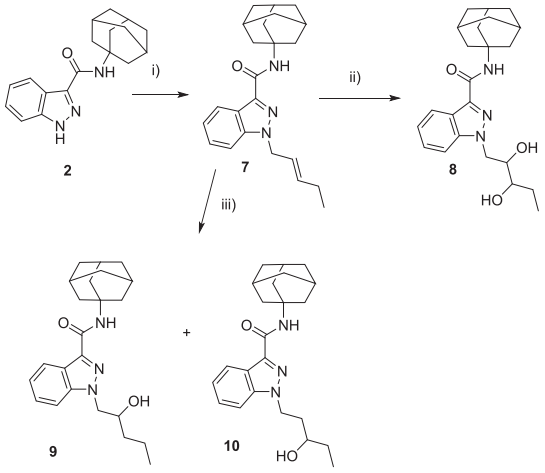
Although the tertiary carbons of the adamantyl moiety were much easier to be oxidized compared to the secondary ones with concentrated H_2SO_4/HNO_3, it's difficult to say whether it will be the same in vivo. Compound **25** was used as starting material to synthesize other potential metabolites with a secondary hydroxyl group. Conditions for a modified Ritter reaction reported in the literature were used.[9] The synthesis of compounds **25**–**31** was reported in our previous study.[5] However, it was not discussed in detail. But the synthetic route is shown in Scheme 5 for a better overview.

Compound **32** was also synthesized from compound **19** with Jones reagent in 59% yield (Scheme 6).

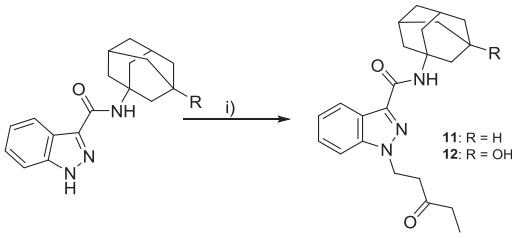
As described previously,[6] compound **30** was shown by LC-MS/



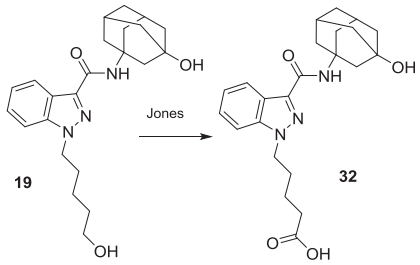
Scheme 1. i) EDC, HOBt, Et_3N, DMF, rt, overnight, 48%; ii) 5-bromopent-1-ene, *t*-BuOK, DMF/THF (1:5), rt, overnight, 87%; iii) OsO_4 (aq), NMO, THF/H_2O (3:1), rt, overnight, 66%. Compounds **4** and **5**, commercially available.



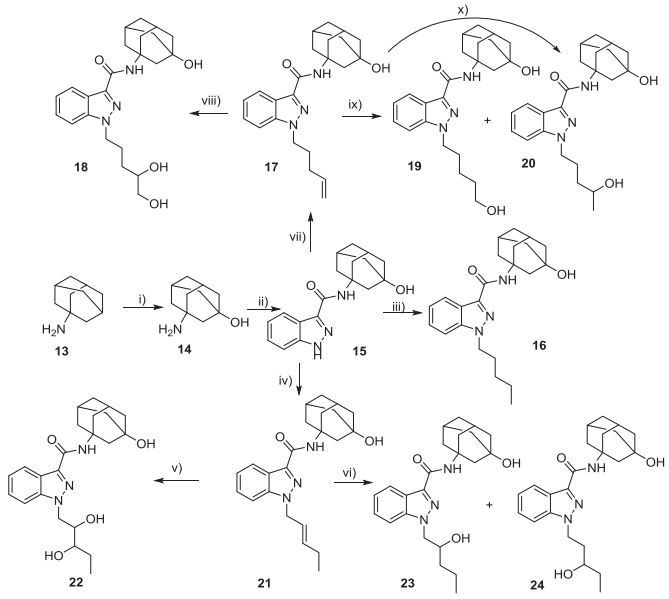
Scheme 2. i) 1-bromopent-2-ene, t-BuOK in DMF/THF (1:5), rt, overnight, 98%; ii) OsO4 (aq), NMO, THF/H2O (3:1), rt, overnight, 80%; iii) a) BH3·THF, N2 (g), 0 °C, 2 h. b) NaOH (aq), H2O2, rt, overnight, 9, 69%; 10, 8%.



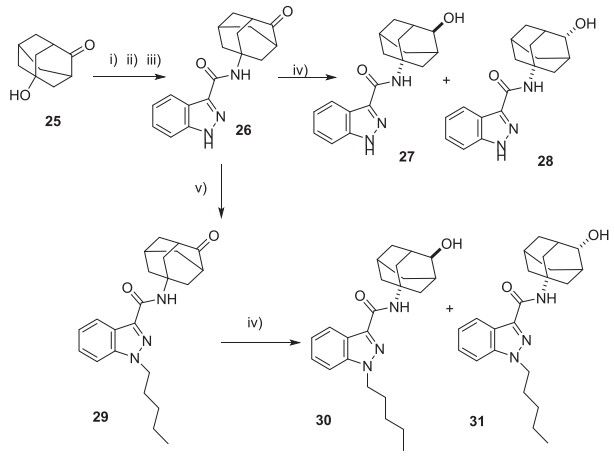
Scheme 3. i) 1-chloro-3-pentanone, *t*-BuOK, THF, MW irradiation, 120 °C, 1 h, **11**, 24%; **12**, 28%.



Scheme 6. Jones reagent, acetone, RT, 59%.



Scheme 4. i) Conc. H_2SO_4, conc. HNO_3, 2 h, 10 °C, 74%; ii) TBTU, 1*H*-indazole-3-carboxylic acid, Et_3N, THF, rt, overnight, 81%; iii) 1-bromopentane, *t*-BuOK, THF/DMF (5:1), rt, overnight, 90%; iv) 1-bromo-2-pentene, *t*-BuOK in THF/DMF (5:1), rt, overnight, 82%; v) OsO_4 (aq), NMO, THF/H_2O (3:1), rt, overnight, 82%; vi) a) BH_3·THF, N_2, 0 °C, 2 h; b) NaOH(aq), H_2O_2, rt, overnight, **23**, 64%; **24**, 7%; vii) 5-bromo-1-pentene, *t*-BuOK, THF/DMF (5:1), rt, overnight, 79%; viii) OsO_4(aq), NMO, THF/H_2O (3:1), rt, overnight, 82%; ix) a) BH_3·THF, N_2, 0 °C 2 h; b) NaOH(aq), H_2O_2, rt, overnight, **19**, 32%; **20**, 3%; x) a) m-CPBA, 66%; b) $NaBH_4$, *i*PrOH, 60 °C, 3 h, 75%.



Scheme 5. i) CH_3CN, BF_3·Et_2O, TFA, 70 °C, 3 h; ii) Conc. HCl, 150 °C MW irradiation 1 h; iii) TBTU, 1*H*-indazole-3-carboxylic acid, Et_3N, THF, rt, overnight, 30% over 3 steps; iv) $NaBH_4$, MeOH, rt, 1 h; v) 1-Bromopentane, *t*-BuOK, DMF/THF (1:5), rt, overnight, **30**, 36%; **31**, 20%.

MS to be the major metabolite, hydroxylated at the adamantyl moiety of AKB-48. In a similar experiment compounds **4**, **5**, **9** and **10** were analyzed with the urine sample (Fig. 3), indicating that the major metabolite was **5** with minor contribution of **10** and traces of **9**. Small amounts of **4** cannot be ruled out. Compound **2** was identified unambiguously by MS/MS spectra as a minor metabolite based on previous results.[5] Although **5** was the major identified mono-hydroxyl metabolite of the pentyl chain, it is unfortunately less suitable as a biomarker for the detection of AKB-48 intake as it is also a major metabolite of 5F-AKB-48.[5]

In summary, Compounds **2**, **5**, **9**, **10**, **30** and **31** were identified as metabolites of AKB-48 in the urine sample and compound **10** and **30** have the potential to be used as biomarkers of AKB-48 intake with strong intensities and structures uniquely identifying AKB-48 from 5F-AKB-48.

Interestingly, studies of the metabolites of the substances containing an adamantine ring suggest that the tertiary carbon is normally easier to be oxidized than the secondary carbon unless the tertiary carbons are substituted, for example: 1-amino-3-hydroxyadamantane is the only detected metabolite of amantadine.[10–13] Our results highlight the importance of having access to all the potential metabolites for accurate metabolic study.

3. Conclusion

In conclusion, 19 potential metabolites of AKB-48 have been synthesized (Fig. 2), and six metabolites were identified by comparison with the metabolites in an authentic urine sample (Fig. 4). Compounds **10** and **30** are of most interest with strong intensities and structures uniquely identifying AKB-48 from 5F-AKB-48, therefore they are prime candidates to be used as biomarkers for detection of AKB-48 intake in routine urine sample analysis. The developed synthesis methods could be applied to other synthetic cannabinoids with alkyl adamantyl or pentyl chain moiety. The findings could also be of use in elucidating the metabolism of novel synthetic cannabinoids.

4. Experimental section

4.1. General information

TLC was performed using 0.25 mm precoated silica-gel plates (Merck 60 F_{254}), detection by UV-abs at 254 nm. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian Mercury 300 MHz instrument (25 °C in $CDCl_3$, MeOH-d_4 or acetone-d_6). ^{19}F NMR analyses were compared with an external standard (TFA). HPLC-MS was performed on a Gilson system (Column: Phenomenex C-18, 5 μm, 100 × 21, 20 mm and Waters X-Bridge C-8 2.5 μm, 50 × 4.6 mm or C-8 2.5 μm, 50 × 4.6 mm for preparative and analytical experiments respectively; using acetonitrile and deionized water with ammonium acetate (10 mM) as mobile phase. Pump: Gilson gradient pump 322; UV/VIS detector: Gilson 155, detection at 215 nm; MS

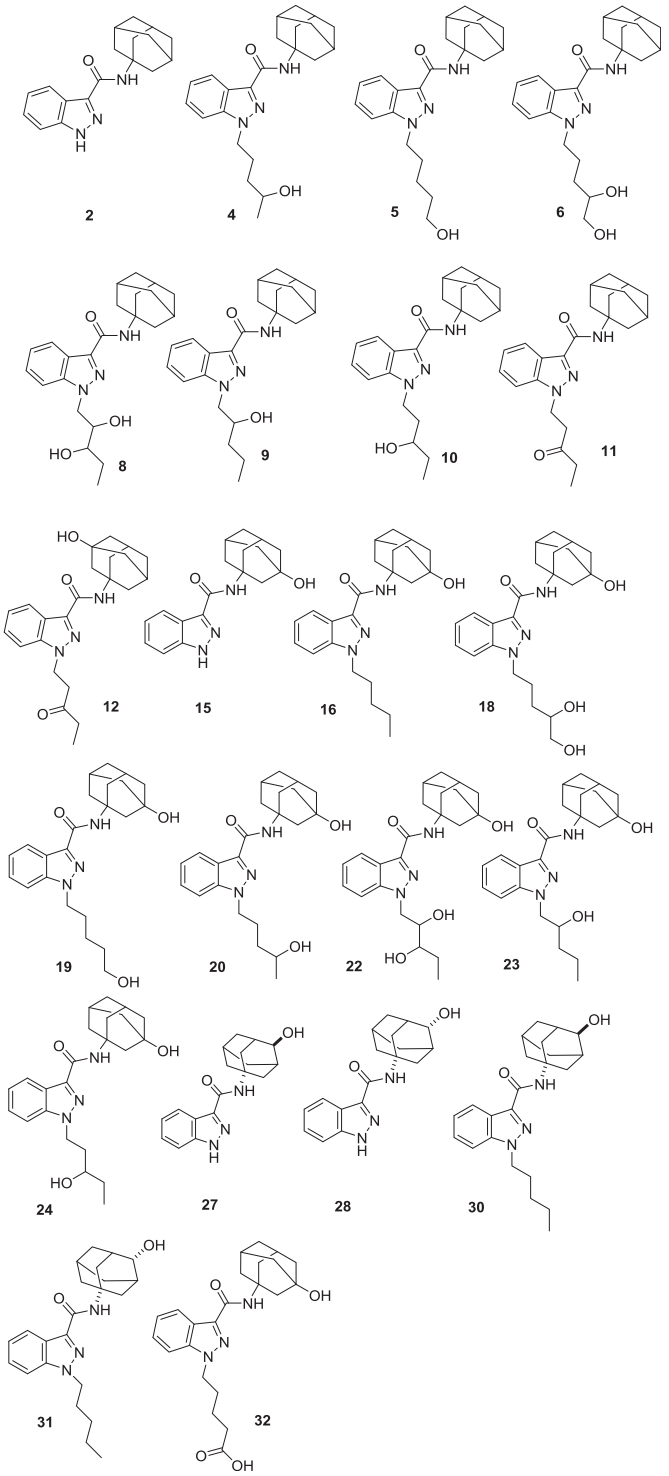


Fig. 2. List of structures of 19 synthesized and two (**4** and **5**) commercial potential metabolites of AKB-48.

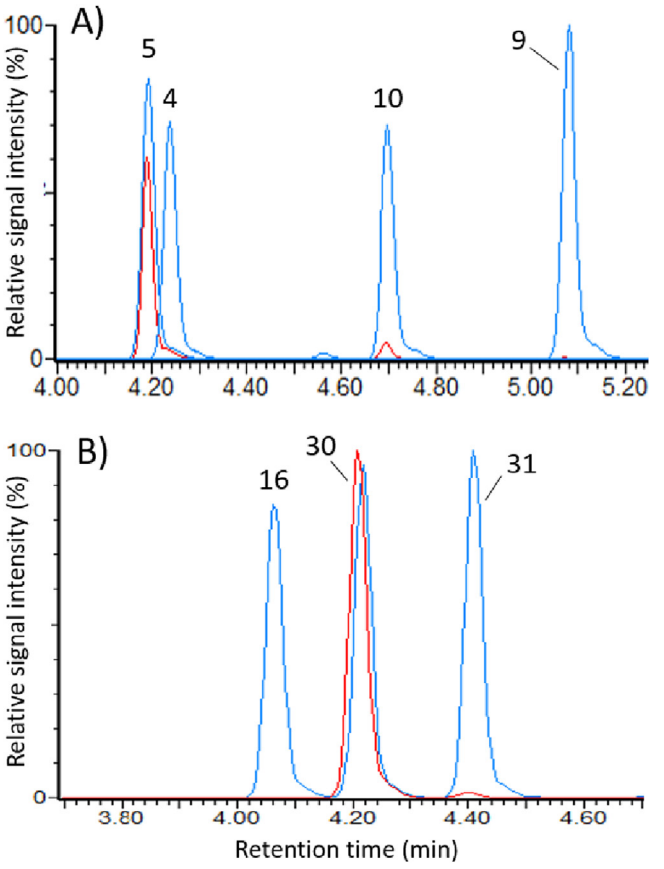


Fig. 3. Analysis of reference materials **4**, **5**, **9**, **10**, **16**, **30**, **31** and an authentic urine sample (red) by LC-MS. A) *m/z* 382 -> *m/z* 135 B) 382 -> *m/z* 151; LC gradient: 26–67% MeCN in 10 mM ammonium acetate, 100 × 2.1 mm Cortecs UPLC C18 column (Waters), 7.5 min.

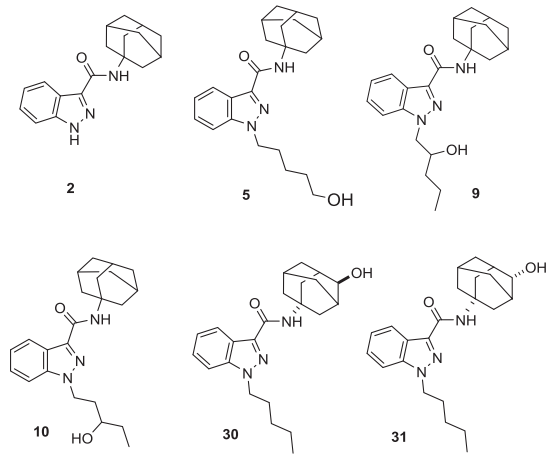


Fig. 4. Identified synthesized metabolites from authentic urine samples with possible intake of only AKB-48.

detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204).Flash chromatography was performed using the following silica gel: High purity grade (Merck Grade 9385), pore size 60 Å, 230–240 mesh particle size. The reagents were purchased from Sigma-Aldrich, Merck (EDC and NMO), Fluka (TBTU and $BF_3(OEt)_2$) and Alfa Aesar (1-bromo-5-fluoropentane). Compounds **4** and **5** were purchased from Chiron AS. Compounds **14**–**16**, **26**, **29**–**31**[6] have been reported in our previous publication in Tetrahedron Letters, but they were also reported here for convenience of the readers. ^{1}H NMR and ^{13}C NMR spectra of the synthesized potential metabolites can be found in the appendix while the results of the high-resolution mass measurements by RMV using LC-QTOF-MS can be found in the experimental.

4.2. Synthesis

4.2.1. N-(adamantan-1-yl)-1H-indazole-3-carboxamide (**2**)

Compound **1** (1.0 g, 6.17 mmol) was dissolved in DMF (57 mL). To this solution EDC (1.74 g, 9.57 mmol), HOBt (1,03 g, 7.6 mmol) and Et_3N (3.7 mL, 26.3 mmol) was added. The solution was left to stir for 10 min in room temperature, thereafter compound **13** (1.32 g, 7.05 mmol) was added to the solution and it was left to stir overnight at room temperature. The progression of the reaction was monitored by TLC. The reaction mixture was then diluted with EtOAc (30 mL) and washed with water (100 mL). The water phase was extracted with EtOAc (3 × 20 mL). The collected organic phases were dried using magnesium sulfate, filtered and concentrated *in vacuo*. The residue was thereafter chromatographed on silica using a mobile phase of EtOAc/n-Hep (3:7) and the fractions containing **2** were concentrated *in vacuo* to afford compound **2** (880 mg, 48%). R_f = 0.44 (3:7 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{18}H_{22}N_3O_2^+$: 296.1758. Found: 296.1754. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.1 (s, 1H), 8.42 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.50–7.40 (m, 2H), 7.30 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 6.85 (s, 1H), 2.20–2.15 (m, 6H), 2.15–2.10 (m, 3H), 1.80–1.70 (m, 6H). ^{13}C NMR (CD_3OD 75.4 MHz) δ: 164.4 (CONH), 143.0, 140.3, 127.9, 123.4, 122.9, 111.4 (aromatic C), 53.0, 42.7, 37.5, 31.0 (aliphatic C).

4.2.2. N-(adamantan-1-yl)-1-(pent-4-en-1-yl)-1H-indazole-3-carboxamide (**3**)

Compound **2** (50 mg, 0.17 mmol) was dissolved in DMF/THF (1:5, 6 mL). The solution was cooled to 0 °C and stirred for 5 min. To the solution t-BuOK (30 mg, 0.26 mmol) was added and stirred for 15 min. The mixture was brought to room temperature and 5-bromopent-1-ene (40 μl, 0.34 mmol) was added to the solution which was left to stir over the weekend. Progression of the reaction was monitored on TLC. The solvents were evaporated, water (20 mL) was added and the solution was extracted using EtOAc (3 × 20 mL). The combined organic phases were then dried using magnesium sulfate, filtered and concentrated *in vacuo*. The residue was thereafter chromatographed on silica using a mobile phase of EtOAc/n-Hep (1:4) and the fractions containing **3** were concentrated *in vacuo* to yield compound **3** (54 mg, yield 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.42 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.50–7.40 (m, 2H), 7.30 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 6.85 (s, 1H), 5.81 (m, 1H), 5.05 (m, 2H), 4.36 (t, J = 7 Hz, 2H), 2.20–2.15 (m, 6H), 2.15–2.10 (m, 3H), 2.05 (dt, J = 7 Hz, 6 Hz, 2H), 2.00 (q, J = 7 Hz, 2H), 1.80–1.70 (m, 6H).

4.2.3. N-(adamantan-1-yl)-1-(4,5-dihydroxypentyl)-1H-indazole-3-carboxamide (**6**)

Compound **3** (110.9 mg, 0.30 mmol) was dissolved in a solvent mixture containing THF/H_2O (3:1, 20 mL). To the solution OsO_4 (4 wt% in H_2O, 186 μl, 0.03 mmol) and NMO (61.9 mg, 0.46 mmol) was added. The solution was left to stir at room temperature overnight. The progression of the reaction was monitored on TLC. When the reaction had run its course, saturated $NaHCO_3$(aq) (10 mL) was added to the reaction mixture and the reaction mixture was then extracted using EtOAc (3 × 20 mL). The organic layers were collected and concentrated *in vacuo*. The remaining residue was purified by column chromatography with EtOAc as the eluent. The fractions which contained **6** were collected and concentrated *in vacuo* to give compound **6** (79.2 mg, yield 66%). R_f = 0.37 (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_3^+$: 398.2439. Found: 398.2440. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.33 (td, J = 8.4 Hz, 2.4 Hz, 1.2 Hz, 1H), 7.40–7.30 (m, 2H), 7.21 (m, 1H), 6.81 (s, 1H), 4.36 (t, J = 7.2 Hz, 2H), 3.68 (m, 1H), 3.55 (dd, J = 11.1 Hz, 3 Hz, 1H), 3.38 (dd, J = 11.1 Hz, 6.9 Hz, 1H), 2.68 (s, 2H), 2.20–2.15 (m, 6H), 2.14–2.08 (m, 3H), 2.10–1.90 (m, 2H), 1.80–1.66 (m, 6H), 1.46–1.38 (m, 2H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2 (CONH), 141.0, 138.2, 126.8, 123.1, 122.8, 122.6, 109.2 (aromatic C), 71.7, 66.7 (COH), 52.1, 49.2, 42.0, 36.5, 30.1, 29.6, 26.1 (aliphatic C).

4.2.4. N-(adamantan-1-yl)-1-(pent-2-en-1-yl)-1H-indazole-3-carboxamide (**7**)

Compound **2** (150 mg, 0.51 mmol) was dissolved in DMF/THF (1:5, 6 mL). The solution was put to 0 °C using an ice bath and left to stir for 5 min. To the solution t-BuOK (85 mg, 0.76 mmol) was added and the solution was stirred for 15 min. As the mixture was brought to room temperature 1-bromo-2-pentene (79 μl, 0.76 mmol) was added to the solution and the reaction mixture was left to stir over the weekend. The development of the reaction was monitored on TLC. Water (20 mL) was added to the solution and thereafter the solution was extracted using EtOAc (3 × 20 mL). The organic phases were collected and concentrated *in vacuo*. The remaining residue was applied to a silica column with a mobile phase composition of EtOAc/n-Hep (1:2) and chromatographed. The fractions containing **7** were concentrated *in vacuo* to give compound **7** (182.5 mg, yield 98%). R_f = 0.68 (1:2 EtOAc/n-Hep). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.38 (td, J = 8.1 Hz, 1.8 Hz, 0.9 Hz, 1H), 7.40–7.30 (m, 2H), 7.22 (m, 1H), 6.82 (s, 1H), 5.78–5.56 (m, 2H), 4.93 (dd, J = 5.1 Hz, 1.2 Hz, 2H), 2.21–2.16 (m, 6H), 2.16–2.08 (m, 3H), 2.08–1.98 (m, 2H), 1.80–1.65 (m, 6H), 0.96 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.0 (CONH), 140.8, 138.2, 136.7, 126.6, 123.2, 123.1, 122.9, 122.4, 109.6, 51.9, 51.8, 41.9, 36.5, 29.6, 25.2, 13.2.

4.2.5. N-(adamantan-1-yl)-1-(2,3-dihydroxypentyl)-1H-indazole-3-carboxamide (**8**)

Compound **7** (43 mg, 0.12 mmol) was dissolved in a solvent system comprised of THF/H_2O (3:1, 8 mL). OsO_4 (4 wt% in H_2O, 73 μl, 0.012 mmol) and NMO (24 mg, 0.177 mmol) was added to the solution and left to stir overnight at room temperature. The development of the reaction was observed on TLC. When the reaction was finished, saturated $NaHCO_3$(aq) (1.5 mL) was added to the reaction mixture prior to extraction using EtOAc (3 × 10 mL). The organic layers were gathered and concentrated using a rotary evaporator. The remaining residue was applied to a silica column and chromatographed using a mobile system of 2:1 (EtOAc/n-Hep). The fraction vials containing **8** were gathered and concentrated *in vacuo* to afford compound **8** (37.7 mg, yield: 80%). R_f = 0.34 (2:1 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_3^+$: 398.2439. Found: 398.2440. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.34 (dd, J = 8.4 Hz, 1.2 Hz, 1H), 7.50–7.35 (m, 2H), 7.23 (m, 1H), 6.73 (s, 1H), 4.49 (dd, J = 7.2 Hz, 1.2 Hz, 2H), 4.06 (m, 1H), 3.43 (m, 1H), 2.88 (m, 1H), 2.30 (m, 1H), 2.20–2.16 (m, 6H), 2.16–2.10 (m, 3H), 1.80–1.66 (m, 6H), 1.70–1.58 (m, 2H), 0.99 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 161.8 (CONH), 141.9, 139.1, 127.2, 123.2, 122.8, 122.7, 109.5 (aromatic C), 73.3, 72.5 (COH), 52.2, 52.1, 42.0, 36.6, 29.7, 26.8, 10.2 (aliphatic C).

4.2.6. N-(adamantan-1-yl)-1-(2-hydroxypentyl)-1H-indazole-3-carboxamide (**9**) and N-(adamantan-1-yl)-1-(3-hydroxypentyl)-1H-indazole-3-carboxamide (**10**)

Compound **7** (139 mg, 0.382 mmol) was put into a round bottom flask which was purged with nitrogen. To the flask BH_3·THF (1 M, 3 mL) was added and the reaction mixture was left for 2 h at 0 °C. The progression of the reaction was monitored on liquid chromatography. As the reaction mixture was brought to room temperature, it was quenched using water (2 mL). Following the quenching NaOH (31 mg, 0.764 mmol) together with H_2O_2 (1 mL) was added and the reaction mixture was left to react overnight. When the reaction was done, checked using liquid chromatography, the reaction mixture was extracted using EtOAc (3 × 20 mL). The organic layers were collected and filtered before being concentrated *in vacuo*. The crude mixture was then dissolved in DCM and applied to

a silica column and chromatographed using an eluent system of 1:2 (EtOAc/n-Hep). The fractions containing **9** were gathered and dried *in vacuo* to give compound **9** (101 mg, yield 69%). R_f = 0.56 (1:2 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_2^+$: 382.2490. Found: 382.2505. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.36 (ddd, J = 8.1 Hz, 2.4 Hz, 1.2 Hz, 1H), 7.46–7.34 (m, 2H), 7.23 (m, 1H), 6.73 (s, 1H), 4.40–4.25 (m, 2H), 4.15 (m, 1H), 2.58 (s, 1H), 2.20–2.16 (m, 6H), 2.16–2.10 (m, 3H), 1.80–1.66 (m, 6H), 1.62–1.40 (m, 4H), 0.96 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 161.8 (CONH), 141.8, 138.9, 127.1, 123.2, 122.7, 109.5 (aromatic C), 70.9 (COH), 55.0, 52.1, 42.0, 36.7, 36.6, 29.7, 18.8, 14.1 (aliphatic C).

The fractions containing **10** were combined and purified once again on a silica column using EtOAc as the mobile phase. The fraction vials containing **10** were collected and dried *in vacuo* to afford (11.4 mg, yield: 8%). R_f = 0.85 (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_2^+$: 382.2490. Found: 382.2507. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.38 (td, J = 8.1 Hz, 2.1 Hz, 1.2 Hz, 1H), 7.50–7.35 (m, 2H), 7.24 (m, 1H), 6.76 (s, 1H), 4.64–4.46 (m, 2H), 3.45 (m, 1H), 2.22–2.16 (m, 6H), 2.16–2.10 (m, 3H), 2.14 (m, 1H), 1.92 (m, 1H), 1.80–1.65 (m, 6H), 1.56–1.44 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.1 (CONH), 141.2, 138.5, 126.9, 123.3, 122.9, 122.6, 109.3 (aromatic C), 70.4 (COH), 52.0, 46.1, 42.1, 36.6, 36.5, 30.7, 29.7, 9.9 (aliphatic C).

4.2.7. N-(adamantan-1-yl)-1-(3-oxopentyl)-1H-indazole-3-carboxamide (**11**)

Compound **2** (52.5 mg, 0.18 mmol) was dissolved in THF (10 mL). To the solution t-BuOK (30 mg, 0.27 mmol) and 1-chloro-3-pentanone (31 μl, 0.27 mmol) were added. The solution was heated to 120 °C using microwave irradiation and stirred for one hour. Progress of the reaction prior to purification was monitored using LC as well as TLC. The solvents were evaporated, water (20 mL) was added and the solution was extracted using EtOAc (3 × 20 mL). The combined organic phases were concentrated *in vacuo*, dissolved in MeOH and purified with the aid of preparative LC. The fractions containing **11** were concentrated *in vacuo* to yield compound 11 (16.4 mg, yield 24%). R_f = 0.23 (1:3 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{30}N_3O_2^+$: 380.2333. Found: 380.2329. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.36 (dd, J = 8.1 Hz, 1,2 Hz, 1H), 7.50–7.35 (m, 2H), 7.26 (m, 1H), 6.73 (s, 1H), 4.64 (t, J = 7.2 Hz, 2H), 3.08 (t, J = 7.2 Hz, 2H), 2.44 (q, J = 7.8 Hz, 2H), 2.22–2.18 (m, 6H), 2.18–2.10 (m, 3H), 1.82–1.66 (m, 6H), 1.05 (t, J = 7.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 208.7 (CO), 161.9 (CONH), 141.1, 138.7, 127.0, 123.2, 122.9, 122.7, 109.4 (aromatic C), 52.0, 43.6, 42.0, 41.6, 36.6, 29.7, 7.8 (aliphatic C).

4.2.8. N-(3-hydroxyadamantan-1-yl)-1-(3-oxopentyl)-1H-indazole-3-carboxamide (**12**)

Compound **15** (30 mg, 0.09 mmol) was dissolved in THF (10 mL). To the solution t-BuOK (18 mg, 0.15 mmol) and 1-chloro-3-pentanone (12 μl, 0.09 mmol) were added. The solution was heated to 120 °C for one hour using microwave irradiation. The progression of the reaction was checked using liquid chromatography prior to purification. The solvents were evaporated, water (20 mL) was added and the solution was extracted using EtOAc (3 × 20 mL). The combined organic phases were concentrated *in vacuo*, the remaining residue was dissolved in MeOH and purified using preparative liquid chromatography. The fractions containing **12** were collected and concentrated to give (10.9 mg, yield 28%). R_f = 0.60 (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{30}N_3O_3^+$: 396.2282. Found: 396.2282. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.33 (dd, J = 8.4 Hz, 1,2 Hz, 1H), 7.50–7.38 (m, 2H), 7.25 (m, 1H), 6.80 (s, 1H), 4.64 (t, J = 6.6 Hz, 2H), 3.08 (t, J = 6.9 Hz, 2H), 2.44 (q, J = 7.8 Hz, 2H), 2.38–2.30 (m, 2H), 2.20–2.16 (m, 2H), 2.13–2.08 (m, 4H), 1.82–1.54 (m, 6H), 1.05 (t, J = 7.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 208.6 (CO), 162.0 (CONH), 141.2, 138.5, 127.1, 123.1, 122.9, 122.8, 109.5 (aromatic C), 69.4 (COH), 54.4, 49.6, 44.3, 43.7, 41.6, 40.7, 36.6, 35.1, 30.9, 7.8 (aliphatic C).

4.2.9. 3-Amino-1-adamantol (**14**)

To a round bottom flask containing compound **13** (100 mg, 0.53 mmol) a mixture of concentrated sulfuric acid (2 mL, 37.1 mmol) and concentrated nitric acid (0.2 mL, 4.46 mmol) was added at 0 °C. The mixture was left to stir for two hours at 10 °C. Ice-cold water was then carefully added to the reaction mixture, following this addition the reaction mixture was made basic using solid sodium hydroxide until precipitation could be seen. The solution was thereafter filtered, and the wet solid collected and dissolved in DCM and left to stir for 30 min. Next, the solid was removed from the solution by filtration and washed with DCM. The DCM-solution containing **14** was concentrated *in vacuo* to afford compound **14** (70 mg, yield 81%). ^{1}H NMR ($CDCl_3$ 300 MHz) δ: 2.24–2.16 (m, 2H), 1.62–1.58 (m, 4H), 1.54–1.50 (m, 2H), 1.49–1.43(m, 6H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 69.6 (HOC), 53.9, 50.5, 44.9, 44.2, 34.9, 31.1 (aliphatic C).

4.2.10. N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**15**)

Compound **14** (41 mg, 0.246 mmol) was dissolved in THF (15 mL). To the solution Et_3N (69 μl, 0.5 mmol), compound **1** (27 mg, 0.164 mmol) and TBTU (79 mg, 0.246 mmol) were added. The reaction mixture was left to stir overnight at room temperature. Progression of the reaction was monitored on TLC. Following the evaporation of solvents, the remaining residue was chromatographed on silica using a mobile phase system of EtOAc/n-Hep (4:1). The fractions which contained **15** were pooled and concentrated to yield compound **15** (41.5 mg, yield 81%). R_f = 0.38 (4:1 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{18}H_{22}N_3O_2^+$: 312.1707. Found: 312.1709. ^{1}H NMR (CD_3OD 300 MHz) δ: 8.18 (dd, J = 9.6 Hz, 1.2 Hz, 1H), 7.55 (dd, J = 9.3 Hz, 1.2 Hz, 1H), 7.47 (s, 1H), 7.40 (m, 1H), 7.23 (m, 1H) 2.34–2.26 (m, 2H), 2.18–2.14 (m, 2H), 2,14–2.08 (m, 4H), 1.80–1.56 (m, 6H). ^{13}C NMR (CD_3OD, 75.4 MHz) δ: 164.4 (CONH), 143.0, 140.2, 127.9, 123.4, 122.8, 111.4 (aromatic C), 69.7 (HOC), 55.6, 55.5, 49.8, 49.7, 44.9, 41.4, 36.1, 32.2 (aliphatic C).

4.2.11. N-(3-hydroxyadamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide (**16**)

Compound **15** (32 mg, 0.1 mmol) was dissolved in DMF/THF (1:5, 7.2 mL) and left to stir at 0 °C. Following 5 min of stirring t-BuOK (17 mg, 0.15 mmol) was added and the solution was stirred for an additional 15 min. Finally, 1-Bromopentane (13 μl, 0.1 mmol) was added to the solution, brought to room temperature and stirred overnight. The progression of the reaction was monitored by TLC. Water (30 mL) was added to the solution and extracted using EtOAc (3 × 20 mL). The combined organic phases were concentrated *in vacuo* and the residue was chromatographed on silica using an eluent composition of 2:1 EtOAc/n-Hep. The fractions which contained **16** were gathered and concentrated to afford compound **16** (35.4 mg, yield 90%). R_f = 0.44 (2:1 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_2^+$: 382.2490. Found: 382.2483. ^{1}H NMR ($CDCl_3$ 300 MHz) δ: 8.36 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.42–7.37 (m, 2H) 7.25 (m, 1H), 6.88 (s, 1H) 4.35 (t, J = 6.9 Hz, 2H) 2.37–2.30 (m, 2H) 2.21–2.18 (m, 2H) 2.14–2.10 (m, 4H) 1.93 (dt, J = 7 Hz, 2H) 1.80–1.50 (m, 6H), 1.42–1.24 (m, 4H) 0.89 (t, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.3 (CONH), 141.0, 137.9, 126.7, 123.1, 122.9, 122.5, 109.3 (aromatic C), 69.4 (COH), 54.4, 49.6, 49.5, 44.3, 40.7, 35.1, 30.9, 29.6, 29.0, 22.4, 14.1 (aliphatic C).

4.2.12. 1-(pent-4-en-1-yl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**17**)

Compound **15** (130 mg, 0.42 mmol) was dissolved in DMF/THF (1:5, 6 mL). The solution was put to 0 °C using an ice bath and left to stir for five minutes. t-BuOK (70 mg, 0.63 mmol) was added to the solution which was stirred for an additional 15 min. As the mixture was brought to room temperature 5-bromopent-1-ene (50 μl, 0.42 mmol) was added to the solution and the reaction mixture was left to stir over the weekend. The progression of the reaction was checked on TLC. Water (20 mL) was added to the solution and thereafter the solution was extracted using EtOAc (3 × 20 mL). The organic phases were collected and concentrated *in vacuo*. The remaining residue was applied to a silica column with a mobile phase composition of EtOAc/n-Hep (3:1) and chromatographed. The fractions containing **17** were concentrated *in vacuo* to afford compound **17** (125.3 mg, yield 79%). R_f = 0.38 (3:1 EtOAc/n-Hep). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.35 (d, J = 8.1 Hz, 1H), 7.38–7.32 (m, 2H), 7.23 (m, 1H), 6.88 (s, 1H), 5.78 (m, 1H), 5.04 (m, 1H), 5.00 (m, 1H), 4.34 (t, J = 6.9 Hz, 2H), 2.34–2.26 (m, 2H), 2.20–2.16 (m, 2H), 2.15–1.96 (m, 8H), 1.82–1.50 (m, 6H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.1 (CONH), 140.9, 137.9, 137.1, 126.7, 123.1, 122.8, 122.5, 115.9, 109.2, 69.3 (COH), 54.3, 49.5, 48.5, 44.2, 40.6, 35.0, 30.80, 30.76, 28.8.

4.2.13. 1-(4,5-dihydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**18**)

Compound **17** (30.3 mg, 0.08 mmol) was dissolved in a solvent mixture of THF:H_2O (3:1, 8 mL). To the solution OsO_4 (4 wt% in H_2O, 49 μl, 0.008 mmol) and NMO (16.2 mg, 0.12 mmol) were added and left to stir overnight at room temperature. The development of the reaction was monitored on TLC. When the reaction was completed a saturated $NaHCO_3$ solution (1 mL) was added to the reaction mixture. Following this, the reaction mixture was extracted using EtOAc (3 × 10 mL). The organic phases were combined and concentrated *in vacuo*. The residue was then chromatographed on silica using EtOAc as the eluent. The fractions containing **18** were gathered and dried to afford compound **18** (27 mg, yield 82%). R_f = 0.08 (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_4^+$: 414.2388. Found: 414.2384. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.34 (dd, J = 8.4 Hz, 1.2 Hz, 1H), 7.42–7.35 (m, 2H), 7.24 (m, 1H), 6.87 (s, 1H), 4.40 (t, J = 7.2 Hz, 2H), 3.73 (m, 1H), 3.62 (m, 1H), 3.42 (m, 1H), 2.38–2.28 (m, 2H), 2.20–2.16 (m, 2H), 2.16–2.06 (m, 4H), 2.10–2.00 (m, 2H), 1.82–1.50 (m, 6H) 1.45 (q, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.3 (CONH), 141.0, 138.0, 126.9, 123.1, 122.9, 122.7, 109.3 (aromatic C), 71.8, 69.4, 66.7 (COH), 54.5, 49.5, 49.2, 44.2, 40.6, 35.1, 30.9, 30.1, 26.1 (aliphatic C).

4.2.14. 1-(5-hydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**19**) and 1-(4-hydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**20**) mixture

Compound **17** (95 mg, 0.25 mmol) was put into a round bottom flask which was flushed with nitrogen. BH_3·THF (1 M, 2 mL) was added to the flask and the reaction mixture was left to stir for three hours at 0 °C. The progression of the reaction was monitored using TLC. As the reaction mixture was brought to room temperature, it was first quenched using water (2 mL). Following the quenching NaOH (20 mg, 0.5 mmol) together with H_2O_2 (1 mL) were added to the reaction mixture which was then left to react overnight. When the reaction was finished, which was checked with the use of liquid chromatography, the reaction mixture was extracted using EtOAc (3 × 10 mL). The organic layers were gathered before being concentrated *in vacuo*. The crude mixture was then dissolved in DCM and applied to a silica column and chromatographed using EtOAc as the eluent. The fractions containing the mixture of **19** and **20** were accumulated, concentrated and dissolved in methanol for further purification using preparative LC. The fractions containing the mixture of **19** (91%) and **20** (9%) were collected and dried *in vacuo* to afford compound **19** and **20** (34.8 mg, yield 35%). R_f = 0.20 (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_3^+$: 398.2439. Found: 398.2434. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.34 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.40–7.36 (m, 2H), 7.24 (m, 1H), 6.89 (s, 1H), 4.34 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H), 2.36–2.30 (m, 2H), 2.22–2.16 (m, 2H), 2.14–2.08 (m, 4H), 1.96 (quin, J = 7.5 Hz, 2H), 1.82–1.50 (m, 8H), 1.46–1.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2 (CONH), 141.0, 137.9, 126.8, 123.1, 122.9, 122.6, 109.2 (aromatic C), 69.4, 62.6 (COH), 54.4, 49.5, 49.3, 44.3, 40.6, 35.1, 32.2, 30.8, 29.6, 23.2 (aliphatic C).

4.2.15. 1-(4-hydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**20**)

To the solution of compound **17** (29 mg, 0.077 mmol) in 3 mL DCM was added 70% m-CPBA (21 mg, 0.085 mmol) of and stirred overnight (about 18 h). The reaction was then quenched with saturated NH_4Cl (aq) and extracted with ethyl acetate (3 × 10 mL). The solvent was then evaporated, and the product was dissolved in MeOH (1.25 mL) and purified using preparative LC to give epoxide (21 mg, yield 66%). ^{1}H NMR ($CDCl_3$ 300 MHz) δ: 8.36 (dt, J = 8.1 Hz, 0.9 Hz, 1H), 7.43–7.36 (m, 2H), 7.28–7.21 (m, 1H), 6.87 (br s, 1H), 4.51–4.33 (m, 2H), 2.99–2.87 (m, 1H), 2.75 (dd, J = 5.1, 3.9 Hz, 1H), 2.47 (dd, J = 5.1, 2.7 Hz, 1H), 2.37–2.29 (m, 2H), 2.21–2.08 (m, 8H), 1.82–1.40 (m, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2, 141.1, 138.2, 126.9, 123.2, 122.9, 122.7, 109.2, 69.5, 54.4, 51.8, 49.5, 48.9, 47.0, 44.3, 40.7, 35.1, 30.9, 29.7, 26.5.

To the resulted epoxide (21 mg, 0.053 mmol) added 2-propanol (1.2 mL) and $NaBH_4$ (4 mg, 0.106 mmol). The mixture was stirred at 60 °C for about 3 h. Then quenched with 10 mL saturated NH_4Cl (aq) and extracted with ethyl acetate (3 × 10 mL), concentrated and purified on silica gel with EtOAc to give compound **20** (15.7 mg, 75% yield).

^{1}H NMR ($CDCl_3$ 300 MHz) δ: 8.36 (dt, J = 8.1, 1.0 Hz, 1H), 7.43–7.37 (m, 2H), 7.24 (m, 1H), 6.86 (br s, 1H), 4.39 (t, J = 8.1 Hz, 2H), 3.83 (m, 1H), 2.36–2.30 (m, 2H), 2.22–1.93 (m, 7H), 1.82–1.42 (m, 9H), 1.18 (d, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2, 141.0, 138.0, 126.8, 123.2, 123.0, 122.6, 109.3, 69.4, 67.7, 54.4, 49.6, 49.3, 44.3, 40.7, 36.2, 35.1, 30.9, 26.2, 23.9.

4.2.16. 1-(pent-2-en-1-yl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**21**)

Compound **15** (150 mg, 0.48 mmol) was dissolved in a solvent mixture containing DMF/THF (1:5, 6 mL). The solution was cooled to 0 °C and stirred for five minutes before addition of t-BuOK (81 mg, 0.72 mmol) followed by 15 min of stirring. As 1-bromo-2-pentene (57 μl, 0.48 mmol) was added to the solution the solution was brought to room temperature and was left to stir over night. The development of the reaction was monitored on TLC. The reaction mixture was diluted with water (20 mL) and extracted using EtOAc (3 × 20 mL). The combined organic phases were concentrated *in vacuo* and chromatographed on a silica column using a mobile phase system composed of 3:1 EtOAc/n-Hep. The fractions containing **21** were gathered and dried to give compound **21** (149.2 mg, yield 82%). R_f = 0.35 (3:1 EtOAc/n-Hep). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.36 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.40–7.30 (m, 2H), 7.24 (m, 1H), 6.88 (s, 1H), 5.80–5.55 (m, 2H), 4.94 (dd, J = 6.6 Hz, 1.2 Hz, 2H), 2.35–2.28 (m, 2H), 2.20–2.18 (m, 2H), 2.14–1.98 (m, 4H), 2.10–2.00 (m, 2H), 1.80-1-50 (m, 6H), 0.97 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2 (CONH), 140.9, 138.0, 136.8, 126.7, 123.2, 123.1, 122.9, 122.6, 109.7, 69.4 (COH), 54.4, 52.0, 49.6, 44.3, 40.6, 35.1, 30.9, 25.2, 13.2.

4.2.17. 1-(2,3-dihydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**22**)

Compound **21** (34.4 mg, 0.091 mmol) was dissolved in a solvent mixture of THF/H_2O (3:1, 8 mL). To the solution osmium tetroxide (OsO_4, 4 wt % in H_2O) (55 μl, 0.0091 mmol) and 4-Methylmorpholine N-oxide monohydrate (NMO) (18.4 mg, 0.136 mmol) were added and left to stir overnight at room temperature. The progress of the reaction was monitored on TLC. When the reaction was completed saturated $NaHCO_3$ solution (1 mL) was added to the reaction mixture. Following this, the reaction mixture was extracted using EtOAc (3 × 10 mL). The organic phases were combined and concentrated *in vacuo*. The residue was then chromatographed on silica using EtOAc as the eluent. The fractions containing **22** were gathered and dried to afford compound **22** (30.7 mg, yield 82%). $R_f = 0.26$ (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_4^+$: 414.2388. Found: 414.2383. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.31 (dd, $J = 8.1$ Hz, 1.2 Hz, 1H), 7.50–7.35 (m, 2H), 7.23 (m, 1H), 6.82 (s, 1H), 4.52–4.46 (m, 2H), 4.06 (m, 1H), 3.44 (m, 1H), 3.02 (m, 1H), 2.40 (m, 1H), 2.34–2.26 (m, 2H), 2.15–2.10 (m, 2H), 2.10–2.04 (m, 4H), 1.80–1.50 (m, 6H), 1.70–1.50 (m, 2H), 0.99 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 161.9 (CONH), 141.9, 138.7, 127.3, 123.0, 122.9, 122.8, 109.7 (aromatic C), 73.6, 72.5, 69.4 (COH), 54.5, 52.3, 49.4, 44.2, 40.62, 40.59, 35.1, 30.8, 26.8, 10.3 (aliphatic C).

4.2.18. 1-(2-hydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**23**) and 1-(3-hydroxypentyl)-N-(3-hydroxyadamantan-1-yl)-1H-indazole-3-carboxamide (**24**)

Compound **21** (107 mg, 0.282 mmol) was put into a round bottom flask which was flushed with nitrogen. To the flask $BH_3 \cdot THF$ (1 M, 2.5 mL) was added and the reaction mixture was left to stir for three hours at 0 °C. The progression of the reaction was checked using liquid chromatography. As the reaction mixture was brought to room temperature, it was first quenched with water (1.5 mL). Following the quenching NaOH (23 mg, 0.764 mmol) together with H_2O_2 (1 mL) was added and the reaction mixture was left to react overnight. When the reaction was over, which was checked with the use of liquid chromatography, the reaction mixture was extracted using EtOAc (3 × 10 mL). The organic layers were collected and filtered before being concentrated *in vacuo*. The crude mixture was then dissolved in DCM and applied to a silica column and chromatographed using EtOAc as the eluent. The fractions containing **23** were collected and dried *in vacuo* to yield compound **23** (72 mg, yield 64%). $R_f = 0.61$ (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_3^+$: 398.2439. Found: 398.2437. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.35 (dd, $J = 8.1$ Hz, 1.2 Hz, 1H), 7.48–7.38 (m, 2H), 7.25 (m, 1H), 6.80 (s, 1H), 4.44–4.24 (m, 2H), 4.16 (m, 1H), 2.53 (m, 1H), 2.35–2.25 (m, 2H), 2.18–2.14 (m, 2H), 2.10–2.05 (m, 4H), 1.80–1.40 (m, 10H), 0.96 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 161.9 (CONH), 141.9, 138.7, 127.2, 123.1, 122.9, 109.6 (aromatic C), 70.9, 69.4 (COH), 55.1, 54.5, 49.5, 44.3, 40.6, 36.7, 35.1, 30.8, 18.8, 14.1 (aliphatic C).

The fractions containing **24** were combined and dried *in vacuo* to give compound **24** (8 mg, yield 7%). $R_f = 0.45$ (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_3^+$: 398.2439. Found: 398.244. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.36 (d, $J = 7.5$ Hz, 1H), 7.50–7.36 (m, 2H), 7.25 (m, 1H), 6.83 (s, 1H), 4.64–4.46 (m, 2H), 3.45 (m, 1H), 2.38–2.30 (m, 2H), 2.22–2.16 (m, 2H), 2.17–2.10 (m, 5H), 1.92 (m, 1H), 1.82–1.44 (m, 8H), 0.92 (t, $J = 7.5$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.2 (CONH), 141.2, 138.2, 127.0, 123.1, 122.9, 122.7, 109.4 (aromatic C), 70.4, 69.5 (COH), 54.5, 49.6, 46.1, 44.3, 40.7, 36.6, 35.1, 30.9, 30.7, 9.9 (aliphatic C).

4.2.19. N-(4-oxoadamantan-1-yl)-1H-indazole-3-carboxamide (**26**)

Compound **25** (50 mg, 0.3 mmol) was dissolved in MeCN (220 μl, 4.2 mmol). To the solution boron trifluoride diethyl etherate (114 μl, 0.9 mmol) and TFA (184 μl, 2.4 mmol) were added and the vial was sealed with a cap. The solution was heated at 70 °C for three hours before being let cool to room temperature and left overnight. The solvents were evaporated prior to the addition of a saturated $NaHCO_3$(aq) solution (3 mL). Additional water was added, and the solution was extracted using chloroform (3 × 20 mL). The organic phases were concentrated and the crude carboxamide was dissolved in concentrated HCl (3 mL). The solution was heated for one hour at 150 °C using microwave irradiation. The solvent was then evaporated. The crude 5-aminoadamantan-2-one was dissolved in THF (10 mL). Compound **1** (32 mg, 0.2 mmol), TBTU (96 mg, 0.3 mmol) and Et_3N (84 μl, 0.6 mmol) were added to the solution. The reaction mixture was left to stir overnight at room temperature. The advancement of the reaction was followed using TLC. Following the evaporation of solvents the remaining residue was chromatographed on silica using a mobile phase system of EtOAc/n-Hep (4:1). The fractions which contained **26** were pooled and concentrated to give **26** (27.8 mg, yield 30%). $R_f = 0.57$ (4:1 EtOAc/n-Hep). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.56 (s, 1H), 8.27 (m, 1H), 7.54–7.40 (m, 2H), 7.29 (m, 1H), 6.98 (s, 1H), 2.72–2.64 (m, 2H), 2.58–2.42 (m, 6H), 2.32 (m, 1H), 2.16–1.94 (m, 4H). ^{13}C NMR (CD_3OD, 75.4 MHz) δ: 219.0 (CO), 164.7 (CONH), 143.0, 139.9, 127.9, 122.6, 122.5, 122.4, 111.5 (aromatic C), 52.1, 48.1, 42.9, 41.3, 39.4, 30.2 (aliphatic C).*

**Results from a crude ^{13}C-NMR where the strongest signals thought to originate from the product were recorded.*

4.2.20. N-((1s,3R,4s,5S,7s)-4-hydroxyadamantan-1-yl)- 1H-indazole-3-carboxamide (**27**) and N-((1s,3R,4r,5S,7s)-4-hydroxyadamantan-1-yl)- 1H-indazole-3-carboxamide (**28**)

Compound **26** (37.7 mg, 0.122 mmol) was dissolved in methanol (10 mL). To the solution $NaBH_4$ (25 mg, 0.66 mmol) was added, the solution was stirred for one hour at room temperature. The advancement of the reaction was monitored using liquid chromatography. Once the reaction had finished the reaction mixture was concentrated before addition of HCl(aq) (1 M, 10 mL). The acidic solution was extracted using EtOAc (3 × 10 mL). After concentrating the combined organic layers, the resulting residue was dissolved in DMF and purified by preparative liquid chromatography. The fractions containing **27** were combined and concentrated to afford **27** (11.7 mg, yield 31%).$R_f = 0.75$ (EtOAc). 2D-NMR were recorded for structural elucidation. HRMS (ESI, $[M+H]^+$): Calcd. for $C_{18}H_{22}N_3O_2^+$: 312.1707. Found: 312.1708. ^{1}H NMR (CD_3OD, 300 MHz) δ: 8.18 (dd, $J = 8.4$ Hz, 1.2 Hz, 1H), 7.56 (m, 1H), 7.48 (m, 1H), 7.24 (m, 1H), 3.95 (m, 1H), 2.28–2.00 (m, 11H), 1.58–1.48 (m, 2H). ^{13}C NMR (CD_3OD, 75.4 MHz) δ: 164.5 (CONH), 143.0, 140.1, 127.9, 123.4, 122.8, 111.4 (aromatic C), 74.2 (COH), 52.4, 42.8, 41.2, 36.5, 31.0, 30.3 (aliphatic C).

The fractions containing **28** were combined and concentrated to afford compound **28** (8 mg, yield 21%). $R_f = 0.79$ (EtOAc). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{18}H_{22}N_3O_2^+$: 312.1707. Found: 312.1709. ^{1}H NMR (CD_3OD, 300 MHz) δ: 8.19 (dd, $J = 8.4$ Hz, 1.2 Hz, 1H), 7.56 (m, 1H), 7.40 (m, 1H), 7.24 (m, 1H), 3.78 (m, 1H), 2.46–2.00 (m, 9H), 1.92–1.66 (m, 4H). ^{13}C NMR (CD_3OD, 75.4 MHz) δ: 164.4 (CONH), 143.0, 140.2, 127.9, 123.4, 122.8, 111.4 (aromatic C), 73.6 (COH), 52.6, 42.3, 37.2, 36.3, 36.2, 29.8 (aliphatic C).

4.2.21. N-(4-oxoadamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide (**29**)

Compound **26** (27.8 mg, 0.09 mmol) was dissolved in DMF/THF (1:5, 7.2 mL) and left to stir at 0 °C for five minutes. t-BuOK (15 mg,

0.135 mmol) was added and the solution was stirred for 15 min. As the solution was brought to room temperature 1-bromopentane (11 μl, 0.09 mmol) was added and the solution was left to stir overnight. The progression of the reaction was monitored by TLC. Water (20 mL) was added to the solution which was extracted using EtOAc (3 × 20 mL). The combined organic phases were concentrated *in vacuo* and the residue was chromatographed on silica using an eluent composition of 1:1 EtOAc/n-Hep. The fractions which contained **29** were gathered and concentrated to afford compound **29** (24.6 mg, yield 72%). R_f = 0.53 (1:1 EtOAc/n-Hep). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{30}N_3O_2^+$: 380.2333. Found: 380.2331. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.33 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.42–7.36 (m, 2H), 7.25 (m, 1H), 6.87 (s, 1H), 4.34 (t, J = 7.1 Hz, 2H) 2.70–2.62 (m, 2H) 2.58–2.38 (m, 6H), 2.31 (m, 1H), 2.16–1.95 (m, 4H), 2.00–1.85 (m, 2H), 1.40–1.24 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 216.7 (CO), 162.5 (CONH), 141.0, 137.5, 126.8, 123.0, 122.8, 122.7, 109.3 (aromatic C), 50.9, 49.5, 46.7, 42.3, 40.7, 38.5, 29.6, 29.0, 28.9, 22.4, 14.1 (aliphatic C).

*4.2.22. N-((1s,3R,4s,5S,7s)-4-hydroxyadamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide (**30**) and N-((1s,3R,4r,5S,7s)-4-hydroxyadamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide (**31**)*

Compound **29** (21.9 mg, 0.058 mmol) was dissolved in methanol (10 mL). To the solution $NaBH_4$ (15 mg, 0.4 mmol) was added, the solution was left to stir for one hour at room temperature. The progression of the reaction was monitored using liquid chromatography. Once the reaction had finished the reaction mixture was concentrated prior to the addition of HCl(aq) (1 M, 10 mL). The acidic solution was extracted using EtOAc (3 × 10 mL). After combination and concentration of the organic phases, the residue was dissolved in methanol, filtered through a pipette using cotton and purified by preparative liquid chromatography. The fractions containing **30** were combined and concentrated to give compound **30** (8 mg, yield 36%). R_f = 0.38 (1:1 EtOAc/n-Hep). 2D-NMR were recorded for structural elucidation. HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_2^+$: 382.2490. Found: 382.2489. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.36 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 7.42–7.38 (m, 2H), 7.24 (m, 1H), 6.81 (s, 1H), 4.35 (t, J = 7.4 Hz, 2H), 4.02 (m, 1H), 2.34–2.04 (m, 11H), 1.98–1.86 (m, 2H), 1.66–1.50 (m, 2H), 1.42–1.24 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.4 (CONH), 141.0, 137.9, 126.7, 123.1, 122.9, 122.5, 109.3 (aromatic C), 73.6 (COH), 51.2, 49.5, 42.1, 40.4, 35.5, 30.1, 29.6, 29.1, 28.9, 22.4, 14.1 (aliphatic C).

The fractions containing **31** were combined and concentrated to yield compound **31** (4.5 mg, yield 20%). R_f = 0.40 (1:1 EtOAc/n-Heptane). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_2^+$: 382.2490. Found: 382.2492. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.37 (d, J = 8.1 Hz, 1H), 7.42–7.36 (m, 2H), 7.24 (m, 1H), 6.82 (s, 1H), 4.35 (t, J = 7.4 Hz, 2H), 3.83 (m, 1H), 2.50–2.00 (m, 9H), 2.00–1.86 (m, 2H), 1.92–1.82 (m, 2H), 1.72–1.60 (m, 2H), 1.42–1.24 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ: 162.3 (CONH), 141.0, 137.9, 126.6, 123.2, 122.9, 122.5, 109.3 (aromatic C), 73.1 (COH), 51.3, 49.5, 41.6, 36.2, 35.6, 35.3, 29.6, 29.0, 28.3, 22.4, 14.1 (aliphatic C).

*4.2.23. 5-(3-((3-hydroxyadamantan-1-yl)carbamoyl)-1H-indazol-1-yl)pentanoic acid (**32**)*

Chromium trioxide (CrO_3) (40 mg, 0.4 mmol) was dissolved in a solvent mixture containing H_2O (1.17 mL) and concentrated H_2SO_4 (0.33 mL). This solution, called Jones reagent was added drop wise to a flask containing **19** (15 mg, 0.038 mmol) in acetone (5 mL) at 0 °C until the orange color persisted. Isopropanol was added to the orange solution until the solution became a green suspension. Following the color change HCl (aq) (1 M) was added until the solution became clear. The clear solution was extracted using DCM (3 × 10 mL). The organic phase was collected and then extracted using NaOH (aq) (1 M, 3 × 10 mL). The basic water phases were gathered and acidified to pH = 1 using concentrated HCl (aq) before being extracted using DCM (3 × 10 mL). The organic phases were collected, concentrated *in vacuo* and the remaining residue was dissolved in DCM and purified by flash chromatography using a mobile phase system containing EtOAc with <0.5% formic acid. The fractions containing **32** were, accumulated and concentrated *in vacuo* to afford compound **32** (9.0 mg, yield 58%). R_f = 0.39 (EtOAc, >0.5% formic acid). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{30}N_3O_4^+$: 412.2231. Found: 412.2227. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.35 (d, J = 8.1 Hz, 1H), 7.42–7.36 (m, 2H), 7.25 (m, 1H), 6.90 (s, 1H), 4.38 (t, J = 6.9 Hz, 2H), 2.40 (t, J = 6.9 Hz, 2H), 2.36–2.30 (m, 2H), 2.21–2.16 (m, 2H), 2.16–2.08 (m, 4H), 2.06–1.94 (m, 2H), 1.83–1.52 (m, 8H). ^{13}C NMR ($CDCl_3$, *75.4 MHz)* δ: 177.6 (COOH), 162.2 (CONH), 141.0, 138.0, 126.9, 123.2, 122.9, 122.7, 109.1 (aromatic C), 69.6 (COH), 54.4, 49.5, 48.8, 44.3, 40.6, 35.1, 33.2, 30.9, 29.1, 22.0 (aliphatic C).

4.3. Confirmation of analytes in authentic urine samples

The method used for liver microsome incubations was presented in detail earlier.[5] In brief, AKB-48 (1 μg/ml) was incubated with human liver microsomes and the incubation was terminated by addition of acetonitrile after 60 min. The samples were analyzed by liquid chromatography Time-of-Flight mass spectrometry (LC-QTOF-MS).

To optimize the separation of analogs a separation on a Cortecs UPLC C18 column (100 × 2.1 mm, Waters) was developed. A 7.5 min gradient from 26 to 67% ACN in 10 mM ammonium acetate was used. Retention times of synthesized analogs were compared to those of a urine sample treated with glucuronidase/arylsulfatase.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.04.026.

References

1. Canazza I, Ossato A, Trapella C, et al. *Psychopharmacology*. 2016;233:3685–3709.
2. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y. *Forensic Toxicol*. 2012;30:114–125.
3. Gandhi AS, Zhu M, Pang S, et al. *AAPS J*. 2013;15:1091–1098.
4. Holm NB, Nielsen LM, Linnet K. *AAPS J*. 2015;17:1237–1245.
5. Vikingsson S, Josefsson M, Gréen H. *J Anal Toxicol*. 2015;39:426–435.
6. Wallgren J, Vikingsson S, Johansson A, et al. *Tetrahedron Lett*. 2017;58:1456–1458.
7. a) Wanka L, Iqbal K, Schreiner PR. *Chem Rev*. 2013;113:3516–3604;
b) Kajbaf M, Rossato P, Barnaby JR, Pellegatti M. *Xenobiotica*. 1998;28:167–178;
c) Su H, Boulton DW, Barros Jr A, et al. *Drug Metab Dispos*. 2012;40:1345–1356.
8. Lavrova LN, Indulen MK, Ryazantseva GM, Korytnyi VS, Yashunskii VG. *Pharm Chem J*. 1990;24:35–39.
9. Averina NV, Borisova GS, Zefirova ON, Selyunina EV, Zyk NV, Zefirov NS. *Russ J Org Chem*. 2004;40:497–501.
10. Wesemann w, Schollmeyer W, Sturm G. *Arzneim-Forsch/Drug Res*. 1977;27:1471–1477.
11. Nakada N, Oda K. *Xenobiotica*. 2015;45:757–765.
12. Fura A, Khanna A, Vyas V, et al. *Drug Metab Dispos*. 2009;37:1164–1171.
13. Liu JY, Tsai HJ, Morisseau C, et al. *Biochem Pharmacol*. 2015;98:718–731.