2,4-diphenylthiophene induces mainly base pair mutation in *Salmonella Typhimurium*

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ABSTRACT

Heterocyclic aromatic compounds containing sulfur (S-HET), have been detected in air, soil, marine environment and freshwater sediment. Toxicity and mutagenicity data of this class of substances are scarce. The present study focuses on implications of two aryl thiophenes and their mutagenic properties in *Salmonella/microsome* test. In our experiment only 2,4-diphenylthiophene showed little mutagenic effect in both variants of activation (±S9) in strain TA100. Thiophene ring joined to K-region of phenanthrene did not change the biological activity of 3,6-dimetoxyphenanthro [9,10-c]thiophene and this compound did not show mutagenic potency.

INTRODUCTION

Aromatic compounds are widely distributed pollutants in soil, air, sediments and water, as well as in biota (Brack and Schirmer 2003). The majority originate from anthropogenic sources. During the process of industrialization, semisolid tar oil pollutants became ubiquitous, and currently oils and sediments are major sources of polycyclic aromatic hydrocarbons (PAHs) (Peddinghaus et al. 2012). Creosote represents a complex mixture of over 10,000 single organic substances which are formed by thermal processes (Peddinghaus et al. 2012).

Heterocycles are also present in dyestuff, pesticides and pharmaceuticals (Broughton and Watson 2004; Cripps et al. 1990). Heterocyclic compounds are substituted PAHs, where one carbon atom of the aromatic ring is replaced by a nitrogen, sulfur or oxygen atom (NSO-HET). In tar oil, NSO-HET are present at lower concentration than their nonsubstituted analogues, but their increased water solubility leads to greater bioavailability and potential for toxic effects (Feldmannová et al. 2006). Because of their high mobility, heterocyclic compounds can leach into the water and contaminate both groundwater and drinking water (Zamfirescu and Grathwohl 2001).

The present study focuses on the possible implications of the mutagenicity of sulfur heterocyclic compounds. Except of carbon and hydrogen, sulfur is the most important element in crude oils. One of the main forms of organosulfur compounds is the thiophene moiety, where sulfur is incorporated into polycyclic aromatic hydrocarbons (PAHs) to form sulfur heterocycles (S-HET) (Li et al. 2012). For determining the mutagenic potential of heterocyclic compounds the *Salmonella/microsome* test was conducted. This is the preliminary study and a first step in risk assessment of the studied compounds. Thus, further investigations are needed to evaluate the toxicity of these compounds with a special focus on human health and bioaccumulation in aquatic animals.
MATERIAL AND METHODS

2,4-diphenylthiophene was synthesized from 3,4-diphenylthiophene via rearrangement in the presence of AlCl₃ in 80%. Synthesis of 3,4-diphenylthiophene has been described in the paper by Budzikur et al. (2011).

3,6-dimetoxyphenanthro[9,10-c]thiophene (Figure 1) was obtained in two-step procedure described by Shields et al. (1975). Synthesis involved Hinsberg condensation of 3,6-dimetoxyphenanthrenequinone with diethyl thiodiglycolate followed by decarboxylation from dicarboxylic acid by sublimation.

![Chemical structure of S-HET used in this study.](image)

All chemicals were especially synthesized anew for this experiment because they were not available commercially. Purity of samples was confirmed by NMR spectroscopy and was greater than 95%. Gas chromatography (GC-FID) analysis showed no impurities. Yield of isolated diarylthiophene was within the range of 81-95%. All chemicals were synthesized at the Faculty of Chemistry in Jagiellonian University in the research group of Chemistry of Carbocyclic Compounds.

Salmonella mutagenicity assays

Histidine dependent strains TA98 and TA100 of Salmonella Typhimurium have been purchased from TRINOVA Biochem GmbH Germany.

The Ames mutagenicity test was conducted with both Salmonella strains of (TA98 and TA100) with and without metabolic activation (S9 microsome fraction, S9-mix). The S9 microsome fraction is induced with Aroclor 1254 (Trinova Biochem GmbH Germany) and is able to stimulate eukaryotic processes that cannot be conducted by microorganisms themselves. The strains were cultured as described by Maron and Ames (1983) and Mortelmans and Zeiger (2000). Confirming genotypes of the tester strains were routinely carried out, including crystal violet, UV and ampicillin sensitivities. The number of spontaneous revertants and induced revertants following exposure to such diagnostic mutagens as NQNO (10µg·plate⁻¹) and B[a]P (10µg·plate⁻¹) were measured in each experiment and compared to control values. In these studies, the tested compound was dissolved in 80µl of DMSO and added to 2.5ml molten top agar (at 42°C) with 18h of nutrient broth culture of appropriate strain of S. Typhimurium and 0.5ml of S9 mix. The final mixture was poured on minimal glucose agar plates. Mutations from histidine-dependent to histidine-independent bacteria were assessed 48h after plating by counting the colonies of bacteria on the Petri dishes. The compounds were assayed in triplicate at each dose level.

Statistical analyses

The results are reported as mean numbers of revertant colonies per plate with the standard deviation for the test chemicals and the controls. Non-parametric method (Mann-Whitney) was applied for detection the differences between successive doses of the compound and the number of revertants in the corresponding negative control.

The dose-response relationship between the number of revertants of the test strain and dose of chemicals was also examined.

Mutagenic rate of studied compounds (MR) was calculated which is the ratio of the number of revertants netto in the given dose and the average number of revertants in the negative control. The compound is considered mutagenic when its value of MR equals or exceeds 2 (MR≥2).

RESULTS

Only 2,4-diphenylthiophene showed a little mutagenic effect in both variants of activation (±S9) in case of the strain TA100 (Figure 2). In a variant without metabolic activation (-S9) there was a significant increase of number of revertants for dose 0.1µg per plate, but mutagenic rate did not exceed a value of 2. For doses 50 and 200µg per plate decrease in the number of revertants was noticed. In the strain TA98 a little toxic effect of 2,4-diphenylthiophene in the variant without metabolic activation for doses 50 and 200µg per plate was observed.

3,6-dimetoxyphenanthro[9,10-c]thiophene did not show mutagenic properties in case of both strains. However in variant with metabolic activation in the strain TA98 statistically significant increase in the number of revertants in dose 10, 50 and 200mg was observed. In the strain TA100 small toxic effect was observed for highest doses of chemicals.

DISCUSSION

The present study contains preliminary evaluation of mutagenic activity of rarely studied sulfur heterocyclic compounds commonly found in tar oil-contaminated sites. At present most studies have focused on heterocyclic compounds with an incorporated nitrogen atom belonging to the class of azaarenes (Grant et al. 1992; Lübcke-von Varel et al. 2012). Even though sulfur heterocycles are the third most abundant element in crude oils and condensed thiophenes
are the most common form in which sulfur is present (Xu et al. 2009) there is limited information available about their biological effects (Eisentraeger et al. 2008).

Predominant route of biotransformation of S-HET is metabolic oxidation of the sulfur atom leading to sulfone, which is less mutagenic (Valadon et al. 1996). Our results may confirm this principle. 2,4-diphenylthiophene showed small mutagenic activity in variant without metabolic activation in strain TA100. Some sulfur heterocycles can be bioactivated to DNA adduct-forming species (Devanaboyina et al. 1993; King et al. 2001). Ashby et al. (1993) have demonstrated that 6,11-dimethylbenzo[b]naphtho[2,3-d]thiophene binds covalently to mouse skin DNA. The adducts formed by this compound may be more mutagenic than those of the mouse skin carcinogen 7,12-dimethylbenzo[a]anthracene compound without sulfur ring. Sivak et al. (1997) demonstrated that most biologically active fractions in bitumen fumes are those containing S-HETs.

Schreiner (2011) reports that the ability of chemicals to bind DNA and persist to form mutations depends on the conformation of adducts within the DNA nucleotide sequence and the effectiveness of excision and repair the lesion. Pure compounds often produced higher numbers of DNA adducts than the mixtures (Baird et al. 2005). For example benzo[a]pyrene produced highest numbers of DNA adducts in cultures of human diploid lung fibroblasts, when co-administered with other carcinogen of lesser potency in producing DNA adducts, resulted in significantly lower DNA adducts level (Binková and Šrám 2004).

It is possible that the location of phenyl in thiophene ring has an impact on mutagenic potency of a compound. 3,4-diphenylthiophene did not show any mutagenic potency in contrast to 2,4-diphenylthiophene (Budzikur et al. 2011). Dibenzothiophene is similar to diphenylthiophene and was characterized as non-competitive inhibitor of CYP1A activity in in vitro conditions (Wassenberg et al. 2005).

Sulfur analogs of benzo[c]phenanthrene were examined by Swartz et al. (2009). In this study phenanthro[3,4-b]thiophene was mutagenic in strain TA100. In our

Figure 2. Mean numbers of revertants induced by 2,4-diphenylthiophene and by 3,6-dimetoxyphenanthro[9,10-c]thiophene in Salmonella Typhimurium strains TA98 and TA100. Each mean is based on three measurements (plates); vertical lines show minimum and maximum numbers.
experiment 3,6-dimetoxyphenanthro[9,10-c]-thiophene did not show any mutagenic potency. Thiophene ring joined to K-region of phenenantherene did not change the biological activity of this compound (Ioanides 2007).

Since the review by Jacob (1990) on the biological activity of SPAH very little has been published on these compounds. Several of these compounds are mutagenic in Salmonella Typhimurium strains TA98 and TA100. Some SPAH have lower activity than their isosteric PAH, whereas others are very potent carcinogens. There is, however, no simple correlation between the carcinogenic potential of carboxylic systems and that of their isosteric SPAH.

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