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Research Paper

Identification of novel polar aryl hydrocarbon receptor agonists accumulated in liver of black-tailed gulls in Korea using advanced effect-directed analysis

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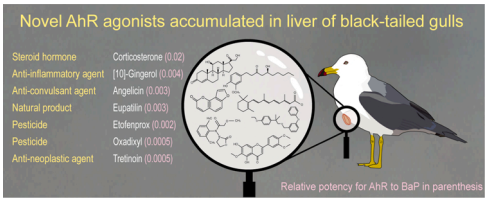
HIGHLIGHTS

- Seven AhR agonists were identified in livers of seagull using effect-directed analysis.
- Novel AhR agonists have been mainly used for pharmaceuticals and pesticides.
- Novel AhR agonists significantly contributed to total induced AhR-mediated potencies.
- Some polar AhR agonists could be biomagnified to top predators through the food web.

GRAPHICAL ABSTRACT



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ABSTRACT

Although bioaccumulation of persistent organic pollutants in seabirds has been examined, few studies have been conducted to identify previously unidentified substances. Here, aryl hydrocarbon receptor (AhR) agonists were identified in livers of black-tailed gulls from South Korea using effect-directed analysis combined with full-scan screening analysis. Significant AhR-mediated potencies were observed in the polar fractions of liver extracts using H4IIE-*luc* bioassay. Eight known polar AhR agonists accounted for 11–20% of the total AhR-mediated potencies in the polar fractions; hydrocortisone and rutaecarpine were the major contributors. Twenty-two AhR agonist candidates in the polar fractions were identified using liquid chromatography–quadrupole time-of-flight mass spectrometry during a six-step selection process. Of these, [10]-gingerol, angelicin, corticosterone, eupatilin, etofenprox, oxadixyl, and tretinoin were identified as novel AhR agonists. The contribution to potencies increased with inclusion of novel AhR agonists (27–52%); corticosterone and [10]-gingerol contributed significantly. Quantitative structure-activity relationship suggested that the novel AhR agonists have other potential toxic effects, including carcinogenicity and mutagenicity. Polar AhR agonists have been used for pharmaceuticals and pesticides. Some novel AhR agonists have log $K_{OW} > 2$ and log $K_{OA} \geq 6$, which indicates that these compounds can be biomagnified in air-breathing organisms, such as seabirds.

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1. Introduction

Currently available effect-directed analysis (EDA) technique is useful for identification of key toxicants in environmental samples (Brack et al., 2016; Hong et al., 2016). Effect-based assessment using in vitro and/or in vivo bioassays is conducted to screen major toxic fractions in extracts of environmental samples (Brack, 2003). Multi-step fractionations enable separation of thousands of chemicals in complex environmental mixtures into fractions according to physico-chemical properties, such as polarity, log K_{OW}, and/or molecular mass (Brack, 2003; Simon et al., 2013). Fractionation of environmental sample extracts enables isolation of major causative compounds into a few fractions and facilitates chemical analysis by reducing sample complexity. Based on relative potencies (RePs) of target compounds with respect to the reference compound and concentrations in the fraction, contributions of individual compounds to specific effects can be evaluated quantitatively (Simon et al., 2013). However, known toxic substances sometimes less explain the total toxic potencies of samples; thus, identification of previously unidentified toxic substances in environmental samples is necessary (Muz et al., 2017; Simon et al., 2013).

Full-scan screening analysis (FSA) has become a powerful tool to identify the previously unmonitored toxicants in environmental samples with the combination of the EDA (Cha et al., 2021; Lee et al., 2020). Recently, FSA has been conducted using high-resolution mass spectrometry (HRMS) techniques, such as quadrupole time-of-flight mass spectrometry (QTOFMS) and Orbitrap mass spectrometry (Muz et al., 2017). FSA with HRMS followed by sophisticated data analysis enables detection of numerous compounds in complex environmental samples (Cha et al., 2019, 2021; Muz et al., 2017; Zwart et al., 2020). Numerous detected compounds can be prioritized through application of selection criteria by use of data- and experiment-based strategies (Tian et al., 2020). Consequently, performance of advanced EDA (i.e., EDA combined with FSA) could lead to the successful identification of previously unmonitored toxic chemicals in environmental samples (Cha et al., 2019, 2021; Muz et al., 2017; Simon et al., 2013).

Several advanced EDA studies have been conducted, primarily with abiotic samples, such as sediment, soil, wastewater, or river water (Cha et al., 2019, 2021; Muz et al., 2017; Zwart et al., 2020). Few studies have involved identification and confirmation of unknown toxicants in biotic samples, since collection, extraction, clean-up, and instrumental analysis can be more complicated for these samples than for abiotic samples (Simon et al., 2015). However, EDA studies of biological samples can provide information on bioavailability, bioaccumulation, and internal exposure pathways of previously unidentified substances; thus, these studies are being increasingly recommended (Simon et al., 2015).

The black-tailed gull (*Larus crassirostris*) is a top predator in marine and adjacent terrestrial food webs, feeding on large zooplankton, squirrels, cephalopods, small fish, and other terrestrial food sources (Choi et al., 2001; Hong et al., 2014; Yamashita et al., 2018). Due to its high lipid content, long lifespan, wide habitat range, and diversity of food sources, it is an excellent model organism for biomonitoring of toxic substances (Choi et al., 2001; Hong et al., 2014; Yamashita et al., 2018). Feeding ranges of black-tailed gulls on the eastern coast of South Korea have a radius of approximately 73 km (KNPS, 2015). Black-tailed gulls acclimate to environmental conditions and resources of food, with ecological responses occurring at the individual and population levels (Robuck et al., 2020). Samples from this species provide useful information on contamination, bioavailability, and the trophic transfer of persistent toxic substances in the environment, as well as overall ecosystem health (Furness and Camphuysen, 1997; Hazen et al., 2019; Hobson et al., 1994).

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that can bind to environmental and dietary ligands and subsequently initiate pleiotropic signal transduction pathways, that can affect fitness of individuals (Mimura and Fujii-Kuriyama, 2003). Abnormal gene expression by AhR-active compounds can cause developmental toxicity, carcinogenicity, and reproductive toxicity (Mimura and Fujii-Kuriyama, 2003). Compounds capable of triggering AhR activation are commonly known as dioxin-like compounds and polycyclic aromatic hydrocarbons (PAHs), which generally have hydrophobic properties (Larsson et al., 2014). A recent report revealed that even polar compounds could bind to AhR (Cha et al., 2021; Lee et al., 2020). For example, canrenone, rutaecarpine, ciprofloxacin, mepanipyrim, genistein, protopine, hydrocortisone, enoxolone, and medroxyprogesterone, which have been used as pharmaceuticals and fungicides, were recently identified as novel AhR agonists in sediment from an industrialized area (Cha et al., 2021; Lee et al., 2020).

In the present study, AhR-active compounds in livers from black-tailed gulls inhabiting the southeastern coast of Korea were investigated, by use of advanced EDA. Classical and emerging persistent organic pollutants (POPs) and PAHs accumulated in livers were investigated, and AhR-mediated activities were measured using H4IIE-*luc* bioassays. FSA was performed on more potent fractions, and tentative AhR agonists were identified. Novel AhR agonists were identified through chemical and toxicological confirmation, and the contributions of existing and novel AhR agonists were evaluated. In addition, in silico modeling was applied to identify the other potential toxicities of polar AhR agonist candidates. Finally, biomagnification potentials of polar AhR agonist candidates in liver samples of black-tailed gulls were evaluated. To our knowledge, this report is the first to describe the identification of previously unidentified AhR agonists in the seabird liver by use of advanced EDA.

2. Materials and methods

2.1. Sample collection and preparation

The design of the study for identification of novel AhR agonists in the liver of black-tailed gulls was presented in Fig. S1 of the Supplementary Materials. Three carcasses of adult black-tailed gulls (S1 −S3) were provided by the Nakdong Estuary Eco Centre in Busan City, and two (S4 and S5) were provided by the Wildlife Rescue and Management Center of Ulsan City (Fig. 1a). The birds were brought to the rescue centers in 2014 (Busan) and 2016 (Ulsan). They failed to rehabilitate and were stored frozen in the centers. Seagulls used in this study were killed by accident or euthanized due to severe injuries (Table S1). Because the habitat ranges of these black-tailed gulls collected in Busan and Ulsan overlapped, regional differences were not considered in this study. Detailed biological information for these samples is provided in Table S1.

Liver tissue was separated from each black-tailed gull body and stored at − 20 ℃ until analysis. Samples of liver were homogenized using a Tekmar tissuemizer (Ultra-Turrax T25; Janke & Kunkel Inc., Staufen, Germany), dehydrated by the addition of anhydrous sodium sulfate. Raw organic extracts (RE) were extracted with 350 mL 80% hexane (Honeywell, Charlotte, NC) in methylene chloride (MC; J.T. Baker, Phillipsburg, NJ) on a Soxhlet extractor for 16 h. Lipids were removed from the eluent using high-performance liquid chromatography (HPLC; 250 × 22.5-mm I.D. size-exclusion column packed with Phenogel 100 Å; Phenomenex Co., Torrance, CA).

2.2. POPs and PAHs analyses

Concentrations of historically known and emerging POPs and PAHs in liver were quantified as described previously (Target compounds are listed in Tables S2 and S3) (Hong et al., 2005, 2010, 2014). In brief, organic extracts of liver were cleaned up using silica gel and alumina-multilayer-column chromatography. Instrumental analysis was conducted using an Agilent 6890 series gas chromatograph coupled to an Agilent 5975 quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA) in electron impact ionization mode for polychlorinated biphenyls (PCBs), chlordane-related compounds (CHLs),

dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), pentachlorobenzene (PeCB), and PAHs. Negative chemical ionization mode was used for polybrominated diphenyl ethers (PBDEs). For column separation, a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA) was used for PCBs, CHLs, DDTs, HCHs, HCB, PeCB, and PAHs, and a DB-1 column (15 m × 250 μm i.d., 0.25 μm film thickness; J&W Scientific) was used for PBDEs. Hexabromocyclododecanes (HBCDs) in extracts were analyzed as described previously (Hong et al., 2014). HBCD concentrations were measured with an Agilent 1200 HPLC system (Agilent Technologies) coupled with a triple-quadrupole mass spectrometer (API 3200; SCIEX, Toronto, Canada) (Hong et al., 2014). For quality control, isotopically labeled surrogate standards were added before extraction. The recovery rates were generally acceptable with ranging from 56% to 132% for POPs and PAHs analyses (Details in Table S4).

2.3. Silica gel column fractionation

REs of liver were separated using silica gel column chromatography (8 g activated silica gel, 70–230 mesh; Sigma-Aldrich, Saint Louis, MO). The REs (2 mL) were separated into non-polar, mid-polar, and polar fractions. The non-polar fraction was collected using 30 mL hexane. The mid-polar fraction was obtained using 60 mL 20% MC in hexane. The polar fraction was eluted using 50 mL 60% MC in acetone (J.T. Baker). All eluents were concentrated to 2 mL using a rotary evaporator followed by a gentle stream of N_2 gas. One milliliter of each fraction was completely evaporated to ensure that extracts in the bioassay had no biological effects, and was exchanged to the solvent with dimethyl sulfoxide (DMSO; Sigma-Aldrich) for H4IIE-*luc* bioassay. The remaining 1 mL was exchanged for methanol for targeted and nontargeted instrumental analyses.

2.4. Targeted polar AhR agonists analysis

Targeted compounds (i.e., known polar AhR agonists) are listed in Table S5. For example, eight compounds include hydrocortisone, genistein, medroxyprogesterone, ciprofloxacin, canrenone, rutaecarpine, protopine, and mepanipyrim were quantified in livers samples of black-tailed gulls. Concentrations of previously identified polar AhR agonists were measured using a 1290 infinity II series HPLC (Agilent Technologies) coupled to a QTRAP 6500 series tandem mass spectrometer (AB Sciex, Framingham, MA) (Cha et al., 2021). The sample to be analyzed was polar fractions of S1–S5, and a ZORBAX Eclipse XDB-C18 (150 mm × 2.1 mm i.d., 5 μm film thickness) column was used to separate chromatography. The column temperature was 40 ℃, the sample corresponding to 3 μL was injected. Mobile phase A: 0.1% formic acid and 10 mM ammonium formate in water, B: 0.1% formic acid in acetonitrile was used, and the flow rate was set to 0.4 mL min^{-1}. Electrospray ionization (ESI) positive mode was used. Ion source gas 1, ion source gas 2, and curtain gas were set to 50 psi, 50 psi, and 30 psi, respectively. It was set to 5500 V in positive mode using a DuoSpray ion source. Detailed instrumental conditions are presented in Table S6.

2.5. In vitro H4IIE-luc bioassays

A recombinant H4IIE-*luc* cell line is rat hepatoma cells stably transfected with a luciferase reporter gene under the regulation of the dioxin-sensitive factor (Sanderson et al., 1996). AhR-mediated potencies were measured in REs and silica gel fractions of extracts from liver, by use of the H4IIE-*luc* transactivation bioassay (4 h exposure). The bioassay methodology has been described in detail elsewhere (Hong et al., 2016). In brief, trypsinized cells were seeded into 96-well plates at ~74,000 cells mL^{-1} (250 μL/well). H4IIE-*luc* cells were grown at 37 ℃ in a 5% CO_2 humified atmosphere incubator for 24 h. The test wells were dosed

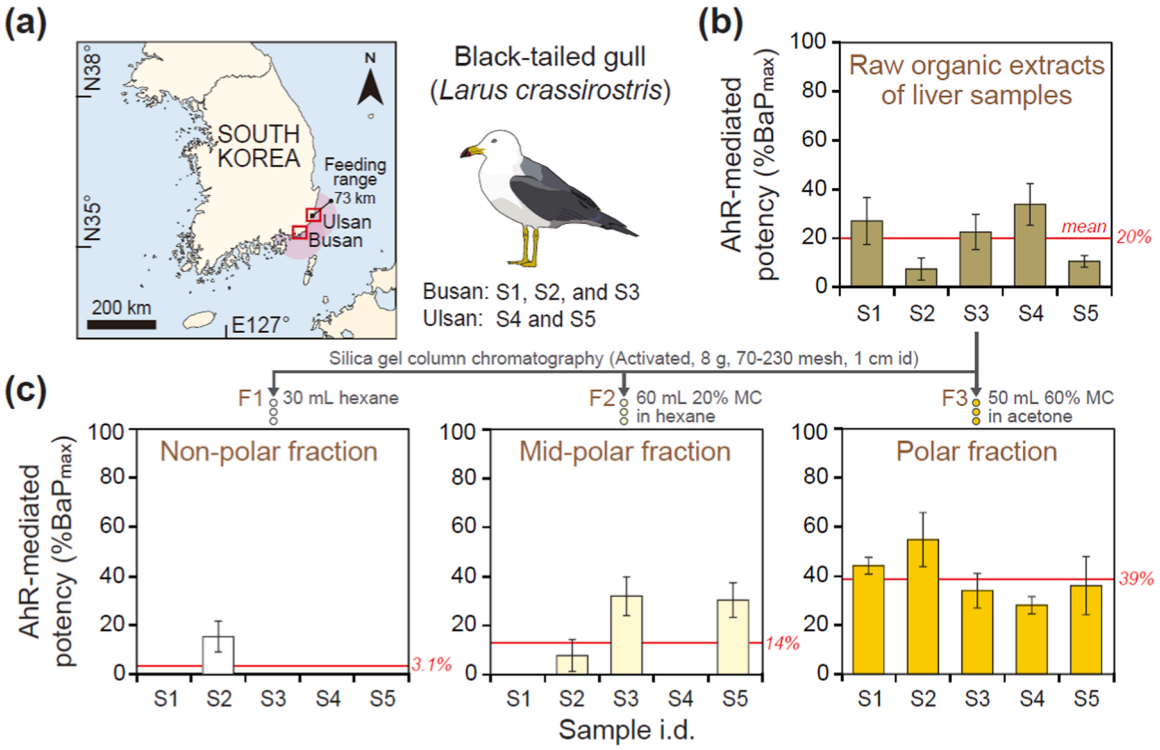

Fig. 1. (a) Map showing the sampling sites of black-tailed gulls (*Larus crassirostris*) on the southeastern coast of South Korea. The feeding range (~73 km) of black-tailed gulls on the east coast of South Korea was investigated in the previous study (KNPS, 2005). (b) AhR-mediated potencies in raw organic extracts. Red solid lines represent the mean value of AhR-mediated activities measured for five liver samples. To reduce the complexity of the samples, the raw organic extracts of liver samples were fractionated using polarity-based silica gel column chromatography. (c) AhR-mediated potencies in silica gel fractions of liver extracts from black-tailed gulls (Error bar: mean ± SD; n = 3).

with the positive standards (benzo[*a*]pyrene [BaP]; 0.1% dosing), samples (liver REs and silica gel fractions), and control wells (blank and 0.1% DMSO). Because organic solvents, such as hexane, MC, and acetone, used for silica gel fractionation were completely evaporated, DMSO was selected as the only negative control in H4IIE-*luc* bioassay. The luminescence activity was measured using a Victor multilabel plate reader (PerkinElmer, Waltham, MA). The bioassay responses in samples were calculated as percentages of the value observed in the BaP positive standard (50 nM). In this study, the AhR-mediated activity in the samples was compared with that of BaP. In the previous studies, when AhR activity was evaluated for non-polar and poorly metabolized substances, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), PCBs, and PBDEs, using a long exposure time (72 h), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was used as a reference material (Eljarrat and Barceló, 2003; Van den Berg et al., 2006). In this study, H4IIE-*luc* bioassay with a shorter exposure time (4 h) was mainly focused on mid-polar and polar with metabolizable AhR agonists, and thus, BaP was used as reference material (Cha et al., 2021; Lee et al., 2020). To exclude the effect of DMSO solvent (negative control) on the bioassay results, the relative luminescence units (RLU) expressed in the positive standard and samples were calculated by subtracting the RLU expressed in 0.1% DMSO. In addition, control well (blank) did not show AhR-mediated activities. AhR-mediated potencies in the sample extracts were expressed as BaP equivalent concentration (BaP-EQ_{bio}). BaP-EQ_{bio} was measured from dose-response relationships of the polar fractions of liver extracts. All samples were tested in triplicate.

2.6. Nontargeted instrumental analyses and data processing

FSA was conducted on more potent polar fractions of extracts from liver, by use of a modification of a previously described method (Table S7) (Cha et al., 2021). Full mass scan was conducted to identify and check all peaks corresponding to compounds in the liver of black-tailed gulls (Cha et al., 2021; Lee et al., 2020). Potential AhR agonists in the polar fractions were selected through six-step criteria application. The first step was to detect all peaks from the LC-QTOFMS results. Meanwhile, chromatogram peaks commonly detected in both liver and blank samples were removed. In the second step, tandem mass spectrometry (MS/MS) data for chromatogram peaks were acquired. In the third step, the mass spectrometric data of chemicals were matched with known chemicals in the TCM library 1.0 metabolite software (Cha et al., 2021). In the fourth step, chemicals with the library matching score $\geq$ 70 were selected (Muz et al., 2017). In the fifth step, compounds with aromatic rings were selected (Mekenyan et al., 1996). Finally, in the sixth step, compounds detected in at least four samples were selected as tentative AhR agonist candidates. All available standard materials were purchased from Sigma-Aldrich for toxicological and chemical confirmation.

2.7. Toxicological and chemical confirmation and potency balance analysis

The AhR-mediated transcriptional activity of candidate compounds was evaluated by use of the H4IIE-*luc* bioassay. Using three-fold serial dilution, authentic standards of compounds were prepared at six concentrations (1000, 333, 111, 37, 12, and 4.1 μg mL^{-1}). ReP values for the AhR-active compounds were calculated based on effective concentrations (ECs) at the 20% level observed in BaP. Concentrations of selected compounds in the polar fractions of the liver extracts were quantified using HPLC-MS/MS (Table S8). Results of the AhR agonist candidates and positive standard measured from the bioassay were fitted and plotted using SigmaPlot 10.0 (four-parameter sigmoid function). The sum of chemical concentrations multiplied by their assay-specific ReP values (BaP-EQ_{chem}) and concentrations of BaP-EQ_{bio} for the polar fractions of the liver extracts was compared to determine contributions of individual AhR agonists to the overall bioassay results (Cha et al., 2021). Concentrations of BaP-EQ_{chem} were calculated by multiplying chemical concentrations and their assay-specific ReP values (Eq. 1).

$$\text{BaP-EQ}_{chem} = \sum[(\text{concentrations of AhR agonist}_i) \times \text{ReP}_i] \quad (1)$$

Where [concentrations of AhR $agonists_i$] is the concentrations of AhR $agonists_i$ in the sample of the liver, and ReP_i is the calculated ReP values of the compounds.

2.8. Quantitative structure-activity relationship modeling

VEGA quantitative structure-activity relationship (QSAR) is a set of programs to predict toxic potencies that contains models for various toxicological endpoints (Marzo et al., 2016). The VEGA QSAR models were derived primarily from application of a data-driven knowledge discovery approach, and their results are obtained using different methods (Benfenati et al., 2013). The androgen receptor (AR) activity, carcinogenicity, developmental toxicity, estrogen receptor (ER) activity, mutagenicity, and thyroid hormone receptor (TR) activity of AhR agonist candidates were evaluated (Benfenati et al., 2013). In addition, the AhR, AR, ER, and TR predicted binding affinities of AhR agonist candidates were evaluated using VirtualToxLab in silico modeling (Muster et al., 2008). VirtualToxLab could estimate the toxic potential of compounds using 4D Boltzmann scoring, through standardized individual binding affinities for a set of protein models known to induce adverse effects (Vedani et al., 2015).

3. Results and discussion

3.1. Concentrations of POPs in livers of black-tailed gulls

Classical and emerging POPs were detected in all samples of liver from black-tailed gulls collected from the southeastern coast of South Korea (Table S2). DDTs and PCBs were found to accumulate in the greatest concentrations, followed by PBDEs, HBCDs, CHLs, HCB, HCHs, and PeCB. The concentrations of POPs in the samples of liver examined in the present study were compared with previously reported concentrations in tissues from black-tailed gulls and various seabirds inhabiting other regions (Table S9) (Choi et al., 2001; Fromant et al., 2016; Helgason et al., 2008; Hong et al., 2014; Jaspers et al., 2013; Mello et al., 2016; Yamashita et al., 2018). The concentrations of all compounds except DDTs were greater in the livers of the black-tailed gulls than in previously described muscle samples (Hong et al., 2014). In addition, concentrations of POPs in the samples of liver were greater than those reported for preen gland oil, subcutaneous fat, and egg samples (Choi et al., 2001; Hong et al., 2014; Yamashita et al., 2018). Concentrations of PCBs, DDTs, and HCB, but not CHLs, were greater in the samples of liver than in various tissues, including muscle, preen oil, kidney, and blood, of white-tailed eagles in Greenland (Jaspers et al., 2013). POPs concentrations were significantly greater in the samples of liver than in eggs of herring gull, black-labeled kittiwake, Atlantic puffin, and common murre in Norway and south polar skua in Antarctica (Fromant et al., 2016; Helgason et al., 2008). Concentrations of POPs in samples of liver from Antarctic prion inhabiting Kerguelen Island were about 6–460 times less than those in the black-tailed gull livers (Mello et al., 2016). These results indicate that black-tailed gulls collected from the southeastern coast of South Korea have greater concentrations of anthropogenic toxic substances than seabirds inhabiting other countries and regions. In addition, compared with accumulation in other organs, concentrations of synthetic organic substances were greater in livers. In general, livers accumulate various exogenous pollutants introduced from environmental components, such as prey, water, and air; these pollutants undergo redistribution and metabolic transformation and can be transported to the brain via blood (Falkowska et al., 2013, 2016; Kubota et al., 2013). Thus, the liver is considered to be a suitable tissue for evaluation of chemical stress by contamination of persistent toxic

substances. This study focused on identifying unknown toxic substances accumulated in liver of black-tailed gulls.

3.2. AhR-mediated potencies in livers of black-tailed gulls

All liver REs from black-tailed gulls showed significant AhR-mediated potency, which was greater in samples S1, S3, and S4 (Fig. 1b). Variation occurred among individuals rather than between sampling areas. AhR-mediated potencies were greater in the polar fractions (mean = 39%) than in the non-polar (mean = 3.1%) and mid-polar (mean = 14%) fractions (Fig. 1c). This result, obtained by 4 h exposure in the H4IIE-*luc* bioassay, revealed that polar compounds are the major AhR agonists in the livers of black-tailed gulls. AhR-mediated potencies were generally greater in silica gel fractions than in REs, particularly in samples S2 and S5 (Figs. 1b and 1c). This phenomenon might be attributable to mixture effects (i.e., antagonism), given the great complexity of the biological samples (Bittner et al., 2009).

Concentrations of BaP-EQ_{bio} based on EC_{20}s were calculated for samples with observed BaP_{max} values > 20%. EC_{20} values for extracts were obtained from the dose-response relationships for AhR-mediated activities of mid-polar and polar fractions. Concentrations of BaP-EQ_{bio} in the mid-polar fractions of liver extracts from S3 and S5 were 9.2 ng g^{-1} and 10 ng g^{-1}, respectively. Concentrations of BaP-EQ_{bio} could not be calculated for mid-polar fractions of S1, S2, or S4 because their AhR-mediated potencies were < 20% BaP_{max}. Concentrations of BaP-EQ_{bio} in polar fractions were greater than those in mid-polar fractions; they were 42 ng g^{-1} in S1, 28 ng g^{-1} in S2, 25 ng g^{-1} in S4, 14 ng g^{-1} in S5, and 12 ng g^{-1} in S3. Overall, AhR-mediated activities of polar compounds in extracts of livers of black-tailed gulls were greater than those of mid-polar compounds.

3.3. Contributions of known AhR agonists to total AhR-mediated potencies

Concentrations of BaP-EQ_{chem} were calculated to determine the contributions of known AhR agonists to the total observed biological effects. PCDD/Fs, PCBs, and PBDEs are well-known AhR agonists, but they require relatively long durations of exposure of approximately 72 h for maximally activating AhR in the H4IIE-*luc* bioassays (Lee et al., 2013; Suzuki et al., 2006; Villeneuve et al., 2002). On the other hand, PAHs and polar AhR agonists rapidly bind to AhR and show maximum binding capacity at about 4–6 h, and then tend to decrease due to metabolism (Masunaga et al., 2004). That is, considering the concentrations of the PAHs in the samples and the great RePs values in the 4-h exposure, PAHs are stronger AhR agonists at 4 h exposure of H4IIE-*luc* bioassays than PCDD/Fs (Kim et al., 2019). It has been reported that PCBs and PBDEs are mainly eluted in the non-polar fraction (F1), and PCDD/Fs and PAHs are mainly eluted in the mid-polar fraction (F2) (Khim et al., 1999). The small AhR-mediated activity in F1 may be due to insufficient bindings of PCBs and PBDEs. PAHs were considered to be the major AhR agonists in the mid-polar fractions of extracts, because 4 h exposure experiments in the H4IIE-*luc* bioassays were conducted in the present study (Larsson et al., 2014). Concentrations of PAHs in liver ranged from 220 to 1400 ng g^{-1} lipid mass (lm) (mean = 590 ng g^{-1} lm; Table S3). Concentrations of BaP-EQ_{chem} for aromatic AhR agonists in the mid-polar fractions of the liver extracts ranged from 0.2 to 1.0 ng g^{-1} wet mass (wm) (mean = 0.4 ng g^{-1} wm). The contributions of aromatic AhR agonists to the BaP-EQ_{bio} in the mid-polar fractions from samples S3 and S5 were 2.8% and 9.5%, respectively (Fig. S2).

All known polar AhR agonists were detected in polar fractions of samples of liver (Fig. 2a and Table S5). Hydrocortisone, which has been used as an anti-inflammatory agent (Sprung et al., 2008), occurred at the greatest concentrations, with a mean of 19 ng g^{-1} wm, in liver. Concentrations of genistein, an anti-cancer agent (Banerjee et al., 2008), ranged from not detectable to 8.7 ng g^{-1} wm (mean = 3.9 ng g^{-1} wm). In addition, medroxyprogesterone (a uterine cancer agent) (Prior et al., 1994) was detected in the polar fractions, with an average concentration of 3.9 ng g^{-1} wm. Other polar AhR agonists, such as the pharmaceutical agents ciprofloxacin (Forrest et al., 1993), canrenone (Derosa et al., 2014), rutaecarpine (Sheu et al., 1996), and protopine (Jiang et al., 2004), were widely detected in extracts of liver. The mean concentration of mepanipyrim, used as an insecticide (Nakamura et al., 2003), was 0.2 ng g^{-1} wm. The greatest BaP-EQ_{chem} concentration in a polar fraction was from sample S1 (5.3 ng g^{-1} wm), followed by those in samples S4 (4.9 ng g^{-1} wm), S2 (4.0 ng g^{-1} wm), S3 (1.8 ng g^{-1} wm), and S5 (1.5 ng g^{-1} wm; Fig. 2b and Table 1). The polar AhR agonists accounted for 11–20% (mean = 15%) of BaP-EQ_{bio} concentrations in the polar fractions of the liver extracts; hydrocortisone and rutaecarpine were major contributors. These results suggest that a variety of unknown polar AhR agonists are present in the livers of black-tailed gulls.

3.4. Identification of novel polar AhR agonists in livers of black-tailed gulls

In the first step of identification of AhR agonist, all chromatogram peaks derived from the LC-QTOFMS analysis were identified, and 1096, 807, 744, 922, and 772 compounds were detected in samples S1, S2, S3, S4, and S5, respectively (Fig. 3a). In the second step, 676, 554, 504, 591, and 548 compounds were selected in each sample. In the third step, compounds matched with the TCM library 1.0 were selected (Cha et al., 2021). In the fourth step, 101, 95, 80, 88, and 85 compounds, respectively, with library scores ≥ 70 were selected (Muz et al., 2017). In the fifth step, 66, 58, 50, 49, and 49 compounds with aromatic rings, were selected, respectively (Table S10) (Mekenyan et al., 1996). Because the five samples of livers exhibited similar, significant AhR-mediated potencies, it was assumed that AhR agonists were commonly present in these samples. In the sixth step, 22 compounds were selected as tentative AhR agonist candidates (Fig. S3 and Table S11). Of these, nine compounds ([10]-gingerol, angelicin, corticosterone, enrofloxacin, eupatilin, etofenprox, oxadixyl, tretinoin, and triamterene) were available as standard materials and used for chemical and toxicological confirmation. Seven of the nine candidate compound (corticosterone, [10]-gingerol, angelicin, eupatilin, etofenprox, oxadixyl, and tretinoin) had significant AhR-mediated potencies; their EC_{20} values were 36, 200, 250, 300, 338, 1600, and 1600 nM, respectively (Fig. 3b). Angelicin was previously reported to bind to the AhR (Vrzal et al., 2013). Potencies of AhR activation for corticosterone, [10]-gingerol, eupatilin, etofenprox, oxadixyl, and tretinoin are reported for the first time here.

In the present study, the efficacies of 7 novel AhR agonists were evaluated using the H4IIE-*luc* bioassays. When AhR agonists bind to receptors, AhR enters the nucleus and binds to ARNT, acting as a transcription factor (Tanguay et al., 1999). This transcription factor promotes expressions of genes by recognizing and binding to dioxin response elements found in promoters of responsive genes (Tanguay et al., 1999). New proteins are formed, which can lead to abnormal expressions of genes in the process (Englert et al., 2012). Activation of binding ligands to the receptor is caused by a series of complex processes. Thus, additional studies for validation of AhR-mediated activities of these novel substances in livers of seagulls should be conducted.

3.5. Concentrations of novel polar AhR agonists in livers of black-tailed gulls

All of the novel polar AhR agonists in polar fractions of liver extracts except etofenprox had measurable concentrations in the quantitative HPLC-MS/MS analysis (Fig. 4a and Table S12). Corticosterone, a steroid hormone (Kitaysky et al., 2003), was detected at the greatest concentrations (240–390 ng g^{-1} wm). Corticosterone has critical effects on growth and development of black-tailed gulls, because it interferes with synthesis of proteins and can affect behavior, including food instincts and aggression (Kitaysky et al., 2003). In addition, corticosterone is known to be the main glucocorticoid hormone in birds (Cockrem, 2007).

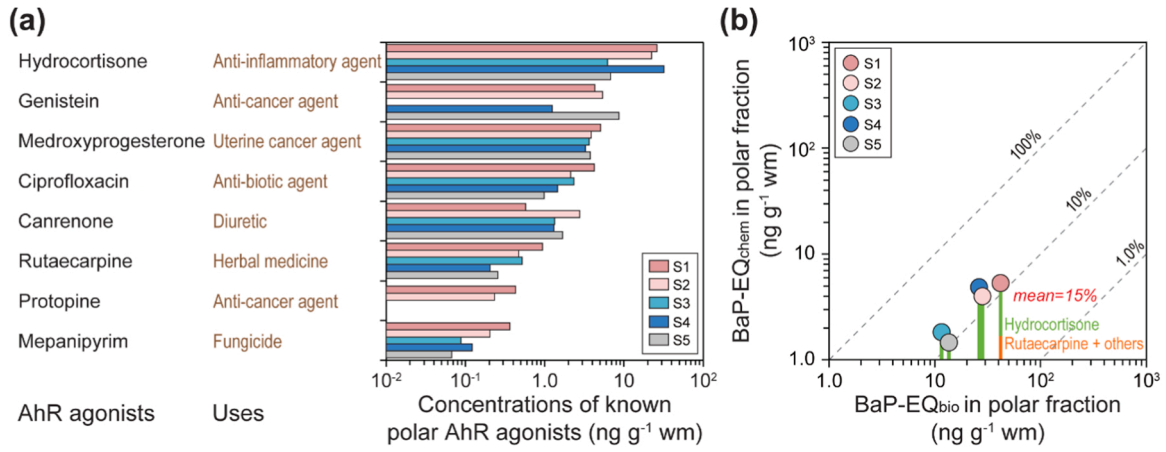

Fig. 2. (a) Concentrations of known AhR agonists in polar fractions from livers of black-tailed gulls. The known polar AhR agonists include 7 pharmaceuticals and 1 fungicide. (b) comparison of instrument-derived BaP-EQ$_{chem}$ and bioassay-derived BaP-EQ$_{bio}$ concentrations in polar fractions. The length of the vertical line under the circle indicates the contribution of each compound.

Table 1
Potency balance between BaP-EQ$_{chem}$ and BaP-EQ$_{bio}$ concentrations in polar fractions of the livers from black-tailed gulls from the southeastern coast of South Korea.

Compounds	S1	S2	S3	S4	S5
Known polar AhR agonists (BaP-EQ$_{chem}$, ng g^{-1} wm)					
Canrenone	0.003	0.01	0.01	0.006	0.007
Ciprofloxacin	0.02	0.01	0.01	0.01	0.01
Hydrocortisone	3.6	3.1	0.8	4.5	0.9
Genistein	0.004	0.01	ND[a]	0.001	0.01
Medroxyprogesterone	0.07	0.06	0.05	0.05	0.05
Mepanipyrim	0.0002	0.0001	0.00004	0.0001	0.00003
Protopine	0.00001	0.000003	ND	ND	ND
Rutaecarpine	1.6	0.8	0.9	0.4	0.4
Novel polar AhR agonists (BaP-EQ$_{chem}$, ng g^{-1} wm)					
[10]-Gingerol	0.2	0.1	0.6	0.08	0.09
Angelicin	ND	ND	0.05	0.06	ND
Corticosterone	5.7	4.3	3.6	4.0	4.5
Etofenprox	ND	ND	ND	ND	ND
Eupatilin	0.01	0.01	0.003	0.002	0.001
Oxadixyl	0.001	0.001	0.001	0.001	0.001
Tretinoin	0.01	0.003	0.003	0.002	0.005
BaP-EQ$_{chem}$ (ng g^{-1} wm)[b]	11	8.4	6.0	9.0	6.1
BaP-EQ$_{bio}$ (ng g^{-1} wm)	42	28	12	25	14
Contribution (%)	27	30	52	36	45

[a] ND: not detected.
[b] Calculated by multiplying the concentrations of known and novel polar AhR agonists by their ReP values.

In most vertebrates, including black-tailed gulls, glucocorticoid receptors can be activated by steroid hormones, which have been reported to induce chronic stress and various other adverse effects (Macikova et al., 2014). Endogenous substances, such as corticosterone and hydrocortisone, in living organisms are essential for physiology, growth, development, and maintenance of homeostasis (Nguyen and Bradfield, 2008). Studies on the AhR activation of endogenous ligands in living organisms as well as exogenous ligands in environment matrices have been reported. For instance, the AhR binding ability of indigoids, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester, equilenin, arachidonic acid metabolites, heme metabolites, and tryptophan metabolite has been confirmed (Nguyen and Bradfield, 2008). Thus, further investigation is needed to AhR activation that could be expressed by the unmonitored endogenous ligands in black-tailed gulls. [10]-Gingerol, which has been used as an anti-inflammatory agent and binds to ER (Bernard et al., 2017; Dugasani et al., 2010), was detected at relatively great concentrations (mean = 77 ng g^{-1} wm) in all samples of liver. Concentrations of tretinoin, used as an anti-neoplastic agent (Collado-Borrell et al., 2016) and to treat melasma in South Korea (Suh et al., 2011), ranged from 4.6 to 17 ng g^{-1} wm.

Angelicin, which has been used as an anti-convulsant agent and binds to ER (Ge et al., 2019; Pazos-Navarro et al., 2013), was detected only in samples S3 and S4. Although the US Environmental Protection Agency's Office of Water has identified selected pharmaceuticals as contaminants of emerging concern (EPA, 1998), these drugs continue to be released into environments due to the lack of management and regulation. Eupatilin, a natural plant product and peroxisome proliferator-activated receptor alpha–active compound (Choi et al., 2015; Seo and Surh, 2001), was detected in the samples of liver at an average concentration of 2.5 ng g^{-1} wm. Oxadixyl, used to prevent pests and diseases in crops, including tomatoes, in South Korea (Kwon et al., 2015), was detected at concentrations of 1.2–2.3 ng g^{-1} wm. In a previous study, oxadixyl was detected in the river water of an Australian horticultural-production catchment, possibly originating from pesticide use in the surrounding area (Wightwick et al., 2012). Etofenprox, another pesticide (Szabó et al., 2019), was not detected in all samples of liver in the present study. As etofenprox binds to TR and is known as ER antagonist (Du et al., 2010), further studies of its potential toxic effects are needed.

Overall, the novel polar AhR agonists detected in the livers of black-tailed gulls in this study have been reported to have various potential toxicities and are distributed widely in the environment. Polar AhR agonists seem to be discharged from industrial and pharmaceutical manufacturing complexes and wastewater treatment plants, and aquatic organisms could be exposed to them, followed by transfer to top predators, such as the black-tailed gull, through the marine and terrestrial food webs.

3.6. Contributions of novel polar AhR agonists to total AhR-mediated potencies

For determination of relative contributions to BaP-EQ$_{bio}$, concentrations of BaP-EQ$_{chem}$ were recalculated to include novel AhR agonists identified in liver. The contribution to potencies increased significantly with inclusion of novel AhR agonists (27–52%, mean = 38%; Fig. 4b and Table 1). Corticosterone was the greatest contributor to AhR-mediated potencies in most samples (S1–S3 and S5), due to its greater concentrations than other compounds. [10]-Gingerol explained about 1.4% of the BaP-EQ$_{bio}$ concentrations in the samples of liver. Although contributions of AhR agonists differed among the five samples of livers of black-tailed gulls, concentrations of the BaP-EQ$_{chem}$ did not differ significantly (Fig. S4). However, novel AhR agonists were unable to

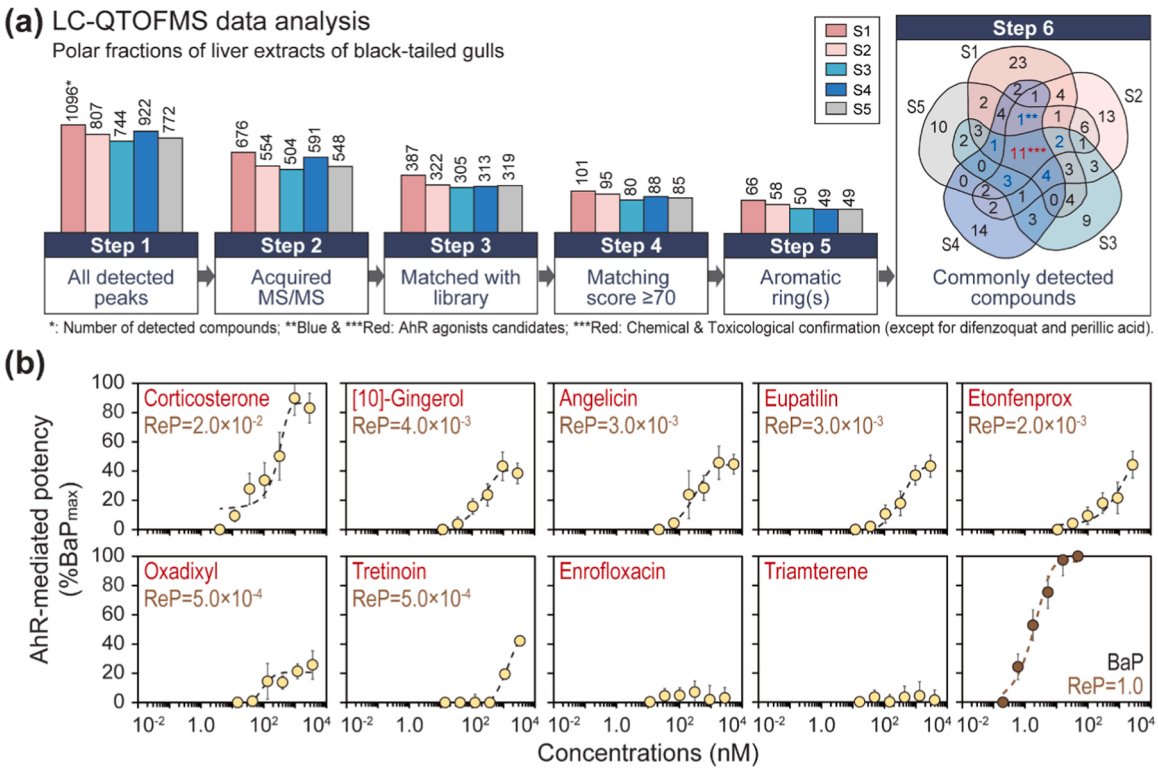

Fig. 3. (a) The stepwise approach for LC-QTOFMS data analysis used to select AhR agonist candidates present in five polar fractions of liver extracts from black-tailed gulls (S1–S5). (b) Dose-response curves for the AhR-mediated potency of nine AhR agonist candidates (yellow circles) with six concentration gradients and benzo[*a*] pyrene (brown circles) in the H4IIE-*luc* bioassay at 4 h exposures (Error bar: mean ± SD; n = 3). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

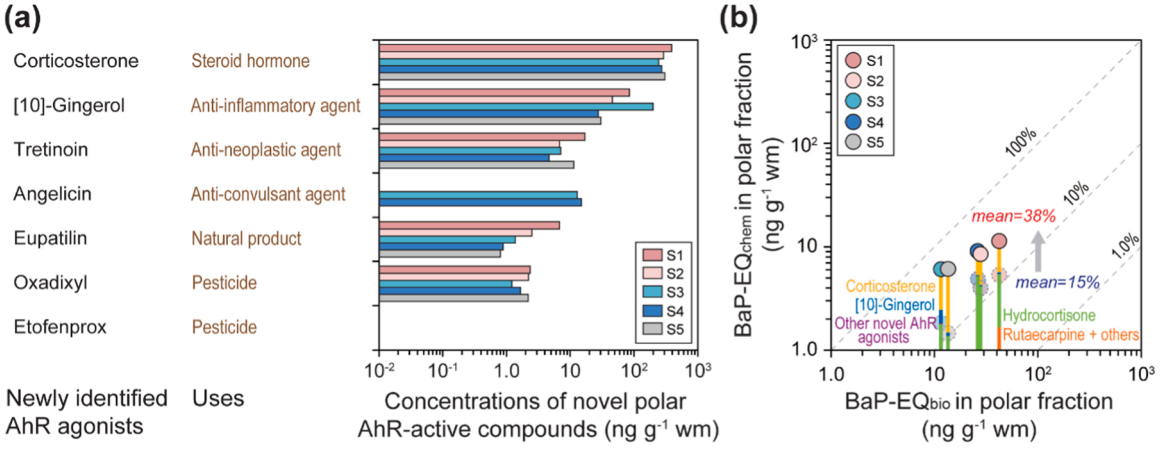

Fig. 4. (a) Concentrations of novel polar AhR agonists and (b) improved potency balance between BaP-EQ_{chem} and BaP-EQ_{bio} concentrations in polar fractions from livers of black-tailed gulls. The length of the vertical line under the circle indicates the contribution of each compound.

explain the observed biological responses fully. Additional toxicological confirmation of AhR agonist candidates may further explain the results. In addition, studies of the AhR efficacies of polar metabolites produced by ingested hydrophobic AhR agonists should be performed. Meanwhile, in the present study, FSA for highly potent fractions was conducted using only ESI positive mode. Previous studies revealed that unmonitored toxic substances in environmental samples were screened using ESI negative mode as well (Lee et al., 2020; Simon et al., 2013). For example, enoxolone was identified as a novel polar AhR agonist in sediment extracts of industrial areas (Lee et al., 2020), and transthyretin-binding compounds were identified in the blood plasma of polar bear cubs (Simon et al., 2013). Thus, it is expected that the use of both the positive mode and the negative mode will be effective in identifying unknown toxic substances and improving the portion that could explain the biological effects.

3.7. *Prediction of other potential toxicities*

For novel AhR agonist candidates, additional toxicities were predicted using VEGA QSAR and VirtualToxLab. Polar AhR agonists of endogenous or/and exogenous ligands were identified as endocrine disruptors capable of binding to estrogen, glucocorticoid, and thyroxine receptors (Beberok et al., 2018; Du et al., 2010). Endogenous ligands from living organisms and exogenous ligands from environmental

samples could adversely affect organisms by their ability to bind to multiple receptors rather than uniquely to a specific receptor (Hashmi et al., 2020; Whirledge et al., 2015). Thus, it was evaluated whether the AhR agonist candidates accumulated in the liver of black-tailed gulls could express other potential toxicities by identifying the binding ability to other hormone receptors. VEGA QSAR analysis predicted that angelicin, corticosterone, eupatilin, fenhexamid, and isorhamnetin could bind to the AR (Table S13). The carcinogenicity model showed that all compounds were predicted to be carcinogens by at least one model. Developmental toxicity was predicted for all compounds except difenzoquat, oxadixyl, cyprodinil, and xylazine. [10]-Gingerol, etofenprox, eupatilin, daphnoretin, fenhexamid, isorhamnetin, and xylazine were identified as potential ER-active compounds. Eleven compounds (angelicin, corticosterone, difenzoquat, enrofloxacin, eupatilin, oxadixyl, cyprodinil, daphnoretin, fenazaquin, flavin mononucleotide, and isorhamnetin) were found to have potential mutagenicity toxicity. Of these compounds, only triamterene was predicted to bind to TR. Enrofloxacin and triamterene, which were predicted to not bind to the AhR, were accumulated at mean concentrations of 6.4 and 0.2 ng g^{-1} wm, respectively, in liver (Table S12), and could have potential toxic effects in black-tailed gulls. Enrofloxacin has been confirmed to be present in various environmental matrices, such as farmed fishery products and river water (Kang et al., 2018; Kim et al., 2016), in South Korea.

Enrofloxacin, atenolol, buturon, flavin mononucleotide, and methsuximide could not be included in the VirtualToxLab analysis. Angelicin, etofenprox, oxadixyl, tretinoin, and fenazaquin had moderate AhR biological activity, and the other compounds had weak or no AhR activity (Table S14). Binding affinities in the VirtualToxLab were slightly different from the RePs of the chemicals, but it was confirmed that all 7 novel AhR agonists were able to bind to AhR. VirtualToxLab predicted that [10]-gingerol could bind strongly to AR and that angelicin, corticosterone, eupatilin, and daphnoretin could bind moderately to AR. Only [10]-gingerol exhibited moderate ER biological activity. [10]-Gingerol, angelicin, corticosterone, oxadixyl, tretinoin, and fenazaquin were identified as capable of moderate TR activation. Overall, the VEGA QSAR and VirtualToxLab analyses produced somewhat differing results regarding compound–receptor biological activity, as the VEGA QSAR approaches are based on compound structures, and VirtualToxLab relies on the consideration of compound–receptor thermodynamics. Empirical verification, such as with the performance of multiple bioassays to predict the potential toxicities of compounds, is needed.

3.8. Biomagnification potentials of polar AhR agonists

Biomagnification potentials in air-breathing organisms, such as seabirds and marine mammals, are affected by the octanol-air partition coefficient (K_{OA}) rather than the octanol-water partition coefficient (K_{OW}) of chemicals because compounds with high K_{OA} values are relatively non-volatile and tend to split into lipids rather than being released into the air (Kelly and Gobas, 2003; Kelly et al., 2007). Previous studies have revealed that compounds accumulated in air-breathing organisms have biomagnification potentials when log $K_{OA} \geq 6$ and log $K_{OW} > 2$ in marine-mammalian food webs (Kelly et al., 2007). Of the known polar AhR agonists, canrenone, mepanipyrim, medroxyprogesterone, rutaecarpine, and genistein are known to have log $K_{OA} \geq 6$ and log $K_{OW} > 2$. Among the novel AhR agonists, tretinoin, etofenprox, [10]-gingerol, and eupatilin were identified as biomagnifiable compounds (Fig. 5). Of the candidate AhR agonists detected in five samples, enrofloxacin and triamterene, which were predicted to not bind to the AhR, showed no biomagnification potential. Alternatively, perillic acid and difenzoquat were identified as biomagnifiable compounds. Cyprodinil, buturon, fenazaquin, xylazine, phenazepam, and fenhexamid, which are AhR agonist candidates detected in four samples, were confirmed to have biomagnification potential. Etofenprox, tretinoin, and fenazaquin have been found to have great biomagnification potential (log $K_{OA} \geq 8$ and log $K_{OW} \geq 5.5$) in air-breathing organisms in marine-mammalian food webs (Kelly et al., 2007). In the present study, several polar AhR agonists with low K_{OW} values and high K_{OA} values were found to be accumulated in the livers of black-tailed gulls from coastal areas of South Korea. These AhR agonists were transferred to top predators through the marine food web. These compounds are actively and commercially used and could adversely affect marine organisms. Meanwhile, it is possible that AhR agonists candidates for which, due to the limitation of standards, chemical structures and toxicological responses could not be confirmed, could be transferred in seagulls through the food chain. In addition, although this study focused on polar AhR agonists accumulated in livers of seagulls, there might be toxic substances that are specifically accumulated in other organs, such as POPs that are selectively accumulated in the fat. Thus, follow-up studies on bioaccumulation and trophic transfer of these toxic substances are needed.

4. Conclusions

In this study, an advanced EDA approach was applied successfully to identify the distribution, biological activity, toxicity contributions, and biomagnification potentials of AhR agonist candidates in livers of black-tailed gulls from South Korea. Much environmental science research has been undertaken recently to better understand the relationships between environmental exposure and ecosystem health effects during the lifetimes of individual organisms. For the examination of toxicokinetic processes, such as bioavailability and internal intake routes of environmental pollutants, biotic samples are more appropriate than samples from external sources, including sediment, river water, and wastewater. The analytical monitoring of pollutants in environmental samples cannot adequately determine toxicological effects unless supplemented with biological analyses. Further investigation of the metabolism and excretion of AhR agonists accumulated in black-tailed gulls is needed. In addition, the advanced EDA approach could be applied to various toxicological endpoints, and it is expected to identify the potential toxic substances in biological samples for each endpoint.

CRediT authorship contribution statement

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

Statement of novelty

"Effect-directed analysis (EDA) combined with full-scan screening (FSA)" is useful for identifying unknown toxic substances in environmental samples. In this study, for the first time, EDA with FSA was applied to identify the AhR agonists in livers of black-tailed gulls. Seven novel AhR agonists were successfully identified, and these compounds have been used as pharmaceuticals and pesticides.

The novel toxic substances were capable of biomagnification through the food chain; they can be transferred to top predators, such as seabirds. To date, there are few reports of these substances in the marine ecosystems; thus, follow-up monitoring and management are urgently needed.

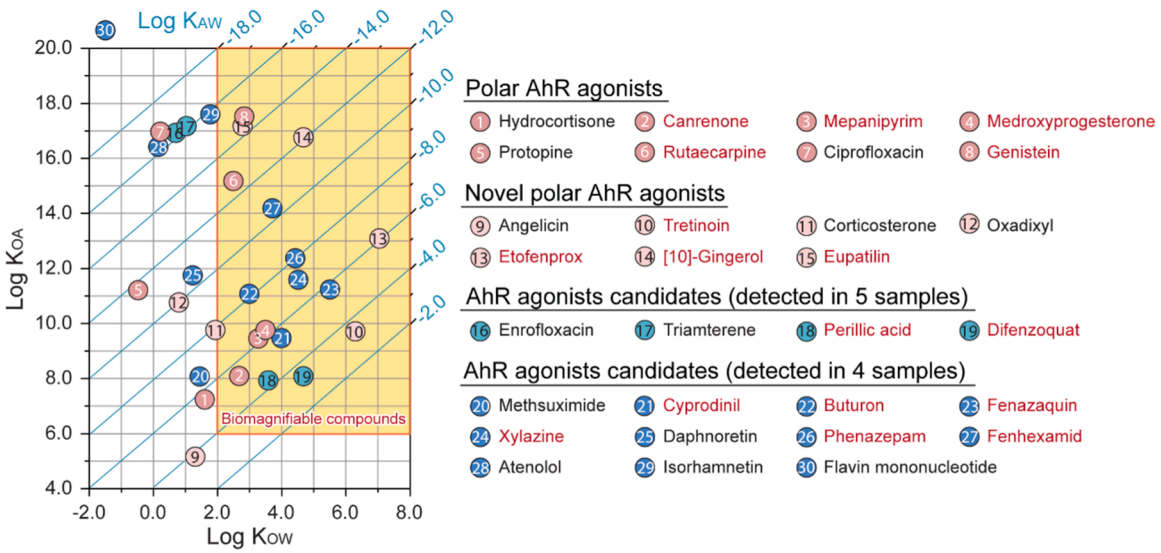

Fig. 5. Evaluation of biomagnification potential of polar AhR agonists. Chemical space map for polar AhR agonists and candidate substances based on partition coefficients [log K_{OA}, log K_{AW}, and log K_{OW} were obtained from ChemSpider (ChemSpider, 2021)].

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.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.128305.

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Supplementary materials for

Identification of novel polar aryl hydrocarbon receptor agonists accumulated in liver of black-tailed gulls in Korea using advanced effect-directed analysis

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References

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Supplementary Tables

Table S1. Biological information of black-tailed gulls from the southeastern coast of Korea.

Samples	Region	Body length (cm)	Body mass (g)	Lipid content in liver (%)	Moisture content in liver (%)	Cause of death
S1	Busan	38	460	2.0	73	Gunshot wound
S2	Busan	36	390	1.8	74	Building crash
S3	Busan	38	330	2.2	71	Fish hook entanglement (Euthanized)
S4	Ulsan	40	400	1.2	77	Car crash (Euthanized)
S5	Ulsan	46	320	1.1	76	Car crash (Euthanized)

Table S2. Concentrations of classical and emerging persistent organic pollutants (POPs) in livers of black-tailed gulls from the southeastern coast of Korea.

Compounds	Abb.[a]	S1	S2	S3	S4	S5
				(ng g^{-1} lipid mass)		
Polychlorinated biphenyls (PCBs)						
2,4'-Dichlorobiphenyl	CB 8	0.5	ND[b]	ND	ND	ND
2,2',5-Trichlorobiphenyl	CB 18	ND	ND	0.1	ND	ND
2,4,4'-Trichlorobiphenyl	CB 28	110	340	78	130	120
2,4,5-Trichlorobiphenyl	CB 29	ND	ND	ND	ND	ND
2,2',3,5'-Tetrachlorobiphenyl	CB 44	ND	ND	ND	ND	ND
2,2',5,5'-Tetrachlorobiphenyl	CB 52	8.7	2.4	9.0	23	3.2
2,3',4,4'-Tetrachlorobiphenyl	CB 66	430	1700	340	630	720
2,2',3,4,5'-Pentachlorobiphenyl	CB 87	1700	15000	3700	4200	4600
2,2',4,5,5'-Pentachlorobiphenyl	CB 101	120	460	160	210	270
2,3,3',4,4'-Pentachlorobiphenyl	CB 105	1400	5700	1000	1600	2100
2,3,3',4',6-Pentachlorobiphenyl	CB 110	110	83	59	200	60
2,3',4,4',5-Pentachlorobiphenyl	CB 118	6000	21000	4500	6800	8300
2,2',3,3',4,4'-Hexachlorobiphenyl	CB 128	900	4100	1000	1400	1800
2,2',3,4,4',5'-Hexachlorobiphenyl	CB 138	8400	35000	9300	12000	15000
2,2',4,4',5,5'-Hexachlorobiphenyl	CB 153	12000	60000	14000	17000	22000
2,2',3,3',4,4',5-Heptachlorobiphenyl	CB 170	1500	14000	2400	3400	3300
2,2',3,4,4',5,5'-Heptachlorobiphenyl	CB 180	3900	38000	5500	7800	7900
2,2',3,4',5,5',6-Heptachlorobiphenyl	CB 187	1700	15000	3700	4220	4600
2,2',3,3',4,4',5,6-Octachlorobiphenyl	CB 195	110	1400	190	280	280
2,2',3,3',4,5,6,6'-Octachlorobiphenyl	CB 200	36	320	93	100	90
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	CB 206	98	780	170	250	350
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	CB 209	110	370	160	410	930
Polybrominated diphenyl ethers (PBDEs)						
2,4-Dibromo-1-(2-bromophenoxy)benzene	BDE 17	ND	6.5	ND	ND	4.7
2,4-Dibromo-1-(4-bromophenoxy)benzene	BDE 28	11	28	11	6.3	9.3
1,1'-Oxybis(2,4-dibromobenzene)	BDE 47	760	6200	2400	1400	1600
1,2-Dibromo-4-(2,4-dibromophenoxy)benzene	BDE 66	16	76	21	ND	ND
1,2,4-Tribromo-5-(2,4-dibromophenoxy)benzene	BDE 99	290	1100	700	460	610
1,3,5-Tribromo-2-(2,4-dibromophenoxy)benzene	BDE 100	190	1700	820	560	740
1,2,3-Tribromo-4-(2,4,5-tribromophenoxy)benzene	BDE 138	ND	ND	ND	4.9	10
1,1'-Oxybis(2,4,5-tribromobenzene)	BDE 153	670	2400	820	820	1200
1,3,5-Tribromo-2-(2,4,5-tribromophenoxy)benzene	BDE 154	210	600	440	320	390
1,2,3,5-Tetrabromo-4-(2,4,5-tribromophenoxy)benzene	BDE 183	16	93	73	52	110
1,1'-Oxybis(pentabromobenzene)	BDE 209	23	1000	830	220	1200

Chlordane related compounds (CHLs)						
Alpha-Chlordane	α-chlordane	11	ND	4.9	6.2	16
Gamma-Chlordane	γ-chlordane	ND	ND	ND	ND	ND
Oxychlordane	Oxychlordane	400	940	290	310	960
Heptachlor	Heptachlor	ND	ND	ND	ND	ND
Heptachlor epoxide	Heptachlor epoxide	60	110	48	350	280
Cis-nonachlor	*Cis*-nonachlor	210	43	40	170	94
Trans-nonachlor	*Trans*-nonachlor	800	1600	460	700	1330
Dichlorodiphenyltrichloroethane (DDTs)						
2,2-(2-Chlorophenyl-4'-chlorophenyl)-1,1-dichloroethene	o,p'-DDE	ND	ND	ND	ND	ND
2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene	p,p'-DDE	36000	110000	250000	23000	50000
2,4'-(dichlorodiphenyl)-2,2-dichloroethane	o,p'-DDD	ND	ND	ND	ND	ND
Dichlorodiphenyldichloroethane	p,p'-DDD	3.3	5.8	22	3.9	2.8
1-Chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene	o,p'-DDT	0.7	ND	0.5	ND	ND
Dichlorodiphenyltrichloroethane	p,p'-DDT	1.0	ND	ND	ND	ND
Hexabromocyclododecanes (HBCDs)						
1,2,5,6,9,10-Hexabromocyclododecane	α-HBCD	2700	5100	4300	1200	2500
Beta-hexabromocyclododecane	β-HBCD	ND	ND	ND	92	ND
Gamma-hexabromocyclododecane	γ-HBCD	ND	ND	ND	ND	ND
Hexachlorocyclohexanes (HCHs)						
1,2,3,4,5,6-hexachloro-1,2,3,4,5,6-hexadeuteriocyclohexane	α-HCH	ND	ND	ND	ND	ND
1,2,3,4,5,6-Hexachlorocyclohexane	β-HCH	1200	2400	390	530	2800
Lindane	γ-HCH	0.4	ND	ND	ND	ND
Hexachlorobenzene (HCB)						
Hexachlorobenzene	HCB	1800	2500	790	560	1920
Pentachlorobenzene (PeCB)						
Pentachlorobenzene	PeCB	53	46	24	13	28

[a] Abb.: Abbreviations.

[b] ND: Not detected.

Table S3. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in livers of black-tailed gulls from the southeastern coast of Korea.

Compounds	Relative potency[a]	S1	S2	S3	S4	S5
		(ng g^{-1} lipid mass)				
Acenaphthylene	–[b]	22	ND[c]	13	200	330
Acenaphthene	–	ND	ND	18	ND	250
Fluorene	–	71	60	45	220	200
Phenanthrene	–	73	140	88	190	320
Anthracene	–	ND	ND	ND	16	19
Fluoranthene	–	18	47	30	71	98
Pyrene	–	19	44	34	ND	ND
Benzo[*a*]anthracene	3.2×10^{-1}	23	42	34	ND	88
Chrysene	8.5×10^{-1}	ND	ND	ND	22	46
Benzo[*b*]fluoranthene	5.0×10^{-1}	ND	ND	ND	ND	19
Benzo[*k*]fluoranthene	4.8×10^{-1}	ND	ND	ND	ND	15
Benzo[*a*]pyrene	1.0	ND	ND	ND	ND	ND
Indeno[*1,2,3-cd*]pyrene	5.8×10^{-1}	ND	ND	ND	ND	ND
Dibenz[*a,h*]anthracene	6.6×10^{-1}	ND	ND	ND	ND	ND
Benzo[*g,h,i*]perylene	–	ND	ND	ND	ND	ND
∑PAHs		220	340	260	720	1400

[a] ReP values of PAHs were reported in Kim et al. (2019).
[b] –: Not significant.
[c] ND: Not detected.

Table S4. Recovery rates of surrogate standards for analyses of POPs and PAHs in livers of black-tailed gulls.

Surrogate standards	S1	S2	S3	S4	S5
HCH, CHLs, DDT, and HCB					
$^{13}C_6$ *α*-HCH	69	64	80	60	52
$^{13}C_6$ HCB	59	57	58	55	48
$^{13}C_{10}$ trans-nonachlor	81	85	90	68	81
$^{13}C_{12}$ *p,p′*-DDT	93	107	121	108	103
PCBs					
$^{13}C_{12}$ CB9	69	70	76	53	50
$^{13}C_{12}$ CB52	71	72	72	67	64
$^{13}C_{12}$ CB101	85	90	95	98	76
$^{13}C_{12}$ CB138	119	137	149	134	102
$^{13}C_{12}$ CB194	112	144	155	137	111
PBDEs					
$^{13}C_{12}$ BDE139	83	85	76	70	62
$^{13}C_{12}$ BDE209	110	141	102	77	82
HBCDs					
^{13}C α-HBCD	51	70	83	89	76
^{13}C-β-HBCD	58	81	53	128	85
^{13}C-γ-HBCD	57	81	69	102	80
PAHs					
Acenaphthene-*d10*	71	78	58	47	49
Phenanthrene-*d10*	114	115	100	54	52
Chrysene-*d12*	153	145	120	74	70
Perylene-*d12*	148	152	135	82	74

Table S5. Concentrations of known polar AhR agonists in livers of black-tailed gulls from the southeastern coast of Korea.

Compounds	Relative potency	S1	S2	S3	S4	S5	Reference
		(ng g^{-1} wet mass)					
Canrenone	6.0×10^{-3}	0.6	2.8	1.3	1.3	1.7	Cha et al. (2021)
Ciprofloxacin	5.0×10^{-3}	4.2	2.1	2.3	1.5	1.0	
Genistein	1.0×10^{-4}	4.3	5.4	ND[a]	1.3	8.7	
Hydrocortisone	2.0×10^{-1}	26	22	6.2	32	6.8	
Medroxyprogesterone	2.0×10^{-2}	5.1	3.9	3.6	3.3	3.7	
Mepanipyrim	4.0×10^{-4}	0.4	0.2	0.1	0.1	0.1	
Protopine	2.0×10^{-5}	0.4	0.2	ND	ND	ND	
Rutaecarpine	2.0	0.9	0.5	0.5	0.2	0.3	

[a] ND: Not detected.

Table S6. Instrumental conditions for analyzing polar AhR-active compounds using HPLC-MS/MS.

Instrument	HPLC: Agilent Infinity 1290 II, MS/MS: SCIEX Qtrap 6500
Samples	Polar fractions from S1–S5
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	

Time (min)	Solvent	
	A	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 mode
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

Table S7. 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	Polar fractions from S1–S5
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	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 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 ℃
Ion source	DuoSpray Ion Source
Ion spray voltage	Positive: 5,500 V
Software	All-in-One_HRMS/MS TCM library 1.0 metabolite software

Table S8. Conditions of HPLC-MS/MS for quantification of AhR agonists candidates identified in livers of black-tailed gulls from the southeastern coast of Korea.

Compounds	MRM transition Parent ion → Daughter ion (m/z)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
[10]-Gingerol	351.05 → 333.30 (ESI+)	61	10	5	6
Angelicin	186.91 → 131.00 (ESI+)	106	10	31	18
Corticosterone	347.06 → 329.10 (ESI+)	1	10	21	16
Enrofloxacin	365.05 → 321.10 (ESI+)	15	10	25	10
Etofenprox	377.07 → 301.10 (ESI+)	81	10	11	8
Eupatilin	344.99 → 330.00 (ESI+)	136	10	33	14
Oxadixyl	278.99 → 219.10 (ESI+)	106	10	13	10
Tretinoin	300.98 → 284.00 (ESI+)	106	10	69	8
Triamterene	253.80 → 237.00 (ESI+)	86	10	37	16

Table S9. Distributions of POPs in seabird samples obtained from the present study and previous studies.

Common name	Region	Tissue	DDTs	PCBs	PBDEs	HBCDs	CHLs	HCB	HCHs	PeCB	Reference
			(ng g^{-1} lipid mass)								
Black-tailed gull (*Larus crassirostris*)	Korea	Liver	92000	86000	6300	2600	1800	1500	1500	33	This study
		Muscle	101000	54000	3500	1100	720	800	1100	23	Hong et al. (2014)
	Japan	Preen gland oil	2700	1660					13		Yamashita et al. (2018)
		Subcutaneous fat	1900	4900			100	104	190		Choi et al. (2001)
		Egg	1500	2300			74	74	150		
White-tailed eagle (*Haliaeetus albicilla*)	Greenland	Muscle	18000	36000			7800	1200			Jaspers et al. (2013)
		Preen oil	12000	14000			5200	1100			
		Liver	7200	11000			3000	780			
		Kidney	6300	9100			2600	740			
		Blood	5100	6900			2100	880			
Herring gull (*Larus argentatus*)	Norway	Egg	2400	10600			940	480	21		Helgason et al. (2008)
Black-legged Kittiwake (*Rissa tridactyla*)		Egg	1200	7600			480	560	27		
Atlantic puffin (*Fratercula arctica*)		Egg	970	3800			640	370	23		
Common murre (*Uria aalge*)		Egg	490	2300			68	490	14		
South polar skua (*Stercorarius maccormicki*)	Antarctica	Egg	1400	1700	66						Mello et al. (2016)
Antarctic prion (*Pachyptila desolata*)	Kerguelen island	Muscle	110	350	59		11	110	5.1		Fromant et al. (2016)
		Liver	200	280	980		11	160	4.4		
		Kidney	76	150	290		5.5	110	1.7		

Table S10. List of compounds with aromatic ring(s) (Step 5, see Figure 3 in the main text) detected in livers of black-tailed gulls using LC-QTOFMS.

Compounds	Molecular formula	CAS number	Molecular mass	S1	S2	S3	S4	S5
Compounds detected in 5 samples								
[10]-Gingerol	$C_{21}H_{34}O_4$	23513-15-7	350.492	+[a]	+	+	+	+
Angelicin	$C_{11}H_6O_3$	523-50-2	186.163	+	+	+	+	+
Corticosterone	$C_{21}H_{30}O_4$	50-22-6	346.461	+	+	+	+	+
Difenzoquat	$C_{17}H_{17}N_2$	49866-87-7	249.330	+	+	+	+	+
Enrofloxacin	$C_{19}H_{22}FN_3O_3$	93106-60-6	359.395	+	+	+	+	+
Etofenprox	$C_{25}H_{28}O_3$	80844-07-1	376.488	+	+	+	+	+
Eupatilin	$C_{18}H_{16}O_7$	22368-21-4	344.315	+	+	+	+	+
Oxadixyl	$C_{14}H_{18}N_2O_4$	77732-09-3	278.304	+	+	+	+	+
Perillic acid	$C_{10}H_{14}O_2$	7694-45-3	166.217	+	+	+	+	+
Tretinoin	$C_{20}H_{28}O_2$	302-79-4	300.435	+	+	+	+	+
Triamterene	$C_{12}H_{11}N_7$	396-01-0	253.263	+	+	+	+	+
Compounds detected in 4 samples								
Atenolol	$C_{14}H_{22}N_2O_3$	29122-68-7	266.336	+	+	+	+	
Buturon	$C_{12}H_{13}ClN_2O$	3766-60-7	236.697	+	+	+		+
Cyprodinil	$C_{14}H_{15}N_3$	121552-61-2	225.289	+	+	+	+	
Daphnoretin	$C_{19}H_{12}O_7$	2034-69-7	352.294	+	+	+	+	
Fenazaquin	$C_{20}H_{22}N_2O$	120928-09-8	306.401	+		+	+	+
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	126833-17-8	302.196	+	+		+	+
Flavin mononucleotide	$C_{17}H_{21}N_4O_9P$	146-17-8	456.344		+	+	+	+
Isorhamnetin	$C_{16}H_{12}O_7$	480-19-3	316.262		+	+	+	+
Methsuximide	$C_{12}H_{13}NO_2$	77-41-8	203.237		+	+	+	+
Phenazepam	$C_{15}H_{10}BrClN_2O$	51753-57-2	349.610	+	+	+		+
Xylazine	$C_{12}H_{16}N_2S$	7361-61-7	220.334	+	+	+	+	
Compounds detected in 3 samples								
16-Dehydroprogesterone	$C_{21}H_{28}O_2$	1096-38-4	312.446	+			+	+
Betaxolol	$C_{18}H_{29}NO_3$	63659-18-7	307.428	+	+			+
Buprenorphine	$C_{29}H_{41}NO_4$	52485-79-7	467.640	+			+	+
Cinnamaldehyde	C_9H_8O	104-55-2	132.159	+			+	+
Dextromethorphan	$C_{18}H_{25}NO$	125-71-3	271.397	+		+		+
Diethyltoluamide	$C_{12}H_{17}NO$	134-62-3	191.270	+			+	+
Isoscopoletin	$C_{10}H_8O_4$	776-86-3	192.168	+		+		+
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	846-49-1	321.158	+	+	+		
Morphine	$C_{17}H_{19}NO_3$	52-27-2	285.338		+	+		+
Normeperidine	$C_{14}H_{20}ClNO_2$	24465-45-0	269.767	+	+	+		

Penoxsulam	$C_{16}H_{14}F_5N_5O_5S$	219714-96-2	483.370	+	+	+		
Scopolamine	$C_{17}H_{21}NO_4$	51-34-3	303.353		+		+	+
Strychnine	$C_{21}H_{22}N_2O_2$	57-24-9	334.412		+	+	+	
Thiothixene	$C_{23}H_{29}N_3O_2S_2$	3313-26-6	443.625		+		+	+
Uniconazole	$C_{15}H_{18}ClN_3O$	83657-22-1	291.776	+	+		+	
Vitamin A	$C_{20}H_{30}O$	68-26-8	286.452	+		+		+
Compounds detected in 2 samples								
7-Aminonitrazepam	$C_{15}H_{13}N_3O$	4928-02-3	251.283	+		+		
7-Hydroxycoumarin	$C_9H_6O_3$	93-35-6	162.142		+			+
Alprenolol	$C_{15}H_{23}NO_2$	13655-52-2	249.349			+	+	
Amphetamine	$C_9H_{13}N$	300-62-9	135.206	+	+			
Baquiloprim	$C_{17}H_{20}N_6$	102280-35-3	308.381		+	+		
Carvone	$C_{10}H_{14}O$	99-49-0	150.218	+	+			
Clonidine	$C_9H_9Cl_2N_3$	4205-90-7	230.094	+		+		
Danofloxacin	$C_{19}H_{20}FN_3O_3$	112398-08-0	357.379	+			+	
Desmethylcitalopram	$C_{19}H_{19}FN_2O$	62498-67-3	310.365	+				+
Dimefuron	$C_{15}H_{19}ClN_4O_3$	34205-21-5	338.789			+		+
Donepezil	$C_{24}H_{29}NO_3$	120014-06-4	379.492			+		+
EDDP	$C_{20}H_{23}N$	136765-23-6	277.403	+		+		
Fenthion-sulfoxide	$C_{10}H_{15}O_4PS_2$	3761-41-9	294.328		+			+
Fenpropidin	$C_{19}H_{31}N$	67306-00-7	273.456			+	+	
Fluridone	$C_{19}H_{14}F_3NO$	59756-60-4	329.316			+	+	
Hydrocinnamic acid	$C_9H_{10}O_2$	501-52-0	150.175	+	+			
Hydroxygenkwanin	$C_{16}H_{12}O_6$	20243-59-8	300.263		+		+	
Imipramine	$C_{19}H_{24}N_2$	50-49-7	280.407	+				+
Inabenfide	$C_{19}H_{15}ClN_2O_2$	82211-24-3	338.788		+	+		
Indoxyl	C_8H_7NO	480-93-3	133.147	+	+			
Methaqualone	$C_{16}H_{14}N_2O$	72-44-6	250.295	+			+	
Naltrexone	$C_{20}H_{23}NO_4$	16590-41-3	341.409	+		+		
Neburon	$C_{12}H_{16}Cl_2N_2O$	555-37-3	275.174		+			+
O-Methylsinapic acid	$C_{12}H_{14}O_5$	90-50-6	238.237		+			+
Psoralidin	$C_{20}H_{16}O_5$	18642-23-4	336.338		+			+
Triclabendazole sulfone	$C_{14}H_9Cl_3N_2O_3S$	106791-37-1	391.657		+		+	
Tryptophanol	$C_{11}H_{14}N_2O$	2899-29-8	190.242		+			+
Compounds detected in 1 sample								
11a-Hydroxyprogesterone	$C_{21}H_{30}O_3$	321-90-3	330.461					+
17-Hydroxyprogesterone	$C_{21}H_{30}O_3$	68-96-2	330.461			+		
17a-Methyltestosterone	$C_{20}H_{30}O_2$	58-18-4	302.451	+				
2-hydroxyhippuric acid	$C_9H_9NO_4$	16555-77-4	195.172			+		

3-Methylindole	C_9H_9N	83-34-1	131.174	+					
4-Ethylamphetamine	$C_{11}H_{17}N$	1334811-39-0	136.259	+					
4-Hydroxycoumarin	$C_9H_6O_3$	1076-38-6	162.142						+
5-Hydroxyindoleacetic acid	$C_{10}H_9NO_3$	54-16-0	191.183	+					
6-beta-naltrexol	$C_{20}H_{25}NO_4$	49625-89-0	343.417	+					
6-Methylcoumarin	$C_{10}H_8O_2$	92-48-8	160.169		+				
6,7-Dimethoxycoumarin	$C_{11}H_{10}O_4$	120-08-1	206.195					+	
7-Aminoflunitrazepam	$C_{16}H_{14}FN_3O$	34084-50-9	283.300					+	
Amygdalin	$C_{20}H_{27}NO_{11}$	29883-15-6	457.428	+					
Arenobufagin	$C_{24}H_{32}O_6$	464-74-4	416.507					+	
Aripiprazole	$C_{23}H_{27}Cl_2N_3O_2$	129722-12-9	448.385		+				
Butorphanol	$C_{21}H_{29}NO_2$	42408-82-2	327.461					+	
Benzamide	C_7H_7NO	55-21-0	121.137				+		
Beberrubine	$C_{19}H_{16}ClNO_4$	15401-69-1	357.788	+					
Cannabinol	$C_{21}H_{26}O_2$	521-35-7	310.430		+				
Campesterol	$C_{28}H_{48}O$	474-62-4	400.680					+	
Chloridazon	$C_{10}H_8ClN_3O$	1698-60-8	221.643		+				
Chrysin	$C_{15}H_{10}O_4$	480-40-0	254.238		+				
Cinnamic acid	$C_9H_8O_2$	140-10-3	148.159						+
Cortexolone	$C_{21}H_{30}O_4$	152-58-9	346.461						+
Corydaline	$C_{22}H_{27}NO_4$	518-69-4	369.454					+	
Coumatetralyl	$C_{19}H_{16}O_3$	5836-29-3	292.328				+		
Cyphenothrin	$C_{24}H_{25}NO_3$	39515-40-7	375.460				+		
Enrofloxacin-D5	$C_{19}H_{17}D_5FN_3O_3$	1173021-92-5	364.426						+
Esmolol	$C_{16}H_{25}NO_4$	81147-92-4	295.374	+					
Estrone	$C_{18}H_{22}O_2$	53-16-7	270.366						+
Ethiprole	$C_{13}H_9Cl_2F_3N_4OS$	181587-01-9	397.203	+					
Fenthion-sulfone	$C_{10}H_{15}O_5PS_2$	3761-42-0	310.327	+					
Fentin	$C_{18}H_{17}OSn$	76-87-9	367.029					+	
Fenvalerate	$C_{25}H_{22}ClNO_3$	51630-58-1	419.900						+
Flunixin	$C_{14}H_{11}F_3N_2O_2$	38677-85-9	296.245		+				
Flunixin-D3	$C_{14}H_8D_3F_3N_2O_2$	1015856-60-6	299.263		+				
Formononetin	$C_{16}H_{12}O_4$	485-72-3	268.264					+	
Fonofos	$C_{10}H_{15}OPS_2$	944-22-9	246.329	+					
Glycyrrhetinic acid	$C_{30}H_{46}O_4$	471-53-4	470.684	+					
Hematoporphyrin	$C_{34}H_{38}N_4O_6$	14459-29-1	598.689	+					
Hexaconazole	$C_{14}H_{17}Cl_2N_3O$	79983-71-4	314.210	+					
Hymecromone	$C_{10}H_8O_3$	90-33-5	176.169	+					
Indigo Dye	$C_{16}H_{10}N_2O_2$	482-89-3	262.263					+	

Ketotriclabendazole	$C_{13}H_7Cl_3N_2O_2$	1201920-88-8	329.566	+						
Lamotrigine	$C_9H_7Cl_2N_5$	84057-84-1	256.091	+						
Marmesin	$C_{14}H_{14}O_4$	13849-08-6	246.259	+						
Metconazole	$C_{17}H_{22}ClN_3O$	125116-23-6	319.829					+		
Metosulam	$C_{14}H_{13}Cl_2N_5O_4S$	139528-85-1	418.255					+		
Mianserin	$C_{18}H_{20}N_2$	24219-97-4	264.365			+				
Nalbuphine	$C_{21}H_{27}NO_4$	20594-83-6	357.443		+					
Naloxone	$C_{19}H_{21}NO_4$	465-65-6	327.474	+						
Naringenin	$C_{15}H_{12}O_5$	207-550-2	272.253							+
Nitrazepam	$C_{15}H_{11}N_3O_3$	146-22-5	281.266			+				
Norbuprenorphine	$C_{25}H_{35}NO_4$	75715-23-8	413.550					+		
o-Tyrosine	$C_9H_{11}NO_3$	2370-61-8	181.189	+						
Olanzapine-D3	$C_{17}H_{27}D_3N_4S$	786686-79-1	315.451					+		
Orientin	$C_{21}H_{20}O_{11}$	28608-75-5	448.377							+
Paraoxon-methyl	$C_8H_{10}NO_6P$	950-35-6	247.142	+						
Procymidone	$C_{13}H_{11}Cl_2NO_2$	32809-16-8	284.138							+
Progesterone	$C_{21}H_{30}O_2$	57-83-0	314.462		+					
Propranolol	$C_{16}H_{21}NO_2$	525-66-6	259.343					+		
Retinal	$C_{20}H_{28}O$	116-31-4	284.436			+				
Sanguinarine	$C_{20}H_{14}NO_4$	2447-54-3	332.329			+				
Scutellarin	$C_{21}H_{18}O_{12}$	27740-01-8	462.360	+						
Tadalafil	$C_{22}H_{19}N_3O_4$	171596-29-5	389.404		+					
Tebufenpyrad	$C_{18}H_{24}ClN3O$	119168-77-3	333.856	+						
Triazoxide	$C_{10}H_6ClN_5O$	72459-58-6	247.641		+					
Triclabendazole	$C_{14}H_9Cl_3N_2OS$	68786-66-3	359.658		+					
Triptonide	$C_{20}H_{22}O_6$	38647-11-9	358.385		+					

[a] +: detected.

Table S11. AhR agonist candidates (Step 6, see Figure 3 in the main text) in livers of black-tailed gulls using LC-QTOFMS.

Compounds	Confidence level[a]	AhR-active	Uses/references
Compounds detected in 5 samples			
[10]-Gingerol	1	+[b]	Anti-inflammatory agent Dugasani et al. (2010)
Angelicin	1	+	Anti-convulsant agent Pazos-Navarro et al. (2013)
Corticosterone	1	+	Steroid hormone Kitaysky et al. (2003)
Difenzoquat	2a		Pesticide Núnez et al. (2002)
Enrofloxacin	1	−[c]	Anti-bacterial agent Ballesteros et al. (2004)
Etofenprox	1	+	Pesticide Szabó et al. (2019)
Eupatilin	1	+	Flavonoid Seo and Surh. (2001)
Oxadixyl	1	+	Pesticide Kwon et al. (2015)
Perillic acid	2a		Anti-cancer agent Ezennia et al. (1997)
Tretinoin	1	+	Anti-neoplastic agent Collado-Borrell et al. (2016)
Triamterene	1	−	Diuretic Nisha and Kumar. (2019)
Compounds detected in 4 samples			
Atenolol	2a		Anti-hypertensive agent Yousefpour et al. (2015)
Buturon	2a		Herbicide Liu et al. (2015)
Cyprodinil	2a		Fungicide Cabras et al. (1997)
Daphnoretin	2a		Herbal medicine Ho et al. (2010)
Fenazaquin	2a		Insecticide Joo and Keum. (2018)
Fenhexamid	2a		Fungicide Billard et al. (2012)
Flavin mononucleotide	2a		Natural product Cioni et al. (2009)
Isorhamnetin	2a		Anti-viral agent Dayem et al. (2015)
Methsuximide	2a		Anti-convulsant agent Orton and Nicholls. (1972)
Phenazepam	2a		Benzodiazepine drug Dargan et al. (2016)
Xylazine	2a		Analgesic Grubb et al. (2002)

[a] Schymanski et al. (2014)
[b] +: Significant.
[c] −: Not significant.

Table S12. Concentrations of nine AhR agonist candidates in livers of black-tailed gulls from the southeastern coast of Korea.

Compounds	S1	S2	S3	S4	S5
			(ng g^{-1} wet mass)		
[10]-Gingerol	85	46	200	27	30
Angelicin	ND[a]	ND	13	15	ND
Corticosterone	390	290	240	270	310
Enrofloxacin	15	6.4	3.1	3.7	3.9
Etofenprox	ND	ND	ND	ND	ND
Eupatilin	6.8	2.5	1.4	0.9	0.8
Oxadixyl	2.3	2.2	1.2	1.6	2.2
Tretinoin	17	6.7	7.1	4.6	11
Triamterene	0.5	0.2	0.2	0.2	0.2

[a] ND: Not detected.

Table S13. Predicted other potential toxicity of AhR agonist candidates in livers of black-tailed gulls using VEGA QSARs.

Compounds	Androgen receptor activity[a]	Carcinogenicity[b]	Developmental toxicity[c]	Estrogen receptor activity[d]	Mutagenicity[e]	Thyroid receptor activity[f]
Compounds detected in 5 samples						
[10]-Gingerol	−[g]	− + − −	+ +	+ −	− − − − −	− −
Angelicin	+[h]	+ − + +	+ +	− −	− − + − −	− −
Corticosterone	+	+ − + +	+ +	− −	− − + − −	− −
Difenzoquat	−	− + − −	− −	− −	− + − + +	− −
Enrofloxacin	−	+ − + +	+ +	− −	+ + + + +	− −
Etofenprox	−	+ − − +	+ −	− +	− − − − −	− −
Eupatilin	+	+ − − +	+ +	+ +	+ − − − −	− −
Oxadixyl	−	+ + + +	− −	− −	+ + + − +	− −
Perillic acid	−	+ + − +	+ −	− −	− − − − −	− −
Triamterene	−	− − + +	+ +	− −	− − − − −	− +
Tretinoin	−	− − − +	+ +	− −	− − − − −	− −
Compounds detected in 4 samples						
Atenolol	−	+ − − +	+ +	− −	− − − − −	− −
Buturon	−	+ − + +	+ +	− −	− − − − −	− −
Cyprodinil	−	+ + − +	− −	− −	− + − + −	− −
Daphnoretin	−	+ − + +	+ −	+ −	− − + − −	− −
Fenazaquin	−	+ − − +	+ −	− −	− + − + −	− −
Fenhexamid	+	− − − +	+ −	− +	− − − − −	− −
Flavin mononucleotide	−	+ − − +	+ +	− −	− + − + +	− −
Isorhamnetin	+	+ − + +	+ +	+ +	− − + + −	− −
Methsuximide	−	+ − − −	+ −	− −	− − − − −	− −
Phenazepam	−	+ − + +	+ +	− −	− − − − −	− −
Xylazine	−	+ + − +	− −	− +	− − − − −	− −

[a] Predicted androgen receptor activity of AhR agonists candidates by use of VEGA QSAR with one model (IRFMN/COMPARA).
[b] Predicted carcinogenicity of AhR agonists candidates by use of VEGA QSAR with four model (IRFMN/Antares, CAESAR, ISS, IRFMN/ISSCAN-CGX).
[c] Predicted developmental toxicity of AhR agonists candidates by use of VEGA QSAR with two model (CAESAR, PG).
[d] Predicted estrogen receptor activity of AhR agonists candidates by use of VEGA QSAR with two model (IRFMN/CERAPP, IRFMN).
[e] Predicted mutagenicity of AhR agonists candidates by use of VEGA QSAR with five model (CAESAR, CONSENSUS, ISS, KNN/Read-Across, SarPy/IRFMN).
[f] Predicted thyroid receptor alpha and beta activity of AhR agonists candidates by use of VEGA QSAR with one model (NRMEA).
[g] −: Not active.
[h] +: Active.

Table S14. Predicted other receptor binding potencies of AhR agonist candidates in livers of black-tailed gulls using VirtualToxLab in silico modeling.

Compounds	AhR	AR	ER	TR
Compounds detected in 5 samples				
[10]-Gingerol	0.377 (weak[a])	0.657 (strong)	0.548 (moderate)	0.589 (moderate)
Angelicin	0.402 (moderate)	0.425 (moderate)	0.350 (weak)	0.433 (moderate)
Corticosterone	0.314 (weak)	0.425 (moderate)	0.350 (weak)	0.433 (moderate)
Difenzoquat	Not binding	Not binding	Not binding	Not binding
Enrofloxacin	NA[b]	NA	NA	NA
Etofenprox	0.506 (moderate)	0.399 (weak)	0.235 (weak)	0.350 (weak)
Eupatilin	0.248 (weak)	0.402 (moderate)	0.278 (weak)	0.346 (weak)
Oxadixyl	0.403 (moderate)	0.319 (weak)	0.319 (weak)	0.473 (moderate)
Perillic acid	Not binding	Not binding	Not binding	0.319 (weak)
Triamterene	Not binding	Not binding	Not binding	Not binding
Tretinoin	0.403 (moderate)	0.319 (weak)	0.317 (weak)	0.473 (moderate)
Compounds detected in 4 samples				
Atenolol	NA	NA	NA	NA
Buturon	NA	NA	NA	NA
Cyprodinil	0.323 (weak)	0.193 (weak)	Not binding	0.300 (weak)
Daphnoretin	0.356 (weak)	0.418 (moderate)	0.296 (weak)	0.286 (weak)
Fenazaquin	0.492 (moderate)	0.375 (weak)	0.246 (weak)	0.439 (moderate)
Fenhexamid	0.208 (weak)	0.366 (weak)	0.369 (weak)	0.286 (weak)
Flavin mononucleotide	NA	NA	NA	NA
Isorhamnetin	0.207 (weak)	0.382 (weak)	0.331 (weak)	0.251 (weak)
Methsuximide	NA	NA	NA	NA
Phenazepam	0.350 (weak)	0.318 (weak)	0.374 (weak)	0.318 (weak)
Xylazine	Not binding	Not binding	Not binding	Not binding

[a] Toxpotential score < 0.4 is weak, 0.4–0.6 is moderate, > 0.6 is strong binding affinity to receptor.

[b] NA: Not analyzed using VirtualToxLab in silico modeling.

Supplementary Figures

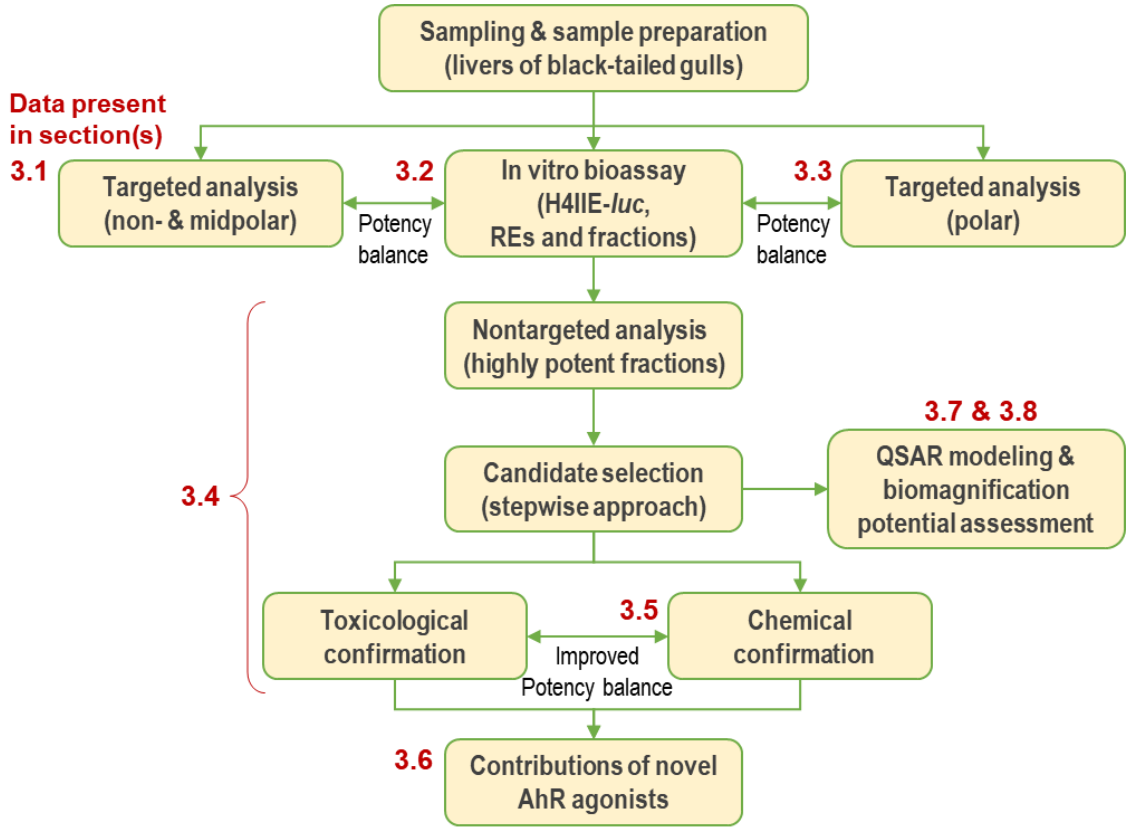

Fig. S1. Flowchart for the identification of novel AhR agonists in the livers of black-tailed gulls from the southeastern coast of Korea.

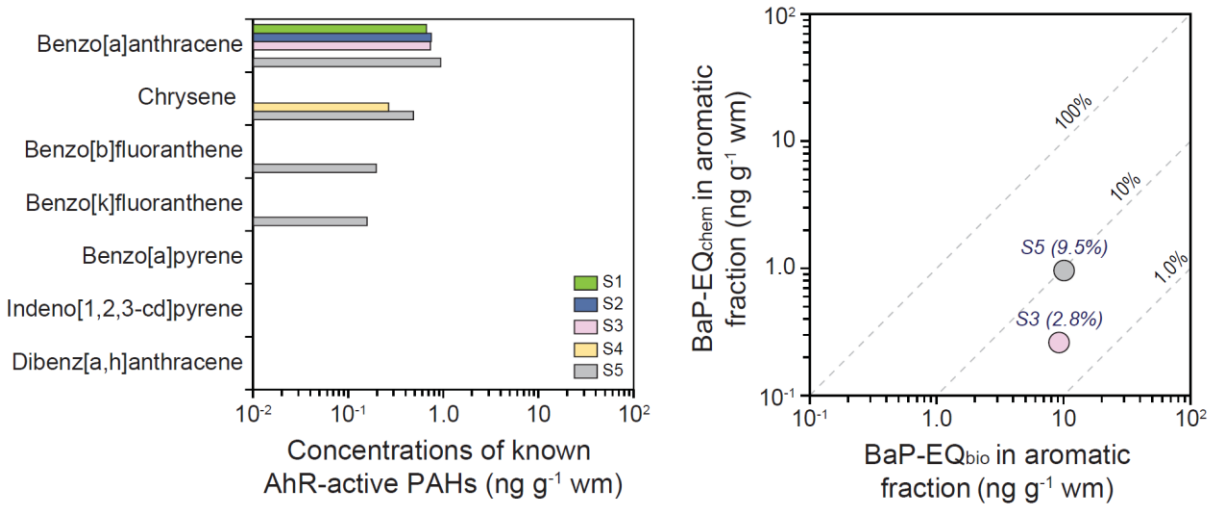

Fig. S2. Concentrations of aromatic AhR agonists and their contributions ($BaP\text{-}EQ_{chem}$) to $BaP\text{-}EQ_{bio}$ in mid-polar fractions of livers of black-tailed gulls from the southeastern coast of Korea.

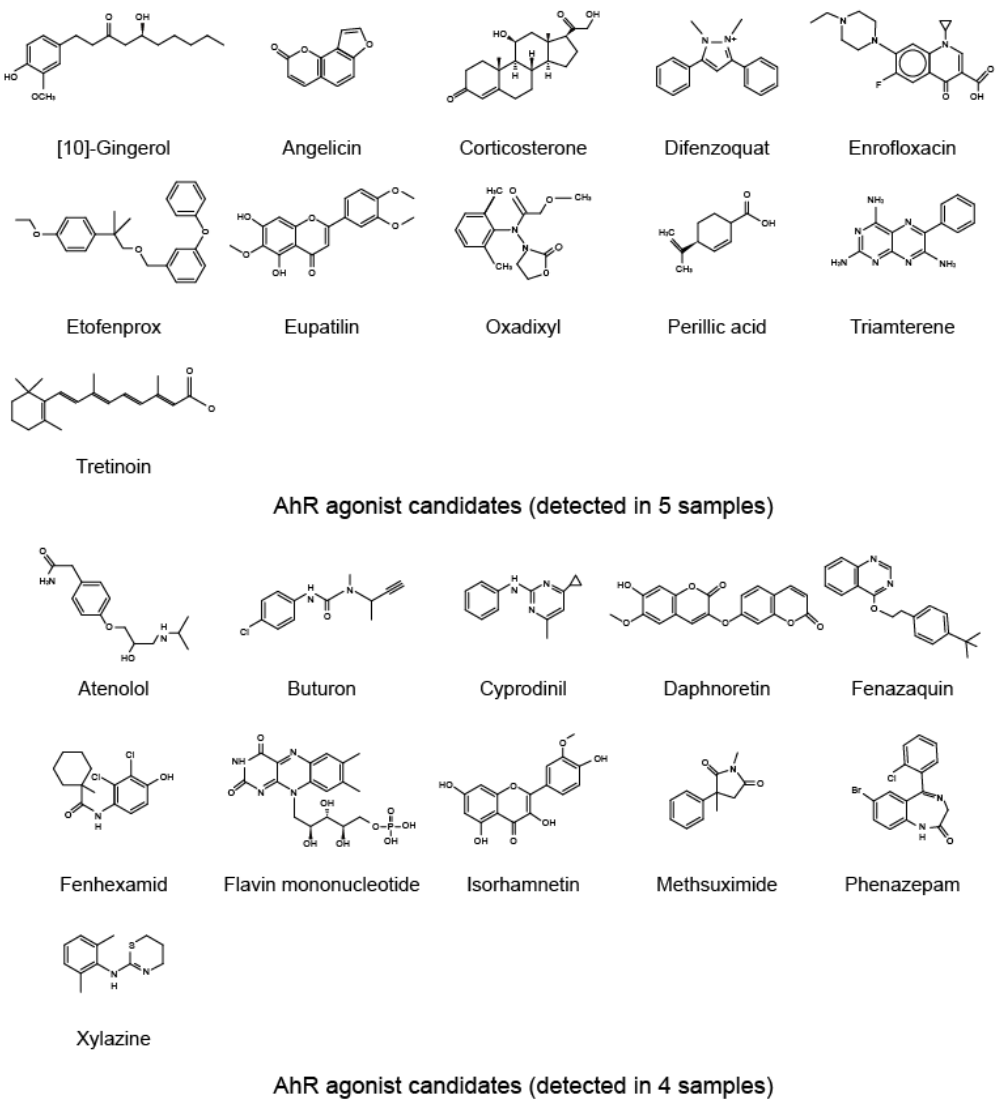

Fig. S3. Chemical structures of 22 AhR agonist candidates in polar fractions of livers of black-tailed gulls from the southeastern coast of Korea.

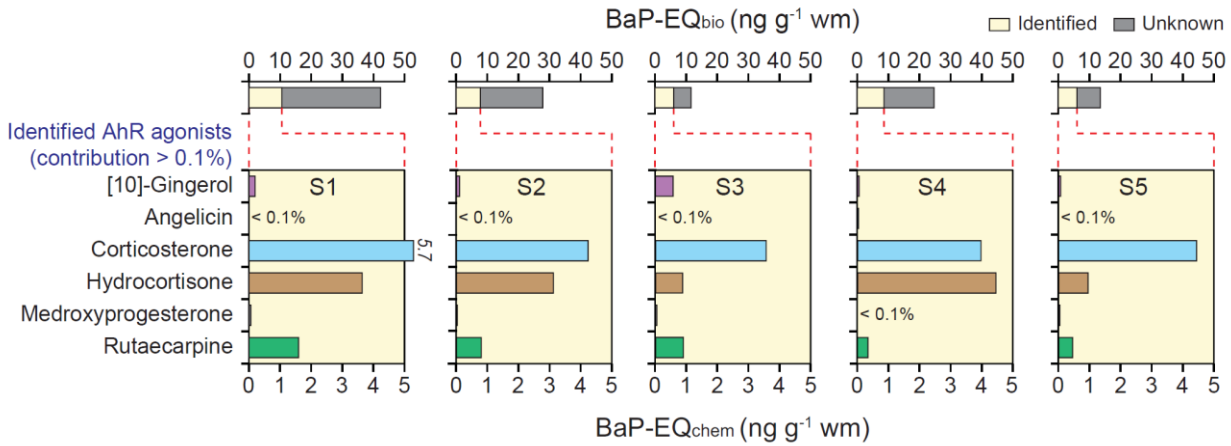

Fig. S4. Contributions of polar AhR agonists (BaP-EQ_{chem}, >0.1%) to total induced AhR-mediated potencies (BaP-EQ_{bio}) in polar fractions of livers of black-tailed gulls the southeastern coast of Korea.

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