Estrogenic potency of 42 polycyclic aromatic compounds and their hydroxylated, oxygenated and alkylated derivatives in human breast cancer cells.

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Introduction

The substance group of polycyclic aromatic compounds (PACs) include compounds such as polycyclic aromatic hydrocarbons (PAHs), oxygenated, hydroxylated, alkylated PAHs and heterocyclic aromatic compounds. Several PACs have been studied for their mutagenicity, carcinogenicity, AhR activating potential and estrogenic activity. However the number of compounds in this group is enormous and there is still a lack of information of the estrogenic potential of PACs. PAHs are known to bind to the AhR, resulting in the induction of the CYP1A1 and increased biotransformation of the inducing compounds. In the present study the VM7Luc4E2 cell line was used to screen 42 PACs for their estrogenic activity. In addition, luciferase activity and EROD activity of each estrogenic active compound were assessed in the absence or presence of the AhR antagonist/CYP1A1-inhibitor α-naphthoflavone (α-NF), to assess if the estrogenic activity was elicited by the compound itself and/or by its metabolites.

Methods

- 42 PACs were tested for their estrogenic potency in the VM7Luc4E2 Transactivation assay (TA) (Fig.1)
- Relative potency factors (REPs) were calculated for all estrogenic active compounds
- Luciferase activity and EROD activity of each estrogenic active compound were measured in the absence or presence of the α-NF (2μM) and ICI182,780 (100 nM)
- Luciferase activity was normalized against E2-maximum induction (EMI) and results of EROD activity presented as the fold-induction (FI) of EROD above that of DMSO
- Significant differences were determined using ANOVA followed by Dunnett's test (*=p<0.05, **=p<0.01 ***=p<0.001).
- Experiments were carried out three times independently

REP Calculation: $REP_i = \frac{Standard\ EC_x}{Substance\ EC_x}$ EC= Effect concentration REP=Relative potency

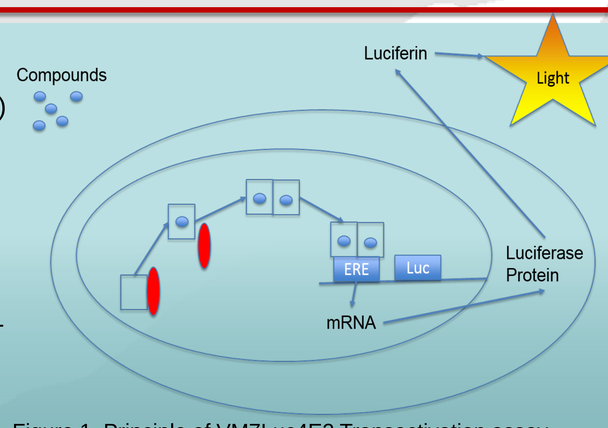


Figure 1. Principle of VM7Luc4E2 Transactivation assay

Results

Substance	REP25	REP50	REP20-80
Benzo[a]anthracene	1.2E-06	1.5E-06	1.2E-06 - 2.9E-06
Benzo[a]anthracene-7,12-dione	2.8E-06	5.2E-06	2.1E-06 – 7.9E-06
7,12-dimethylbenzo[a]anthracene	2.3E-06	3.5E-06	2.0E-06 – 1.1E-05
1,2,6-trimethylphenanthrene	4.9E-05	2.8E-05	5.4E-05 - nq
1,2,8-trimethylphenanthrene	2.5E-06	2.4E-06	2.7E-06 - nq
Benzo[a]apyrene	2.7E-06	2.3E-06	2.7E-06 - nq
Chrysene	2.2E-06	nq	3.4E-06 -nq
1-methylchrysene	8.3E-05	nq	1.1E-04 -nq
2-hydroxychrysene	2.5E-05	2.2E-05	1.7E-05 - 5.4E-05
2-methoxychrysene	9.2E-06	1.2E-05	8.7E-06 - 1.6E-05
2-methylchrysene	7.6E-06	nq	6.6E-06 - nq
3-methylchrysene	6.3E-05	2.5E-05	7.1.E-05 - nq
2-methylanthracene	nq	nq	1.4E-06 - nq
2,3-dimethyl-9,10-anthraquinone	2.3E-05	nq	2.2E-05 - nq
2-hydroxy-9,10-anthraquinone	4.6E-06	nq	1.2E-05 - nq
Acridine	1.1E-05	7.4E-06	2.0E-05 - 9.9E-06
9(10H)-acridone	5.0E-04	3.6E-04	6.0E-04 - 4.1E-04
9-methylacridine	4.4E-04	3.3E-04	4.9E-04 - 3.5E-04

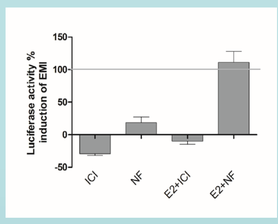
- REPs were determined for 18 tested PACs that were estrogenic active in the VM7luc4E2 TA.
- 9-Methylacridine and 9(10H)-acridone obtained highest REPs
- Anthracene and phenanthrene had no quantifiable estrogenic potencies, while derivatives had similar REPs to other compounds tested in this study
- Derivatives of Benzo[a]anthracene had slightly higher potencies than parent compound
- Estrogenic active derivatives of chrysene were 2-30 times more potent than the parent compound

Phenanthrene, 2-methylphenanthrene, 2,4-dimethylphenanthrene, 7-methylbenzo[a]pyrene, 1,4-chrysenequinone, 6-ethylchrysene, anthracene, 2-methyl-9,10-anthraquinone, 9,10-anthraquinone, fluorene, 9-fluorenone, dibenzothiophene, 2-methyldibenzothiophene, 2,8-dibenzothiophene, 1-indanone, 7h-benz[de]anthracen-7-one, benzo[h]quinoline, carbazole, dibenz[a,h]acridine, naphthacene, naphtho[2,3-a]pyrene, quinoline, fluoranthene and perylene had **no quantifiable estrogenic activity**

Luciferase activity

In absence or presence of the AhR antagonist/CYP1A1-inhibitor α-naphthoflavone and ER-antagonist ICI182,780

Figure 2. Luciferase activity of ICI and α-NF on basal and E2-induced luciferase activity. The dashed line indicates 100% activity induced by the reference compound E2.



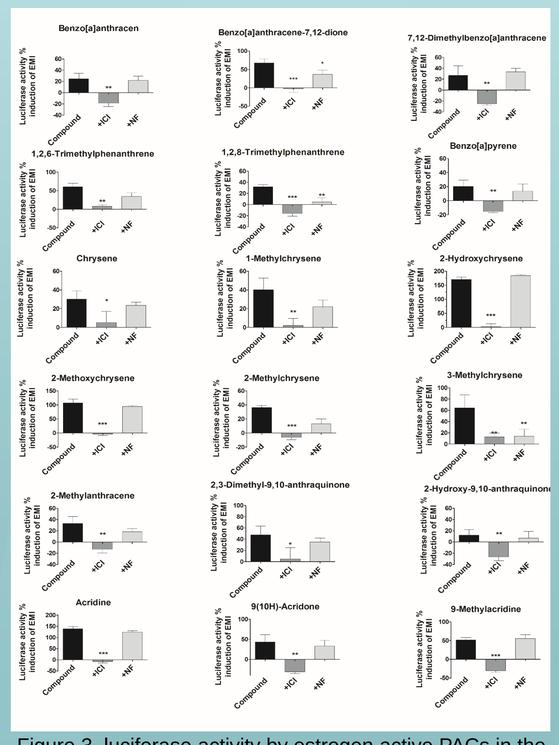
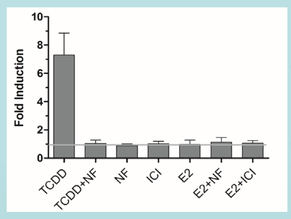


Figure 3. luciferase activity by estrogen active PACs in the absence or presence of ICI and α-NF.

- Luciferase activity of the ICI and the mixture of E2 and ICI was below the DMSO-control (-30% and -10% of EMI) (Fig. 2).
- α-NF induced 19% of EMI.
- Luciferase activity of PACs decreased by addition of ICI (Fig. 3).
- Addition of α-NF decreased the induction of luciferase activity by benzo[a]anthracene-7,12-dione, 1,2,8-trimethylphenanthrene and 3-methylchrysene, 1,2,6-trimethylphenanthrene, 1-methylchrysene and 2-methylanthracene
- Luciferase activity induced by the remaining compounds was not significantly decreased (<15%) in the presence of α-NF.

EROD activity

Figure 4. EROD activity of TCDD or E2 in the absence or presence of ICI and α-NF. The dashed line indicates EROD activity obtained with DMSO.



- ICI alone had no significant effect on basal EROD activity (FI: 1.02)
- α-NF decreased basal EROD activity slightly below DMSO-level with a FI of 0.88 (Figure 4).
- EROD activity of resulted was 7.29-fold induction of EROD above that with DMSO.
- Induction of EROD by TCDD was reduced to basal (DMSO) levels (FI: 1.04) by the addition of α-NF.

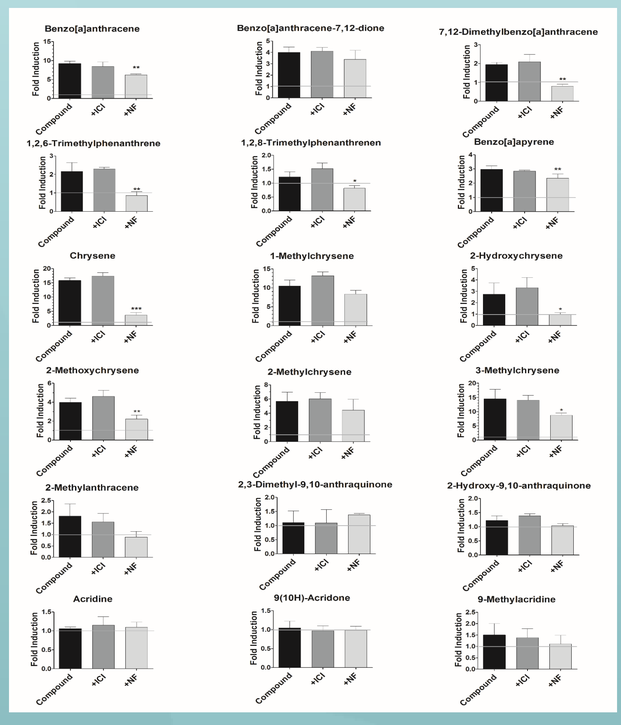


Figure 5. EROD activity of PACs in combination with α-NF or ICI. The dashed line indicates the relative activity of the DMSO-control.

- ICI did not have a significant effect on the EROD activity produced by these compounds
- Acridine, 9(10H-)acridone, 9-methylacridine, 2-hydroxy-9,10-anthraquinone, 2,3-dimethyl-9,10-anthraquinone, 2-methylanthracene, and 1,2,8-trimethylphenanthrene failed to induce EROD (FI ranged from 1.05 to 1.50) (Fig. 5).
- EROD activity was induced by all the other compounds, and their FI values ranged from 2.16 to 15.81.
- Addition of α-NF reduced EROD activity induced by benzo[a]anthracene, 1,2,6-trimethylphenanthrene, benzo[a]pyrene, chrysene, 2-hydroxychrysene, 2-methoxychrysene and 3-methylchrysene, benzo[a]anthracene-7,12-dione, 1-methylchrysene, 2-methylchrysene, 2-methylanthracene

Conclusions

Our data suggests:

- Derivatives have higher potency than parent compounds
- REP values ranged between 10^{-4} - 10^{-6}, similiar to known environmental xenoestrogens such as bisphenol a
- Acridine and its derivatives, along with benzo[a]anthracene, benzo[a]pyrene, chrysene, 2,3-dimethyl-9,10-anthraquionone, 2-hydroxy-9,10-anthraquinone, 2-methoxychrysene and 7,12-dimethylbenzo[a]anthracene are direct ER agonists.
- Additional studies are necessary to investigate possible ER activation through other pathways affected by these chemicals.
- The derivatives benzo[a]anthracene-7,12-dione, 1,2,6-trimethylphenanthrene, 1,2,8-trimethylphenanthrene, 1-methylchrysene, 2-methylchrysene, 3-methylchrysene and 2-methylanthracene seem to stimulate ER-dependent reporter gene expression indirectly (i.e. they appear to require metabolism to be able to activate ER signaling). Both 2-hydroxychrysene and possible formed metabolites might be able to activate the ER.

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