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Human Toxicity Caused by Indole and Indazole Carboxylate Synthetic Cannabinoid Receptor Agonists: From Horizon Scanning to Notification

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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 synthesized 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 analyzed plasma and urine samples from 160 patients presenting to emergency departments with severe toxicity after suspected NPS use during 2015 to 2016 for these and other NPS using data-independent LC-MS/MS.

RESULTS: We successfully synthesized 5 carboxylate SCRAs using established synthetic and analytical chemistry methodologies. We identified at least 1 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 and aggression (82%), reduced consciousness (76%), acidosis (47%), hallucinations and 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.

The emergence of novel psychoactive substances (NPS)[8] during recent decades, and particularly synthetic cannabinoid receptor agonists (SCRA), 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 professionals, 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

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[8] Nonstandard abbreviations: NPS, novel psychoactive substances; SCRA, synthetic cannabinoid receptor agonists; EMCDDA, European Monitoring Centre for Drugs and Drug Addiction; IONA, Identification Of Novel psychoActive Substances study; ALT, alanine transaminase; TOF, time of flight; SWATH, Sequential Window Acquisition of all Theoretical fragment-ion spectra; MRM, multiple reaction monitoring; LOD, limit of detection; LOQ, limit of quantification.

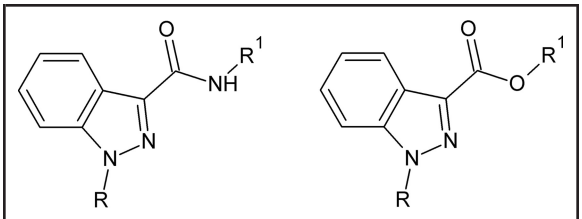

Fig. 1. Comparison of the chemical structure of indazole carboxamide (left) and indazole carboxylate (right) SCRAs.

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 characterize 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*) synthesizing appropriate reference standards, (*c*) analyzing 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, with 1 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 SCRAs 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, 11, 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 *(15–17)*, and BB-22 *(18)*. Although 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 SCRAs BB-22 and 5F-PB-22 *(11)*. Therefore, it appeared probable that toxicity associated with indole or indazole carboxylate SCRAs would become increasingly frequent in clinical practice.

To investigate this expectation, we synthesized 2 indole (FUB-PB-22, NM-2201) and 3 indazole (FUB-NPB-22, 5F-NPB-22, 5F-SDB-005) carboxylate SCRAs that were commercially unavailable at the time. These compounds were added to reference standards used for analysis of biological samples obtained from a multicenter 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 Supplemental Material file that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue2.

PARTICIPANT RECRUITMENT

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

CLINICAL SURVEILLANCE

The IONA study recruited patients >16 years of age presenting to participating emergency departments in 16 hospitals in England, Wales, and Scotland with severe toxicity suspected to be caused by exposure to a novel psychoactive substance. Severe toxicity criteria included fever (>38.5 °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) behavioral disturbance, arrhythmia, chest pain, electrocardiogram evidence of cardiac ischemia or myocardial infarction, acidosis (arterial or venous pH <7.35 or bicarbonate <20 mmol/L),

severe electrolyte or fluid disturbances, hypoglycemia (<1.7 mmol/L), methemoglobinemia (>50%), tachycardia (>140 beats/min), systolic hypertension or hypotension (>180 or <80 mmHg), acute kidney injury, increased creatine kinase (>1000 IU/L), alanine transaminase (ALT) or aspartate transaminase (>300 IU/L), prothrombin time (>15 s), or international normalized ratio (>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 a linked-anonymized format identified by a specific code number that could be linked with only 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) preconditioned 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 2-stage elution. We first eluted with 500 μL of 1:1:1 methanol/acetonitrile/ethyl acetate, followed by elution with 500 μL of 5% ammonia in 1:1:1 methanol/acetonitrile/ethyl acetate. We combined the 2 eluates, evaporated them to dryness under a stream of nitrogen at 45 °C in a Zymark TurboVap (Biotage), reconstituted the dried extract in 25 μL mobile phase 90:10 (v/v) 0.1% formic acid water/0.1% formic acid acetonitrile, vortex-mixed and centrifuged at 4000*g* for 5 min, and then transferred to amber autosampler vials containing 300-μL glass inserts. We injected 1 μL per analysis.

INSTRUMENTATION

We analyzed and identified NPS by LC-MS/MS. The system consisted of a TripleTOF 5600^+ high-resolution quadrupole time-of-flight (TOF) mass spectrometer (Sciex) equipped with a DuoSpray ion source operated in positive electrospray mode, coupled to an Eksigent Nano LC 420 system, using nontargeted 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 liquid chromatography column (100 mm × 300 μm × 3 μm; HighChrom) fitted with a 0.25-μm column saver precolumn 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 1 min, 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 autosampler temperatures were 25 °C and 8 °C, respectively.

MASS SPECTROMETRY

We analyzed NPS qualitatively using nontargeted 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, which used the fast scanning speeds of quadrupole quadrupole time-of-flight mass spectrometers. SWATH mass spectrometry (Sciex) is a form of data-independent analysis that repeatedly cycles through consecutive preset 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 tandem mass spectrometry mass spectra of all analytes. We detected protonated molecular ions via a TOF mass spectrometry scan covering the 100- to 800-Da mass range. We followed the TOF mass spectrometry scan by SWATH tandem mass spectrometry acquisition in high sensitivity mode at a mass resolution of at least 20 000, with a collision energy spread of 30 ± 15 V over a mass range of 30 to 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). We identified compounds by software-assisted library searching against reference spectra. We performed library searching and analyte identification on tandem mass spectrometry data with LibraryView version 1.0 (Sciex) and ChemSpider Library version 2.0 (Royal Society of Chemistry), integrated within MasterView software. We extracted synthesized NPS (see the online Data Supplement) from plasma and urine and collected SWATH mass spectrometry data. We incorporated TOF mass spectrometry and tandem mass spectrometry data obtained for these compounds into LibraryView software, joining other preexisting 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, utilized to re-

Table 1. MRM transitions of synthesized 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

Q1, quadrupole 1; Q3, quadrupole 3; CE, collision energy; DP, declustering potential; EP, entrance potential; CXP, collision cell exit potential.

move small peaks under a specified intensity, to 5. We used a library match with a purity score >65% and the presence of the molecular ion and 3 characteristic tandem mass spectrometry fragment ions as criteria for identification. We checked positive matches obtained from this search by manual review.

We quantified identified synthetic cannabinoids 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 liquid chromatograph. We used 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 liquid chromatography column (100 mm × 2.1 m × 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 400 μL/min. We held gradient conditions at 5% B, for 1 min, 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 autosampler temperatures were 50 °C and 8 °C, respectively. We optimized tandem mass spectrometry 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 3 sets of samples as described by Matuszewski et al. *(19)*, with 6 data points for each set. We fortified sample set 1, urine and plasma with analytes and internal standards, before solid-phase extraction. We fortified sample set 2, urine and plasma with analytes and internal standards, after solid-phase extraction. 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.

LIMIT OF DETECTION, LIMIT OF QUANTIFICATION, AND LINEARITY

For MRM-based quantification, we evaluated the limit of detection (LOD) over 3 runs with duplicates from 3 different urine and plasma extractions. We defined the LOD as the lowest concentration producing a peak eluting within ±0.1 min of the analyte retention time with a signal-to-noise ratio ≥3:1 and 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 the LOQ as the lowest concentration meeting LOD criteria with a signal-to-noise ratio ≥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 data independent acquisition 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 5 TOF mass spectrometry and SWATH tandem mass spectrometry scans across each peak, reliably acquiring representative spectra for each analyte. We used the following criteria for analyte identification and specificity: (*a*) mass error for the molecular ion <5 ppm, (*b*) retention time error <5%, (*c*) isotopic pattern difference from theoretical <80%, and (*d*) LibraryView library fit score >65%. All 4 criteria had to be fulfilled. We combined this with visual verification of at least 2 extracted ion chromatograms for each analyte, including the molecular ion and the most intense tandem mass spectrometry fragment, plus at least 2 common fragments in the sample and

Table 2. LOD, LOQ, mean extraction efficiencies, and matrix effects for indole/indazole synthetic cannabinoids extracted from urine and plasma by cation-exchange solid-phase extraction.

Analyte	MRM LOD, pg on-column	MRM LOQ, pg on-column	Intraassay precision, % low/high	Interassay 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

reference tandem mass spectrometry spectra. We evaluated LOD over 3 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 analyzing drug-free urine and plasma samples spiked with the low and high concentrations of analytes. We analyzed 5 sets of samples over 5 different days. We estimated repeatability, between-day precision, and intermediate precision, expressed as %CV of calculated concentrations, using 1-way 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 intraassay and interassay analytical recovery and imprecision from 4 replicates at 2 different quality control concentrations, 1 ng/mL and 10 ng/mL of urine or plasma, across the linear dynamic range of the assay. We evaluated interassay imprecision and recovery on 5 different runs with 4 replicates in each run, analyzed on 5 separate days ($n = 20$). We expressed imprecision as %CV of calculated concentrations. We conducted ANOVA on low and high quality controls to evaluate interassay and intraassay differences in analyte concentrations.

Results

The results of the validation procedure with the LOD/LOQ, precision, recovery, and matrix effect are summarized in Table 2 for data-independent SWATH and targeted MRM approaches.

We launched the IONA study in March 2015 and by December 31, 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 1 SCRA in 49 of 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; BB-22, 1) and indazoles in 12 (5F-NPB-22, 10; FUB-NPB-22, 1; 5F-SDB-005, 2). Thus, we detected 4 of the 5 synthesized indole or indazole carboxylate SCRAs in clinical practice. We identified both indole and indazole carboxylate compounds in samples from 6 participants. Quantification of the 5 synthesized SCRAs are reported in Table 3.

The first indole or indazole SCRAs detected in the IONA study were from an exposure in July 2015 with 7 cases recorded in 2015 and 10 cases in 2016. Of the 17 cases in which 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 and 13 were male, with a median age of 30 years (range, 18–58 years). We identified additional substances other than indole and indazole carboxylate SCRA in samples from all but 1 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 (1), 3,4 methylenedioxy-*N*-ethylamphetamine (1), morphine (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 in whom we detected an indole and/or indazole carboxylate SCRA included confusion, agitation, or aggression (14; 82%), reduced consciousness (13; 76%), acidosis (8; 47%), hallucinations and paranoid features (7; 41%), tachycardia (6; 35%), hypertension (5; 29%), raised cre-

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

Patient (age, years/sex)	Reported exposure	Route	Timing	Source	Clinical features	Treatment	Outcome	LoS	Synthesized SCRA detected with quantification	Other substances detected
1 (19/F)	New cannabis, LSD, mushroom tea	Ingestion	3 h earlier	Unknown	Reduced GCS (9/15), seizure, mydriasis, hypertonia, hyperreflexia, clonus, confusion, tachycardia (120 beats/min), hypertension (185/103 mmHg), 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 mmHg), arrhythmia, breathlessness, confusion, acidosis	Anesthetized, 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 beats/min), hypertension (160 mmHg), palpitations, breathlessness, agitation, raised creatine kinase, methemoglobinemia (37%)	Methylene blue	Transferred to another unit	7 h	FUB-PB-22 (urine, 1.4 ng/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, 2.2 ng/mL), 5F-SDB-005 (urine, 0.9 ng/mL)	5F-PB-22, 5F-ADB, methadone, EDDP, diazepam
6 (35/M)	Legal high	Smoked	Chronic	Friend	Tachycardia (116 beats/min), dizziness, agitation, confusion, paranoid ideation	None	Discharged	N/A	5F-NPB-22 (plasma, 0.7 ng/mL)	5F-ADB, methadone
7 (38/F)	Pandora's box	Smoked	Chronic, last 1 h earlier	Shop	Reduced GCS, confusion, paranoid ideation	None	Discharged	4 h	5F-NPB-22 (plasma, 4.6 ng/mL)	5F-PB-22, 5F-ADB
8 (22/M)	Cherry bomb, diazepam	Smoked, ingested	Acute	Friend, dealer	Reduced GCS (11/15), bradycardia (46 beats/min), hypotension (91 mmHg), bradypnea (8 breaths/min), agitation, confusion, hallucination, suicidal ideation, acidosis (pH 7.31), raised creatine kinase (1365), trauma to face	None	N/A	N/A	5F-NPB-22 (plasma, 3.1 ng/mL; urine, 1.1 ng/mL)	5F-AKB-48, 5F-ADB
9 (42/M)	Cherry bomb	Smoked	Acute	Friend	Reduced GCS (3/15), tachycardia (114 beats/min), hypertension (161 mmHg), acidosis (pH 7.21)	Naloxone	Discharged	N/A	5F-NPB-22 (plasma, 0.8 ng/mL)	5F-AKB-48, 5F-ADB
10 (52/M)	Unknown	Unknown	Unknown	Friend	Reduced GCS, mydriasis, tachycardia (max 120 beats/min), breathing difficulties, agitation, confusion	ITU for intubation and ventilation	Discharged	44 h	5F-NPB-22 (plasma, 0.4 ng/mL; urine, 0.7 ng/mL)	NNEI, 5F-ADB, methamphetamine, methadone

Continued on page 352

Table 3. Demographic, clinical, and analytical results of 17 patients with analytically confirmed exposure to indole or indazole carboxylate SCRA. *(Continued from page 351)*

Patient (age, years/sex)	Reported exposure	Route	Timing	Source	Clinical features	Treatment	Outcome	LoS	Synthesized SCRA detected with quantification	Other substances detected
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, 3.1 ng/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, 0.9 g/mL; urine, 1.1 ng/mL)	5F-PB-22, 5F-ADB
13 (30/F)	Legal high, smack, benzodiazepine	Oral (benzo), others not known	Benzo, chronic; others, acute	Dealer	Hypothermia (34.3 (C), reduced GCS (3/15), mydriasis, nystagmus, metabolic acidosis (pH 7.34), hepatic dysfunction (ALT, 89), raised creatine kinase (1357)	None	Discharged	22 h	5F-SDB-005 (plasma, 0.5 ng/mL)	5F-ADB, 5F-PB-22, methadone
14 (24/M)	Black wrapper with picture	Smoked	Acute	Friend	Reduced GCS (14/15), bradycardia (43 beats/min), confusion, metabolic acidosis (pH 7.33)	None	Discharged	24 h	5F-NPB-22 (plasma, 5.1 ng/mL; urine, 8.9 ng/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, 7.1 ng/mL)	5F-PB-22, MDMA, MDEA
16 (58/M)	Exodus	Smoked	Chronic	Unknown	Abdominal pain, reduced GCS (7/15), seizure, tachycardia (119 beats/min), hypertension (235 mmHg systolic), agitation, confusion, acidosis (7.09, lactate 10), hepatic dysfunction (ALT, 64)	Anesthetized, intubated and ventilated	Discharged	77 h		BB-22, AB-CHMICA, 5F-AKB-48
17 (18/M)	Incense	Smoked	Acute	Unknown	Vomiting, abdominal pain, bleeding, reduced GCS (5/15), bradycardia (40 beats/min), chest pain, confusion	None	Discharged	16 h		5F-PB-22, 25I-NBOMe

LoS, length of stay; F, female; GCS, Glasgow Coma Score; 5F-NPB-22, 1-(5-fluoropentyl)-8-quinolinyl ester-1*H*-indazole-3-carboxylic acid; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; 5F-PB-22, 1-(5-fluoropentyl)-8-quinolinyl ester-1*H*-indole-3-carboxylic acid; M, male; FUB-PB-22, 1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxylic acid, 8-quinolinyl ester; 5F-AKB-48, *N*-((3s,5s,7s)-adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide; BE, base excess; 5F-SDB-005, naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate; N/A, not available; ITU, intensive therapy unit; MDMA, 3,4 methylenedioxymethamphetamine; MDEA, 3,4 methylenedioxy-*N*-ethylamphetamine; BB-22, 1-(cyclohexylmethyl)-8-quinolinyl ester-1*H*-indole-3-carboxylic acid; 25I-NBOMe, 4-iodo-2,5-dimethoxy-*N*-(2-methoxybenzyl)phenethylamine; 5F-ADB, Methyl 2-{[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate; NNEI, Pentyl-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide; AB-CHMICA, *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indole-3-carboxamide; 5F-AB-PINACA, *N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide.

atine kinase (4; 24%), and seizures (2; 12%); 3 patients required intubation and ventilation in critical care units. All patients recovered and were discharged from hospital after a median length of stay of 20 h (range, 3–77 h).

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 July 8, 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 analog of methamphetamine. The other 4 indole or indazole carboxylate SCRAs synthesized 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 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 preexisting 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 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 notify 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, whereas depressant compounds including opioids or benzodiazepines may cause or 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, in whom 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 synthesized SCRAs, and we are not aware of published quantified clinical cases. It is not possible to correlate the severity of toxicity to concentration because of the small sample size, uncertainty regarding exposure-to-sample time, and the contribution of coexposures.

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 preexisting appropriately approved mechanism for the collection of appropriate biological samples from users experiencing suspected NPS toxicity, while synthesis of appropriate standards allows early accurate detection and quantification of emerging NPS. Such a comprehensive toxicovigilance program is valuable for the early detection of emerging NPS and for understanding the harms associated with their use.

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