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Synthesis and biological evaluation of fused thio- and selenopyrans as new indolocarbazole analogues with aryl hydrocarbon receptor affinity

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ABSTRACT

A series of thio- and selenopyrans having two fused indole units, structurally related to indolocarbazoles, have been prepared and evaluated for aryl hydrocarbon receptor (AhR) affinity, leading to the identification of several new significant AhR ligands. In particular, the parent thiopyrano[2,3-*b*:6,5-*b*′]diindole and its derivative having a methyl group in the central ring, as well as the two corresponding selenopyrans, displayed the highest potencies of the compounds tested.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent intracellular receptor that, when activated by binding of a ligand, can stimulate gene transcription of numerous genes. The best-characterized classes of ligands for the receptor include environmental contaminants such as a variety of polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs), of which the environmental toxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (**1**) (Fig. 1) exerts the highest activating capacity. Based on structure–activity relationship (SAR) analysis utilizing HAHs and PAHs, a "best-fit model" of an AhR ligand has been proposed. According to this model an AhR ligand would be a planar, aromatic and lipophilic molecule with maximal dimensions of 14 Å × 12 Å × 5 Å. In addition, other properties, such as hydrophobicity, electronegativity and hydrogen binding also contribute to the receptor interaction.[1] However, the AhR binds a large number of structurally diverse substances, both of exogenous and endogenous origin. While the relative potency of these ligands is typically much lower compared to that of HAHs, some of them stand out as having high affinities. Among these are several indolocarbazoles,[2] most notably 6-formylindolo[3,2-*b*]carbazole (FICZ) (**2**) and indolo[3,2-*b*]carbazole (ICZ) (**3**), both exhibiting a greater capacity to activate the AhR than TCDD.[3] While ICZ is formed from the dietary tryptophan (Trp) derivative indole 3-carbinol (I3C) under acidic conditions (for example in contact with stomach juice),[4] FICZ is formed from Trp when irradiated with UVA, UVB and visible light.[3,5]

Despite the tremendous amount of effort invested in studies of the potent indolo[3,2-*b*]carbazoles mentioned above, as well as the intensely scrutinized family of indolo[2,3-*a*]carbazoles, which embodies numerous members with powerful and diverse biologi-

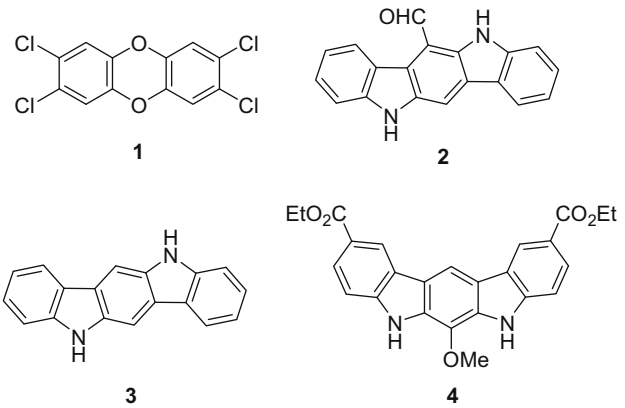

Figure 1.

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cal effects,[2c,d] relatively little is known about the biological properties of the other possible isomeric indolocarbazoles, or indolocarbazole analogues. Nevertheless, very recently, the indolo[2,3-*b*]carbazole derivative **4** has been identified as a potent orally available anticancer agent,[6] demonstrating that even this class of indolocarbazoles may display powerful biological effects. In addition, there are some chemical and biological studies devoted to indolocarbazole analogues incorporating additional heteroatoms in the pentacyclic framework,[7] or related fused systems featuring a central seven-membered ring.[8]

With this background, it was envisaged that design and synthesis of indolocarbazole analogues incorporating one sulfur or selenium atom as a replacement for one of the carbon atoms in the central ring may provide access to a new class of biologically active fused heterocyclic systems containing two indole units, enabling further studies of SAR of these types of compounds, for instance as ligands for the AhR. Here we describe the synthesis of several thio- and selenopyranodiindoles, and compare their AhR activating capacities to those of TCDD (**1**), ICZ (**3**) and FICZ (**2**).

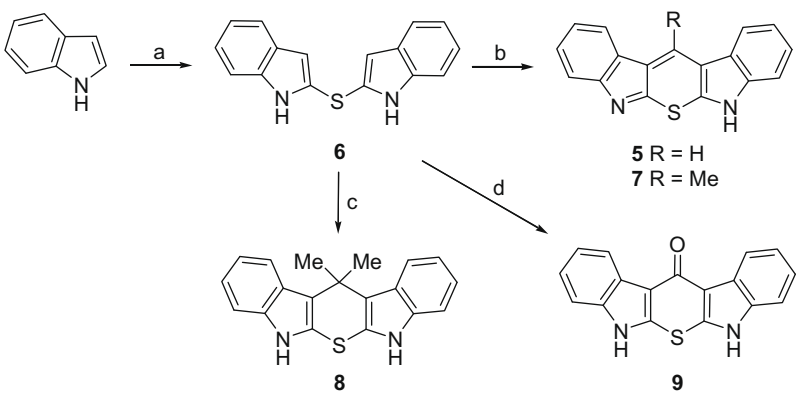
2. Results and discussion

2.1. Chemistry

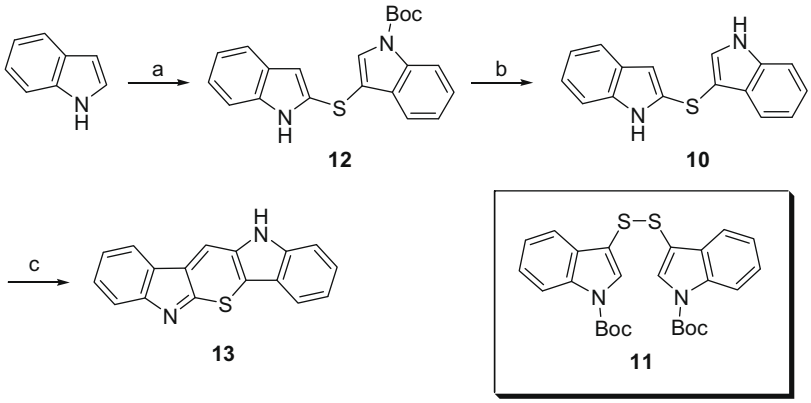
Our synthetic work commenced with development of a new approach to derivatives of the known system **5**, which has previously been observed as a product originating from dimerization of indoline-2-thione under Vilsmeier formylation conditions.[9] As we needed a more general route, which would allow introduction of substituents in the central ring, the bis(indol-2-yl) sulfide **6** was selected as the starting compound. Based on our previous experience of preparation of bisindolyl sulfides[10] by exposure of metalated indoles to bis(phenylsulfonyl) sulfide, indole was lithiated according to Katritzky,[11] followed by introduction of bis(phenylsulfonyl) sulfide,[12] affording the desired intermediate **5** in acceptable yield (Scheme 1). Although the initial cyclization experiments involving triethyl orthoformate as the reagent in 1,4-dioxane in the presence of methanesulfonic acid gave the target heteroaromatic molecule **5**, the yields were rather low (typically around 20%). On the other hand, changing the solvent to acetonitrile resulted in a substantial improvement, rendering the product **5** in 42% yield. A similar annulation reaction using triethyl orthoacetate gave the methyl derivative **7**. Both thiopyrano[2,3-*b*:6,5-*b*′]diindoles **5** and **7** displayed a fast prototropy, and only one set of aromatic resonances could be discerned in their ^{1}H NMR spectra.[13] The sulfide **6** could also serve as a precursor for further related thiopyran derivatives, and gave the dimethyl derivative **8** upon treatment with acetone under acidic conditions, whereas reaction of **6** with phosgene afforded the keto derivative **9**, demonstrating the scope of this approach, which should allow construction of many additional sulfur analogues of indolo[2,3-*b*]carbazole. Moreover, it should be noted in this context that the behaviour of **6** in these reactions was rather similar to that of 1,2-bis(indol-2-yl)ethane, a useful precursor for synthesis of cycloheptabisindole systems.[8c]

The synthetic approach to the isomeric series of sulfur analogues of indolo[3,2-*b*]carbazole required the sulfide **10**, which has been previously prepared in modest overall yield by lithiation of indole following the Katritzky protocol, subsequent reaction of the intermediate lithioindole with 3,3′-dithiobis(1-phenylsulfonyl)indole, and final removal of the phenylsulfonyl unit.[10a,14] However, using instead the Boc-protected 3,3′-dithiobisindole **11**[14] in this route provides the intermediate unsymmetrical disulfide **12** in good yield (Scheme 2). Removal of the protecting group present in **12** was thereafter accomplished by treatment with aqueous potassium hydroxide in ethanol, affording the key precursor **10** in good overall yield from indole. It is also noteworthy that attempted deprotection experiments using TFA in CH_2Cl_2 under standard conditions proved to be disappointing, leading to complex mixtures of several products. Next, annulation experiments involving the sulfide **10** were undertaken, bearing in mind that its reactivity may resemble that of 2,3′-diindolylmethane, which has previously been used for direct conversion to indolo[3,2-*b*]carbazoles.[15] Indeed, exposure of **10** to triethyl orthoformate in the presence of methanesulfonic acid gave the novel ring system **13** in moderate yield. However, attempted annulation of **10** with triethyl orthoacetate failed, giving an intractable mixture. This might be due to the fact that the substrate **10** is less reactive than the 2,2′-connected indolic sulfide **6**, and application of more forcing acidic conditions will eventually give extensive side reactions, for instance rearrangements. Such behaviour under acidic conditions is well documented for thioindoles.[16]

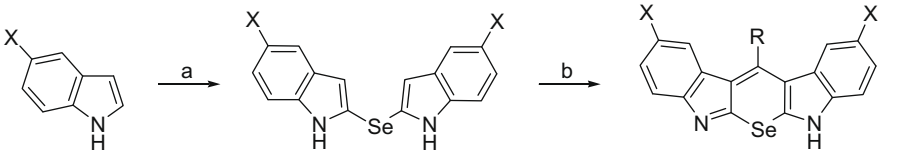
Having established useful conditions for synthesis of the sulfur-containing systems outlined in Scheme 1, we turned our attentions to the preparation of a related series of selenium-based molecules. Hence, metalation of indole or 5-methoxyindole according to Katritzky,[11] and subsequent quenching of the resulting organometallic intermediates with the electrophile bis(phenylsulfonyl) selenide,[17] yielded the desired precursors **14** and **15** in good yield (Scheme 3). Subsequent exposure of **14** or **15** to triethyl orthoformate or triethyl orthoacetate under acidic conditions in 1,4-dioxane (acetonitrile in the case of compound **17**), gave, just as expected, the target compounds **16**–**19** in moderate yields.



Scheme 1. *Reagents and conditions.* (a) (i) BuLi, THF; (ii) CO_2; (iii) *t*-BuLi; (iv) $(PhSO_2)_2S$, 63%; (b) $HC(OEt)_3$, $MeSO_3H$, CH_3CN (for **5**), 42%; $MeC(OEt)_3$, $MeSO_3H$, CH_3CN (for **7**), 76%; (c) acetone, $MeSO_3H$, 1,4-dioxane, 78%; (d) $COCl_2$, 1,4-dioxane, 69%.



Scheme 2. *Reagents and conditions.* (a) (i) BuLi, THF; (ii) CO_2; (iii) *t*-BuLi; (iv) **11**, 81%; (b) KOH, H_2O, EtOH, 83%; (c) $HC(OEt)_3$, $MeSO_3H$, CH_3CN, 66%.

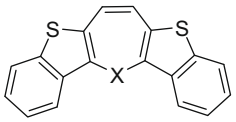


Scheme 3. *Reagents and conditions.* (a) (i) BuLi, THF; (ii) CO_2; (iii) *t*-BuLi; (iv) $(PhSO_2)_2Se$; (b) $HC(OEt)_3$ or $EtC(OEt)_3$, $MeSO_3H$, 1,4-dioxane or CH_3CN.

2.2. AhR activation

Compounds **5**, **7, 13, 16** and **17** demonstrated the highest AhR activating capacities of the thio- and selenopyranodiindoles tested using the Ah-IMMUNOASSAY® (Fig. 2), with relative capacities of 0.13–0.38 compared to that of TCDD (Table 1). The activating capacities of the other compounds tested were between 0.003 and 0.055. As expected, both FICZ (**2**) and ICZ (**3**) demonstrated higher potencies compared to TCDD (**1**), 1.9 and 1.5 times, respectively.[3b]

The compounds tested are aromatic, small and lipophilic – thus displaying good qualities for AhR ligand candidates. In general the structural differences between the compounds tested include different substituents in the central and outer rings, different atoms at position six in the central ring, as well as different orientations of the indole-carbazole ring fusion ([2,3-*b*] and [3,2-*b*]), providing a representative set of sulfur- and selenium analogues of two indolocarbazole frameworks with well-established biological effects. In addition, two previously prepared pentacyclic systems incorporating a central thiepin motif, or a selenepin ring, in conjunction with two benzo[*b*]thiophene units, namely **20**[10b] and **21**[17a] (Fig. 2), were also tested. Compared to other ligands, both xenobiotic and endogenous, the thio- and selenopyranodiindoles tested constitute a novel group of relatively potent AhR ligands. Although their activating capacity depends on the substituents in the central- and outer rings, it can also be effected by the geometry of the ring system. An increase in activity was noted by introduction of a methyl group in the central ring of the slightly bent molecule **5** (i.e., compound **7**), while lower activity was observed for the linear system **13**, a sulfur analogue of ICZ (**3**) itself. An increase in activity by introduction of a methyl group in the central ring was also observed for the selenium analogues **16** and **18** (i.e., compounds **17** and **19**, respectively). The presence of the sulfur or selenium atom in the central ring makes the molecules more polar compared to FICZ (**2**), ICZ (**3**), and the parent indolo[2,3-*b*]carbazole, which all have a carbon atom in that position. It is likely that this increase in polarity contributes to the generally lower activating capacities detected for the thio- and selenopyranodiindoles. However, the



20 X = S
21 X = Se

Figure 2.

Table 1
AhR-activating capacities[a]

Compound	Relative capacity to activate the AhR[b]
TCDD (**1**)	1
5	0.29 ± 0.01
7	0.36 ± 0.02
8	0.0033 ± 0.0003
9	0.039 ± 0.004
13	0.132 ± 0.004
16	0.32 ± 0.02
17	0.38 ± 0.03
18	0.009 ± 0.001
19	0.013 ± 0.003
20	0.055 ± 0.005
21	0.018 ± 0.002
ICZ (**3**)	1.48 ± 0.07[c]
FICZ (**2**)	1.89 ± 0.04[c]

[a] The values presented are means ± SEM, $n = 14$ for FICZ (**2**) and TCDD (**1**) and 4–6 for the other compounds.
[b] Activation by TCDD (**1**) was set as 1.
[c] Data taken from Ref. 3b.

capacities did not differ significantly wether there was a sulfur or selenium atom at that position. Unlike the other thiopyranodiindole compounds tested, compound **8** is not planar due to its geminal methyl groups in the central ring, which is probably the cause of its low potency. The oxygenated compounds **9**, **18** and **19** are planar but more polar compared to the other compounds, also leading to a decrease of the AhR activating capacity, although not to the same extent as with compound **8**. In addition to the increased polarity of compounds **18** and **19**, the steric effect due to the bulk of the –OMe groups in the outer rings most likely plays a crucial role in the decreased capacities observed. Finally, it should be noted that compounds **20** and **21**, which both have bent geometry, also displayed some activity, in particular the thiepin **20**. The hydrophobic characteristics of these compounds, together with their fused pentacyclic core may account for the observed effects. However, these molecules are not planar due to the usually preferred conformation of the central seven-membered rings. Their binding capacities further illustrate that not only planar molecules can display significant affinity to the AhR.[1]

These results bring forward a new and unexplored group of potent AhR ligands. However, in order to establish the importance of the geometry of the ring system, as well as the different functional groups in respect of AhR activation, further studies need to be performed. In addition, the method we used for measuring the AhR activating potencies is a cell free assay meaning that the compounds ability to cross the cell membrane and entering the cytoplasm where the AhR resides is not taken into account. This would also be of importance to investigate.

3. Experimental

3.1. Chemistry

3.1.1. General information

NMR spectra were recorded on a Bruker DPX 300 instrument operating at 300.1 MHz for ^{1}H and 75.5 MHz for ^{13}C, using the residual solvent signal as reference, unless otherwise stated. IR spectra were acquired on a Thermo Nicolet Avatar 330 FT-IR instrument. Melting points were determined in open capillary tubes on a Büchi B-545 melting point apparatus. Chemicals and solvents were obtained from commercial sources and used as received, except THF, which was distilled from sodium and benzophenone, and acetonitrile, which was stored over activated 4 Å molecular sieves. All reactions were performed under nitrogen atmosphere. Chromatography was performed using silica gel (40–63 μm).

3.1.2. Bis(1*H*-indol-2-yl)sulfide (6)

BuLi (2.5 M in hexanes, 14.4 mL, 36.0 mmol) was added dropwise to a solution of indole (3.51 g, 30.0 mmol) in dry THF (80 mL) at −78 °C. The resulting solution was kept at −78 °C for 30 min, followed by introduction of CO_2 (g) during 15 min. The solvents were thereafter evaporated under reduced pressure (during that time the temperature was allowed to rise to 20 °C), leaving a solid residue. N_2 was introduced into the vessel. The solids were dissolved in dry THF (80 mL), the solution was cooled to −78 °C, and *t*-BuLi (1.7 M in heptane; 21.2 mL, 36.0 mmol) was then added dropwise (over ca. 15 min) at −78 °C. The resulting mixture was stirred for 30 min at −78 °C, followed by addition of bis(phenylsulfonyl) sulfide[12] (4.80 g, 15.3 mmol) in THF (40 mL) during 20 min. The temperature was allowed to rise to 20 °C over 17 h. Acetic acid (2 mL) was added and stirring was continued for 15 min, followed by addition of satd aq NH_4Cl (50 mL). The mixture was extracted with Et_2O (2× 50 mL), and the combined organic phases were washed with water (2× 50 mL), brine (60 mL) and dried (Na_2SO_4). Evaporation of the solvents gave a yellow oil, which was subjected to column chromatography [*n*-heptane/CH_2Cl_2 (3:1)], affording compound **6** (2.50 g, 63%) as a white solid, mp: 144–146 °C; IR (neat) 3379, 1443, 1406, 1338, 1316, 1275, 1228, 1091, 933, 808, 778, 744, 731 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.47 (s, 1H), 7.50–7.48 (m, 2H), 7.33–7.30 (m, 2H), 7.12–6.97 (m, 4H), 6.64 (m, 2H); ^{13}C NMR (DMSO-d_6) 137.4, 127.8, 126.5, 122.0, 119.7, 119.4, 111.1, 106.8; HRMS (FAB) *m/z* 264.0726 [M$^+$], $C_{16}H_{12}N_2S$ requires 264.0721.

3.1.3. 5*H*-Thiopyrano[2,3-*b*:6,5-*b*′]diindole (5)

A solution of methanesulfonic acid (0.05 mL) in dry CH_3CN (1 mL) was added to a solution of triethyl orthoformate (0.10 g, 0.7 mmol) and compound **6** (0.13 g, 0.50 mmol) in dry CH_3CN (5 mL) at room temperature. The reaction mixture was stirred for 4 days at room temperature. The resulting precipitate was collected by filtration, washed with CH_3CN, and purified by column chromatography [CH_2Cl_2/MeOH (49:1 → 9:1)] to give the thiopyran **5** (65 mg, 42%) as an orange solid. The spectral and physical data for **5** were in excellent agreement with those reported previously.[9]

3.1.4. Compound 7

This material was prepared following the procedure for thiopyran **5**, but using triethyl orthoacetate (0.11 g, 0.7 mmol). Purification of the crude product by column chromatography [CH_2Cl_2/MeOH (49:1 → 9:1)] to give the thiopyran **7** (0.11 g, 76%) as an orange solid, mp >300 °C; IR (neat) 1546, 1492, 1446, 1394, 1355, 1302, 1256, 1241, 1206, 1174, 1105, 899, 841, 737, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6) 13.20 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.44–7.30 (m, 4H), 3.41 (s, 3H); HRMS (FAB) *m/z* 288.0728 [M$^+$], $C_{18}H_{12}N_2S$ requires 288.0721.

3.1.5. Compound 8

To a solution of compound **6** (0.26 g, 1.0 mmol) and acetone (80 mg, 1.4 mmol) in 1,4-dioxane (5 mL) added $MeSO_3H$ (0.08 mL). The mixture was stirred at room temperature for 1 h and was thereafter heated at reflux for 2.5 h. After cooling to room temperature, silica gel (2 g) was added, and the solvent was evaporated. The residue was subjected to column chromatography [*n*-heptane/EtOAc (2:1)], yielding the product **8** (0.24 g, 78%) as an off-white solid, mp >300 °C; IR (neat) 3377, 1445, 1420, 1338, 1165, 909, 844, 758, 736 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.32 (s, 2H), 7.75–7.72 (m, 2H), 7.33–7.30 (m, 2H), 7.09–6.99 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 137.3, 129.3, 125.4, 120.4, 119.6, 119.1, 118.7, 114.2, 110.8, 34.5, 28.8; HRMS (FAB) *m/z* 304.1032 [M$^+$], $C_{19}H_{16}N_2S$ requires 304.1034.

3.1.6. Compound 9

Phosgene (20% solution in toluene, 0.32 mL, 0.6 mmol) was added to a solution of compound **6** (0.13 g, 0.5 mmol) in dry 1,4-dioxane (5 mL), and the reaction mixture was stirred for 24 h at room temperature. The precipitate was collected by filtration, washed with cold Et_2O (15 mL), and dried to give compound **9** (0.10 g, 69%) as a yellow solid, mp >300 °C; IR (neat) 2884, 1548, 1520, 1494, 1445, 1360, 1213, 1170, 1157, 1108, 928, 871, 858, 769, 739, 719 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.70 (s, 2H), 8.49–8.46 (m, 2H), 7.57–7.54 (m, 2H), 7.34–7.24 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 175.7, 136.9, 135.5, 124.8, 123.2, 121.0, 120.8, 112.7, 111.1; HRMS (FAB) *m/z* 290.0512 [M$^+$], $C_{17}H_{10}N_2OS$ requires 290.0514.

3.1.7. 1′-(*tert*-Butoxycarbonyl)-2,3′-diindolylsulfide (12)

t-BuLi (1.7 M in pentane, 4.5 mL, 7.7 mmol) was added dropwise to a solution of indole (0.82 g, 7.0 mmol) in dry THF (20 mL) at −78 °C. The resulting solution was kept at this temperature for

30 min followed by introduction of CO_2 (g) during 15 min. The solvent was evaporated under reduced pressure (during that time the temperature was allowed to rise to 20 °C). N_2 was introduced into the vessel, and the resulting crystalline residue was dissolved in dry THF (20 mL), and cooled to −78 °C. *t*-BuLi (1.7 M in heptane; 4.4 mL, 7.7 mmol) was then added dropwise (over 15 min) at −78 °C. The mixture was stirred for 30 min at −78 °C followed by addition of a solution of 3,3′-dithiobisindole **11**[14] (3.5 g, 7.0 mmol) in THF (25 mL) during 15 min. The mixture was allowed to warm to 20 °C over 17 h. Acetic acid (1 mL) was added and stirring was continued for 15 min, followed by addition of satd aq NH_4Cl (30 mL). The mixture was extracted with Et_2O (2× 25 mL), and the combined organic layers were washed with water (2× 25 mL), brine (30 mL) and dried (Na_2SO_4). Evaporation of the solvents gave a yellow oil which was subjected to column chromatography [*n*-heptane/ether (9:1)] to afford compound **12** (2.06 g, 81%) as an off-white solid, mp 56–58.5 °C; IR (neat) 1733, 1445, 1368, 1354, 1336, 1311, 1247, 1217, 1147, 1060, 742 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.07 (d, J = 8.27 Hz, 1H), 7.99 (s, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.39–7.23 (m, 3H), 7.08–6.93 (m, 2H), 6.61 (dd, J = 2.0, 0.6 Hz, 1H), 1.64 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 148.5, 137.3, 134.8, 130.0, 129.7, 127.8, 127.3, 125.2, 123.3, 121.8, 119.6, 119.38, 119.35, 115.1, 111.0, 109.7, 106.1, 84.6, 27.6; HRMS (FAB) *m*/*z* 364.1241 [M^+], $C_{21}H_{20}N_2O_2S$ requires 364.1245.

3.1.8. 2,3′-Diindolylsulfide (10)

A mixture of compound **12** (1.7 g, 4.7 mmol), ethanol (80 mL) and 1 M KOH (50 mL) was heated at 90 °C during 30 min. The reaction mixture was cooled, and extracted with CH_2Cl_2 (2× 40 mL). The combined organic phases were washed with water (3× 50 mL), brine (40 mL) and dried (Na_2SO_4). Evaporation of the solvents gave a brownish solid, which was recrystallized from $CHCl_3$/*n*-hexane to give **10** (1.1 g, 83%) as off-white crystals with spectroscopic and physical data identical to those reported previously.[10a]

3.1.9. Compound 13

This product was prepared following the procedure for compound **4**, using 2,3′-diindolylsulfide (**10**) (0.13 g, 0.50 mmol) triethyl orthoformate (0.10 g, 0.7 mmol). Reaction time: 48 h. The crude product was purified by gradient column chromatography [CH_2Cl_2/MeOH (49:1 → 19:1)] to give the thiopyran **13** (90 mg, 66%) as a dark red solid, mp >300 °C; IR (neat) 1589, 1383, 1330, 1300, 1235, 1210, 1176, 1127, 1097, 899, 864, 731 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.14 (s, 1H), 8.87 (s, 1H), 8.29–8.27 (m, 1H), 7.98–7.95 (m, 1H), 7.72–7.63 (m, 2H), 7.55–7.47 (m, 2H), 7.33–7.27 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 158.2, 153.9, 138.6, 128.9, 128.8, 127.8, 126.3, 125.3, 123.1, 121.1, 120.6, 120.5, 120.0, 118.6, 117.4, 112.4, 111.7; HRMS (FAB) *m*/*z* 274.0563 [M^+], $C_{17}H_{10}N_2S$ requires 274.0565.

3.1.10. Synthesis of bis(1*H*-indol-2-yl)selenides 14 and 15

The selenides **14** and **15** were prepared in a similar manner to that for compound **6** from appropriate indoles, using bis(phenylsulfonyl) selenide [$(PhSO_2)Se$][17] as the electrophile. The reactions were performed on 15 mmol scale for compound **14** and 5 mmol scale for compound **15**.

3.1.11. Bis(1*H*-indol-2-yl)selenide (14)

The crude product was subjected to column chromatography using *n*-heptane/EtOAc (3:1 → 1:1), to give compound **14** (1.65 g, 71%) as light yellow solid, mp 142–144.5 °C; IR (neat) 2988, 2901, 1433, 1394, 1338, 1312, 1273, 1228, 1077, 1042, 804, 751, 781, 732 cm^{-1}; ^1H NMR (400.1 MHz, DMSO-d_6) δ 11.51 (s, 2H), 7.50–7.46 (m, 2H), 7.34–7.31 (m, 2H), 7.12–6.94 (m, 4H), 6.63–6.62 (m, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 138.0, 128.1, 121.8, 120.9, 119.6, 119.3, 111.1, 108.4; HMRS (FAB) *m*/*z* 312.0160 [M^+], $C_{16}H_{12}N_2Se$ requires 312.0166.

3.1.12. Bis(5-methoxy-1*H*-indol-2-yl)selenide (15)

The crude product was subjected to column chromatography using *n*-heptane/EtOAc (3:1 → 1:1), to yield compound **15** (640 mg, 69%) as an off-white solid, mp 127.5–130.5 °C; IR (neat) 3369, 2972, 2901, 1448, 1430, 1401, 1335, 1210, 1157, 1147, 1018, 847, 831, 811, 793, 776, 749 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.33 (s, 2H), 7.22–7.19 (m, 2H), 6.98 (d, J = 2.5 Hz, 2H), 6.72 (dd, J = 8.8, 2.5 Hz, 2H), 6.54–6.53 (m, 2H), 3.71 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 153.4, 133.1, 128.4, 121.2, 112.1, 111.8, 108.2, 101.0, 55.2; HMRS (FAB) *m*/*z* 372.0371 [M^+], $C_{18}H_{16}N_2O_2Se$ requires 372.0377.

3.1.13. General procedure for synthesis of the selenopyrans 16–19

$MeSO_3H$ (0.05 mL) was added to a solution of triethyl orthoacetate (0.11 g, 0.7 mmol) or triethyl orthoformate (0.10 g, 0.7 mmol) and the selenides **14** or **15** (0.50 mmol) in dry 1,4-dioxane (CH_3CN for compound **17**) (8 mL) at rt under N_2. The reaction mixture was stirred for 3 days at rt. The solvent was evaporated, and the resulting solid was subjected to column chromatography, eluting with CH_2Cl_2/MeOH (99:1 → 95:5), yielding the selenopyrans **16–19**.

3.1.14. 5*H*-Selenopyrano[2,3-*b*:6,5-*b*′]diindole (16)

Orange solid (70 mg, 44%), mp 291 °C; IR (neat) 1565, 1444, 1384, 1351, 1302, 1281, 1239, 1177, 1118, 1102, 736 cm^{-1}; ^1H NMR (400.1 MHz, DMSO-d_6) δ 9.66 (s, 1H), 8.31–8.29 (m, 2H), 7.73–7.71 (m, 2H), 7.47–7.37 (m, 4H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 144.0, 131.0, 125.9, 125.5, 121.7, 119.4, 116.5, 114.2; HMRS (FAB) *m*/*z* 323.0069 [M+H]$^+$, $C_{17}H_{10}N_2Se$ + H requires 323.0087.

3.1.15. Compound 17

Orange solid (100 mg, 60%); mp >300 °C; IR (neat) 1389, 1347, 1309, 1238, 1201, 1179, 1166, 1154, 1105, 816, 736 cm^{-1}; ^1H NMR (400.1 MHz, DMSO-d_6) δ 8.30–8.28 (m, 2H), 7.69–7.67 (m, 2H), 7.43–7.32 (m, 4H), 3.41 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 149.6, 145.1, 126.1, 124.6, 122.3, 121.6, 119.1, 116.6, 114.6, 20.4; HMRS (FAB) *m*/*z* 337.0243 [M+H]$^+$, $C_{18}H_{12}N_2Se$ + H requires 337.0244.

3.1.16. Compound 18

Red solid (90 mg, 47%); mp >300 °C; IR (neat) 1594, 1566, 1474, 1436, 1384, 1347, 1305, 1240, 1178, 1117, 1103, 1025, 801, 724 cm^{-1}; ^1H NMR (400.1 MHz, DMSO-d_6) δ 9.57 (s, 1H), 7.83 (s, 2H), 7.60–7.58 (m, 2H), 7.04–7.02 (m, 2H), 3.91 (s, 6H); (100.6 MHz, DMSO-d_6) δ 155.2, 146.6, 138.8, 130.3, 126.9, 115.1, 115.0, 114.0, 102.3, 55.6; HMRS (FAB) *m*/*z* 383.0282 [M+H]$^+$, $C_{19}H_{14}N_2O_2Se$ + H requires 383.0299.

3.1.17. Compound 19

Red solid (90 mg, 45%); mp 268 °C; IR (neat) 1465, 1432, 1394, 1342, 1213, 1192, 1157, 1136, 1039, 785, 723 cm^{-1}; ^1H NMR (400.1 MHz, DMSO-d_6) δ 7.70 (d, J = 1.5 Hz, 2H), 7.54 (d, J = 6.5 Hz, 2H), 7.02 (dd, J = 6.5, 1.5 Hz, 2H), 3.89 (s, 3H), 3.34 (s, 6H); HMRS (FAB) *m*/*z* 397.0449 [M+H]$^+$, $C_{20}H_{16}N_2O_2Se$ + H requires 397.0455.

3.2. Biological evaluation

The AhR activating capacities of compounds **5**, **7**, **8**,**9, 13, 16, 17, 18, 19, 20** and **21** were compared to that of TCDD (**1**), ICZ (**3**) and FICZ (**2**) using the ELISA based Ah-IMMUNOASSAY®, (Biosense Laboratories, Norway), according to the manufacturer's protocol (Fig. 3). The assay utilizes guinea pig cytosolic fractions to which

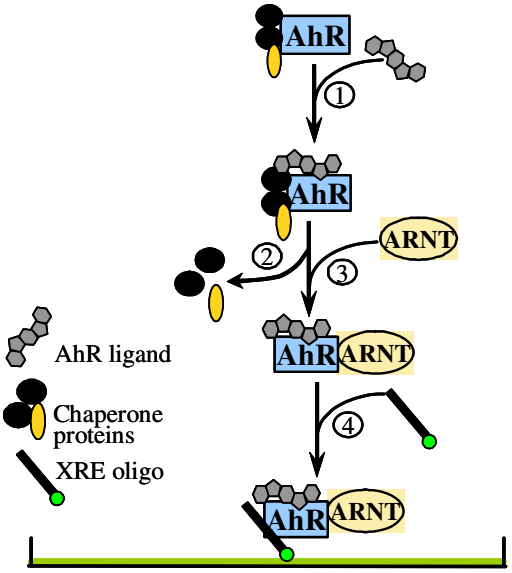


Figure 3. Schematic outline of AhR activation using the Ah-IMMUNOASSAY®. In the absence of a ligand the AhR resides inactive in the cytosol in complex with chaperone proteins. Upon ligand binding (1) the chaperone proteins are released (2) and the AhR heterodimerizes with ARNT (3). The heterodimer recognizes the XRE oligo and binds to it. Subsequently, the ligand:AhR:ARNT:XRE oligo complex is bound and fixed into the matrix of the ELISA plate (4). Antibodies are added in two steps and detection of bound complexes is in turn detected colorimetrically at 405 nm. The amount of complexes formed is proportional to the color intensity.

Ah receptor nuclear translocator (ARNT) extracts, xenobiotic responsive element (XRE) oligos, and test compound is added. The ligand:AhR:ARNT:XRE complexes formed are subsequently immobilized into the matrix of the ELISA plate. The amount of complexes formed is quantified and translated into the activating capacity of the compound added.

The compounds were dissolved in DMSO and the same range of concentrations was used (10–3500 pM). Each substance was analyzed at least four times as a series of dilutions. OD405 was plotted against the different concentrations giving a dose–response curve for each compound. The activating capacity of the respective compound was determined by comparing the linear regression of these curves to that of TCDD.

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