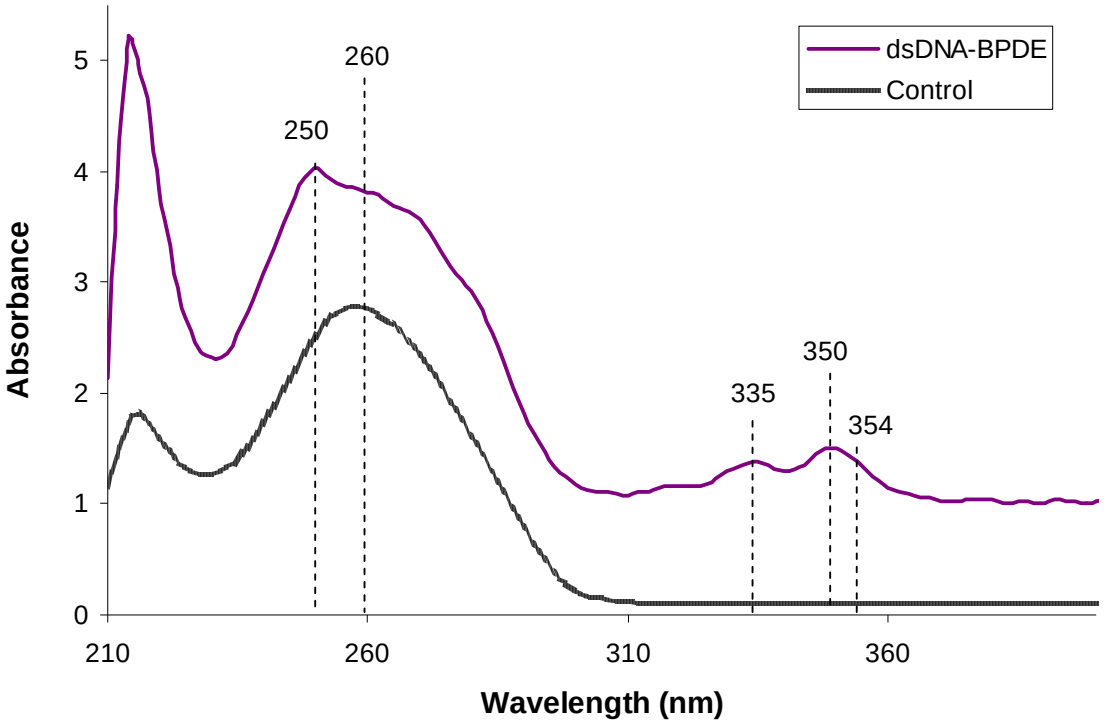
ELECTRONIC SUPPLEMENTARY INFORMATION

1. Confirmation of adducts of BPDE, SO and PGE with DNA

PGE, SO and BPDE previously reported to form covalent adducts by reacting with ss-DNA [1]. We observed the same results in this study. The difference is that we repeated in the presence of an internal standard in this study. Therefore, the results in this study will be able to compare to ds-DNA interaction results. Adducts of BPDE, covalently bonded with ds-DNA, were verified by the UV spectra of adduct peaks (**ESI Figure 1).** The spectra contain typical absorbent wavelengths of BPDE (250, 335 and 350 nm) and the characteristic absorption of DNA at 260 nm. For putative adducts of PGE and SO, although there is no characteristic spectra to support the formation of adducts in the zone of putative ds-DNA adducts, UV spectra of these peaks revealed a strong absorption at λ = 260 and 270 nm, (peaks at $t_R \sim 30$ min for ds-DNA reacted with SO and PGE, respectively). The red-shift from 260 to 270 nm in the UV spectra of the proposed PGE-DNA adduct further could support covalent modification of the DNA probe with PGE. Based on comparison to the DNA-negative control, these appearances were not due to residual chemical remaining after the extraction. This conclusion was supported by HPLC-MS analysis which confirmed the presence of PGE- and SO-adducts.

Data were obtained from the mass spectra of stable colvalent chemical adducts formed by reaction of BPDE, SO and PGE with ds-DNA as a paired series of multiply charged ions representing 5-HTT and RC-5-HTT. Adducts were deconvoluted with reference to unmodified ds-DNA in MS spectra. The double-stranded DNA adducts showed a profile consistent with adduct formation on both strands. For example, both strands (5-HTT and RC-5-HTT) were bonded to BPDE with a series of multiple charged ions as $[M-4]^{-4}$ (*m/z* 1511.0, 1546.7; 1586.6, 1622.2; 1662.0, 1697.4), $[M-5]^{-5}$ (*m/z* 1208.5, 1236.9; 1269.3, 1297.1; 1329.4, 1357.5), $[M-6]^{-6}$ (*m/z* 1007.1, 1030.7; 1057.4, 1081.2; 1107.7, 1131.2). The molecular mass of each strand was deconvoluted from the characteristic charge series of multiply charged ions produced by each strand and the total mass of the covalent adduct of BPDE and ds-DNA was the sum of two strands. Deconvolution of the first set of paired strands (*m/z* 1511.0, 1208.5, 1007.1; 1546.7, 1236.9, 1030.7) yielded molecular masses which correspond to the molecular masses of RC-5-HTT (6047 Da) and 5-HTT (6189 Da). The second series of pairs (*m/z* 1586.6, 1269.3, 1057.4; 1622.2, 1297.1, 1081.2) deconvoluted to masses of 6349 and 6491 Da which correspond to adduction of one BPDE molecule (molecular mass of 303 Da) while the third series of pairs (*m/z* 1662.0, 1329.4, 1107.7; 1697.4, 1357.5, 1131.2) deconvoluted to masses of 6651 and 6793 Da which corresponds to the adduction of two molecules of BPDE (molecular mass difference of 604 Da). Similarly, covalent ds-DNA adducts were observed for SO and PGE. For SO, single SO adducts were observed for SO-RC-5-HTT (6167 Da) and SO-5-HTT (6309 Da) by deconvolution of *m/z* 1540.8, 1233.4, 1027.6 and 1576.6, 1261.7, 1050.7. For PGE adducts single adducts were observed for PGE-RC-5-HTT (6197 Da) and PGE-5-HTT (6339 Da) by deconvolution of *m/z* 1548.1, 1239.5, 1031.5 and 1583.7, 1266.8, 1055.9. Adducts with multiple molecules of PGE were also observed. The covalent PGE adducts 2PGE-RC-5-HTT (6347 Da), 2PGE-5-HTT (6489 Da), 3PGE-RC-5-HTT (6497 Da) and 3PGE-5-HTT (6639 Da) were deconvoluted from *m/z* 1586, 1266.8, 1055.9; 1621.9, 1297.4, 1081.0 for 2PGE-adducts and *m/z* 1081.0, 926.4, 811.1; 1105.0, 947.9, 829.9 for 3PGE-adducts.

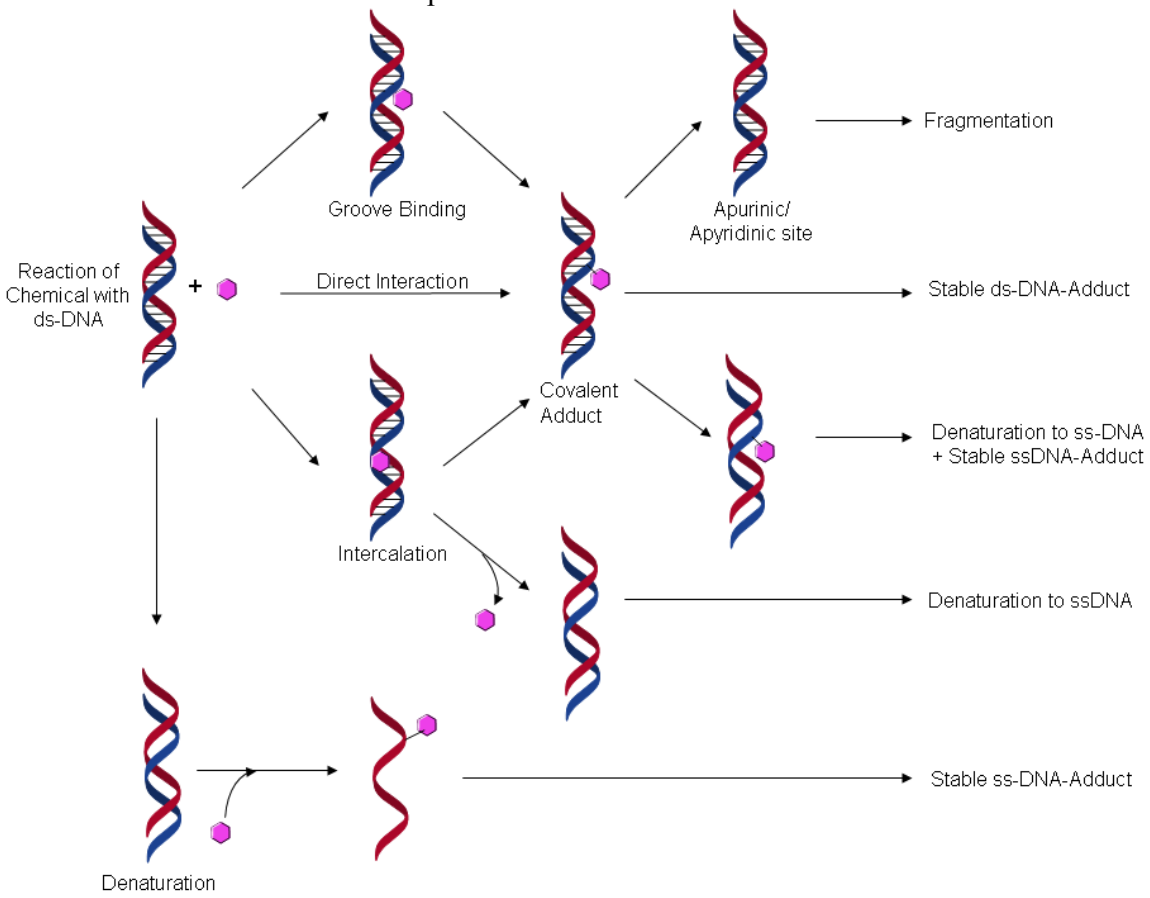


ESI Figure 1. UV absorption spectra of adducts formed during reaction of ds-DNA probe (5-HTT) with BPDE compared to control ds-DNA. Adduct spectra represents a summed average of peak at t_R = 30 min. Spectra are offset for clarity.

2 Possible routes of chemical-probe interaction

Given that our system was devoid of potentially complicating metabolic and repair systems, the major variable affecting both potency and reaction rate was the chemical nature of both the test chemicals and the DNA probes. Probe reduction includes adduct formation, DNA damage as well as association of the chemicals with DNA, and could occur by a variety of pathways for the ds-DNA probe, as illustrated in **ESI Figure 1**. Chemicals may interact directly with ds- DNA by forming covalent adducts, or may require ss-DNA prior to interaction. Furthermore, non-covalent interactions may also play a significant role in the observed rate of reaction and overall potency. Although non-covalent association may not directly contribute to reduction of the probe peak, pre-association of a chemical with the DNA target increases the likelihood of reaction and therefore increases the observed rate constant. This is supported by the observation that BPDE had greater potency and kinetic rate constants compared to the other chemicals studied and is known to intercalate into ds-DNA. Therefore, in our method, chemicals with higher affinity for DNA show greater interaction potencies compared to direct-acting chemicals that do not pre-association. Other non-covalent interactions may be capable of contributing directly to the reduction of the DNA probe should the interaction be great enough to alter the retention behaviour of the probe in the HPLC column, such as unwinding of the double helix, or if chemical interaction leads to denaturation of the probe. Finally, interaction may also

lead to downstream processes, such as denaturation or fragmentation, since reaction with some chemicals are known cause depurination to apurinic sites and frequency of strand breaks increases with the introduction of apurinic sites.



ESI Figure 2. Possible routes of chemical interaction with ds-DNA.

3 References

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