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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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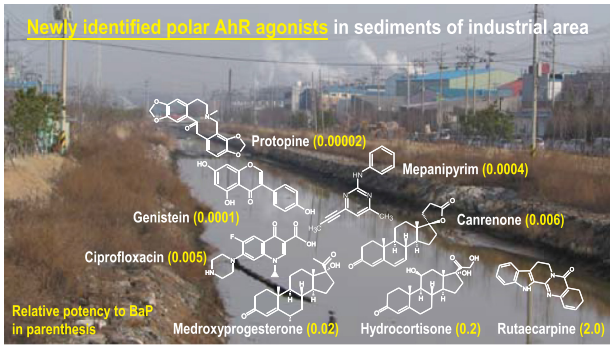
## HIGHLIGHTS

- 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.

## GRAPHICAL ABSTRACT

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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.



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## 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., 2015). 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; Redman 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; J. Lee et al., 2017; 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 (Y. Lee et al., 2017). 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 °C 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 50 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 (~$7.0 \times 10^4$ cells $mL^{-1}$) were seeded in the 96 micro-well plate at 250 μL per well. After seeding, cells were incubated at 37 °C 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; J.

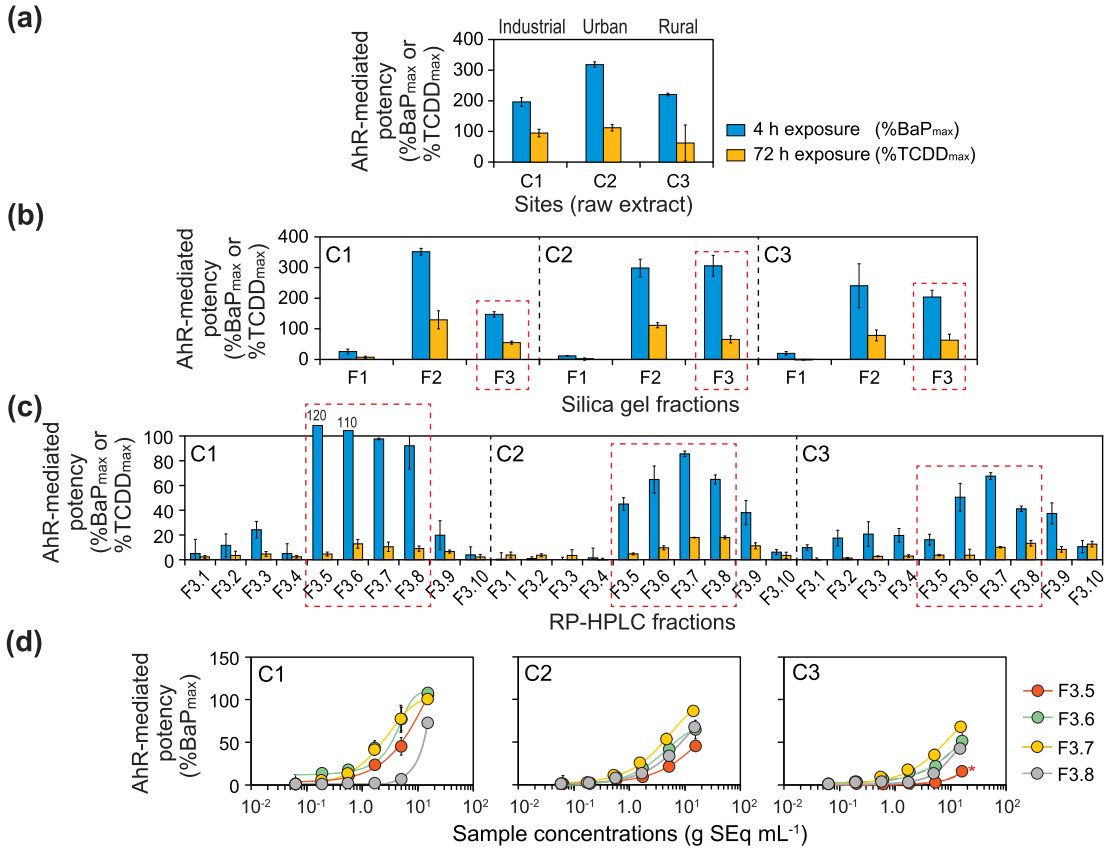


**Fig. 1.** (a) AhR-mediated potencies of raw extracts, (b) silica gel fractions, (c) RP-HPLC fractions of inland creeks (C1–C3) after 4 h and 72 h exposure, and (d) dose-response curves for AhR-mediated potency of selected HPLC-fractions (F3.5–F3.8 of C1–C3 sediment extracts) from the inland creeks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

Lee et al., 2017); 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).

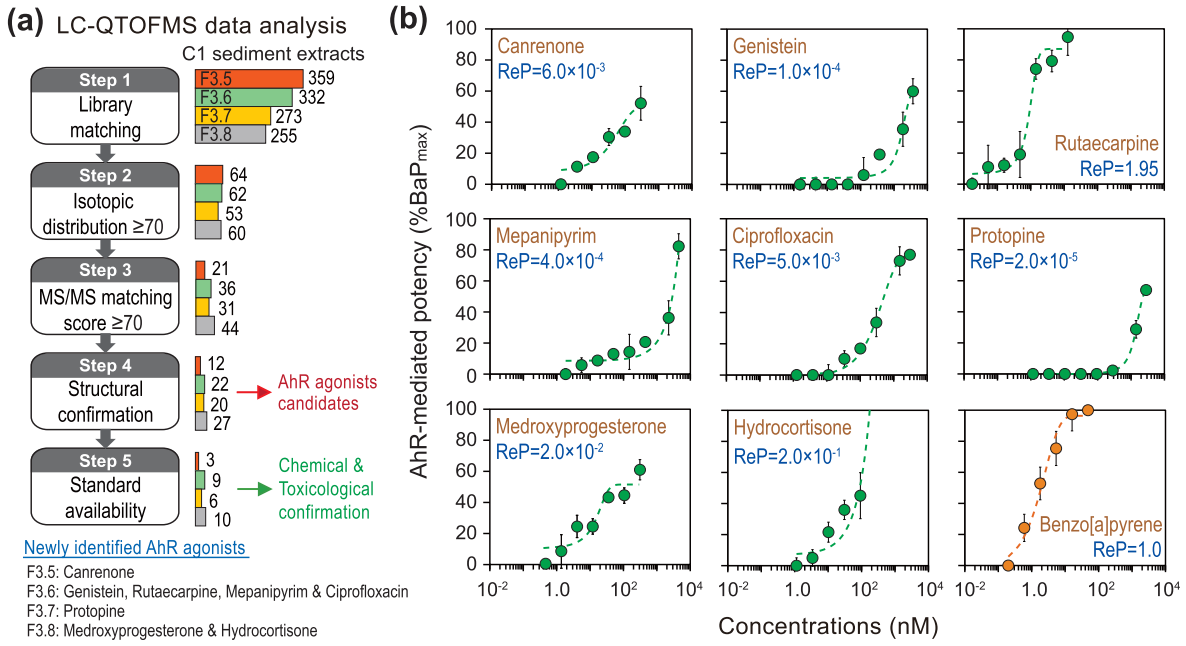


**Fig. 2.** (a) Five-step selection process for LC-QTOFMS data analysis to select potential AhR agonists, and (b) dose-response relationships for AhR-mediated potency of eight tentative AhR agonists and benzo[*a*]pyrene in the H4IIE-*luc* bioassay (error bar: mean ± SD (n = 3); ReP: relative potency value).

### 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)

**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[a] | $C_{22}H_{28}O_3$ | 213-554-5 | 340.46 | 100,529 | Diuretic | Romanelli and Gentilini (2004) |
| Triphenyl phosphate | $C_{18}H_{15}O_4P$ | 115-86-6 | 326.28 | 1,736,175 | Plasticizer, fire retardant | Stapleton et al. (2009) |
| Daidzein | $C_{15}H_{10}O_4$ | 486-66-8 | 254.24 | 125,412 | Anti-cancer agent | Coward et al. (1993) |
| *F3.6 fraction* | | | | | | |
| Genistein[a] | $C_{15}H_{10}O_5$ | 446-72-0 | 270.24 | 98,801 | Anti-cancer agent | Banerjee et al. (2008) |
| Quercetin | $C_{15}H_{10}O_7$ | 117-39-5 | 302.23 | 79,488 | Dietary supplement | Volate et al. (2005) |
| Ruatecarpine[a] | $C_{18}H_{13}N_3O$ | 84-26-4 | 287.32 | 31,527 | Herbal medicine | Shew et al. (1996) |
| Mepanipyrim[a] | $C_{14}H_{13}N_3$ | 110235-47-7 | 223.27 | 240,686 | Fungicide, pesticide | Nakamura et al. (2003) |
| Glycitein | $C_{16}H_{12}O_5$ | 40957-83-3 | 284.26 | 152,401 | Anti-cancer agent | Shimoda and Hamada (2010) |
| Kaempferol | $C_{15}H_{10}O_6$ | 520-18-3 | 286.24 | 77,339 | Anti-cancer agent | Kim and Choi (2013) |
| Loratadine | $C_{22}H_{23}ClN_2O_2$ | 79794-75-5 | 382.88 | 205,071 | Anti-pruritic agent | Roman and Danzig (1993) |
| Coumarin | $C_9H_6O_2$ | 91-64-5 | 146.14 | 54,360 | Anti-coagulant agent | Cravotto et al. (2001) |
| Ciprofloxacin[a] | $C_{17}H_{18}FN_3O_3$ | 85721-33-1 | 331.34 | 40,577 | Anti-biotic agent | Forrest et al. (1993) |
| *F3.7 fraction* | | | | | | |
| Pyridaben | $C_{19}H_{25}ClN_2OS$ | 96489-71-3 | 364.93 | 1,845,249 | Pesticide | Zhu et al. (2005) |
| Cortisone | $C_{21}H_{28}O_5$ | 53-06-5 | 360.44 | 136,293 | Anti-inflammatory agent | Alsop et al. (2016) |
| Naringenin | $C_{15}H_{12}O_5$ | 67604-48-2 | 272.25 | 35,900 | Anti-ulcer agent | Yamamoto et al. (2004) |
| Protopine[a] | $C_{20}H_{19}NO_5$ | 103-86-9 | 353.37 | 52,206 | Anti-cancer agent | Jiang et al. (2004) |
| Formononetin | $C_{16}H_{12}O_4$ | 485-72-3 | 268.26 | 100,718 | Anti-angiogenic agent | Huh et al. (2009) |
| Clodinafop-propargyl | $C_{17}H_{13}ClFNO_4$ | 105512-06-9 | 349.74 | 94,202 | Herbicide | Baghestani et al. (2008) |
| *F3.8 fraction* | | | | | | |
| Dioctyl phthalate | $C_{24}H_{38}O_4$ | 117-84-0 | 390.56 | 13,784,615 | Plasticizer | Rajendran et al. (2002) |
| Ziprasidone | $C_{21}H_{21}ClN_4OS$ | 146939-27-7 | 412.94 | 1,934,198 | Anti-psychotic agent | Schmidt et al. (2001) |
| Danazol | $C_{22}H_{27}NO_2$ | 17230-88-5 | 337.46 | 227,810 | Endometriosis treatment | Igarashi et al. (1998) |
| Hydrocortisone[a] | $C_{21}H_{30}O_5$ | 50-23-7 | 362.46 | 165,545 | Anti-inflammatory agent | Sprung et al. (2008) |
| Dibutyl phthalate | $C_{16}H_{22}O_4$ | 84-74-2 | 278.34 | 1,971,180 | Insect attractant | Zong et al. (2013) |
| Medroxyprogesterone[a] | $C_{22}H_{32}O_3$ | 520-85-4 | 344.49 | 65,514 | Uterine cancer agent | Prior et al. (1994) |
| Wogonin | $C_{16}H_{12}O_5$ | 632-85-9 | 284.26 | 71,742 | Anti-convulsant drug | Park et al. (2007) |
| Rafoxanide | $C_{19}H_{11}Cl_2I_2NO_3$ | 22662-39-1 | 626.01 | 93,218 | Veterinary drug | Matsubara et al. (2012) |
| 17α-Ethynylestradiol | $C_{20}H_{24}O_2$ | 57-63-6 | 296.40 | 55,738 | Contraceptive | Hua et al. (2016) |
| Thioridazine | $C_{21}H_{26}N_2S_2$ | 50-52-2 | 370.57 | 55,237 | Anti-psychotic agent | Min et al. (2014) |

[a] Newly identified AhR agonists.

with AhR compared to BaP. In addition, RePs of hydrocortisone ($2.0 \times 10^{-1}$), medroxyprogesterone ($2.0 \times 10^{-2}$), canrenone ($6.0 \times 10^{-3}$), ciprofloxacin ($5.0 \times 10^{-3}$), mepanipyrim ($4.0 \times 10^{-4}$), genistein ($1.0 \times 10^{-4}$), and protopine ($2.0 \times 10^{-5}$) 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-active 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% in F3.8. 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

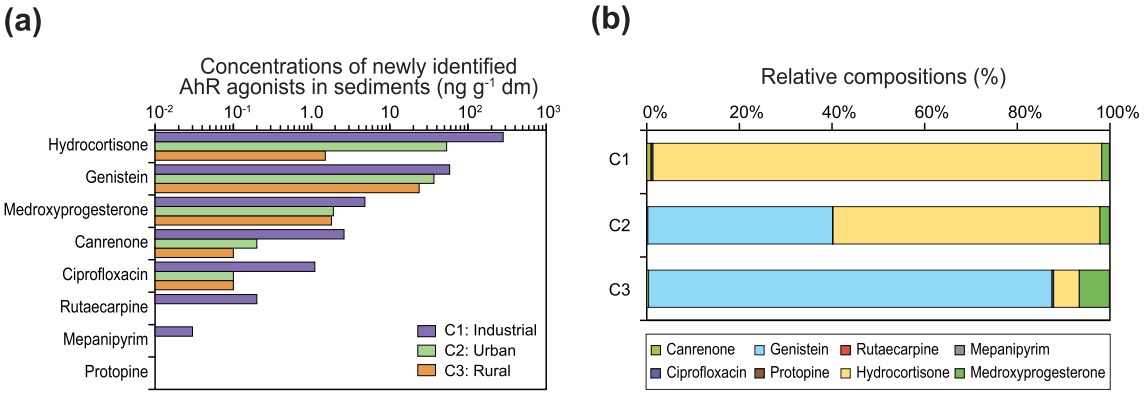


**Fig. 3.** (a) Distributions and (b) relative compositions of newly identified AhR agonists in the organic extracts of sediments from the inland creeks (industrial, urban, and rural areas) of Lake Sihwa, Republic of Korea.

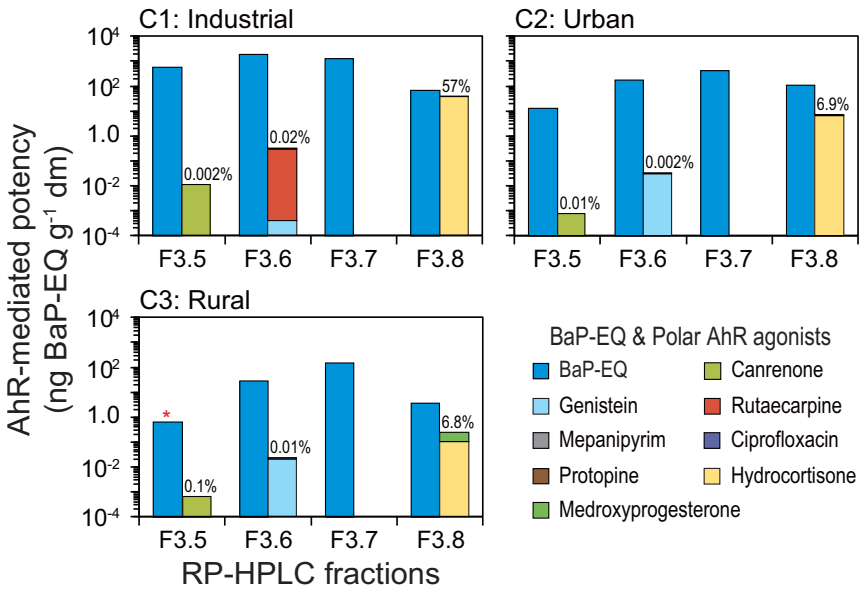


**Fig. 4.** Contribution of instrument-derived BEQs (newly identified AhR agonists) to bioassay-derived BaP-EQs (potency-based) in RP-HPLC fractions (F3.5–F3.8) of sediments from the inland creeks (industrial, urban, and rural areas) of Lake Sihwa, Republic of Korea.

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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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146566.

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<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)<br>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,<br>B: 0.1% Formic acid in acetonitrile |
| Mobile phase gradient | Time (min) / Solvent A / Solvent B<br>0 / 90 / 10<br>1 / 90 / 10<br>15 / 0 / 100<br>24 / 0 / 100<br>25 / 90 / 10<br>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<br>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,<br>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.

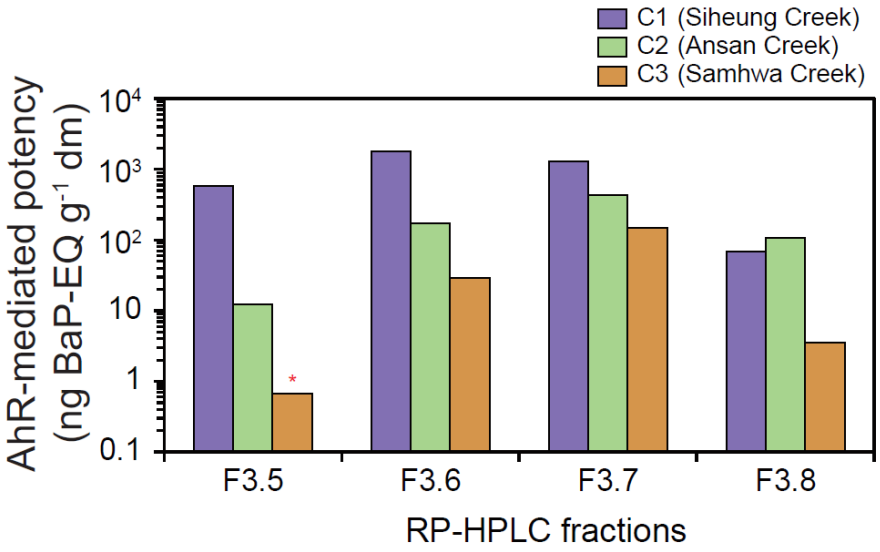


**Fig. S2.** Bioassay-derived BaP-EQs (potency-based) in RP-HPLC fractions (F3.5−F3.8) of sediment organic extracts (*: based on $EC_{20}$ values).

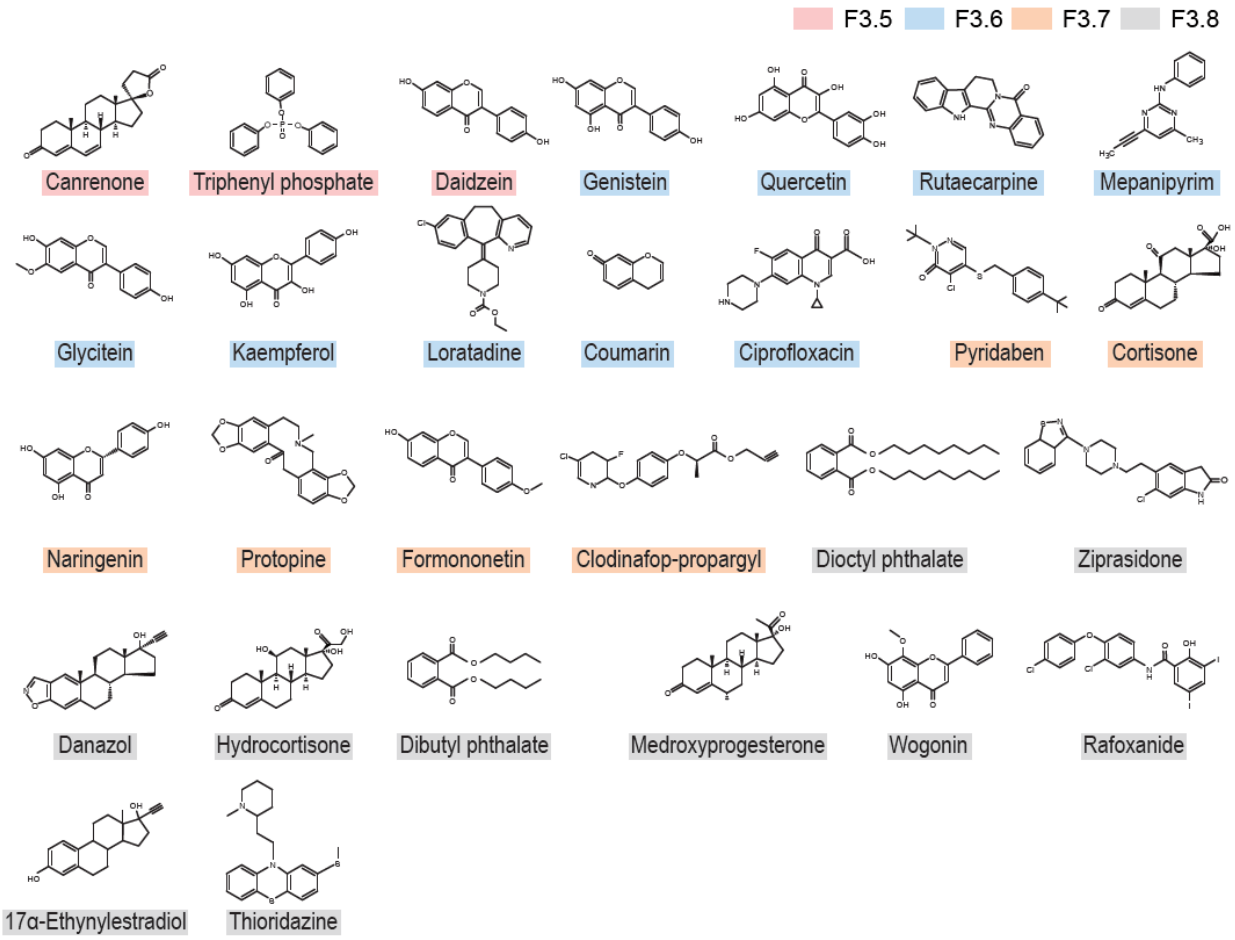


**Fig. S3.** Chemical structures of 28 tentative AhR agonists (for toxicological confirmation) in sediments from the inland creeks of Lake Sihwa, Republic of Korea.

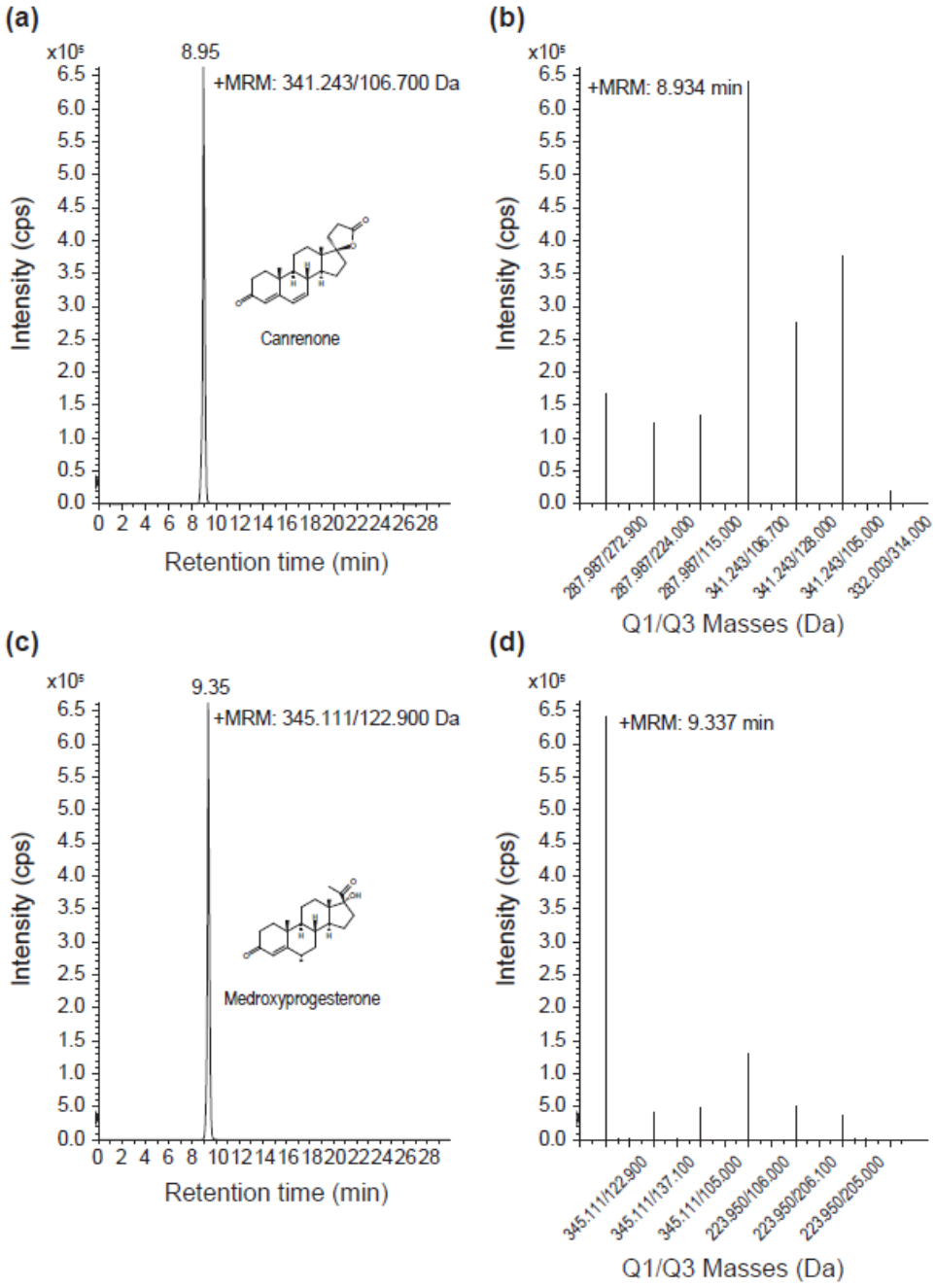


**Fig. S4.** Extracted ion chromatograms (a, c) and Q1/Q3 masses (b, d) of canrenone and medroxyprogesterone.