# Mutagenicity induced in *Salmonella* strains TA98 and TA100 by diphenylthiophenes

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## ABSTRACT

Mutagenic properties of four different diphenylthiophenes: 3,4-diphenylthiophene, 3,4-di(4′-methylphenyl)thiophene, 3,4-di(4′-methoxyphenyl)thiophene and 3,4-di(4′-pentoxyphenyl)thiophene were investigated applying the *Salmonella* test. The research was done on two strains of *Salmonella* Typhimurium: TA98 and TA100, tested in two variants: with (+S9) and without (-S9) enzymatic activation. Only one compound 3,4-di(4′-methylphenyl)thiophene showed mutagenic activity when studied with metabolic activation (+S9) and its mutagenic rate (MR) score was 3.41 for the dose of 10.00$\mu g \cdot plate^{-1}$. Other studied compounds did not show any mutagenic activity (±S9) and their MR score did not exceed the threshold value of 2.0.

## INTRODUCTION

Sulfur heterocycles found in the environment often originate from fossil fuels (Bessler et al. 1997). Some secondary metabolites of microorganisms and plants also constitute another source of sulfur heterocycles, with thiophenes being the most common (Speight 1980).

Developments in organic electronics have caused growing interest in new molecular components for the construction of organic field-effect transistors (OFETs), organic light-emitting diodes (OLEDs) and photovoltaic cells (Perepichka and Perepichka 2009; Skotheim and Reynolds 2007). In this context, materials derived from linear organic π-conjugated monomers, e.g. diphenylthiopenes, are particularly appealing and commonly described (Helgesen and Krebs 2010; Henckens et al. 2005; Parvez et al. 2009). These materials, when suitably modified, enable low-cost fabrication processes of stable, large-area, and lightweight electronic devices (Odaci et al. 2008). Even though the thiophene monomers and polymers are now commonly applied in mobile phone and computer displays, their toxicity or mutagenicity remain unknown.

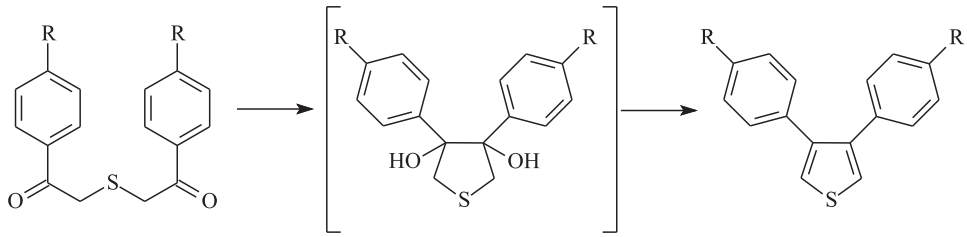
Numerous medicines contain thiophene rings in their structure. There are reports that thiophene compounds were activated to electrophilic intermediates by cytochrome P450 (Lin et al. 2011). The resulting sulfoxides can be then covalently modified by glutathione and other enzymes' compounds containing the thiol group. The mechanism for thiophene toxicity is not known, although it has been suggested that thiophene metabolism may lead to the formation of oxidative intermediates that could function as the ultimate toxicants (Dansette et al. 2005; Du et al. 2008).

## MATERIAL AND METHODS

### Synthesis of diphenylthiophenes

Diphenylthiophenes were synthesized from the corresponding sulfides by McMurry coupling followed by aromatization according to a modified Nakayama protocol (Nakayama et al. 1985). The general experimental procedure is given below (Scheme 1).

Scheme 1



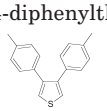
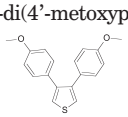
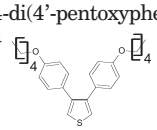

A solution of 10.0mMol of sulfide 1(a-d) in dry THF (40ml) was added to 60.0mMol of activated Zn powder and placed in a flask equipped with a thermometer and gas inlet. The mixture was cooled to -20°C with vigorous stirring and 30.0mMol of $TiCl_4$ was added drop-wise over 3h in an argon atmosphere. The mixture was allowed to warm to 10-15°C and stirred for another 3h. The reaction was then quenched by pouring on crushed ice (10ml), neutralized with a saturated solution of $K_2CO_3$ and extracted with 30cm$^3$ of $CH_2Cl_2$. The organic layer was evaporated and the remaining residue was dissolved in 20ml of benzene and placed in a flask equipped with a Dean-Stark trap. 5.0mMol of *p*-toluenesulfonic acid was added and the solution was refluxed for 4h. The chilled solution was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica gel/toluene). The yields of isolated diarylthiophenes 2(a-d) were within the range 81-95%.

The structures of the synthesized and characterized compounds are given in Table 1. All chemicals were specially synthesized for this experiment because they were not available commercially. The purity of samples was confirmed by NMR spectroscopy and was greater than 95%. Gas chromatography (GC-FID) analysis showed no impurities.

### *Salmonella* mutagenicity assays

Histidine-dependent strains of TA98 and TA100 of *Salmonella* Typhimurium were purchased from TRINOVA Biochem GmbH Germany and were cultured as described by Maron and Ames (1983) and Mortelmans and Zeiger (2000). Confirmation of genotypes of the tester strains were routinely carried out, including crystal violet, UV and ampicillin sensitivities. The number of

**Table 1. The structure and characteristics of the examined compounds.**

| Compound name and formula | Melting point, solvent | Spectral data | Yield |
|---|---|---|---|
| 3,4-diphenylthiophene **2a**  | 110°C (ethanol) (lit. m.p. 112-113°C)[1] | $^1$HNMR (300 MHz, $CDCl_3$): 7.16-7.26 (m, 10H), 7.30 (s, 2H) $^{13}$CNMR (75 MHz, $CDCl_3$): 124.2, 127.0, 128.3, 129.2, 136.7, 141.9 | 81% |
| 3,4-di(4'-methylphenyl)thiophene **2b**  | 69°C (ethanol) (lit. m.p. 70-72°C)[2] | $^1$HNMR (300 MHz, $CDCl_3$): 2.36 (s, 6H), 7.10-7.15 (m, 8H), 7.29 (s, 2H) $^{13}$CNMR (75 MHz, $CDCl_3$): 21.3, 123.7, 129.0, 133.9, 136.6, 141.8 | 85% |
| 3,4-di(4'-metoxyphenyl)thiophene **2c**  | 103-105°C (ethanol) (lit. m.p. 107-108°C)[3] | $^1$HNMR (300 MHz, $CDCl_3$): 3.78 (s, 6H), 6.78-6.80 (m, 4H), 7.10-7.13 (m, 4H), 7.21 (s, 2H) $^{13}$CNMR (75 MHz, $CDCl_3$): 55.3, 113.7, 123.2, 129.4, 130.2, 141.5, 158.7 | 84% |
| 3,4-di(4'-pentoxyphenyl)thiophene **2d**  | 46-48°C (petroleum ether) | $^1$HNMR (300 MHz, $CDCl_3$): 0.91-0.97 (6H, m), 1.35-1.47 (8H, m), 1.74-1.83 (4H, m), 3.94 (t, J=6.6Hz, 4H), 6.77-6.81 (m, 4H), 7.09-7.13 (m, 4H), 7.22 (s, 2H) $^{13}$CNMR (75 MHz, $CDCl_3$): 14.6, 23.1, 28.9, 29.6, 68.6, 114.8, 123.6, 129.7, 130.7, 142.0, 158.8 MS (m/z) 408,72 | 98% |

[1] Grunwell et al. (1977)
[2] Dang and Chen (2007)
[3] Chadwick et al. (1972)

spontaneous revertants and induced revertants following exposure to such diagnostic mutagens as B[*a*]P (10$\mu$g·plate$^{-1}$) and NQNO (10$\mu$g·plate$^{-1}$) were measured in each experiment and compared to control values. The assays were performed with and without an activation system. For activation, we used S9 mix, a "rat liver post-mitochondrial supernatant" (TRINOVA Biochem). For this preliminary study, five concentrations (0.10, 1.00, 10.00, 50.00, 200.00$\mu$g per plate) in triplicate of each compound were fixed. In these studies, the tested compound was dissolved in 80$\mu$l of DMSO and added to 2.5ml molten top agar (at 42°C) with a 18h nutrient broth culture of an 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 for 48h after plating by counting the colonies of bacteria on the Petri dishes. The compounds were assayed in triplicate at each dose level.

### Statistical methods

The results are reported as mean numbers of revertant colonies per plate ± the standard deviation for the test chemicals and the controls. The non-parametric method (Mann-Whitney) was applied for detecting the differences.

In order to compare the mutagenicity of selected compounds, their mutagenic rate (MR) were calculated. MR (as a relative measure of the mutagenic effect) is the ratio of the number of net revertants in a given dose and the average number of revertants in the negative control. A mutagenic effect occurs with a value of MR≥2.

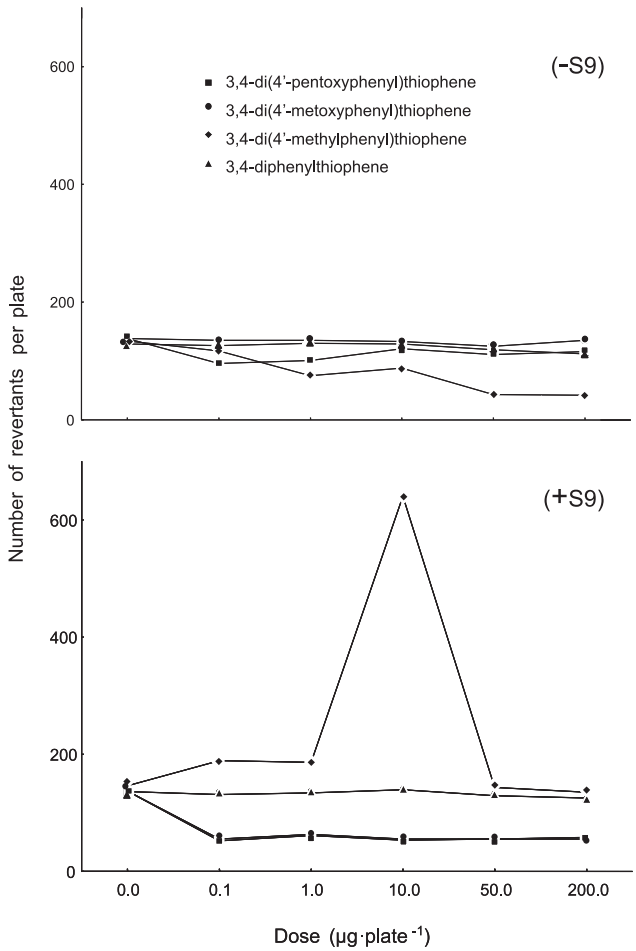



**Figure 1. Mean number of revertants induced by four different diphenylthiophenes in *Salmonella* Typhimurium strain TA100.**

## RESULTS

The compounds showed mutagenic activity only in strain TA100 with metabolic activation. 3,4-di(4'-methylphenyl)-thiophene 2b induced the highest number of TA100 revertants, for the dose of 10.00$\mu$g·plate$^{-1}$ and caused a high mutagenic rate (MR=3.41). For the dose 50$\mu$g·plate$^{-1}$, a significant decrease in the number of revertants was observed. For the next two examined compounds (3,4di(4'-metoxyphenyl) -thiophene 2c and 3,4-di (4'-pentoxyphenyl)thiophene 2d), a weak toxic effect for the lowest concentration was observed. A small toxic effect was observed in both variants (with and without metabolic activation) for 3,4-di(4'-pentoxyphenyl)-thiophene 2d. The combined data for all four compounds for the strain TA100 are shown in Figure 1.

Diphenylthiophens in both variants showed no mutagenic effect in strain TA98. The number of revertants in strain TA98 for each tested dose is presented in Table 2.

## DISCUSSION

In the structure of the examined diphenylthiophenes, different aromatic functionalities or substitutes are attached to the thiophene ring. Our study was intended to determine the relationship between simple modification of the diphenylthiophene structure and mutagenicity or toxicity of the compound. The metabolism of a symmetrically-substituted thiophene molecule may include oxidation of sulfoxides. In contrast to benzothiophene S-oxides, thiophene S-oxides are a very reactive species. Although few of them have been described to date (Dansette et al. 2005), recent results show that oxidation of the thiophene ring leads to adducts. These adducts are obtained in a Michael-type addition of a nucleophilic reagent containing a thiol group to a thiophene-oxide intermediate (Dreiem and Fonnum 2004).

Among the four examined diphenylthiophenes, only 3,4-di(4'-methylphenyl)thiophene induced mutations in the

**Table 1. Mean number of revertants for four tested compounds without (-S9) and with (+S9) activation in *Salmonella* Typhimurium strain TA98.**

| Chemical | Dose ($\mu g \cdot plate^{-1}$) | Mean number of revertants | |
|---|---|---|---|
| | | Strain TA98-S9 | Strain TA98+S9 |
| 3,4-diphenylthiophene **2a** | 0.1 | 32.6 | 34.6 |
| | 1.0 | 35.6 | 38.3 |
| | 10.0 | 39.3 | 35.6 |
| | 50.0 | 32.6 | 33.3 |
| | 200.0 | 29.6 | 29.6 |
| 3,4-di(4'-methylphenyl)thiophene **2b** | 0.1 | 31.3 | 35.6 |
| | 1.0 | 34.6 | 36.0 |
| | 10.0 | 32.0 | 31.0 |
| | 50.0 | 34.3 | 36.0 |
| | 200.0 | 33.0 | 33.0 |
| 3,4-di(4'-metoxyphenyl)thiophene **2c** | 0.1 | 28.6 | 38.6 |
| | 1.0 | 30.0 | 35.6 |
| | 10.0 | 31.6 | 38.6 |
| | 50.0 | 29.6 | 36.6 |
| | 200.0 | 32.6 | 31.6 |
| 3,4-di(4'-pentoxyphenyl)thiophene **2d** | 0.1 | 36.6 | 33.6 |
| | 1.0 | 32.0 | 32.6 |
| | 10.0 | 34.6 | 31.3 |
| | 50.0 | 32.3 | 31.0 |
| | 200.0 | 31.6 | 31.6 |

*Salmonella* Typhimurium strain TA100. It is possible that this compound may be oxidized by cytochrome P450 into a more mutagenic species. Du et al. (2008) have suggested that sulfur compounds could be susceptible to form nucleophilic attack and form DNA adducts. The strain TA100 is sensitive to base-pair mutations which are provoked by small DNA adducts (Benigni and Bossa 2008).

3,4-di(4'-metoxyphenyl)thiophene and 3,4-di(4'-pentoxyphenyl)thiophene were toxic for the strain TA100 in a variant with S9 activation. It is probable that greater functional groups with different conformations could inhibit the formation of DNA adducts (Boctor et al. 1995). It is also possible that derivatives of these two compounds did not match to the binding side of the enzyme. The active site of cytochrome P450 is non-specific but it is only suitable for uniplanar molecules (Oritz de Montellano 2005).

We have not observed any mutagenic rates for *Salmonella* Typhimurium TA98 because the tested compounds and their derivatives were too small to provoke frameshift mutations (Benigni and Bossa 2008).

According to the results of our study, we conclude that derivatives of diphenylthiophenes cause only base-pair-exchange types of mutation and not the frame-shift mutation.

It is difficult to define the metabolic pathways for thiophene derivatives. It is possible that there are different paths correlated with interaction connections (Dansette et al. 2005), molecular shape (Dreiem and Fonnum 2004), branching, symmetry (Du et al. 2008), distribution of charge or quantum-chemical properties of the compound.

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