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Novel polar AhR-active chemicals detected in sediments of an industrial area using effect-directed analysis based on in vitro bioassays with full-scan high resolution mass spectrometric screening

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Abstract

Studies investigating aryl hydrocarbon receptor (AhR)-active compounds in the environment typically focus on non- and mid-polar substances, such as PAHs; while, information on polar AhR agonists remains limited. Here, we identified polar AhR agonists in sediments collected from the inland creeks of an industrialized area (Lake Sihwa, Korea) using effect-directed analysis combined with full-scan screening analysis (FSA; using LC-QTOFMS). Strong AhR-mediated potencies were observed for the polar and latter fractions of RP-HPLC (F3.5−F3.8) from sediment organic extracts in the H4IIE-*luc* in vitro bioassays. FSA was performed on the corresponding fractions. Twenty-eight tentative AhR agonists were chosen using a five-step process. Toxicological confirmation using bioassay revealed that canrenone, rutaecarpine, ciprofloxacin, mepanipyrim, genistein, protopine, hydrocortisone, and medroxyprogesterone were significantly active. The relative potencies of these AhR-active compounds compared to that of benzo[a]pyrene ranged from 0.00002 to 2.0. Potency balance analysis showed that polar AhR agonists explained, on average, ~6% of total AhR-mediated potencies in samples. Some novel polar AhR agonists also exhibited endocrine-disrupting

potentials capable of binding to estrogen and glucocorticoid receptors, as identified by QSAR modeling. In conclusion, the focused studies on distributions, sources, fate, and ecotoxicological effects of novel polar AhR agonists in the environment are necessary.

Keywords: Aryl hydrocarbon receptor, LC-QTOFMS, Nontarget analysis, Sediments, Industrial area.

1. Introduction

In 2020, the number of chemicals registered in the Chemical Abstracts Service (CAS) is about 164 million. Compared to the 20 million registered in 2002, a huge number of new chemicals continue to be created. While chemicals, such as pesticides, industrial chemicals, and pharmaceuticals, have improved the quality of human life, some organic chemicals present their inherent hazard (Escher et al., 2020). Innumerable organic chemicals are introduced into the marine environment through point and non-point sources and can accumulate in sediments and biota (Escher et al., 2020; Hong et al., 2012). Coastal sediment is a major sink for various organic chemicals and can potentially have adverse effects on marine ecosystems (Chiaia-Hernandez et al., 2013; Li et al., 2019; Pal et al., 2014). When evaluating the sediment-related risk, it is important to unravel key toxicants (Escher et al., 2020; Li et al., 2019). Although target analysis is an essential element of risk assessment, it is unable to identify causative chemicals for ecological risk in complex mixtures (Brack et al., 2016; Doyle et al., 2015; Escher et al., 2020; Li et al., 2019; Zhang et al., 2018).

Effect-directed analysis (EDA) combined with full-scan high resolution mass spectrometric screening analysis (FSA) has been widely used to identify previously unmonitored toxic substances in environmental matrices (Cha et al., 2019; Kim et al., 2019). This approach could be applied to various environmental media, including sediments, wastewater, and biota (Brack, 2003; Brack et al., 2016; Cha et al., 2019; Hong et al., 2016; Kim et al., 2019; Muschket et al., 2018). The potential toxicity in samples is measured using in vivo and/or in vitro bioassays. Complexity within samples can be reduced through multi-step fractionation to isolate causative substances (Brack, 2003; Lee et al., 2020; Regueiro et al., 2013; Schmitt et al., 2012; Weller, 2012). Then, high-resolution mass spectrometry, such as time-of-flight mass spectrometry (TOFMS), is used to screen all compounds in toxic fractions. Screening processes are then used to select candidate substances, and chemical and toxicological confirmation is conducted (Cha et al., 2019; Hong et al., 2016; Kim et al., 2019; Simon et al., 2013). These processes are relatively

time-consuming and somewhat complex, but reveal the existence and contribution of previously unidentified toxic substances. Consequently, this approach has revealed a number of novel toxic substances present in environmental samples (Cha et al., 2019; Hong et al., 2016; Kim et al., 2019; Simon et al., 2013).

Previous studies have identified major aryl hydrocarbon receptor (AhR)-active chemicals in sediments of industrialized areas (Cha et al., 2019; Kim et al., 2019; Peng et al., 2016). For example, benz[*b*]anthracene, 11H-benzo[*a*]fluorene, and 4,5-methanochrysene are AhR agonists that have been identified in the sediments of inland creeks of Lake Sihwa, Korea (Cha et al., 2019). In another study, 1-methylchrysene, benzo[*j*]fluoranthene, 3-methylchrysene, 5-methylbenz[*a*]anthracene, 11H-benzo[*b*]fluorene, benzo[*b*]naphtho[*2,3-d*]furan, and benzo[*b*]naphtho[*2,1-d*]thiophene were recently found in Ulsan Bay, Korea (Kim et al., 2019). Due to the addition of these compounds, the explanatory power of total induced AhR-mediated potency in samples was greatly increased. Previous studies searching for AhR-active substances have mainly focused on non-polar and mid-polar compounds, such as PAHs (Cha et al., 2019; Kim et al., 2019). Significant AhR-mediated potencies were observed in the polar fractions of organic extracts from sediments; however, the polar AhR-active compounds remain largely unknown (Cha et al., 2019; Hong et al., 2016; Kim et al., 2019).

Polar compounds generally have relatively high water solubility, bind to membrane transport proteins, and are easily transported into cells (Alharbi et al., 2016; Katayama et al., 2010; Morandi et al., 2016; Redmon et al., 2018). Consequently, polar AhR agonists exhibit greater bioavailability and bioaccessibility compared to non- and mid-polar compounds. Thus, it is necessary to detect polar AhR agonists present in environmental samples. Previous studies have documented the presence of polar AhR-active chemicals in sediments, including (hydroxy-)quinones, keto-, dinitro-, hydroxy-PAHs, and N-heterocycles (Andrysik et al., 2011; Song et al., 2006; Xiao et al., 2016). In addition, benzothiazole and 2-mercaptobenzothiazole, which are used as vulcanization accelerators in rubber production, have been identified as polar AhR agonists in sediments of the Three Gorges Reservoir in China (Xiao et al., 2016). Of note, enoxolone, which is used as an anti-inflammatory agent, was identified as a novel polar AhR agonist in the sediment of Masan Bay, South Korea (Lee et al., 2020). These polar AhR agonists reach coastal sediments via surface runoff and outfall from wastewater treatment plants (WWTPs) (De Wever and Verachtert, 1997; Xiao et al., 2016), and cause acute toxicity to aquatic organisms (Kloepfer

et al., 2005; Xiao et al., 2016).

Lake Sihwa is an artificial lake located on the west coast of Korea. Industrial complexes, including metal, petrochemical, biochemical, pharmaceutical factory, and engineering manufacturing industries, are located adjacent to Lake Sihwa (Cha et al., 2019). Persistent toxic substances (PTSs), such as polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and styrene oligomers (SOs) are widely distributed in the sediments of Lake Sihwa (Hong et al., 2016; Jeon et al., 2017; Lee et al., 2017a; Meng et al., 2017). In particular, the concentrations of PAHs and APs in sediments exceeded interim sediment quality guidelines (ISQGs) established by the Canadian Council of Ministers of the Environment (CCME) (Cha et al., 2019; CCME, 2002). Accordingly, the Korean government designated Lake Sihwa as a special coastal management zone in 2000 and implemented a total pollution load management system in 2013 to regulate the release of land-derived pollutants (Lee et al., 2017b). Since then, the environment in Lake Sihwa has been shown to have improved significantly, but the contamination of sediments of inland creeks flowing into Lake Sihwa is still found to be serious (Cha et al., 2019; Hong et al., 2016).

Here, we investigated polar AhR agonists in the sediments of inland creeks in a highly industrialized area (Lake Sihwa) using EDA with FSA. The specific objectives were to: (i) investigate AhR-mediated potencies in the polar fractions of sediment organic extracts using H4IIE-*luc* bioassay, (ii) identify major AhR agonists in toxic fractions using LC-QTOMFS, and (iii) determine the contribution of polar AhR agonists to total AhR-mediated potencies.

2. Materials and methods

2.1. Sampling and sample preparation

Surface sediments were collected from the inland creeks of industrial (C1) and urban (C2) areas of Lake Sihwa in April 2015, and collected from a rural area (C3) in September 2017 (Fig. S1). Detailed methods on sample preparation for bioassays and chemical analyses are described elsewhere (Cha et al., 2019; Hong et al., 2016). In brief, surface sediments were collected using hand shovels, and were transferred to pre-cleaned glass jars. Sediments were immediately transported to the laboratory, where they were stored at –20 ℃ until analysis. Approximately 60 g of freeze-dried sediments were extracted with 350 mL dichloromethane (DCM, J.T. Baker, Phillipsburg, NJ) on Soxhlet extractor for 16 h. To remove elemental sulfur from extracts, activated copper was added for about 1 h, and organic extracts were concentrated to 4 mL with a

rotary evaporator and N_2 gas flow (~15 g sediment equivalent (SEq) mL^{-1}). Four milliliters of raw extract were divided into 2 mL portions for silica gel column fractionation and bioassays. The solvent of the extract used for H4IIE-*luc* bioassays was exchanged with dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, MO).

2.2. Silica gel and RP-HPLC fractionations

Sediment organic extracts were separated in two-step fractionations, including silica gel column chromatography (8 g activated silica gel, 70–230 mesh, Sigma-Aldrich, Saint Louis, MO) and reverse-phase high-performance liquid chromatography (RP-HPLC, Agilent 1260 HPLC, Agilent Technologies, Santa Clara, CA) (Hong et al., 2015, 2016). Two milliliters of organic extract were placed on the column and separated into non-polar (F1), aromatic (F2), and polar (F3) fractions. The first fraction (F1) was eluted with 30 mL hexane (Honeywell, Charlotte, NC). The aromatic fraction (F2) was collected with 60 mL of 20% DCM in hexane. The third fraction (F3), which contained polar compounds, was eluted with 60 mL of 60% DCM in acetone (J.T. Baker). All elutriates were evaporated on a rotary evaporator and concentrated to 2 mL using N_2 gas flow. To identify polar AhR agonists in sediment organic extracts, the F3 fraction was further separated into 10 subfractions using RP-HPLC (Hong et al., 2016). Separation conditions of RP-HPLC were previously optimized using standard materials of various compounds (34 PCBs, 16 PAHs, 7 alkylphenols, and 5 phthalates), and elution efficiency showed more than 85% for all compounds (Hong et al., 2016; Lee et al., 2020). A C18 column (PrepHT XBD, 21.2 × 250 mm, 7 μm, Agilent Technologies) was used for fractionation. Subfractions were exchanged to hexane or DMSO for further analyses. Detailed instrumental conditions of RP-HPLC were reported previously (Hong et al., 2016, Lee et al., 2020).

2.3. In vitro bioassays

AhR-mediated potencies were measured using H4IIE-*luc* bioassays in raw organic extracts of sediments, silica gel fractions, and RP-HPLC fractions. The H4IIE-*luc* bioassay was performed following the existing methods (Cha et al., 2019; Hong et al., 2016). In brief, trypsinized cells (~7.0×10^4 cells mL^{-1}) were seeded in the 96 micro-well plate at 250 μL per well. After seeding, cells were incubated at 37 ℃ in a 5% CO_2 incubator for 24 h. Dosing was carried out by adding the appropriate standards (benzo[*a*]pyrene (BaP) for 4 h exposure and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 72 h exposure; 0.1% dose), samples (raw extracts, silica gel fractions, RP-HPLC fractions, and tentative AhR agonists; 0.1% dose),

solvent control (0.1% DMSO), and media control. BaP and TCDD standards were diluted three times with 50 nM (=100 %BaP_{max}) and 300 pM (=100 %$TCDD_{max}$) as the first concentration, respectively. After 4 h or 72 h exposure durations, luciferase luminescence was quantified using a Victor X3 multi-label plate reader (PerkinElmer, Waltham, MA). Responses of the H4IIE-*luc* bioassay were converted to percentages of maximum response of BaP and TCDD, respectively. AhR-mediated potency at 4 h exposure was expressed as potency-based BaP-equivalent (EQ) values. Potency-based BaP-EQ values were obtained from sample dose-response curves of the sediment samples at six dilutions. All bioassays were conducted in triplicate. Of note, surrogate standards could not be added in the extraction and fractionation procedures because such chemicals would influence the changes of biological response during the bioassays.

2.4. Full-scan screening analysis

FSA using LC-QTOFMS was performed on highly toxic fractions, including F3.5−F3.8 of the sediment organic extract from Shiheung Creek (C1), where AhR-mediated potencies were greatest. Instrumental conditions are described in Table S1. The liquid chromatography 1290 infinity (Agilent Technologies) coupled with a triple time-of-flight (TripleTOF®) 5600+ mass spectrometer (AB Sciex, Framingham, MA) was used for FSA. An Eclipse XDB-C18 column (150 mm × 2.1 mm i.d. × 5 μm film) was used for separation. The selection criteria for tentative AhR agonists from LC-QTOFMS analysis had five steps. The first step involved matching the compounds with TCM library 1.0 metabolite software (Zedda and Zwiener, 2012). The second step selected compounds with a score of ≥70 by identifying isotope distribution (Lee et al., 2020). The third step involved selecting compounds with a score of ≥70 by confirming library MS/MS matching (Muz et al., 2017). The fourth step involved identifying aromatic compounds (Mekenyan et al., 1996). The fifth step selected only compounds that were commercially available. Finally, 28 tentative AhR agonists including canrenone, triphenyl phosphate, daidzein, genistein, quercetin, rutaecarpine, mepanipyrim, glycetein, kaempferol, loratadine, coumarin, ciprofloxacin, pyridaben, cortisone, naringenin, protopine, formononetin, clodinafop-propargyl, dioctyl phthalate, ziprasidone, danazol, hydrocortisone, dibutyl phthalate, medroxyprogesterone, wogonin, rafoxanide, 17α-ethynylestradiol, and thioridazine were selected. All compounds were purchased from Sigma-Aldrich.

2.5. HPLC-MS/MS analysis

The eight polar AhR agonists (canrenone, rutaecarpine, ciprofloxacin, mepanipyrim,

genistein, protopine, hydrocortisone, and medroxyprogesterone) in the fraction samples were quantified using HPLC-MS/MS. Detailed information on instrumental conditions and methods are described in Table S2. Newly identified AhR agonists were quantified using a 1290 infinity II series HPLC (Agilent Technologies) combined with a QTRAP 6500 series electrospray ionization tandem mass spectrometer (AB Sciex). Compounds were separated with an Eclipse XDB-C18 column. The mobile phase was: (A) 0.1% formic acid and 10 mM ammonium formate in water, and (B) 0.1% formic acid in acetonitrile. The injection volume was 3 μL, and the flow rate was 0.4 mL min^{-1}. Procedural blanks were analyzed concurrently to check for interfering peaks. The polar AhR agonists identified in the present study were not detected in blank samples.

2.6. Relative potency values of putative AhR-active compounds

The relative potency values (RePs) for the AhR-mediated potencies of eight tentative AhR agonists were determined using H4IIE-*luc* bioassays with effective concentrations (EC) at 50% of the maximum level achieved by BaP (EC_{50}). Chemicals were prepared at 10 concentrations using 3-fold serial dilution (viz., 1000, 333, 111, 37, 12, 4.1, 1.4, 0.46, 0.15, and 0.05 μg mL^{-1}), and were tested using the in vitro bioassay method, as described above.

2.7. Potency balance analysis

Potency balance analysis was performed between instrument-derived BaP equivalent concentrations (BEQs) and bioassay-derived BaP-EQs (potency-based) to determine the contribution of each compound to total induced AhR-mediated potency. Instrument-derived BEQs were used to calculate the sum of the products of measured concentrations for individual compounds in sediments multiplied by their RePs (Cha et al., 2019; Kim et al., 2019).

2.8. VirtualToxLab in silico analysis

Other toxic potentials of eight polar AhR agonists were evaluated using quantitative structure-activity relationship (QSAR) modeling. AhR, estrogen receptor (ER), and glucocorticoid receptor (GR) binding affinities with candidates were estimated by VirtualToxLab (Vedani et al., 2015). Combined automated and flexible docking with multidimensional QSAR was used to simulate and quantify toxic potential and how chemicals bind to a set of currently implemented proteins that cause adverse effects.

3. Results and discussion

3.1. AhR-mediated potencies in sediments

All raw extracts of sediments reached saturation efficiency (≥100% BaP_{max}) for AhR-

mediated potency after 4 h exposure, whereas C1 and C2 only showed significant responses after 72 h exposure (Fig. 1a). For the silica gel fractions of the three raw extracts (C1–C3), AhR-mediated potencies were relatively greater in F2 (aromatics) and F3 (polar) compared to F1 (non-polar) after both 4 h and 72 h exposure (Fig. 1b). The causative chemicals of F2 responses are clarified in the previous studies (Cha et al., 2019; Hong et al., 2016; Kim et al., 2019; Lee et al., 2017a); here, the focus was on AhR-mediated potencies in F3. To reduce the complexity of F3, samples were further separated into 10 sub-fractions using RP-HPLC. Significant AhR-mediated potencies were commonly observed in F3.5–F3.8 at 4 h exposure (Fig. 1c). Patterns showing the significant AhR-mediated potencies in F3.5–F3.7 of sedimentary organic extracts were also found in a previous study conducted in Masan Bay, South Korea (Lee et al., 2020). In addition, enoxolone (ReP=0.13), a newly identified AhR agonist, was found to be present in the F3.7 (Lee et al., 2020). F3.5 and F3.6 of C1 extract had high AhR-mediated potencies, indicating that site C1 was contaminated with polar AhR agonists.

Meanwhile, AhR-mediated potencies in the F3 subfractions after 72 h exposure were less than 20% $TCDD_{max}$ in all samples (Fig. 1c). Comparison of AhR-mediated potencies between 4 h and 72 h exposure in the H4IIE-*luc* bioassay provides metabolic information on AhR agonists in environmental samples (Cha et al., 2019; Hong et al., 2016; Xiao et al., 2017). For example, labile compounds such as PAHs tended to be easily metabolized during the longer exposure (Hong et al., 2016; Xiao et al., 2017). The decrease in the relative potency values of PAHs with increasing exposure time could be the metabolic process in the H4IIE-*luc* cells, resulting from the induction of CYP1A1 (Larsson et al., 2014). However, refractory AhR agonists, PCDD/Fs and coplanar-PCBs were relatively stable during exposure of 72 h (Hong et al., 2016; Xiao et al., 2017). The polar AhR agonists in sediment might be easily metabolized in H4IIE-*luc* cells, and have labile characteristics, in general (Andrysik et al., 2011; Song et al., 2006; Xiao et al., 2016). EC_{50} was calculated from dose-response curves for the highly toxic fractions (F3.5–F3.8) (Fig. 1d). For F3.5 of C3, the maximum BaP_{max} was <50%, and EC_{20} was used to calculate BaP-EQ. Potency-based BaP-EQ values ranged from 70 to 1800 ng BaP-EQ g^{-1} dm in C1, 12 to 430 ng BaP-EQ g^{-1} dm in C2, and 0.7 to 150 ng BaP-EQ g^{-1} dm, respectively (Fig. S2). Potency-based BaP-EQs concentrations were used for potency balance analysis.

3.2. Full-scan screening analysis

FSA using LC-QTOFMS was conducted for F3.5–F3.8 of C1. These fractions had

relatively strong AhR-mediated potencies. The data handling strategy involved five steps to select tentative AhR agonists in samples (Fig. 2a). The library software directly matches compounds with chromatograms from LC-QTOFMS results, which can enable it easier to search the identity, generation, and relevance of the mass under investigation (Muz et al., 2017). Thus, library matching in high-resolution mass spectrometry can allow in a time-effective for searching candidate compounds and improve reliability to provide accurate information on unknown compounds. In the first step, 359, 332, 273, and 255 compounds were detected in F3.5, F3.6, F3.7, and F3.8 of C1 extract, respectively (Zedda and Zwiener, 2012). In the second step, compounds with an isotope score of ≥70 were selected, narrowing them down to 64, 62, 53, and 60 compounds (Lee et al., 2020). Out of them, 21, 36, 31, and 44 compounds with a library matching score ≥70 were found (step 3) (Muz et al., 2017). In the fourth step, 12, 22, 20, and 27 compounds with aromatic rings were selected (Mekenyan et al., 1996). Compounds with the structure of aromatic rings and planar tend to bind to the AhR (Cha et al., 2019; Kim et al., 2019; Mekenyan et al., 1996). Eighty-one compounds were identified as tentative candidates for polar AhR agonists in the sediments of C1 (Table S3). Out of these, analytical standards were only available for 28 compounds (Fig. S3), which were purchased for chemical and toxicological confirmation. The candidates included 21 pharmaceuticals, 4 pesticides, 2 plasticizers, and 1 dietary supplement (Table 1).

3.3. Toxicological and chemical confirmation

For toxicological confirmation, dose-response tests for 28 candidates were performed in the H4IIE-*luc* bioassay after 4 h exposure. Out of the 28 compounds, eight compounds (including canrenone, genistein, rutaecarpine, mepanipyrim, ciprofloxacin, protopine, hydrocortisone, and medroxyprogesterone) showed significant AhR-mediated potencies (Fig. 2b). Rutaecarpine (2.0) showed a greater affinity (binding) with AhR compared to BaP. In addition, RePs of hydrocortisone (2.0×10^{-1}), medroxyprogesterone (2.0×10^{-2}), canrenone (6.0×10^{-3}), ciprofloxacin (5.0×10^{-3}), mepanipyrim (4.0×10^{-4}), genistein (1.0×10^{-4}), and protopine (2.0×10^{-5}) for AhR-mediated potency were newly obtained. Out of these, rutaecarpine (Han et al., 2009), hydrocortisone (Abbott et al., 1999), mepanipyrim (Medjakovic et al., 2014), genistein (Piasecka-Srader et al., 2016), and protopine (Vrba et al., 2011) were previously reported as capable of binding to AhR. To the best of our knowledge, medroxyprogesterone and canrenone were newly found as novel polar AhR agonists in sediments. Retention time and mass fragment

ions of eight polar AhR agonists were confirmed using HPLC-MS/MS (positive ionization mode (ESI+) and multiple reaction monitoring (MRM)) (Table S4). The concentrations of these compounds in the fractions were quantified (Table S5). Extracted ion chromatograms and Q1/Q3 masses for canrenone and medroxyprogesterone are shown in Fig. S4.

3.4. Distributions, compositions, and sources of polar AhR agonists

The sedimentary distributions of newly identified polar AhR agonists were site-specific (Fig. 3a and Table S5). Concentrations of polar AhR agonists in the sediment of the industrial area (C1) tended to be greater compared to urban (C2) and rural (C3) areas. Site C1 was previously identified as being highly contaminated by mid-polar AhR agonists, including PAHs and SOs (Cha et al., 2019). The newly identified polar AhR agonists showed different compositions for each site (Fig. 3b). For example, hydrocortisone was the most dominant in C1 sediment. In C2, hydrocortisone and genistein showed similar contributions, and genistein dominated in C3 sediment.

Out of the eight polar AhR agonists, hydrocortisone had the highest concentrations in sediments, and was widely distributed across sampling sites. Hydrocortisone is used as an anti-inflammatory agent (Sprung et al., 2008), and can be adsorbed on the organic phase of suspended particles and sediments, due to its hydrophobic characteristics (Louie, 2010). Genistein is an isoflavone isolated from soybeans, and is widely used as an anti-cancer agent (Banerjee et al., 2008; Coward et al., 1993; Wang et al., 1996). Medroxyprogesterone had detectable concentrations of 4.8 ng g^{-1} dm in C1, 1.9 ng g^{-1} dm in C2, and 1.8 ng g^{-1} dm in C3. It is used as a uterine cancer agent (Prior et al., 1994). In addition, medroxyprogesterone is an endocrine-disrupting compound (EDC) capable of binding to the androgen receptor of organisms (Sauer et al., 2018). Ciprofloxacin is used as an anti-cancer drug. It is introduced to surface waters from hospital and/or pharmaceutical factories (Mater et al., 2014). Canrenone is used as a diuretic (Romanelli and Gentilini 2004). It is released from pharmaceutical factories, and causes the abnormal growth of fish in aquatic ecosystems (Gilbert, 2011; Sanchez et al., 2011; Weizel et al., 2018).

Rutaecarpine and mepanipyrim were only detected in the sediments of the industrial area. Rutaecarpine is a quinazolinocarboline alkaloid that has been used as herbal medicine (Shew et al., 1996). Mepanipyrim is used as fungicide and insecticide (Miura et al., 1994; Nakamura et al., 2003). These compounds might be mainly used in industrial areas, including pharmaceutical

factory, metal, biochemical, and engineering manufacturing industries. Protopine is used as an anti-cancer agent (Jiang et al., 2004). It was less than the limit of quantification at all sampling sites. Even if these AhR agonists are presented less than their own threshold effect and detection limit, they can contribute to toxicity in complex mixtures of sediments (Escher et al., 2020; Kortenkamp and Faust, 2018).

Overall, polar AhR agonists are present at greater concentrations in sediments of the industrial area. These agonists were assumed to originate from various industrial and pharmaceutical complexes. Previous studies on the distribution of polar AhR agonists in the environment were mainly conducted in river water and the effluent of WWTPs (Araujo et al., 2013; Azuma et al., 2017; Creusot et al., 2014; Hajj-Mohamad et al., 2014; Louie, 2010; Weizel et al., 2018; Yarahmadi et al., 2018). In comparison, studies evaluating the distribution of these compounds in sediments are extremely rare. Results of the current study showed that polar AhR compounds are widely distributed in sediments. Thus, follow-up studies on the fate, sources, and potential effects of polar AhR agonists in sediments are needed.

3.5. Potency balance analysis

Potency balance analysis between instrument-derived BEQs and bioassay-derived BaP-EQs was conducted to evaluate the contributions of polar AhR agonists to total induced AhR-mediated potencies (Fig. 4 and Table S6). The results of the potency balance analysis revealed varying contributions among sites and compounds. For example, canrenone accounted for 0.002% of total AhR-mediated potency in F3.5 of C1. In F3.6, the fractions included four polar AhR agonists, BEQs could explain only a small portion (0.002–0.02%) of BaP-EQs. Since protopine was not detected in the F3.7 of sediment extracts, it was excluded from the potency balance analysis. The explanatory power of AhR agonists was relatively high, ranging from 6.8 to 57%. Out of these, hydrocortisone was the greatest contributor, explaining 56% of total induced AhR-mediated potency in the F3.8 of C1. However, the explanatory power of hydrocortisone in urban and rural areas was ~10 times lower, indicating that this compound mainly accumulates in the sediments of industrial areas. Overall, the eight polar AhR agonists had relatively minor contributions to the fractions of sediment extracts in C2 (0–6.9%) and C3 (0–6.8%) sites. Thus, it is necessary to investigate the major polar AhR agonists present in the sediments of rural and urban areas in the future. Furthermore, additional toxicological and chemical confirmation for the remaining candidates (Table S3) might be improved the explanatory power of AhR-mediated

potencies in polar fractions. Overall, the present study successfully applied EDA combined with FSA to identify AhR agonists in polar fractions of sediment organic extracts.

3.6. Additional potential toxicity screening

The newly identified polar AhR agonists had specific potential toxicities in previous studies. For example, canrenone (Fernandez et al., 1983), genistein (Hsieh et al., 1998), and ciprofloxacin (Beberok et al., 2018) are EDCs capable of binding to the ER. In addition, hydrocortisone (Hashmi et al., 2020), medroxyprogesterone (Hashmi et al., 2020), and genistein (Whirledge et al., 2015) are GR-active compounds. However, there are few reports on potential toxicities of some polar AhR agonists, such as rutaecarpine and mepanipyrim. Whether these compounds had other potential toxicities (such as AhR, ER, or GR activity) was further evaluated using QSAR modeling, such as VirtualToxLab (Table S7). VirtualToxLab predicted that canrenone, genistein, protopine, hydrocortisone, and medroxyprogesterone could bind to ER; however, all compounds exhibited binding affinity with GR. Five compounds (canrenone, rutaecarpine, mepanipyrim, medroxyprogesterone, and hydrocortisone) had AhR binding affinity. VirtualToxLab relies solely on thermodynamic considerations when evaluating the potential binding affinity between compounds and receptors; consequently, it might not be consistent with toxicological results from in vitro bioassays. Thus, predictions require careful consideration in combination with empirical verification, such as multiple bioassays.

4. Conclusions

Overall, the present study successfully identified polar AhR agonists in sediments by using EDA with FSA. Various pharmaceuticals, pesticides, and plasticizers had accumulated in sediments near industrial complexes, and were potential AhR-active substances. EDA combined with FSA will be useful for the identification and management of toxic substances in coastal environments. There is a limitation in evaluating the ecotoxicological effects of toxic substances by evaluating the AhR binding potency using H4IIE-*luc* cells applied in the present study. Nevertheless, it has the advantage of being able to select substances with high toxic potential among the numerous unknown compounds present in environmental samples. The present study provides baseline screening data for the establishment of ecological risk assessment. Further investigation on the distribution, sources, fate, and ecotoxicological effects of these unmanaged toxic substances in coastal ecosystems is urgently required in the near future.

CRediT authorship contribution statement

Jihyun Cha: Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft. **Seongjin Hong:** Conceptualization, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition, Supervision. **Junghyun Lee:** Investigation, Formal analysis, Data curation, Writing - review & editing. **Jiyun Gwak:** Investigation, Formal analysis. **Mungi Kim:** Investigation, Formal analysis. **Taewoo Kim:** Investigation, Formal analysis. **Jin Hur:** Writing - review & editing, Project administration, Funding acquisition. **John P. Giesy:** Writing - review & editing, Project administration, Funding acquisition. **Jong Seong Khim:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. AhR agonist candidates in the RP-HPLC fractions (F3.5–F3.8) of sediment extracts from Siheung Creek of Lake Sihwa, Republic of Korea.

Fractions and compounds	**Molecular formula**	**CAS number**	**Molar mass**	**Intensity**	**Uses**	**References**
F3.5 fraction						
Canrenone*	$C_{22}H_{28}O_3$	213-554-5	340.46	100529	Diuretic	Romanelli and Gentilini (2004)
Triphenyl phosphate	$C_{18}H_{15}O_4P$	115-86-6	326.28	1736175	Plasticizer, fire retardant	Stapleton et al. (2009)
Daidzein	$C_{15}H_{10}O_4$	486-66-8	254.24	125412	Anti-cancer agent	Coward et al. (1993)
F3.6 fraction						
Genistein*	$C_{15}H_{10}O_5$	446-72-0	270.24	98801	Anti-cancer agent	Banerjee et al. (2008)
Quercetin	$C_{15}H_{10}O_7$	117-39-5	302.23	79488	Dietary supplement	Volate et al. (2005)
Ruatecarpine*	$C_{18}H_{13}N_3O$	84-26-4	287.32	31527	Herbal medicine	Shew et al. (1996)
Mepanipyrim*	$C_{14}H_{13}N_3$	110235-47-7	223.27	240686	Fungicide, pesticide	Nakamura et al. (2003)
Glycitein	$C_{16}H_{12}O_5$	40957-83-3	284.26	152401	Anti-cancer agent	Shimoda and Hamada (2010)
Kaempferol	$C_{15}H_{10}O_6$	520-18-3	286.24	77339	Anti-cancer agent	Kim and Choi (2013)
Loratadine	$C_{22}H_{23}ClN_2O_2$	79794-75-5	382.88	205071	Anti-pruritic agent	Roman and Danzig (1993)
Coumarin	$C_9H_6O_2$	91-64-5	146.14	54360	Anti-coagulant	Cravotto et al.

					agent	(2001)
Ciprofloxacin*	$C_{17}H_{18}FN_3O_3$	85721-33-1	331.34	40577	Anti-biotic agent	Forrest et al. (1993)
F3.7 fraction						
Pyridaben	$C_{19}H_{25}ClN_2OS$	96489-71-3	364.93	1845249	Pesticide	Zhu et al. (2005)
Cortisone	$C_{21}H_{28}O_5$	53-06-5	360.44	136293	Anti-inflammatory agent	Alsop et al. (2016)
Naringenin	$C_{15}H_{12}O_5$	67604-48-2	272.25	35900	Anti-ulcer agent	Yamamoto et al. (2004)
Protopine*	$C_{20}H_{19}NO_5$	103-86-9	353.37	52206	Anti-cancer agent	Jiang et al. (2004)
Formononetin	$C_{16}H_{12}O_4$	485-72-3	268.26	10[illegible]18	Anti-angiogenic agent	Huh et al. (2009)
Clodinafop-propargyl	$C_{17}H_{13}ClFNO_4$	105512-06-9	349.[illegible]4	[illegible]4202	Herbicide	Baghestani et al. (2008)
F3.8 fraction						
Dioctyl phthalate	$C_{24}H_{38}O_4$	117-84-0	390.56	13784615	Plasticizer	Rajendran et al. (2002)
Ziprasidone	$C_{21}H_{21}ClN_4OS$	146939-27-7	412.94	1934198	Anti-psychotic agent	Schmidt et al. (2001)
Danazol	$C_{22}H_{27}NO_2$	17230-88-5	337.46	227810	Endometriosis treatment	Igarashi et al. (1998)
Hydrocortisone*	$C_{21}H_{30}O_5$	50-23-7	362.46	165545	Anti-inflammatory agent	Sprung et al. (2008)
Dibutyl phthalate	$C_{16}H_{22}O_4$	84-74-2	278.34	1971180	Insect attractant	Zong et al. (2013)
Medroxyprogesterone*	$C_{22}H_{32}O_3$	520-85-4	344.49	65514	Uterine cancer agent	Prior et al. (1994)
Wogonin	$C_{16}H_{12}O_5$	632-85-9	284.26	71742	Anti-convulsant drug	Park et al. (2007)
Rafoxanide	$C_{19}H_{11}Cl_2I_2NO_3$	22662-39-1	626.01	93218	Veterinary drug	Matsubara et al. (2012)
17α-Ethynylestradiol	$C_{20}H_{24}O_2$	57-63-6	296.40	55738	Contraceptive	Hua et al. (2016)
Thioridazine	$C_{21}H_{26}N_2S_2$	50-52-2	370.57	55237	Anti-psychotic	Min et al. (2014)

					agent	

* Newly identified AhR agonists.

Figure captions

Fig. 1. (a) AhR-mediated potencies of raw extracts, (b) silica gel fractions, (c) RP-HPLC fractions of inland creeks (C1–C3) after 4 h and 72 h exposure, and (d) dose-response curves for AhR-mediated potency of selected HPLC-fractions (F3.5–F3.8 of C1–C3 sediment extracts) from the inland creeks of Lake Sihwa, Republic of Korea (Error bar: mean ± SD; n = 3; SEq: sediment equivalents; *: EC_{20} values).

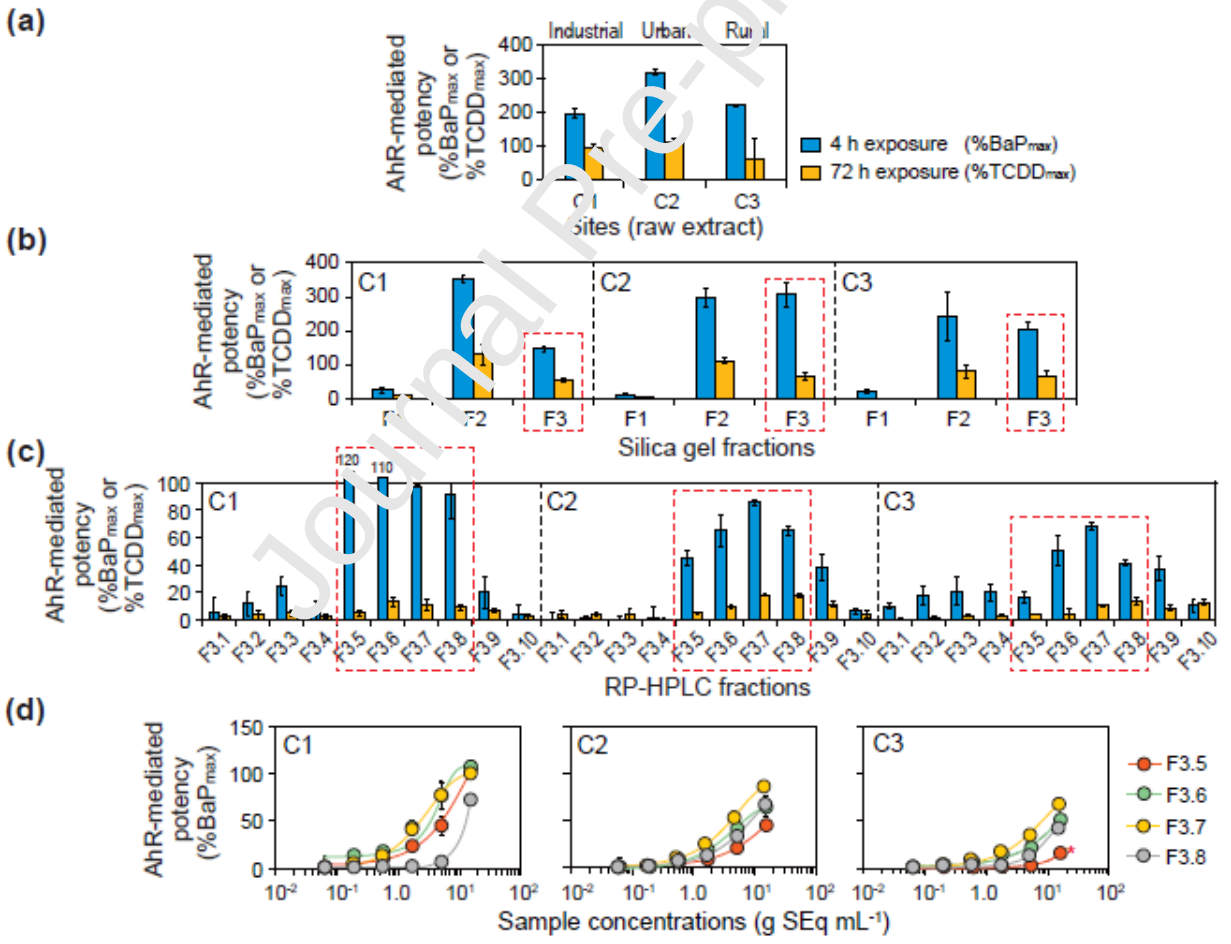


Fig. 2. (a) Five-step selection process for LC-QTOFMS data analysis to select potential AhR

agonists, and (b) dose-response relationships for AhR-mediate potency of eight tentative AhR agonists and benzo[a]pyrene in the H4IIE-*luc* bioassay (Error bar: mean ± SD (n=3); ReP: relative potency value).

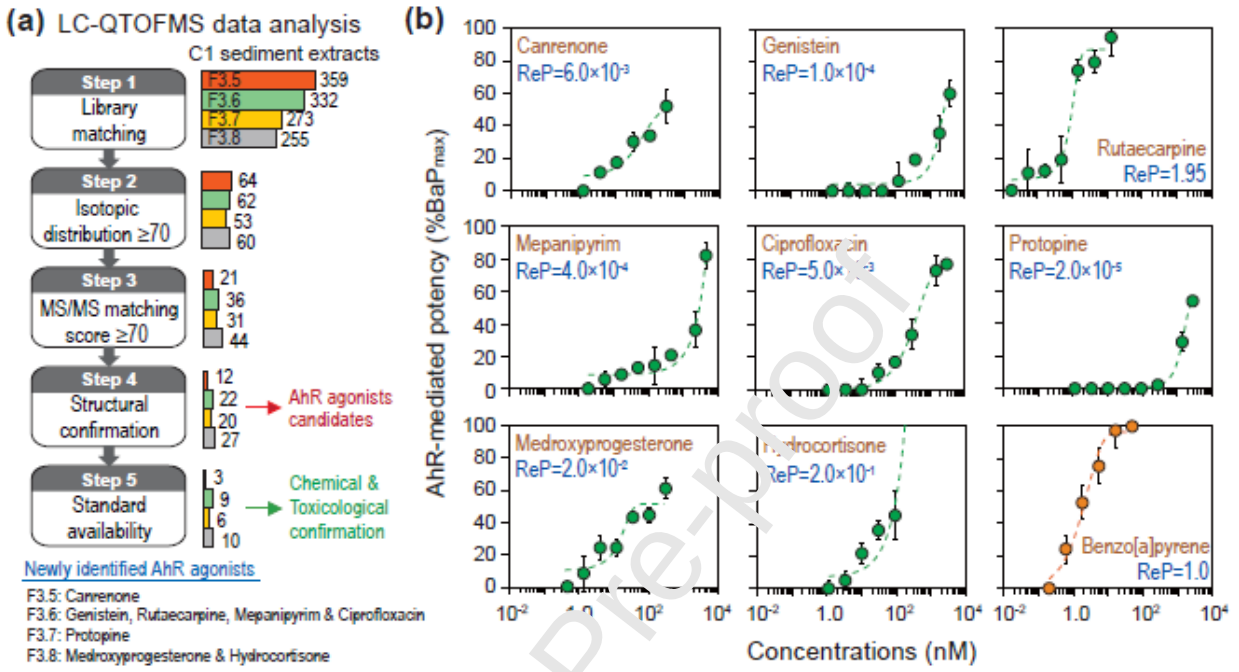


Fig. 3. (a) Distributions and (b) relative compositions of newly identified AhR agonists in the organic extracts of sediments from the inland creeks (industrial, urban, and rural areas) of Lake Sihwa, Republic of Korea.

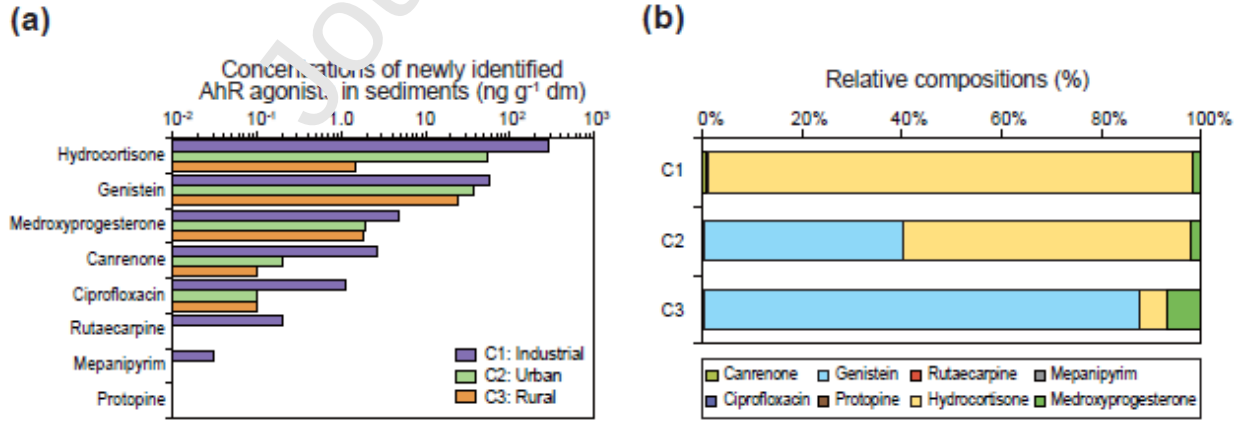
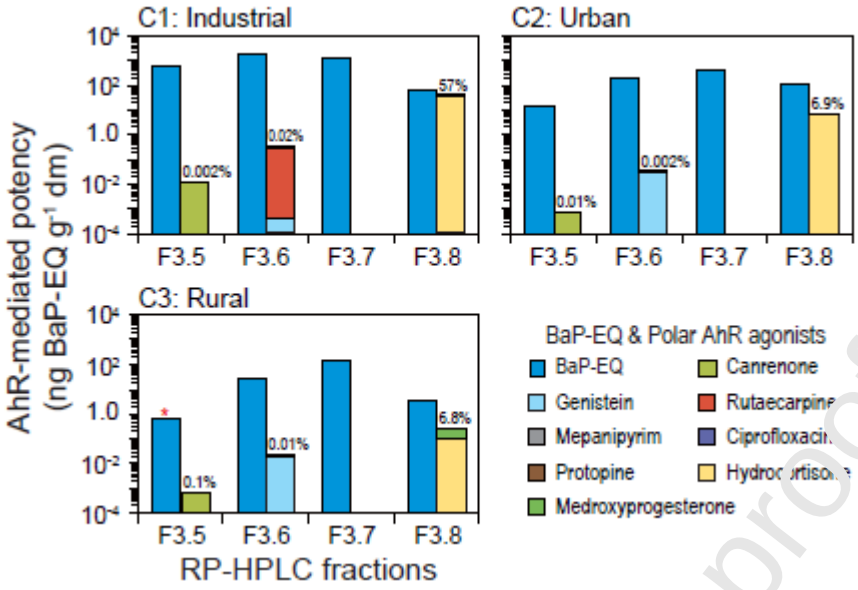
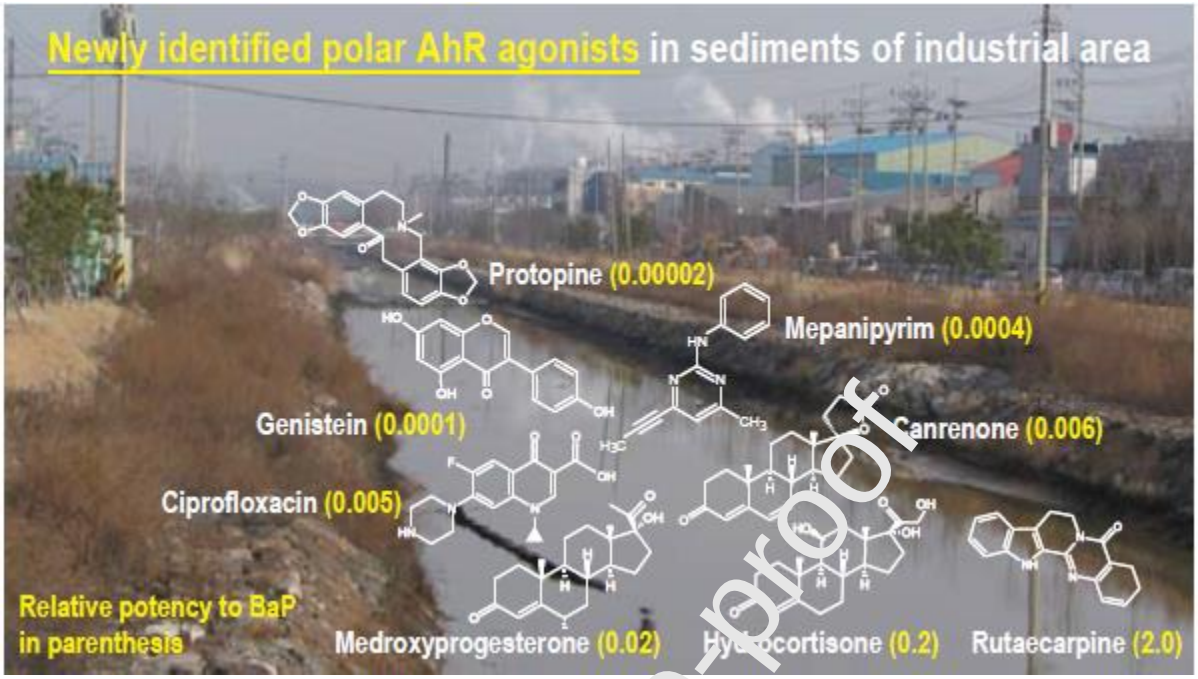


Fig. 4. Contribution of instrument-derived BEQs (newly identified AhR agonists) to bioassay-derived BaP-EQs (potency-based) in RP-HPLC fractions (F3.5–F3.8) of sediments from

the inland creeks (industrial, urban, and rural areas) of Lake Sihwa, Republic of Korea.



Graphical abstract



Highlights

► Novel polar AhR agonists were identified in sediments using EDA combined with FSA.

► A total of 8 compounds were shown significant AhR potencies in the H4IIE-*luc* bioassays.

► Rutaecarpine showed 2-fold greater affinity with AhR compared to benzo[a]pyrene.

► The novel polar AhR agonists are mainly originated from surrounding industrial complexes.

<Supplementary Materials>

Novel polar AhR-active chemicals detected in sediments of an industrial area using effect-directed analysis based on in vitro bioassays with full-scan high resolution mass spectrometric screening

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Supplementary Tables

Supplementary Figures

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Supplementary Tables

Table S1. Instrumental conditions of LC-QTOFMS for full-scan screening analysis.

Instrument	LC: 1290 infinity II (Agilent Technologies, Santa Clara, CA) QTOFMS: Triple time-of-flight (TripleTOF®) 5600+ mass spectrometer (AB Sciex, Framingham, MA)
Samples	F3.5, F3.6, F3.7, and F3.8 RP-HPLC fractions from C1
Analytical column	ZORBAX Eclipse XDB-C18 (150 mm × 2.1 mm i.d. × 5 μm film)
Column temperature	40 °C
Injection volume	3 μL
Flow rate	0.4 mL min^{-1}
Mobile phase	A: 0.1% Formic acid and 10mM ammonium formate in water, B: 0.1% Formic acid in acetonitrile
Mobile phase gradient	Time (min) / Solvent A / Solvent B 0 / 90 / 10 1 / 90 / 10 15 / 0 / 100 24 / 0 / 100 25 / 90 / 10 30 / 90 / 10
Ionization mode	Electrospray ionization (ESI) Positive and Negative mode
Mass scan type	Full scan and Information Dependent Acquisition (IDA) Scanning
TOF masses (Da)	100–2000 Da
Ion source gas 1	50 psi
Ion source gas 2	50 psi
Curtain gas	30 psi
Temperature	500 °C
Ion source	DuoSpray Ion Source
Ion spray voltage	Positive: 5,500 V, Negative -4,500 V
Software	All-in-One_HRMS/MS TCM library 1.0 metabolite software

Table S2. Instrumental conditions for analyzing polar AhR-active compounds using HPLC-MS/MS.

Instrument	HPLC: Agilent Infinity 1290 II, MS/MS: SCIEX Qtrap 6500
Samples	F3.5, F3.6, F3.7, and F3.8 RP-HPLC fractions from C1, C2, and C3
Analytical column	ZORBAX Eclipse XDB-C18 (150 mm × 2.1 mm i.d. × 5 μm film)
Column temperature	40 ℃
Injection volume	3 μL
Flow rate	0.4 mL min^{-1}
Mobile phase	A: 0.1% Formic acid and 10mM ammonium formate in water, B: 0.1% Formic acid in acetonitrile
Mobile phase gradient	See table below
Ionization mode	Electrospray ionization (ESI) Positive mode
TOF masses (Da)	100–2000 Da
Ion source gas 1	50 psi
Ion source gas 2	50 psi
Curtain gas	30 psi
Temperature	500 °C
Ion source	DuoSpray Ion Source
Ion spray voltage	Positive: 5,500 V

Time (min)	Solvent	
	A	B
0	90	10
1	90	10
15	0	100
24	0	100
25	90	10
30	90	10

Table S3. List of candidates for polar AhR-active compounds in the fraction samples (F3.5−F3.8) of organic extracts from C1 sediment using LC-QTOFMS.

Fractions and compounds	Molecular formula	CAS number	Molecular weight	Matching factor	AhR activity
F3.5 fraction					
Canrenone	$C_{22}H_{28}O_3$	213-554-5	340.456	99	+[a]
Triphenyl phosphate	$C_{18}H_{15}O_4P$	115-86-6	326.283	98	–[b]
Diphenoxylate	$C_{30}H_{32}N_2O_2$	915-30-0	452.587	95	
Hydroxygenkwanin	$C_{16}H_{12}O_6$	20243-59-8	300.263	94	
Daidzein	$C_{15}H_{10}O_4$	486-66-8	254.238	89	−
Neburon	$C_{12}H_{16}Cl_2N_2O$	555-37-3	275.174	89	
Scutellarein	$C_{15}H_{10}O_6$	529-53-3	286.236	86	
Eriodictyol	$C_{15}H_{12}O_6$	552-58-9	288.252	86	
Danofloxacin	$C_{19}H_{20}FN_3O_3$	112398-08-0	357.379	79	
Bulleyaconitine A	$C_{35}H_{49}NO_9$	107668-79-1	643.764	77	
Difenzoquat	$C_{17}H_{17}N_2$	49866-87-7	249.330	74	
Strychnine	$C_{21}H_{22}N_2O_2$	57-24-9	334.412	73	
F3.6 fraction					
Isorhamnetin	$C_{16}H_{12}O_7$	480-19-3	316.262	100	
Genistein	$C_{15}H_{10}O_5$	446-72-0	270.237	99	+
Oxadixyl	$C_{14}H_{18}N_2O_4$	77732-09-3	278.304	96	
Quercetin	$C_{15}H_{10}O_7$	117-39-5	302.236	94	−
Rutaecarpine	$C_{18}H_{13}N_3O$	84-26-4	287.315	93	+
Eupatilin	$C_{18}H_{16}O_7$	22368-21-4	344.315	92	
Fenthion-sulfoxide	$C_{10}H_{15}O_4PS_2$	3761-41-9	294.328	91	
Doxycycline	$C_{22}H_{24}N_4O_8$	564-25-0	444.435	91	
Mepanipyrim	$C_{14}H_{13}N_3$	110235-47-7	223.273	89	+
Ellagic acid	$C_{14}H_6O_8$	476-66-4	302.193	85	
Glycitein	$C_{16}H_{12}O_5$	40957-83-3	284.263	85	−
Kaempferol	$C_{15}H_{10}O_6$	520-18-3	286.236	85	−
Loratadine	$C_{22}H_{23}ClN_2O_2$	79794-75-5	382.883	84	−
1,7-Dimethoxyxanthone	$C_{15}H_{12}O_4$	50415-71-9	182.191	83	
3,4,5-Trimethoxycinnamic acid	$C_{12}H_{14}O_5$	90-50-6	238.237	82	
Luteoloside	$C_{21}H_{20}O_{11}$	5373-11-5	448.377	80	
Coumarin	$C_9H_6O_2$	91-64-5	146.143	79	−
Flunixin	$C_{14}H_{11}F_3N_2O_2$	38677-85-9	296.245	79	
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	85721-33-1	331.341	75	+
Baquiloprim	$C_{17}H_{20}N_6$	102280-35-3	308.381	74	
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	846-49-1	321.158	73	
Imipramine	$C_{19}H_{24}N_2$	50-49-7	280.407	72	
F3.7 fraction					
[10]-Gingerol	$C_{21}H_{34}O_4$	23513-15-7	350.492	100	
Amygdalin	$C_{20}H_{27}NO_{11}$	29883-15-6	457.428	100	
Pyridaben	$C_{19}H_{25}ClN_2OS$	96489-71-3	364.933	100	−
Corticosterone	$C_{21}H_{30}O_4$	50-22-6	346.461	99	
Cortisone	$C_{21}H_{28}O_5$	53-06-5	360.444	96	−
Flavin Mononucleotide	$C_{17}H_{21}N_4O_9P$	146-17-8	456.344	93	
Fenazaquin	$C_{20}H_{22}N_2O$	120928-09-8	306.401	92	
Naringenin	$C_{15}H_{12}O_5$	67604-48-2	272.253	92	−
Protopine	$C_{20}H_{19}NO_5$	130-86-9	353.369	91	+
Phenazepam	$C_{15}H_{10}BrClN_2O$	51753-57-2	349.610	89	
Donepezil	$C_{24}H_{29}NO_3$	120014-06-4	379.492	86	
16-Dehydroprogesterone	$C_{21}H_{28}O_2$	1096-38-4	312.446	85	
Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	33171-05-0	308.328	85	
Psoralidin	$C_{20}H_{16}O_5$	18642-23-4	336.388	82	

Ranitidine	$C_{13}H_{22}N_4O_3S$	66357-35-5	314.404	81	
Formononetin	$C_{16}H_{12}O_4$	485-72-3	268.264	77	−
Clodinafop-propargyl	$C_{17}H_{13}ClFNO_4$	105512-06-9	349.741	76	−
Atenolol	$C_{14}H_{22}N_2O_3$	29112-68-7	266.366	75	
Columbianadin	$C_{19}H_{20}O_5$	5058-13-9	328.359	75	
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$	83121-18-0	422.469	71	
F3.8 fraction					
Cinnamic acid	$C_9H_8O_2$	140-10-3	148.159	100	
7-Ketocholesterol	$C_{27}H_{44}O_2$	556-28-9	400.637	100	
Dioctyl phthalate	$C_{24}H_{38}O_4$	117-84-0	390.556	100	−
Etofenprox	$C_{25}H_{28}O_3$	80844-07-1	376.488	99	
Ziprasidone	$C_{21}H_{21}ClN_4OS$	146939-27-7	412.936	99	−
Danazol	$C_{22}H_{27}NO_2$	17230-88-5	337.455	98	−
Hydrocortisone	$C_{21}H_{30}O_5$	50-23-7	362.460	98	+
Dibutyl phthalate	$C_{16}H_{22}O_4$	84-74-2	278.344	97	−
Naringin	$C_{27}H_{32}O_{14}$	10236-47-2	580.535	97	
11a-Hydroxyprogesterone	$C_{21}H_{30}O_3$	312-90-3	330.461	95	
Inabenfide	$C_{19}H_{15}ClN_2O_2$	82211-24-3	338.788	93	
Medroxyprogesterone	$C_{22}H_{32}O_3$	520-85-4	344.488	93	+
Syringin	$C_{17}H_{24}O_9$	118-34-3	372.367	93	
Wogonin	$C_{16}H_{12}O_5$	632-85-9	284.263	92	−
Cortexolone	$C_{21}H_{30}O_4$	152-58-9	346.461	92	
Enrofloxacin-D5	$C_{19}H_{22}FN_3O_3$	1173021-92-5	364.426	90	
Tadalafil	$C_{22}H_{19}N_3O_4$	171596-29-5	389.404	89	
Rafoxanide	$C_{19}H_{11}Cl_2I_2NO_3$	22662-39-1	626.010	82	−
Phthalic acid	$C_8H_6O_4$	88-99-3	166.131	81	
Triclabendazole sulfone	$C_{14}H_9Cl_3N_2O_3S$	106791-37-1	391.657	81	
17α-Ethynylestradiol	$C_{20}H_{24}O_2$	57-63-6	296.403	79	−
Fenthion-sulfone	$C_{10}H_{15}O_5PS_2$	3761-42-0	310.327	78	
Daphnoretin	$C_{19}H_{12}O_7$	2034-69-7	352.294	75	
Norfludiazepam	$C_{15}H_{10}ClFN_2O$	2886-65-9	288.704	72	
Myricetin	$C_{15}H_{10}O_8$	529-44-2	318.235	72	
β-Carotene	$C_{40}H_{56}$	7235-40-7	536.873	72	
Thioridazine	$C_{21}H_{26}N_2S_2$	50-52-2	370.574	70	−

[a] +: Significant response in the H4IIE-*luc* bioassay.

[b] −: Not significant response in the H4IIE-*luc* bioassay.

Table S4. Conditions of HPLC-MS/MS for quantification of polar AhR-active compounds in sediment organic extracts.

Compounds	MRM transition Parent ion → Daughter ion (m/z)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
Canrenone	341.24 → 106.70 (ESI+)	6	10	35	54
Genistein	270.92 → 153.00 (ESI+)	211	10	37	6
Ruatecarpine	287.98 → 272.90 (ESI+)	226	10	43	26
Mepanipyrim	223.95 → 106.00 (ESI+)	1	10	33	10
Ciprofloxacin	332.00 → 314.00 (ESI+)	1	10	27	16
Protopine	353.95 → 189.10 (ESI+)	1	10	41	10
Hydrocortisone	363.04 → 121.00 (ESI+)	76	10	31	10
Medroxyprogesterone	345.11 → 122.90 (ESI+)	121	10	29	20

Table S5. Concentrations of polar AhR-active compounds in the sediments of inland creeks in Lake Sihwa, Republic of Korea.

Sites	Concentrations of polar AhR-active compounds (ng g^{-1} dm)							
	Canrenone	Genistein	Rutaecarpine	Mepanipyrim	Ciprofloxacin	Protopine	Hydrocortisone	Medroxyprogesterone
C1	2.6	58	0.2	0.03	1.1	<LOD	280	4.8
C2	0.2	37	<LOD[a]	<LOD	0.1	<LOD	53	1.9
C3	0.1	24	<LOD	<LOD	0.1	<LOD	1.5	1.8

[a] Below the limit of detection.

Table S6. Potency balance between instrument-derived BEQs and bioassay-derived BaP-EQs in the RP-HPLC fractions (F3.5−F3.8) of selected inland creek sediments (C1–C3).

Compounds	C1				C2				C3			
	F3.5	F3.6	F3.7	F3.8	F3.5	F3.6	F3.7	F3.8	F3.5	F3.6	F3.7	F3.8
Instrument-derived BEQs (ng BEQ g^{-1} dm)												
Polar AhR agonists												
Canrenone	0.01				0.0008				0.0007			
Genistein		0.01				0.003				0.002		
Rutaecarpine		0.26				<LOD				<LOD		
Mepanipyrim		0.00001				<LOD				<LOD		
Ciprofloxacin		0.004				0.0005				0.0005		
Protopine			<LOD[a]				<LOD				<LOD	
Hydrocortisone				39				7.4				0.2
Medroxyprogesterone				0.07				0.03				0.03
BEQ-polar AhR agonists[b]	0.01	0.28	<LOD	39	0.0008	0.004	<LOD	7.4	0.0007	0.002	<LOD	0.2
Bioassay-derived BaP-EQs (ng BaP-EQ g^{-1} dm)												
Potency-based BaP-EQ_{50}[c]	586	1810	1300	69	12	170	430	110	0.66[d]	29	150	3.6
Contribution (%)	0.002	0.02	<LOD	57	0.01	0.002	<LOD	6.9	0.10	0.01	<LOD	6.0

[a] Limit of detection.

[b] BEQ-polar AhR agonists concentrations were calculated from the concentrations of canrenone, genistein, rutaecarpine, mepanipyrim, ciprofloxacin, protopine, hydrocortisone, and medroxyprogesterone multiplied by their RePs values obtained from this study.

[c] Potency-based BaP-EQ_{50} was obtained from sample dose-response relationships elicited by the sediments samples at 6 levels of dilution.

[d] Potency-based BaP-EQ_{20} value.

Table S7. Predicted potential toxicity of eight polar AhR agonists using VirtualToxLab.

Toxicity	Compounds							
	Canrenone	Genistein	Rutaecarpine	Mepanipyrim	Ciprofloxacin	Protopine	Hydrocortisone	Medroxyprogesterone
AhR	6.8 μm[a]	Not binding	2.0 μm	26 μm	Not binding	Not binding	1.2 μm	403 nm
ER[b]	57 μm	2.6 μm	Not binding	Not binding	Not binding	40 μm	46 μm	954 nm
GR[c]	4.3 μm	4.2 μm	4.2 μm	21 μm	72 μm	261 nm	3.2 nm	27 nm

[a] Blue: weak binding, red: moderate binding, black: strong binding.
[b] Estrogenic receptor activity.
[c] Glucocorticoid receptor activity.

Supplementary Figures

Fig. S1. Map showing the sampling sites of surface sediments from the inland creeks in Lake Sihwa, Republic of Korea.

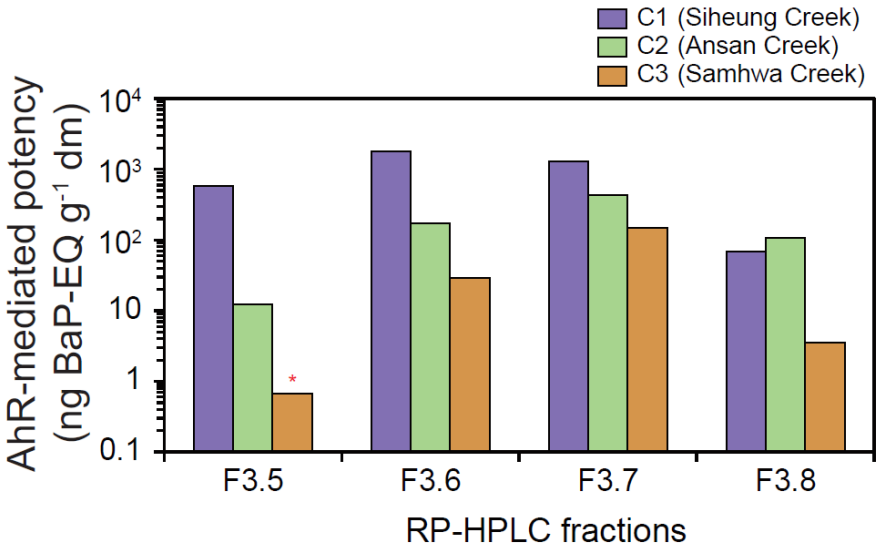

Fig. S2. Bioassay-derived BaP-EQs (potency-based) in RP-HPLC fractions (F3.5−F3.8) of sediment organic extracts (*: based on EC_{20} values).

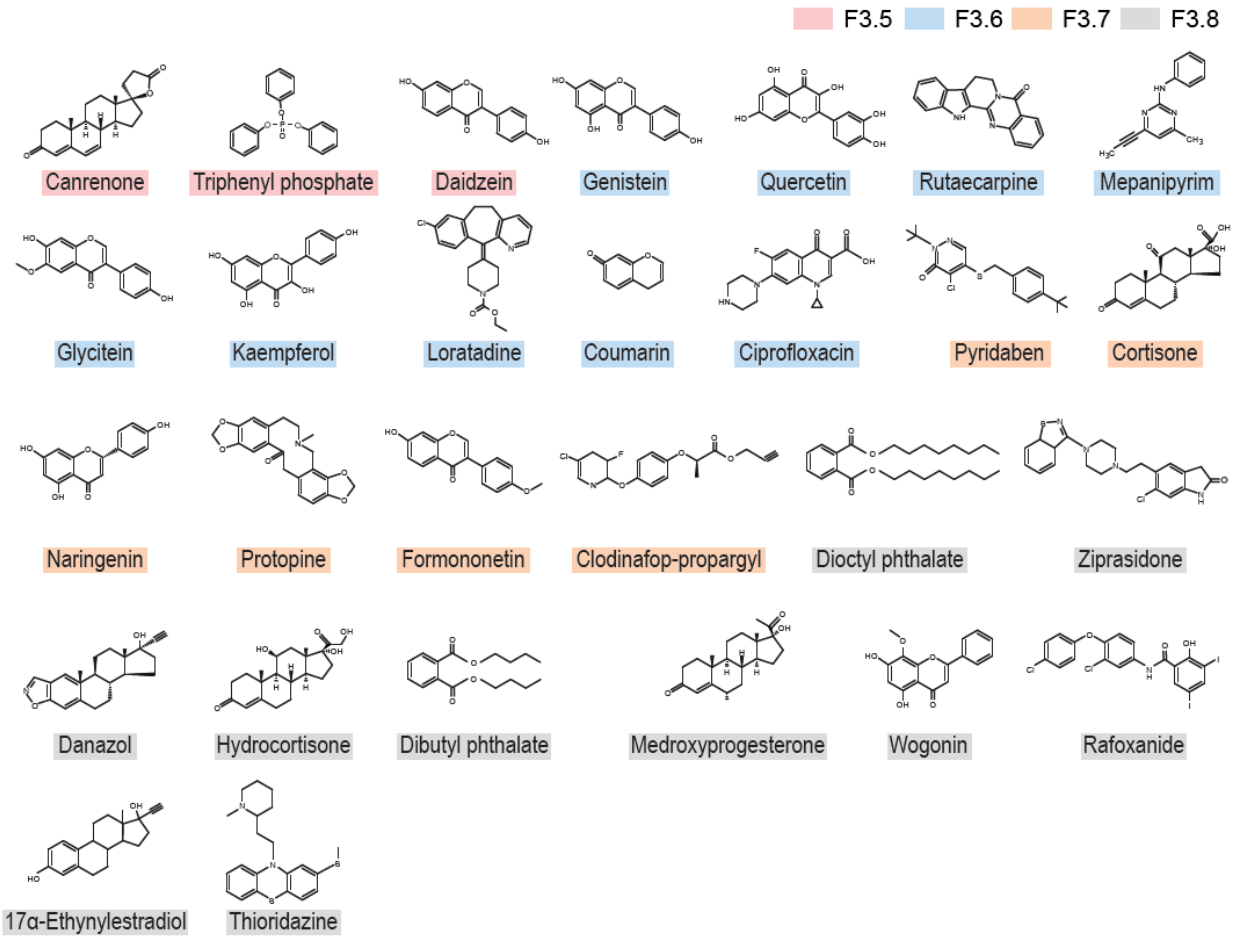

Fig. S3. Chemical structures of 28 tentative AhR agonists (for toxicological confirmation) in sediments from the inland creeks of Lake Sihwa, Republic of Korea.

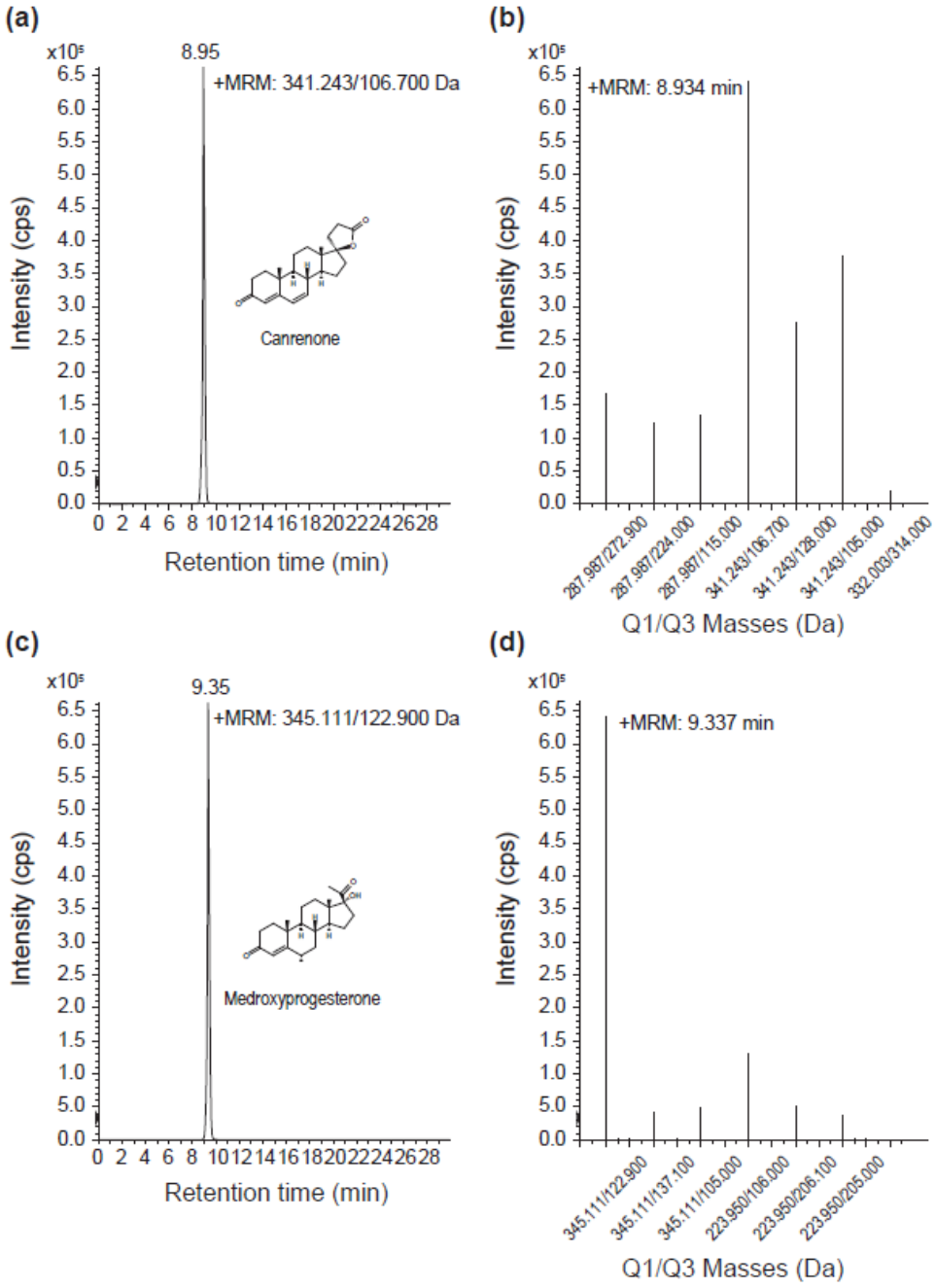

Fig. S4. Extracted ion chromatograms (a, c) and Q1/Q3 masses (b, d) of canrenone and medroxyprogesterone.