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Human toxicity caused by indole and indazole carboxylate synthetic cannabinoid receptor agonists: From horizon scanning to notification.

Running Head: Indole and Indazole SCRA Toxicovigilance

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List of abbreviations

25I-NBOMe	4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine
5F-AKB-48	N-((3s,5s,7s)-adamantan-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
5F-NPB-22	1-(5-fluoropentyl)-8-quinolinyl ester-1H-indazole-3-carboxylic acid
5F-PB-22	1-(5-fluoropentyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid
5F-SDB-005	naphthalen-1-yl 1-(5-fluoropentyl)-1H-indazole-3-carboxylate
ALT	alanine or aspartate transaminase
AKB-48	1-pentyl-N-tricyclo[3.3.1.13,7]dec-1-yl-1H-indazole-3-carboxamide
BB-22	1-(cyclohexylmethyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid
BE	Base Excess
DCC	*N*,*N'*-Dicyclohexylcarbodiimide

DMAP	4-Dimethylaminopyridine
ECG	Electrocardiogram
EDC	*N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESI	ElectroSpray Ionisation
FUB-NPB-22	quinolin-8-yl 1-(4-fluorobenzyl)-1H-indazole-3-carboxylate
FUB-PB-22	1-[(4-fluorophenyl)methyl]-1H-indole-3-carboxylic acid, 8-quinolinyl ester
GCS	Glasgow Coma Score
HPRU	Health Protection Research Unit
IONA	The Identification Of Novel psychoActive Substances study
INR	International Normalised Ratio
ITU	Intensive Therapy Unit
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LoS	Length of Stay
MDEA	3,4 methylenedioxy-N-ethylamphetamine
MDMA	3,4 methylenedioxymethamphetamine
MDMB-CHMICA	methyl (S)-2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate
NM-2201	naphthalen-1-yl 1-(5-fluoropentyl)-1H-indole-3-carboxylate
NPS	Novel Psychoactive Substances

PB-22	1-pentyl-8-quinolinyl ester-1H-indole-3-carboxylic acid
PT	prothrombin time
QqTOF	Quadrupole quadrupole Time-of-Flight Mass Spectrometry
SCRA	Synthetic Cannabinoid Receptor Agonists
SWATH	Sequential Window Acquisition of all Theoretical fragment-ion spectra
t-BuOK	potassium *tert*-butoxide
TOF	Time-of-flight Mass Spectrometry
UK	United Kingdom

Abstract

Background. The emergence of Novel Psychoactive Substances (NPS), particularly Synthetic Cannabinoid Receptor Agonists (SCRA), has involved hundreds of potentially harmful chemicals in a highly dynamic international market challenging users', clinicians' and regulators' understanding of what circulating substances are causing harm. We describe a toxicovigilance system for NPS that predicted the UK emergence and identified the clinical toxicity caused by novel indole and indazole carboxylate SCRA.

Methods. To assist early accurate identification, we synthesised 5 examples of commercially-unavailable indole and indazole carboxylate SCRA (FUB-NPB-22, 5F-NPB-22, 5F-SDB-005, FUB-PB-22, NM-2201). We analysed plasma and urine samples from 160 patients presenting to emergency departments with severe toxicity after suspected NPS use during 2015-2016 for these and other NPS using data-independent liquid chromatography–tandem mass spectrometry.

Results. We successfully synthesised five carboxylate SCRA, using established synthetic and analytical chemistry methodologies. We identified at least one SCRA in samples from 49 patients, including an indole or indazole carboxylate SCRA in 17 (35%), specifically 5F-PB-22 (14%), FUB PB-22 (6%), BB-22 (2%), 5F NPB-22 (20%), FUB NPB-22 (2%), and 5F-SDB-005 (4%). In these 17 patients, there was analytical evidence of other substances in 16. Clinical features included agitation / aggression (82%), reduced consciousness (76%), acidosis (47%), hallucinations / paranoid features (41%), tachycardia (35%), hypertension (29%), raised creatine kinase (24%) and seizures (12%).

Conclusions. This toxicovigilance system predicted the emergence of misuse of indole and indazole carboxylate SCRA, documented associated clinical harms, and notified relevant

agencies. Toxicity appears consistent with other SCRA, including mental state disturbances and reduced consciousness.

Keywords

Synthetic cannabinoid receptor agonists, synthetic cannabinoids, toxicovigilance, Novel Psychoactive Substances

INTRODUCTION

The emergence of novel psychoactive substances (NPS) over recent decades and synthetic cannabinoid receptor agonists (SCRA) in particular is well documented (1-4). Between 2009 and 2016, 157 different novel SCRAs were notified to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), with 24 notified in 2015 alone (4). This large number reflects manufacturers modifying SCRA structures to circumvent legislation. The consequent dynamic market is highly challenging for legislators, healthcare professional and users. There is inadequate information about the SCRAs being taken by users and their possible harms, although there is clear evidence that SCRA can be associated with severe adverse effects (2, 5-8). Accurate identification of SCRA products in biological samples can be challenging because of the complexity of their chemistry and metabolism and the lack of available standards or digital library spectra for emerging compounds (9).

Given the public health importance of the clinical harms caused by NPS, especially SCRAs, it is important that appropriate surveillance activities are performed to detect, identify and characterise the harms of new substances emerging into clinical use. Key components of such a toxicovigilance system include: (a) predicting likely chemical modifications to emerging NPS (horizon scanning); (b) synthesising appropriate reference standards; (c) analysing drug product samples seized by law enforcement agencies and biological samples from users, especially those experiencing adverse effects; and (d) national and international notification of findings. Few other successful toxicovigilance systems currently exist, one example being the STRIDA project in Sweden (10).

Here we describe a complete system for toxicovigilance for novel SCRA established in the UK. Key features include the prediction of novel SCRA expected to enter the UK drug market, the synthesis of reference standards for predicted drugs, availability of relevant biological samples from a clinical research study of patients presenting to hospitals with severe toxicity

suspected to be associated with NPS, detection of these novel SCRA in biological samples and the reporting of the detection of novel SCRA to the UK National Focal Point and subsequently to the EMCDDA.

When this research was conceived in January 2015, indole and indazole carboxamide SCRAs such as AKB-48, 5F-AKB-48 and MDMB-CHMICA were commonly encountered in the UK (6,12). Review of the literature suggested that indole and indazole carboxylate SCRA might supersede indole and indazole carboxamides in the drug market (12). Fig. 1 compares the chemical structure of the indazole carboxamide (left) and indazole carboxylate (right) SCRA.

Some indole carboxylate compounds were detected internationally in patient samples, e.g. PB-22 (13,14), 5F-PB-22 (15,16, 17) and BB-22, (18). While these provided some information about the toxic effects of these compounds, the data were inadequate to allow reliable comparison of harms between these carboxylate SCRAs and previously reported carboxamide compounds. At that time no clinical cases of toxicity associated with carboxylates had been reported in the UK, although some product analysis had identified the indole carboxylate SCRA BB-22 and 5F-PB-22 (11). It therefore appeared probable that toxicity associated with indole or indazole carboxylate SCRAs would become increasingly frequent in clinical practice.

To investigate this expectation we synthesised 2 indole (FUB-PB-22, NM-2201) and 3 indazole (FUB-NPB-22, 5F-NPB-22, 5F-SDB-005) carboxylate SCRA that were commercially unavailable at the time. These compounds were added to reference standards used for analysis of biological samples obtained from a multicentre clinical study of patients presenting to participating UK hospitals with severe toxicity after suspected NPS use.

MATERIALS and METHODS

Synthetic Chemistry

Details of the synthetic pathways are described in the online Supplemental Material file.

Participant Recruitment

The Identification Of Novel psychoActive Substances (IONA) study received ethical approval (REC Reference 15-NE-0023) and all participants, if able, were asked to give fully informed written consent for provision of clinical data and samples for toxicological analysis. Those lacking mental capacity, for example because of severe confusion or being unconscious, could be included on the advice of a personal (usually a family member) or professional (a health professional independent of the study) representative, but were asked to confirm consent when they were able to do so.

Clinical surveillance

The IONA study recruited patients over 16 years of age presenting to participating emergency departments in 16 hospitals in England, Wales and Scotland with severe toxicity suspected to be due to exposure to a novel psychoactive substance. Severe toxicity criteria included; fever (>38.5^{o}C), clinically important hypothermia, unconsciousness (Glasgow Coma Scale <8), critical care or high dependency unit admission, respiratory insufficiency, requirement for intubation and ventilation, seizure, hallucinations or psychosis, extreme agitation, severe or prolonged (>24 h) behavioural disturbance, arrhythmia, chest pain, ECG evidence of cardiac ischemia or myocardial infarction, acidosis (arterial or venous pH <7.35 or bicarbonate <20 mmol/L), severe electrolyte or fluid disturbances, hypoglycaemia (<1.7 mmol/L), methaemoglobinaemia (>50%), tachycardia >140/min, systolic hypertension or hypotension (>180 or<80 mmHg), acute kidney injury, increased creatine kinase (>1000 IU/L), alanine or aspartate transaminase (ALT or AST >300 IU/L), prothrombin time (PT >15 s) or International

Normalised Ratio (INR >1.3), or any other severe manifestation of toxicity as determined and justified by the investigator.

Biological samples (whole blood, serum, plasma and/or urine) were provided to the Health Protection Research Unit at Newcastle University, in linked-anonymised format identified by a specific code number that could only be linked with the participant's identity by the clinicians at the participating hospital, along with demographic and clinical data including details of the reported exposure and clinical features outcomes.

Sample preparation

We extracted psychoactive substances from 500 µL of plasma or 500 µL of urine using cation exchange solid phase extraction. We diluted samples 1:2 with 2% phosphoric acid and centrifuged at 4000 g for 5 min. We transferred the supernatant onto Plexa PCX cation exchange solid phase extraction wells (Agilent), pre-conditioned with 500 µL of methanol and 500 µL of water. After equilibration at ambient pressure for 5 min, we washed the wells with 500 µL of 0.1% formic acid. We followed this with a two-stage elution. We first eluted with 500 µL of 1:1:1 methanol:acetonitrile:ethylacetate, followed by elution with 500 µL of 5% ammonia in 1:1:1 methanol:acetonitrile:ethylacetate. We combined the two eluates, evaporated them to dryness under a stream of nitrogen at 45^{o}C in a Zymark TurboVap (Biotage, Uppsala, Sweden), reconstituted the dried extract in 25 µL mobile phase 90:10 (v/v) 0.1% formic acid water: 0.1% formic acid acetonitrile, vortexed and centrifuged at 4000 g for 5 min, then transferred to amber auto sampler vials containing 300 µL glass inserts. We injected 1 µL per analysis.

Instrumentation

We analysed and identified NPS by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The system consisted of a TripleTOF 5600^{+} high-resolution Quadrupole Time-of-Flight Mass Spectrometer (Sciex, Framingham, MA) equipped with a DuoSpray ion source operated in positive electrospray (ESI^{+}) mode, coupled to an Eksigent Nano LC 420 system, using non-targeted data independent LC-MS/MS techniques. We used AnalystTF version 1.7.1 for instrument control and data acquisition.

Chromatographic conditions

We performed chromatographic separation by gradient elution with an ACE C_{18} capillary LC column (100 mm x 300 µm x 3 µm [HighChrom]) fitted with a 0.25 µm column saver pre-column filter, with (A) 0.1 % formic acid in water and (B) 0.1% formic acid in acetonitrile as mobile phase, at a flow rate of 5 µL/min. Gradient conditions were 5% B, held for 1min, then increased to 95% B over 40 min, held at 95% B until 45 min, returned to 5% B at 45.1 min, and held until 50.0 min. We used a total run time of 50.0 min. The column and auto-sampler temperatures were 25°C and 8°C respectively.

Mass spectrometry

We analysed Novel Psychoactive Substances (NPS) qualitatively using non-targeted data independent LC-MS/MS techniques. We used a data independent analysis method called Sequential Window Acquisition of all Theoretical fragment-ion spectra (SWATH) mass spectrometry (MS), which used the very fast scanning speeds of QqTOF mass spectrometers. SWATH MS (Sciex, Framingham, MA) is a form of data-independent analysis that repeatedly cycles through consecutive pre-set precursor ion isolation windows, detecting all fragment ion

spectra from all precursor ions contained in a specific window at a given time, providing highly selective MS/MS mass spectra of all analytes. We detected protonated molecular ions via a TOF MS scan covering the 100–800 Da mass range. We followed the TOF MS scan by SWATH MS/MS acquisition in high sensitivity mode at a mass resolution of at least 20,000, with a collision energy spread of 30 ± 15V over a mass range of 30–825 Da, using 20 Da SWATH isolation windows. We performed mass calibration on every second sample by injection of a calibration solution through the LC-MS/MS system.

Data processing and identification of NPS

To identify unknown compounds we processed LC-MS/MS data using MasterView software version 2.2. (Sciex, Framingham, MA). We identified compounds by software-assisted library searching against reference spectra. We preformed library searching and analyte identification on MS/MS data with LibraryView version 1.0 (Sciex, Framingham, MA) and ChemSpider Library version 2.0 (Royal Society of Chemistry, Cambridge, UK), integrated within MasterView software. We extracted synthesised NPS (online Supplemental Material file) from plasma and urine and collected SWATH MS data. We incorporated TOF MS and MS/MS data obtained for these compounds into LibraryView software, joining other pre-existing compounds reference standard data, used to identify novel NPS in clinical samples by software-assisted library searching against these reference spectra. We set the intensity factor determining the impact of spectral intensity differences between the acquired and reference spectrum on the purity percentage to 3. We set the intensity threshold, utilised to remove small peaks under a specified intensity, to 5. We used a library match with a purity score greater than 65% and the presence of the molecular ion and three characteristic MS/MS fragment ions as criteria for identification. We checked positive matches obtained from this search by manual review.

We quantified identified SCs by standard multiple reaction monitoring (MRM) using a Q-Trap 5500 hybrid linear ion trap/triple quadrupole mass spectrometer (Sciex) coupled to a Shimadzu Prominence LC. We employed Analyst version 1.6.2 and MultiQuant 2.0 (Sciex) for instrument control/data acquisition and quantitative analysis respectively. We performed chromatographic separation by gradient elution with a Raptor Biphenyl LC column (100 mm x 2.1 m x 2.7 µm [Restek] equipped with a guard column containing identical packing material, with (A) 0.1 % formic acid in water and (B) 0.1% formic acid in methanol as mobile phase, at a flow rate of 400µL/min. We held gradient conditions at 5% B, for 1min, then we increased to 95% B over 25 min, held until 30 min and returned to 5% B at 30.1 min and held until 35.0 min. We used a total run time of 35.0 min. The column and auto-sampler temperatures were 50°C and 8°C respectively. We optimised MS/MS parameters (Table 1) via direct infusion of individual analytes at 50 ng/mL in 50:50 A:B. We used a 2 µL injection volume per sample.

Extraction Efficiency

We evaluated extraction efficiency and matrix effect via three sets of samples as described by Matuszewski et al, (19) with six data points for each set. We fortified sample set 1, urine and plasma with analytes and internal standards prior to solid phase extraction (SPE). We fortified sample set 2, urine and plasma with analytes and internal standards after SPE. Sample set 3 consisted of analytes and internal standards in mobile phase. We calculated extraction efficiency, expressed as a percentage, by dividing mean analyte peak areas of set 1 by set 2. We calculated absolute matrix effect by dividing the mean analyte peak area in set 2 by the mean analyte area in set 3. We then converted the value to a percentage and subtracted from 100 to represent the amount of signal suppressed by the presence of matrix.

LOD, LOQ, and Linearity

For MRM based quantification, we evaluated the limit of detection (LOD) over three runs with duplicates from 3 different urine and plasma extractions. We defined LOD as the lowest concentration producing a peak eluting within ±0.1 min of the analyte retention time with signal-to-noise ≥3:1, Gaussian peak shape and qualifier/quantifier transition peak area ratios ±20% of mean calibrator transition ratios for all replicates. We evaluated limit of quantification (LOQ) in the same manner. We defined LOQ as the lowest concentration meeting LOD criteria with signal-to-noise ≥10:1 and measured concentration within ±20% of target. We confirmed performance at the LOQ in each batch of specimen samples and LOQ was equivalent to the analytes' lowest limit of linearity. We fit calibration curves by linear least squares regression with at least 6 concentrations across the linear dynamic range for each analyte. We required calibrators to quantify within ±20% of the target concentration and correlation coefficients (R^2) to exceed 0.99.

For DIA SWATH based identification of NPS, we determined specificity and LOD according to the method outlined by Scheidweiler et al, (20). We acquired a minimum of five TOF MS and SWATH MS/MS scans across each peak, reliably acquiring representative spectra for each analyte. We used the following criteria for analyte identification and specificity: *i*) mass error for the molecular ion <5 ppm, *ii*) retention time error <5%, *iii*) isotopic pattern difference from theoretical <80%, and *iv*) LibraryView library fit score >65%. All four criteria had to be fulfilled. We combined this with visual verification of at least two extracted ion chromatograms for each analyte, including the molecular ion and the most intense MS/MS fragment, plus at least two common fragments in the sample and reference MS/MS spectra. We evaluated LOD over three runs with 5 different spiked urine and plasma samples and defined LOD as the lowest concentration, fulfilling the identification criteria as detailed for specificity.

Analytical Precision and Recovery

We determined method precision and recovery by analysing drug-free urine and plasma samples spiked with the low and high concentrations of analytes. We analysed five sets of samples over five different days. We estimated repeatability, between-day precision and intermediate precision, expressed as % coefficient of variation (% CV) of calculated concentrations, using one-way analysis of variance (ANOVA) with the grouping variable 'day' at each particular concentration. We calculated recovery as the percentage difference of the grand mean of all 25 measurements and the respective nominal target concentrations at each concentration.

We determined Intra- and inter-assay analytical recovery and imprecision from four replicates at two different QC concentrations, 1ng and 10ng/mL of urine or plasma, across the linear dynamic range of the assay. We evaluated inter-assay imprecision and recovery on five different runs with four replicates in each run, analysed on five separate days (n = 20). We expressed imprecision as % CV of calculated concentrations. We conducted ANOVA on low, and high QCs to evaluate inter- and intra-assay differences in analyte concentrations.

RESULTS

The results of the validation procedure with the limit of detection/quantification, precision, recovery, and matrix effect are summarised in Table 2 for data independent SWATH and targeted MRM approaches.

We launched the IONA study in March 2015 and by 31st December 2016 16 hospitals were participating in the study with 232 patients recruited with suspected NPS-related severe toxicity. We report analytical data from March 2015 to the end of December 2016 for 160 patients with NPS detected in 95 (59%) and conventional drugs of abuse in 90 (56%). These include 49 patients in whom we detected both NPS and conventional drugs. We did not detect psychoactive substances in the remaining 24 patients.

We identified at least one SCRA in 49/160 patients (31%) with the commonest examples being the indole carboxamides 5F-ADB (20), MDMB-CHMICA (8) and 5F-AKB-48 (7). We identified indole or indazole carboxylate SCRA in 17 clinical cases including indoles in 11 (5F-PB 22 (7), FUB-PB-22 (3) and BB-22 (1)) and indazoles in 12 (5F-NPB-22 (10), FUB-NPB-22 (1) and 5F-SDB-005 (2)). Thus we detected 4 of the 5 synthesised indole or indazole carboxylate SCRA in clinical practice. We identified both indole and indazole carboxylate compounds in samples from 6 participants. Quantification of the 5 synthesised SCRA are reported in Table 3.

The first indole or indazole SCRA detected in the IONA study were from an exposure on July 2015 with 7 cases in total recorded in 2015 and 10 cases in 2016. Of the 17 cases where we identified indole or indazole carboxylate SCRA,4 presented in London, 7 in Newcastle-upon-Tyne, 1 in Manchester and 5 in Blackpool; 4 were female, 13 male with a median age of 30

years (range 18-58). We identified additional substances other than indole and indazole carboxylate SCRA in samples from all but one patient (case 9) including other SCRA (8), methiopropamine (2), 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine [25I-NBOMe] (1), cocaine (1), methamphetamine (2), 3,4 methylenedioxymethamphetamine [MDMA] (1), 3,4 methylenedioxy-N-ethylamphetamine [MDEA] (1), morpine (1) diazepam (1) and methadone (4). The route of exposure was reported by 15 cases as smoking in 11, insufflation in 3, both smoking and insufflation in 1 and ingestion in 2. The source of the product was reported as a friend in 7, a dealer in 1 and a shop in 1; no information on the source was provided by the remaining 8 patients.

Clinical features observed in the 17 patients where we detected an indole and/or indazole carboxylate SCRA included confusion / agitation / aggression (14 = 82%), reduced consciousness (13 = 76%), acidosis (8 = 47%), hallucinations / paranoid features (7 = 41%), tachycardia (6 = 35%), hypertension (5 = 29%), raised creatine kinase (4 = 24%) and seizures (2 = 12%).; 3 patients required intubation and ventilation in critical care. All patients recovered and were discharged from hospital after a median length of stay of 20 h (range 3-77 hours).

Table 3 shows the clinical and analytical details for these 17 cases.

Notification and Impact

The identification of FUB-NPB-22 in a clinical sample from London was the first detection of this substance in Europe. We notified the UK Focal Point and the EMCDDA on 8th July 2016. In this case the person (case 1) reported taking ‘new cannabis’, LSD and ‘mushroom tea’ and had neurological, cardiovascular and metabolic toxicity (table 1). In addition to FUB-NPB-22, we identified 5F-PB-22 and methiopropamine, the thiophene analogue of methamphetamine.

The other 4 indole or indazole carboxylate SCRA synthesised had already been notified to the EMCDDA by July 2016.

DISCUSSION

Here we have demonstrated how a comprehensive toxicovigilance can be used to predict structural modifications to current NPS to produce novel compounds and then subsequently detect these compounds in patients experiencing toxicity from their use. Specifically, we anticipated the appearance of several novel indole or indazole carboxylate SCRAs following detection of some of these compounds in drug product testing or in international patient samples (12-18). We facilitated identification and quantification of these novel indole or indazole carboxylate SCRAs by the synthesis of high quality standards and a pre-existing clinical study platform providing appropriate samples from patients experiencing severe toxicity after suspected NPS use with appropriate regulatory and ethical approvals in place.

There were already reports of toxicity associated with the some indole carboxylate SCRAs studied here at the time the research started, and further reports have been published during the conduct of this research (21,22). This research, however, provides further information on the recent and changing prevalence of clinical toxicity associated with SCRA in the UK. Some indazole carboxylate SCRAs (SDB-005 and 5F-SDB-005) were detected by drug product testing in Europe, but there are no previous published reports of toxicity associated with these compounds. We have used this toxicovigilance methodology to rapidly identify and notifiy the novel compound FUB-NPB-22, which had not previously been detected in human samples in Europe or elsewhere.

Although we have identified evidence of severe toxicity in people exposed to indole and indazole SCRA, in almost all cases there is analytical evidence that other NPS have also been used, and these NPS may contribute to the clinical features observed. For example, stimulants like methiopropamine or methamphetamine may cause or enhance mental health disturbances,

tachycardia or hypertension, while depressant compounds including opioids or benzodiazepines may cause of contribute to the reduced level of consciousness. In such cases it is not possible to assess the relative contribution to these features made by the SCRA involved. However, in the single patient exposed to 5F-NPB-22, where there was no evidence of exposure to other drugs of misuse (Case 9), reduced level of consciousness, tachycardia, hypertension and acidosis were all observed. These features have also been observed after exposure to other SCRA in the absence of other types of NPS (5-8).

Concentrations associated with toxicity are not available for the 5 synthesised SCRA, and we are not aware of published quantified clinical cases. It is not possible to correlate the severity of toxicity to concentration due to the small sample size, uncertainty regarding exposure-to-sample time and the contribution of co-exposures.

In conclusion, in this study we demonstrate the feasibility of predicting NPS that may emerge into clinical use. This requires consideration of possible alterations to the structure of currently used NPS, together with horizon scanning involving international information on analysis of seized drug product samples and biological samples from drug users. This process is enhanced by a pre-existing appropriately approved mechanism for the collection of appropriate biological samples from users experiencing suspected NPS toxicity, while synthesis of appropriate standards allowing early accurate detection and quantification of emerging NPS. Such a comprehensive toxicovigilance programme is valuable for the early detection of emerging NPS and for understanding the harms associated with their use.

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TABLE 1. MRM Transitions of Synthesised Indole/Indazole Synthetic Cannabinoids

Analyte	Q1 Mass/amu	Q3 Mass/amu	CE(V)	DP(V)	EP(V)	CXP(V)
NM-2201	376.2	232.1/144.1	32/37	80	12	15/38
5F-SDB-005	377.2	233.1/213.1	35/42	75	14	12/36
5F-NPB-22	378.2	233.1/213.1	29/38	80	14	15/34
FUB-PB-22	397.2	252.1/109.1	34/41	102	9	18/31
FUB-NPB-22	398.2	253.1/109.1	31/39	100	11	16/29

amu - atomic mass unit; V - voltage; MRM - multiple reaction monitoring; DP -declustering potential; EP - entrance potential; CE -collision energy; CXP -collision cell exit potential.

TABLE 2. LODs/LOQs, Mean Extraction Efficiencies and Matrix Effects for Indole/Indazole Synthetic Cannabinoids extracted from urine and plasma by cation exchange SPE.

Analyte	MRM LOD pg on-column	MRM LOQ pg on-column	Intra-day Precision, % Low/High	Inter-day Precision, % Low/High	Recovery, % Low/High	SWATH LOD pg on-column	Extraction Efficiency % Urine/Plasma	Matrix Effect % Urine/Plasma	Linear Range ng/mL
NM-2201	30	70	12.2/4.9	16.8/11.9	16.1/13.8	280	81/88	21/11	0.6 - 20
5F-SDB-005	20	50	10.4/5.8	17.7/10.1	14.9/13.1	225	78/91	16/10	0.4 - 20
5F-NPB-22	5	13	8.2/4.3	12.6./7.9	12.1/13.8	45	96/101	19/12	0.1 - 12
FUB-PB-22	2	4	6.9/4.1	10.4/7.1	9.7/9.1	30	91/102	16/14	0.05 - 12
FUB-NPB-22	4	9	7.6/4.4	11.7/7.8	10.4/12.2	42	89/98	18/12	0.08 - 12

TABLE 3. Demographic, clinical and analytical results of 17 patients with analytically confirmed exposure to indole or indazole carboxylate SCRA.

Patient (Age, y/Sex)	Reported exposure	Route	Timing	Source	Clinical features	Treatment	Outcome	LoS	Synthesised SCRA detected with quantification	Other substances detected
1 (19/F)	new cannabis, LSD, mushroom tea	ingestion	3 hours earlier	unknown	reduced GCS (9/15), seizure, mydriasis, hypertonia, hyperreflexia, clonus, confusion, tachycardia (120/min), hypertension (185/103), dizziness, acidosis (pH 7.25)	none	discharged	17 h	FUB-NPB-22, (Plasma 1.7 ng/mL, Urine 2.6 ng/mL)	5F-PB-22, methiopropamine
2 (35/M)	poppers, clear fluid	insufflation	acute	unknown	reduced GCS (3/15), hypertension (170/), arrhythmia, breathlessness, confusion, acidosis	anaesthetised, intubated and ventilated	discharged	37 h	FUB-PB-22 (Plasma 2.8 ng/mL)	morphine
3 (30/M)	crystal meth, mephedrone, ketamine	insufflation, smoked	chronic ('1 week binge')	unknown	agitation, hallucination, paranoid ideation, depression, raised creatine kinase ((1756)	none	discharged	32 h	FUB-PB-22 (Plasma 2.1 ng/mL)	methamphetamine

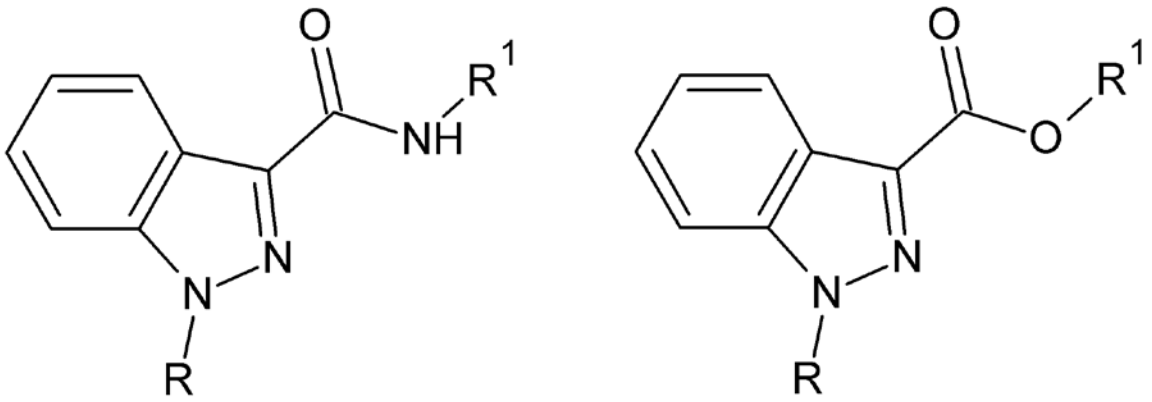
4 (24/M)	**dusk till dawn, poppers**	**insufflation, smoked**	**acute**	**unknown**	**mydriasis, tachycardia (135), hypertension (160/), palpitations, breathlessness, agitation, raised creatine kinase, methaemoglobinaemia (37%)**	**methylene blue**	**transferred to another unit**	**7 h**	**FUB-PB-22 (Urine 1.4ng/mL)**	**5F-AKB-48, methiopropamine,**
5 (28/M)	**legal high**	**smoked**	**acute**	**friend**	**reduced GCS (7/15), metabolic acidosis (pH 7.35, BE -12), hepatic transaminitis (ALT 43)**	**none**	**discharged**	**18 h**	**5F-NPB-22 (Urine 2.2ng/mL), 5F-SDB-005 (Urine 0.9ng/mL)**	**5F-PB-22, 5F-ADB, methadone, EDDP,diazepam**
6 (35/M)	**legal high**	**smoked**	**chronic**	**friend**	**tachycardia (116), dizziness, agitation, confusion, paranoid ideation.**	**none**	**discharged**	**n/a**	**5F-NPB-22 (Plasma 0.7ng/mL)**	**5F-ADB, 5F-AMB, methadone**
7 (38/F)	**Pandora's Box**	**smoked**	**chronic, last 1 hour earlier**	**shop**	**reduced GCS, confusion, paranoid ideation**	**none**	**discharged**	**4 h**	**5F-NPB-22 (Plasma 4.6ng/mL)**	**5F-PB-22, 5F-ADB**
8 (22/M)	**Cherry Bomb, diazepam**	**smoked, ingested**	**acute**	**friend, dealer**	**reduced GCS (11/15), bradycardia (46/min), hypotension (91/), bradypnoea (8/min), agitation, confusion, hallucination, suicidal ideation, acidosis (pH7.31), raised creatine kinase (1365), trauma to face**	**none**	**n/a**	**n/a**	**5F-NPB-22 (Plasma 3.1ng/mL, Urine 1.1ng/mL)**	**5F-AKB-48, 5F-AMB**

9 (42/M)	**Cherry Bomb**	**smoked**	**acute**	**friend**	**reduced GCS (3/15), tachycardia (114/min), hypertension (161/), acidosis (pH 7.21)**	**naloxone**	**discharged**	**n/a**	**5F-NPB-22 (Plasma 0.8ng/mL)**	**5F-AKB-48, 5F-AMB**
10 (52/M)	**unknown**	**unknown**	**unknown**	**friend**	**reduced GCS, mydriasis,tachycardia (max 120/min), breathing difficulties, agitation, confusion,**	**ITU for intubation and ventilation**	**discharged**	**44 h**	**5F-NPB-22 (Plasma 0.4ng/mL Urine 0.7ng/mL)**	**NNEI, 5F-ADB, methamphetamine, methadone**
11 (38/F)	**heroin and Spice**	**unknown**	**unknown**	**unknown**	**vomiting, reduced GCS, hypotension, breathing difficulties, agitation, paranoid ideation, depression,**	**none**	**self-discharged**	**3 h**	**5F-NPB-22 (Plasma 3.1ng/mL)**	**5F-AB-PINACA, 5F-ADB, NNEI**
12 18/M)	**Pandora's Box**	**smoked**	**chronic**	**shop**	**reduced GCS, confusion, paranoid ideation,**	**none**	**discharged**	**4 h**	**5F-NPB-22 (Plasma 0.9g/mL, Urine 1.1ng/mL)**	**5F-PB-22, 5F-ADB**
13 (30/F)	**legal high, smack, benzodiazepine**	**oral (benzo), others not known**	**benzo chronic others acute**	**dealer**	**hypothermia (34.3C), reduced GCS (3/15), mydriasis, nystagmus, metabolic acidosis (pH7.34), hepatic dysfunction (ALT 89), raised creatine kinase (1357)**	**none**	**discharged**	**22 h**	**5F-SDB-005 Plasma 0.5ng/mL)**	**5F-ADB, 5F-PB-22, methadone**
14 (24/M)	**black wrapper with picture**	**smoked**	**acute**	**friend**	**reduced GCS(14/15), bradycardia (43/min), confusion, metabolic acidosis (pH7.33)**	**none**	**discharged**	**24 h**	**5F-NPB-22 (Plasma 5.1ng/mL, Urine 8.9ng/mL)**	**cocaine**

15 (20/M)	**not known**	**smoked**	**unknown**	**unknown**	**vomiting, abdominal pain, reduced GCS, chest pain, confusion,**	**none**	**discharged**	**n/a**	**5F-NPB-22 (Plasma 7.1ng/mL),**	**5F-PB-22, MDMA, MDEA**
16 (58/M)	**Exodus**	**smoked**	**chronic**	**unknown**	**abdominal pain, reduced GCS (7/15), seizure, tachycardia (119/min), hypertension (235 mmHg systolic), agitation, confusion, acidosis (7.09, lactate 10), hepatic dysfunction (ALT 64),**	**anaesthetised, intubated and ventilated**	**discharged**	**77 h**		**BB-22, AB-CHMICA, 5F-AKB48**
17 (18/M)	**Incense**	**smoked**	**acute**	**unknown**	**vomiting, abdominal pain, bleeding, reduced GCS (5/15), bradycardia (40/min), chest pain, confusion,**	**none**	**discharged**	**16 h**		**5F-PB-22, 25I-NBOMe**

FIGURE CAPTIONS:

Fig. 1. Comparison of the chemical structure of indazole carboxamide (left) and indazole carboxylate (right) SCRA.



Indazole carboxamide (left) and indazole carboxylate (right)

Acknowledgements

The authors are grateful to the clinicians and research nurses in hospitals enrolled in the IONA study for their essential contribution in recruiting patients and collecting samples and clinical data. The authors also wish to acknowledge the work of Margaret Knight and Claire Roper who assisted in biological sample assays.

The National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Chemical and Radiation Threats and Hazards funded this research in partnership with Public Health England (PHE). The views expressed are those of the author(s) and not necessarily those of the National Health Service, the NIHR, the Department of Health or Public Health England.

Declaration of interest: The authors report no declarations of interest.

SUPPLEMENTAL MATERIAL

In our approach to synthesising the 5 required indole and indazole carboxylate compounds **(13, 14, 15, 16 and 18)** we envisaged that a general synthetic pathway could be used (Supplemental Fig. 1). Thus, Fisher esterification of the commercially available 1*H*-indole-3-carboxylic acid (**1**) and 1*H*-indazole-3-carboxylic acid (**2**) under standard conditions,(S1), gave the respective methyl carboxylates **3** and **4**. Treatment of **3** and **4** with 4-fluorobenzyl bromide, employing *t*-BuOK as the base, afforded a clean conversion to the *N*-1 linked products **5** and **6** in excellent yields. Similarly, under the same conditions, we introduced 1-bromo-5-fluoropentane to **3** and **4**, to furnish **7** and **8**, respectively. Disappointingly, the conversion of **4** to **8** did not go to completion, even upon addition of further equivalents of reagents, resulting in a moderate yield. We readily obtained the carboxylic acid derivatives **9**-**12** in high yield by hydrolysis of the respective parent carboxylate derivatives. *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated coupling of carboxylic acids **9**-**12**, with the appropriate alcohol in the presence of DMAP, proceeded as expected to furnish the desired indole-3-carboxylates **13** (FUB-PB-22) and **14** (NM-2201) and indazole-3-carboxylates **15** (FUB-NPB-22) and **16** (5F-NPB-22).

We synthesised fluoropentyl derivative **18** (5F-SDB-005) from naphthyl intermediate **17**, which in turn we accessed *via* a Steglich coupling,(S2) employing *N*,*N*′-dicyclohexylcarbodiimide (DCC) and DMAP (Supplemental Fig. 2). Interestingly, the treatment of **17** with 1-bromo-5-fluoropentane, in the presence of Cs_2CO_3 in DMF, gave the *N*-1/*N*-2 fluoropentyl products **18** and **19** in a ratio of 2:1 in 63% yield. Likewise, when we utilised NaH as the base, we made the same observations. We readily separated **18** and **19** by silica gel chromatography.

Experimental Section

General Procedures

We purchased all chemicals from standard suppliers. We obtained anhydrous solvents as septum-sealed bottles and removed them from the bottle under a nitrogen atmosphere. We monitored reaction progress and compound identity by obtaining retardation factors (R_f values) using Thin-Layer Chromatography (TLC). The TLC plates were aluminium sheets with a silica gel coating ($60F_{254}$) from Merck. We employed a variety of eluting solvents, including 40-60 petrol, EtOAc, CH_2Cl_2 and MeOH. We observed most compounds as non-fluorescent spots on a fluorescent background under UV light at 254 nm. Where appropriate we separated, compound mixtures and purified them using Biotage SP4 automated chromatography system with UV monitoring at 254 nm. We aided structural elucidation of compounds primarily by proton and carbon nuclear magnetic resonance (NMR) techniques but this was assisted by fluorine experiments, as well as 2D COSY methods, employing a Bruker Avance III (500 MHz) spectrometer. We reported chemical shifts in parts per million (ppm) and coupling constants (J) in Hertz (Hz). The spin-multiplicity is abbreviated as: s (singlet), d (doublet), t (triplet), m (indistinguishable multiplet) and combinations thereof. We measured samples in deuterated solvents including $CDCl_3$ and DMSO-d_6. We carried out LC-MS (liquid chromatography mass spectrometry) on a Waters Acquity UPLC system with PDA and ELSD operating in positive and negative ion electrospray mode, employing an Acquity UPLC BEH C18, 1.7 mm, 2.1 x 50 mm column with 0.1% formic acid and water–acetonitrile (5–95%) for gradient elution. We obtained FTIR data for neat samples using an Agilent Cary 630 FTIR Spectrometer and determined melting points using a Stuart Scientific SMP3 apparatus. We obtained Ultraviolet (UV) absorption data using a U-2001 Hitachi Spectrophotometer with the

sample dissolved in ethanol. We obtained High-Resolution Mass Spectrometry (HRMS) services from the EPSRC UK National Mass Spectrometry Facility at Swansea University.

Synthetic Methods

We added **Methyl 1*H*-indole-3-carboxylate (3).**(S3)To 1*H*-indole-3-carboxylic acid **1** (745 mg, 4.62 mmol) in methanol (23 mL) catalytic H_2SO_4 and stirred the reaction mixture at reflux for 8 h. After cooling to room temperature, we added EtOAc and partitioned with water. We extracted the aqueous layer with EtOAc (3 × 20 mL), the organics combined and dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by chromatography [silica; petrol-EtOAc (100:0)→(50:50)] afforded **3** (544 mg, 67%) as an off-white solid; R_f 0.54 (50% EtOAc-petrol); mp 145-147 °C (lit. 144-145.6 °C); UV λ_{max} (EtOH)/nm 280, 213; IR (cm^{-1}) 3230, 1662, 1580, 1526, 1442, 1369, 1317; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (3H, s, OC*H*$_3$), 7.26-7.29 (2H, m, 2 × ArH), 7.40-7.44 (1H, m, ArH), 7.92 (1H, d, *J* = 3.0 Hz, ArH), 8.18-8.21 (1H, m, ArH), 8.69 (1H, br s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 51.1, 108.9, 111.5, 121.5, 122.1, 123.3, 125.8, 131.0, 136.1, 165.7; LRMS (ES^-) *m/z* 174.1 $[M-H]^-$; HRMS calculated for $C_{10}H_{10}NO_2$ $[M+H]^+$ 176.0712; found 176.0704.

Methyl 1*H*-indazole-3-carboxylate (4).(S4)To 1*H*-indazole-3-carboxylic acid **2** (1.11 g, 6.88 mmol) in MeOH (34 mL) we added catalytic H_2SO_4 and stirred the reaction mixture at reflux for 4 h. After cooling to room temperature, we added EtOAc and partitioned with water. We extracted the aqueous layer with EtOAc (3 × 20 mL), the organics combined and dried ($MgSO_4$), filtered and concentrated to afford **4** (1.17 g, 96%) as a white solid; R_f 0.26 (50% EtOAc:Petrol); mp 164-165.5 °C (lit. 162-163 °C); UV λ_{max} (EtOH)/nm 294, 257; IR (cm^{-1}) 3156, 3128, 2920, 1727, 1625, 1461, 1400, 1353, 1266, 1228; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.03 (3H, s, C*H*$_3$), 7.27-7.31 (1H, m, H-5), 7.40-7.44 (1H, m, H-6), 7.67 (1H, d, *J* = 8.5 Hz, H-

4), 8.16 (1H, d, J = 8.3 Hz, H-7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.2, 111.1, 121.8, 122.3, 123.6, 127.8, 136.1, 141.2, 163.0; LRMS (ES^-) *m/z* 175.0 $[M-H]^-$; HRMS calculated for $C_9H_9N_2O_2$ $[M+H]^+$ 177.0664; found 177.0654.

Methyl 1-(4-fluorobenzyl)-1*H*-indole-3-carboxylate (5). (S5) To a solution of methyl 1*H*-indole-3-carboxylate **3** (218 mg, 1.24 mmol) in dry THF (6.2 mL) at 0 °C we slowly added *t*-BuOK (154 mg, 1.37 mmol) and stirred the solution at room temperature for 1 h. After recooling to 0 °C, we added 4-fluorobenzyl bromide (0.25 mL, 1.98 mmol) and stirred the reaction mixture at room temperature for 16 h before quenching with H_2O (10 mL). We extracted the aqueous layer with EtOAc (2 × 15 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol- EtOAc (100:0)→(50:50)] afforded **5** (311 mg, 89%) as an off-white solid; R_f 0.76 (50% EtOAc-petrol); mp 80.5-82.0 °C; (lit. 78 °C); UV λ_{max} (EtOH)/nm 288, 213; IR (cm^{-1}) 2946, 1689, 1604, 1533, 1463, 1379, 1223; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.92 (3H, s, C*H*$_3$), 5.31 (2H, s, C*H*$_2$), 7.00-7.03 (2H, m, H-3' and H-5'), 7.12-7.15 (2H, m, H-2' and H-6'), 7.25-7.30 (3H, m, 3 × ArH), 7.81 (1H, s, H-2), 8.18-8.21 (1H, m, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.1, 51.0, 107.8, 110.2, 116.0 (d, J = 21.8 Hz), 122.0 (d, J = 30.9 Hz), 123.1, 126.8, 128.8, 128.6, 131.7 (d, J = 3.3 Hz), 134.3, 136.6, 162.5 (d, J = 247.3 Hz, C-F), 165.4; ^{19}F NMR (470 MHz, $CDCl_3$) -113.8; LRMS (ES^+) *m/z* 306.3 $[M+Na]^+$; HRMS calculated for $C_{17}H_{15}FNO_2$ $[M+H]^+$ 284.1087; found 284.1081.

Methyl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate (6).(S6) To a solution of methyl 1*H*-indazole-3-carboxylate **4** (463 mg, 2.63 mmol) in dry THF (13 mL) at 0 °C we slowly added *t*-BuOK (325 mg, 2.89 mmol) and stirred the solution at room temperature for 1 h. After recooling to 0 °C, we added 4-fluorobenzyl bromide (0.32 mL, 2.61 mmol) and stirred the reaction mixture at room temperature for 16 h before quenching with H_2O (15 mL). We extracted the aqueous layer with EtOAc (2 × 20 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol-EtOAc (100:0)→(50:50)]

afforded **6** (626 mg, 84%) as an off-white solid; R_f 0.64 (50% EtOAc:petrol); mp 82.8-84.3 °C; UV λ_{max} (EtOH)/nm 299, 206; IR (cm^{-1}) 3068, 2952, 1711, 1602, 1505, 1479, 1440, 1403, 1320, 1267, 1233; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.08 (3H, s, CH_3), 5.70 (2H, s, CH_2), 7.01 (2H, t, J = 8.5 Hz, H-4 and ArH), 7.22-7.25 (2H, m, H-3' and H-5'), 7.34-7.41 (3H, m, ArH, H-2' and H-6'), 8.27 (1H, d, J = 8.0 Hz, H-7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.1, 53.4, 109.9, 115.8 (d, J = 21.7 Hz), 122.4, 123.3, 124.2, 127.2, 129.0 (d, J = 8.2 Hz), 131.4 (d, J = 3.2 Hz), 135.2, 140.5, 162.5 (d, J = 245.4 Hz, C-F), 163.0; ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.8; LRMS (ES^+) *m/z* 285.1 $[M+H]^+$;HRMS calculated for $C_{16}H_{14}FN_2O_2$ $[M+H]^+$ 285.1039; found 285.1032.

Methyl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate (7). To a solution of methyl 1*H*-indole-3-carboxylate **3** (243 mg, 1.39 mmol) in dry THF (7.0 mL) at 0 °C we slowly added *t*-BuOK (172 mg, 1.53 mmol) and stirred the solution at room temperature for 1 h. After recooling to 0 °C, we added 1-bromo-5-fluoropentane (0.28 mL, 2.22 mmol) and stirred the reaction mixture at room temperature for 16 h before quenching with H_2O (5 mL). We extracted the aqueous layer with EtOAc (2 × 10 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol-EtOAc (100:0)→(50:50)] afforded **7** (250 mg, 68%) as an off-white solid; R_f 0.76 (50% EtOAc:etrol); mp 130-132 °C; UV λ_{max} (EtOH)/nm 289, 216; IR (cm^{-1}) 2944, 1691, 1531, 1464, 1379, 1204; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42-1.49 (2H, m, $NCH_2CH_2CH_2CH_2CH_2F$), 1.65-1.77 (2H, m, $NCH_2CH_2CH_2CH_2CH_2F$), 1.89-1.96 (2H, m, $NCH_2CH_2CH_2CH_2CH_2F$), 3.91 (3H, s, CH_3), 4.17 (2H, t, J = 7.1 Hz, $NCH_2CH_2CH_2CH_2CH_2F$), 4.37 (1H, t, J = 5.9 Hz, $NCH_2CH_2CH_2CH_2CH_2F$), 4.46 (1H, t, J = 5.9 Hz, $NCH_2CH_2CH_2CH_2CH_2F$), 7.26-7.29 (2H, m, 2 × ArH), 7.34-7.37 (1H, m, ArH), 7.81 (1H, s, H-4), 8.16-8.19 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.8 (d, J = 5.0 Hz), 29.6, 29.9 (d, J = 19.7 Hz), 46.9, 51.0, 83.6 (d, J = 163.8 Hz), 107.1, 110.0, 121.8,

121.9, 122.7, 126.7, 134.1, 136.5, 165.5; ^{19}F NMR (470 MHz, $CDCl_3$) -113.8; LRMS (ES^+) *m/z* 264.1 $[M+H]^+$; HRMS calculated for $C_{15}H_{19}FNO_2$ $[M+H]^+$ 264.1400; found 264.1392.

Methyl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate (8). To a solution of methyl 1*H*-indazole-3-carboxylate **4** (489 mg, 2.78 mmol) in dry THF (14.0 mL) at 0 °C we slowly added *t*-BuOK (343 mg, 3.06 mmol) and stirred the solution at room temperature for 1 h. After recooling to 0 °C, we added 1-bromo-5-fluoropentane (0.55 mL, 4.45 mmol) and stirred the reaction mixture at room temperature for 16 h before quenching with H_2O (10 mL). We extracted the aqueous layer with EtOAc (2 × 20 ml), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol-EtOAc (100:0)→(20:80)] afforded **8** (145 mg, 20%) as a colourless gum (279 mg of unreacted starting material recovered); R_f 0.59 (50% EtOAc:petrol); UV λ_{max} (EtOH)/nm 300, 208; IR (cm^{-1}) 2950, 1708, 1477, 1439, 1407; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42-1.48 (2H, m, $NCH_2CH_2C\mathit{H}_2CH_2CH_2F$), 1.66-1.75 (2H, m, $NCH_2CH_2CH_2C\mathit{H}_2CH_2F$), 1.99-2.05 (2H, m, $NCH_2C\mathit{H}_2CH_2CH_2CH_2F$), 4.12 (3H, s, $C\mathit{H}_3$), 4.35 (1H, t, J = 6.0 Hz, $NCH_2CH_2CH_2CH_2C\mathit{H}_2F$), 4.44-4.50 (3H, m, $NC\mathit{H}_2CH_2CH_2CH_2C\mathit{H}_2F$), 7.30-7.33 (1H, m, ArH), 7.42-7.47 (2H, m, 2 × ArH), 8.23 (1H, d, J = 8.2 Hz, H-7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.7 (d, J = 5.0 Hz), 29.5, 30.0 (d, J = 19.6 Hz), 49.7, 52.0, 83.7 (d, J = 163.9 Hz), 109.5, 122.3, 123.1, 123.8, 126.9, 134.7, 140.5, 163.1; LRMS (ES^+) *m/z* 287.0 $[M+Na]^+$; HRMS calculated for $C_{14}H_{18}FN_2O_2$ $[M+H]^+$ 265.1352; found 265.1346.

1-(4-Fluorobenzyl)-1*H*-indole-3-carboxylic acid (9).(S7) We dissolved Methyl 1-(4-fluorobenzyl)-1*H*-indole-3-carboxylate **5** (452 mg, 1.60 mmoL) in MeOH (3.2 mL) and added 1N NaOH (3.2 mL) at room temperature. We heated the reaction mixture at reflux for 4 h, cooled to room temperature, neutralised with glacial acetic acid and removed the solvent *in vacuo* to afford **9** (390 mg, 91%) as an off-white solid; R_f 0.53 (50% EtOAc:petrol); mp 205.4-206.0 °C (lit. 190-192 °C); UV λ_{max} (EtOH)/nm 288, 212; IR (cm^{-1}) 3105, 1653, 1526, 1507,

1463, 1365, 1276, 1223; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (2H, s, CH_2), 6.99-7.05 (2H, m, H-3' and H-5'), 7.12-7.17 (2H, m, H-2' and H-6'), 7.26-7.32 (3H, m, 3 × ArH), 7.91 (1H, s, H-4), 8.22-8.25 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.2, 107.0, 110.3, 116.1 (d, J = 21.7 Hz), 122.0, 122.5, 123.3, 127.1, 128.9 (d, J = 8.1 Hz), 135.5, 163.7 (not all carbons are visible); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.6; LRMS (ES^-) *m/z* 268.1 $[M-H]^-$; HRMS calculated for $C_{16}H_{13}FNO_2$ $[M+H]^+$ 270.0930; found 270.0924.

1-(4-Fluorobenzyl)-1*H*-indazole-3-carboxylic acid (10).(S6) We dissolved Methyl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate **6** (670 mg, 2.36 mmoL) in MeOH (5.0 mL) and added 1N NaOH (5.0 mL) at room temperature. We heated the reaction mixture at reflux for 4 h, cooled to room temperature, neutralised with glacial acetic acid and removed the solvent *in vacuo* to afford **10** (552 mg, 87%) as a white solid; R_f 0.10 (50% EtOAc:petrol); mp 198.4-200.1 ^{o}C; UV λ_{max} (EtOH)/nm 299, 276, 212; IR (cm^1) 1681, 1600, 1508, 1435, 1376, 1276, 1238; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.68 (2H, s, CH_2), 7.00 (2H, t, J = 8.6 Hz, 2 × ArH), 7.21-7.26 (2H, m, 2 × ArH), 7.32-7.44 (3H, m, 3 × ArH), 8.27 (1H, d, J = 8.2 Hz, H-7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.5, 110.0, 115.9 (d, J = 21.6 Hz), 122.4, 123.8, 124.1, 127.4, 129.2 (d, J = 8.3 Hz), 131.2 (d, J = 3.3 Hz), 134.5, 140.8, 161.6, 163.7 (not all carbons are visible); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.6; LRMS (ES^-) *m/z* 225.2 $[M-COOH]^-$; HRMS calculated for $C_{15}H_{12}FN_2O_2$ $[M+H]^+$ 271.0883; found 271.0878.

1-(5-Fluoropentyl)-1*H*-indole-3-carboxylic acid (11). We dissolved Methyl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate **7** (382 mg, 1.45 mmoL) in MeOH (3.0 mL) and added 1N NaOH (3.0 mL) at room temperature. We heated the reaction mixture at reflux for 4 h, cooled to room temperature, neutralised with glacial acetic acid and removed the solvent *in vacuo* to afford **11** (301 mg, 83%) as an off-white solid; R_f 0.45 (50% EtOAc:Petrol); mp 122.2-123.6 ^{o}C; UV λ_{max} (EtOH)/nm 287, 216; IR (cm^{-1}) 3047, 2935, 1637, 1523, 1465, 1438, 1396, 1271, 1208; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42-1.51 (2H, m, $NCH_2CH_2CH_2CH_2CH_2F$),

1.65-1.78 (2H, m, $NCH_2CH_2CH_2C\mathit{H}_2CH_2F$), 1.89-1.97 (2H, m, $NCH_2C\mathit{H}_2CH_2CH_2CH_2F$), 4.18 (2H, t, J = 7.0 Hz, $NCH_2CH_2CH_2CH_2C\mathit{H}_2F$), 4.37 (1H, t, J = 5.9 Hz, $NC\mathit{H}_2CH_2CH_2CH_2CH_2F$), 4.46 (1H, t, J = 5.9 Hz, $NC\mathit{H}_2CH_2CH_2CH_2CH_2F$), 7.27-7.32 (2H, m, 2 × ArH), 7.35-7.40 (1H, m, ArH), 7.91 (1H, s, H-4), 8.21-8.25 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.8 (d, J = 4.9 Hz), 29.5, 29.9 (d, J = 19.6 Hz), 47.0, 83.6 (d, J = 164.0 Hz), 106.4, 110.0, 122.0, 122.2, 122.9, 127.0, 135.3, 136.6, 169.9; LRMS (ES^-) *m/z* 248.2 $[M-H]^-$; HRMS calculated for $C_{14}H_{17}FNO_2$ $[M+H]^+$ 250.1243; found 250.1236.

1-(5-Fluoropentyl)-1*H*-indazole-3-carboxylic acid (12). We dissolved Methyl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate **8** (145 mg, 0.549 mmoL) in MeOH (1.1 mL) and added 1N NaOH (1.1 mL) at room temperature. We heated the reaction mixture at reflux for 4 h, cooled to room temperature, neutralised with glacial acetic acid and removed the solvent *in vacuo* to afford **12** (120 mg, 87%) as an off-white solid; R_f 0.12 (50% EtOAc:petrol); mp 218 ^oC (dec.); UV λ_{max} (EtOH)/nm 299, 273, 210; IR (cm^{-1}) 2947, 1608, 1484, 1429, 1321, 1221; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21-1.35 (2H, m, $NCH_2CH_2C\mathit{H}_2CH_2CH_2F$), 1.54-1.71 (2H, m, $NCH_2CH_2CH_2C\mathit{H}_2CH_2F$), 1.77-1.91 (2H, m, $NCH_2C\mathit{H}_2CH_2CH_2CH_2F$), 4.27-4.46 (4H, m, $NCH_2CH_2CH_2CH_2C\mathit{H}_2F$ and $NC\mathit{H}_2CH_2CH_2CH_2CH_2F$), 7.07-7.15 (1H, m, H-5), 7.29-7.36 (1H, m, H-6), 7.59 (1H, d, J = 8.4 Hz, H-4), 8.24 (1H, d, J = 8.0 Hz, H-7); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.4 (d, J = 5.4 Hz), 29.6, 29.8 (d, J = 19.1 Hz), 48.7, 84.1 (d, J = 160.8 Hz), 110.2, 121.5, 123.2, 123.5, 126.4, 140.8, 142.0, 166.6; ^{19}F NMR (470 MHz, DMSO-d_6) δ -115.1; LRMS (ES^+) *m/z* 251.2 $[M+H]^+$; HRMS calculated for $C_{13}H_{16}FN_2O_2$ $[M+H]^+$ 251.1196; found 251.1192.

Quinolin-8-yl 1-(4-fluorobenzyl)-1*H*-indole-3-carboxylate (13). We dissolved 1-(4-Fluorobenzyl)-1*H*-indole-3-carboxylic acid **9** (72 mg, 0.267 mmol) in CH_2Cl_2 (2 mL) and added DMF dropwise until dissolved fully. We added 8-Hydroxyquinoline (54 mg, 0.374 mmol) at room temperature, followed by EDC.HCl (89 mg, 0.465 mmol) and DMAP (1.6 mg,

0.01 mmol). We stirred the reaction mixture for 18 h at room temperature then poured into H_2O (5 ml) and extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (5 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo.* Purification by chromatography [silica; petrol-EtOAc (100:0)→(30:70)] afforded **13** (97 mg, 92%) as a white foam; R_f 0.52 (50% EtOAc:petrol); mp 100-102.5 ^oC; UV λ_{max} (EtOH)/nm 300, 229; IR (cm^{-1}) 1704, 1598, 1528, 1508, 1462, 1378, 1311, 1222; ^1H NMR (500 MHz, $CDCl_3$) δ 5.38 (2H, s, C*H*$_2$), 7.01-7.07 (2H, m, H-3' and H-5'), 7.19-7.36 (6H, m, H-2', H-6' and 4 × ArH), 7.42-7.49 (1H, m, ArH), 7.58-7.67 (1H, m, ArH), 7.75-7.78 (1H, m, ArH), 8.20-8.27 (1H, m, ArH), 8.29-8.34 (1H, m, ArH), 8.95 (1H, br s, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.3, 110.3, 116.0 (d, J = 21.6 Hz), 121.6, 122.2, 122.5, 123.3, 125.6, 127.5, 129.0 (d, J = 8.2 Hz), 129.6, 131.6, 136.8 (not all carbons are visible); LRMS (ES^+) *m/z* 397.2 $[M+H]^+$; HRMS calculated for $C_{25}H_{18}FN_2O_2$ $[M+H]^+$ 397.1352; found 397.1340.

Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate (14). We dissolved 1-(5-Fluoropentyl)-1*H*-indole-3-carboxylic acid **11** (67.5 mg, 0.271 mmol) in CH_2Cl_2 (2 mL) and added DMF dropwise until dissolved fully. We added 1-Napthol (55 mg, 0.379 mmol) at room temperature, followed by EDC.HCl (90 mg, 0.471 mmol) and DMAP (1.7 mg, 0.013 mmol). We stirred the reaction mixture for 18 h at room temperature then poured into H_2O (5 ml) and extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (5 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo.* Purification by chromatography [silica; petrol-EtOAc (100:0)→(30:70)] afforded **14** (79 mg, 77%) as a yellow gum; R_f 0.67 (50% EtOAc:petrol); UV λ_{max} (EtOH)/nm 294, 220; IR (cm^{-1}) 3054, 2936, 1711, 1527, 1461, 1374, 1257, 1200; ^1H NMR (500 MHz, $CDCl_3$) δ 1.44-1.51 (2H, m, $NCH_2CH_2CH_2$), 1.64-1.76 (2H, m, CH_2CH_2F), 1.91-1.98 (2H, m, NCH_2CH_2), 4.20 (2H, t, J = 7.2 Hz, NCH_2), 4.35 (1H, t, J = 5.9 Hz, CH_2F), 4.44 (1H, t, J = 5.9 Hz, CH_2F), 7.24-7.35 (3H, m, 3 × ArH), 7.37-7.48 (4H, m, 4 × ArH), 7.70-7.73 (1H, m, ArH), 7.81-7.85 (1H, m, ArH), 7.94-7.98 (1H, m, ArH), 8.06 (1H, s, H-2), 8.23-

8.26 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.9 (d, J = 4.8 Hz), 29.5, 30.0 (d, J = 19.7 Hz), 47.1, 83.6 (d, J = 164.0 Hz), 106.2, 110.2, 118.6, 121.6, 122.1, 122.4, 123.2, 125.6, 125.7, 126.3, 127.0, 127.6, 128.0, 134.8, 135.3, 136.7, 146.9, 163.3; LRMS (ES^+) *m/z* 398.2 $[M+Na]^+$; HRMS calculated for $C_{24}H_{23}FNO_2$ $[M+H]^+$ 376.1713; found 376.1706.

Quinolin-8-yl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate (15). We dissolved 1-(4-Fluorobenzyl)-1*H*-indazole-3-carboxylic acid **10** (144 mg, 0.533 mmol) in CH_2Cl_2 (4.6 mL) and added DMF dropwise until dissolved fully. We added 8-Hydroxyquinoline (108 mg, 0.746 mmol) at room temperature, followed by EDC.HCl (178 mg, 0.927 mmol) and DMAP (3.0 mg, 0.03 mmol). We stirred the reaction mixture for 18 h at room temperature then poured into H_2O (10 ml) and extracted with CH_2Cl_2 (3 × 20 mL), washed with brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo.* Purification by chromatography [silica; petrol-EtOAc (100:0)→(40:60)] afforded **15** (160 mg, 75%) as an off-white solid; R_f 0.48 (50% EtOAc:petrol); mp 136.4-137.7 ºC; UV λ_{max} (EtOH)/nm 301, 227; IR (cm^{-1}) 3053, 1730, 1600, 1509, 1467, 1409, 1388, 1312, 1214; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (2H, s, CH_2), 7.00-7.06 (2H, m, 2 × ArH), 7.29-7.36 (3H, m, 3 × ArH), 7.38-7.46 (3H, m, 3 × ArH), 7.60-7.63 (1H, m, ArH), 7.70 (1H, dd, J = 1.3, 7.5 Hz, ArH), 7.80 (1H, dd, J = 1.3, 8.2 Hz, ArH), 8.23 (1H, dd, J = 1.6, 10.0 Hz, ArH), 8.37-8.39 (1H, m, ArH), 8.92 (1H, dd, J = 1.7, 4.2 Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.7, 110.0, 115.9 (d, J = 21.6 Hz), 121.8, 122.0, 122.7, 123.6, 124.9, 126.1, 126.4, 127.2, 129.2 (d, J = 8.2 Hz), 129.6, 131.4 (d, J = 3.2 Hz), 134.6, 136.2, 140.7, 147.2, 150.5, 161.1, 161.6, 163.5 (not all carbons are visible); ^{19}F NMR (470 Hz, $CDCl_3$) -113.8; LRMS (ES^+) *m/z* 398.1 $[M+H]^+$; HRMS calculated for $C_{24}H_{17}FN_3O_2$ $[M+H]^+$ 398.1305; found 398.1292.

Quinolin-8-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate (16). We dissolved 1-(5-Fluoropentyl)-1*H*-indazole-3-carboxylic acid **12** (120 mg, 0.480 mmol) in CH_2Cl_2 (3.7 mL)

and added DMF dropwise until dissolved fully. We added 8-Hydroxyquinoline (97 mg, 0.672 mmol) at room temperature, followed by EDC.HCl (160 mg, 0.835 mmol) and DMAP (3.0 mg, 0.02 mmol). We stirred the reaction mixture for 18 h at room temperature then poured into H_2O (10 ml) and extracted with CH_2Cl_2 (3 × 20 mL), washed with brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol-EtOAc (100:0)→(40:60)] afforded **16** (125 mg, 69%) as a pale yellow solid; R_f 0.42 (50% EtOAc:petrol); mp 123-125 ^oC; UV λ_{max} (EtOH)/nm 301, 228; IR (cm^{-1}) 2946, 2867, 1726, 1465, 1414, 1388, 1312, 1233, 1202; ^1H NMR (500 MHz, $CDCl_3$) δ 1.17-1.57 (2H, m, $NCH_2CH_2CH_2$), 1.70-1.82 (2H, m, CH_2CH_2F), 2.07-2.13 (2H, m, NCH_2CH_2), 4.40 (1H, t, J = 6.0 Hz, CH_2F), 4.49 (1H, t, J = 6.0 Hz, CH_2F), 4.58 (2H, t, J = 7.3 Hz, NCH_2), 7.32-7.36 (1H, m, ArH), 7.41-7.44 (1H, m, ArH), 7.46-7.50 (1H, m, ArH), 7.52-7.55 (1H, m, ArH), 7.58-7.62 (1H, m, ArH), 7.69 (1H, dd, J = 1.3, 7.5 Hz, ArH), 7.77-7.80 (1H, m, ArH), 8.21 (1H, dd, J = 1.7, 3.3 Hz, ArH), 8.37 (1H, d, J = 8.2 Hz, ArH), 8.90 (1H, dd, J = 1.7, 4.2 Hz, ArH), ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.8 (d, J = 5.1 Hz), 29.5, 30.0 (d, J = 19.7 Hz), 49.9, 83.7 (d, J = 163.8 Hz), 109.6, 121.7, 121.9, 122.6, 123.4, 124.5, 126.0, 126.3, 126.9, 129.6, 134.2, 136.0, 140.8, 141.4, 147.4, 150.6, 161.4; LRMS (ES^+) *m/z* 400.3 $[M+Na]^+$; HRMS calculated for $C_{22}H_{21}FN_3O_2$ $[M+H]^+$ 378.1618; found 378.1610.

Naphthalen-1-yl 1*H*-indazole-3-carboxylate (17). We charged a round bottomed flask with 1*H*-indazole-3-carboxylic acid **2** (183 mg, 1.13 mmol), 1-napthol (326 mg, 2.26 mmol), DMAP (7 mg) and added anhydrous CH_2Cl_2 (5.6 mL) under a nitrogen atmosphere. We stirred the solution and cooled to 0 ^oC in an ice-bath. We added *N*,*N'*-Dicyclohexylcarbodiimide (256 mg, 1.24 mmol) portionwise over 5 min and after a further 5 minutes at 0 ^oC, removed the cooling and stirred the reaction mixture for 18 h at room temperature. We filtered the reaction mixture and washed the filtrate with 0.5 M HCl (2 × 10 mL), followed by saturated aqueous sodium bicarbonate (2 × 10 mL). We dried the organics ($MgSO_4$), filtered and the solvent removed *in*

vacuo. Purification by chromatography [silica; petrol:EtOAc (100:0)→(50:50)] afforded **17** (197 mg, 60%) as an off-white solid; R_f 0.52 (50% EtOAc:petrol); mp 194.0-195.1 °C; UV λ_{max} (EtOH)/nm 293, 220; IR (cm^{-1}) 3274, 2932, 2855, 1695, 1621, 1522, 1444, 1383, 1343, 1299, 1219; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.38-7.42 (1H, m, ArH), 7.52-7.66 (5H, m, 5 × ArH), 7.80 (1H, d, J = 8.5 Hz, ArH), 7.91-7.97 (2H, m, 2 × ArH), 8.07 (1H, d, J = 7.5 Hz, ArH), 8.16-8.18 (1H, m ArH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.9, 119.3, 121.3, 121.4, 123.1, 124.0, 126.3, 126.6, 127.0, 127.2, 127.4, 127.5, 128.6, 134.5, 134.7, 141.7, 146.5, 161.4; LCMS (ES^-) *m/z* 287.0 $[M-H]^-$; HRMS calculated for $C_{18}H_{13}N_2O_2$ $[M+H]^+$ 289.0977; found 289.0966.

Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate (18) and Naphthalen-1-yl 2-(5-fluoropentyl)-2*H*-indazole-3-carboxylate (19)

We dissolved Naphthalen-1-yl 1*H*-indazole-3-carboxylate **17** (184 mg, 0.638 mmol) in anhydrous DMF (3.2 mL) and added 1-bromo-5-fluoropentane (0.09 mL, 0.702 mmol) and Cs_2CO_3 (249 mg, 0.766 mmol) sequentially at room temperature under a nitrogen atmosphere. We stirred the reaction mixture for 18 h then partitioned between water and EtOAc. We washed the organic layer with saturated aqueous NaCl:H_2O (3 × 10:10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol:EtOAc (100:0)→(50:50)] allowed separation of the title compounds **18** (103 mg, *N*-1 linked, slow running) and **19** (47 mg, *N*-2 linked, fast running).

To a stirred solution of naphthalen-1-yl 1*H*-indazole-3-carboxylate **17** (67 mg, 0.232 mmol) in anhydrous DMF (1.2 mL) at 0 °C, we added NaH (60% dispersion in mineral oil, 12.2 mg, 0.302 mmol) portionwise over 5 min. We gradually warmed the reaction mixture to room temperature and stirred for 45 min before cooling again to 0 °C. We added 1-Bromo-5-fluoropentane (0.03 ml, 0.278 mmol) and stirred at room temperature for 16 h. We poured the

reaction mixture into crushed ice, stirred for 10 min and extracted with EtOAc (2 × 20 mL). We washed the combined organics with H_2O (10 mL), brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Purification by chromatography [silica; petrol:EtOAc (100:0)→(50:50)] allowed separation of the title compounds **18** (35 mg, *N*-1 linked, slow running) and **19** (16 mg, *N*-2 linked, fast running).

We combined the *N*-1 linked products and re-purified [silica; CH_2Cl_2-MeOH (100:0)→(95:5)] to afford **18** (106 mg) as an off-white solid; R_f 0.71 (50% EtOAc:petrol); mp 67.7-69.0 °C; UV λ_{max} (EtOH)/nm 249, 200; IR (cm^{-1}) 3059, 2954, 1731, 1598, 1467, 1385, 1222; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51-1.58 (2H, m, $NCH_2CH_2CH_2$), 1.72-1.84 (2H, m, CH_2CH_2F), 2.08-2.16 (2H, m, NCH_2CH_2), 4.41 (1H, t, J = 6.0 Hz, CH_2F), 4.51 (1H, t, J = 6.0 Hz, CH_2F), 4.61 (2H, t, J = 7.3 Hz, NCH_2), 7.35-7.40 (1H, m, ArH), 7.44-7.59 (6H, m, 6 × ArH), 7.81 (1H, d, J = 8.3 Hz, ArH), 7.91 (1H, d, J = 7.5 Hz, ArH), 8.04 (1H, d, J = 8.2 Hz, ArH), 8.34 (1H, d, J = 7.4 Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.8 (d, J = 5.0 Hz), 29.5, 30.0 (d, J = 19.7 Hz), 50.0, 83.7 (d, J = 163.9 Hz), 109.8, 118.6, 121.6, 122.4, 123.6, 124.3, 125.5, 126.2, 126.5, 126.5, 127.1, 127.2, 128.0, 134.8, 140.8, 146.6, 161.2; LRMS (ES^+) *m/z* 377.4 $[M+H]^+$; HRMS calculated for $C_{23}H_{22}FN_2O_2$ $[M+H]^+$ 377.1665; found 377.1653.

We combined the *N*-2 linked products and re-purified [silica; CH_2Cl_2-MeOH (100:0)→(95:5)] to afford **19** (46 mg) as an off-white solid; R_f 0.81 (50% EtOAc:petrol); mp 84.2-85.4 °C; UV λ_{max} (EtOH)/nm 318, 299, IR (cm^{-1}) 3057, 2957, 1723, 1598, 1508, 1456, 1387, 1332, 1275; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47-1.57 (2H, m, $NCH_2CH_2CH_2$) 1.66-1.79 (2H, m, CH_2CH_2F), 2.03-2.11 (2H, m, NCH_2CH_2), 4.36 (1H, t, J = 6.1 Hz, CH_2F), 4.45 (1H, t, J = 6.1 Hz, CH_2F), 4.97 (2H, t, J = 7.4 Hz, NCH_2), 7.35-7.59 (6H, m, 6 × ArH), 7.82-7.98 (4H, m, 4 × ArH), 8.27-8.31 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.5 (d, J = 5.4 Hz), 29.9 (d, J = 19.6 Hz), 30.5, 53.7, 83.8 (d, J = 163.7 Hz), 118.6, 118.6, 121.1, 121.5, 122.6, 124.1, 125.5, 125.9,

126.6, 126.6, 126.7, 126.9, 127.0, 128.2, 134.8, 146.0, 147.6, 158.9; LRMS (ES^+) *m/z* 377.1 $[M+H]^+$; HRMS calculated for $C_{23}H_{22}FN_2O_2$ $[M+H]^+$ 377.1665 found 377.3447.

Supplemental Material References

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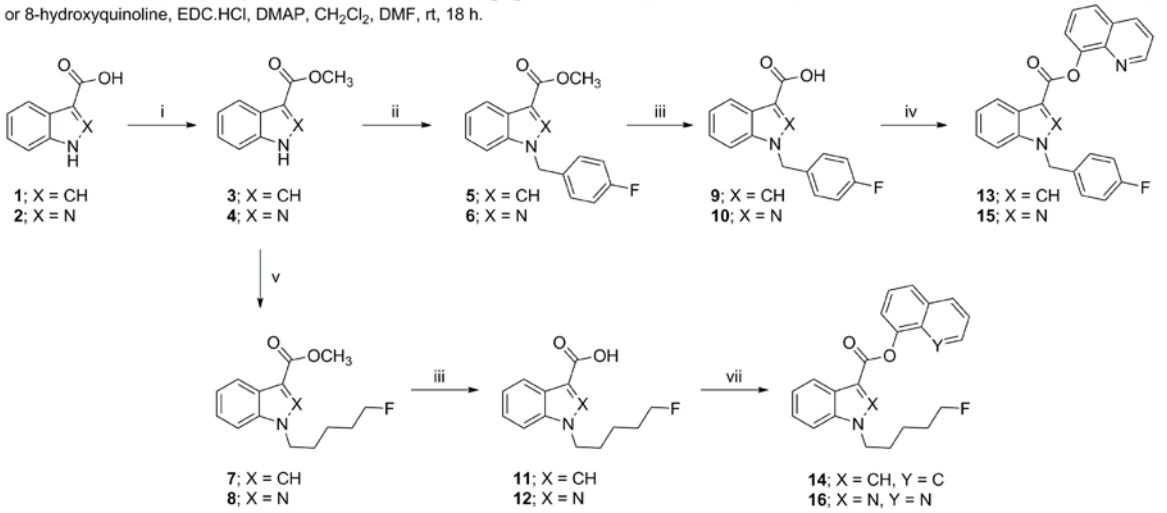
Scheme 1. Reagents and Conditions - (i) catalytic conc. H_2SO_4, MeOH, reflux, 4 h; (ii) 4-fluorobenzyl bromide, *t*-BuOK, THF, 0 °C - rt, 16 h; (iii) 1N NaOH, MeOH, reflux, 4 h; (iv) 8-hydroxyquinoline, EDC.HCl, DMAP, CH_2Cl_2, DMF, rt, 18 h; (v) 1-bromo-5-fluoropentane, *t*-BuOK, THF, 0 °C - rt, 16 h; (vii) 1-napthol or 8-hydroxyquinoline, EDC.HCl, DMAP, CH_2Cl_2, DMF, rt, 18 h.



Scheme 2. Reagents and Conditions - (i) DCC, DMAP, CH_2Cl_2, 0 °C - rt, 18 h; (ii) NaH or Cs_2CO_3, 1-bromo-5-fluoropentane, DMF, 0 °C - rt, 16 h.