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Dioxygenase-catalysed oxidation of monosubstituted thiophenes: sulfoxidation *versus* dihydrodiol formation

Derek R. Boyd,*[a] Narain D. Sharma,[a] Nimal Gunaratne,[a] Simon A. Haughey,[a] Martina A. Kennedy,[a] John F. Malone,[a] Christopher C. R. Allen[b] and Howard Dalton[c]

[a] *School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*

[b] *QUESTOR Centre, The Queen's University of Belfast, Belfast, UK BT9 5AG*

[c] *Department of Biological Sciences, University of Warwick, Coventry, UK CV4 7AL*

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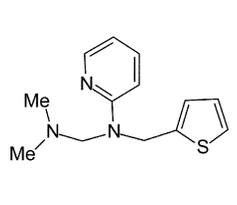
Toluene dioxygenase (TDO)-catalysed sulfoxidation, using *Pseudomonas putida* UV4, was observed for the thiophene substrates **1A**–**1N**. The unstable thiophene oxide metabolites, **6A**–**6G**, **6K**–**6N**, spontaneously dimerised yielding the corresponding racemic disulfoxide cycloadducts **7A**–**7G**, **7K**–**7N**. Dimeric or crossed [4 + 2] cycloaddition products, derived from the thiophene oxide intermediates **6A** and **6D** or **6B** and **6D**, were found when mixtures of thiophene substrates **1A** and **1D** or **1B** and **1D** were biotransformed. The thiophene sulfoxide metabolite **6B** was also trapped as cycloadducts **17** or **18** using stable dienophiles. Preferential dioxygenase-catalysed oxidation of the substituent on the thiophene ring, including exocyclic sulfoxidation (**1H**–**1J**) and *cis*-dihydroxylation of a phenyl substituent (**1G** and **1N**), was also observed.

An enzyme-catalysed deoxygenation of a sulfoxide in *P. putida* UV4 was noticed when racemic disulfoxide cycloadducts **7A**, **7B** and **7K** were converted to the corresponding enantioenriched monosulfoxides **8A**, **8B** and **8K** *via* a kinetic resolution process. The parent thiophene **1A** and the 3-substituted thiophenes **1K**–**1N** were also found to undergo ring dihydroxylation yielding the *cis*/*trans*-dihydrodiol metabolites **9A** and **9K**–**9N**. Evidence is provided for a dehydrogenase-catalysed desaturation of a heterocyclic dihydrodiol (**9K**$_{cis}$/**9K**$_{trans}$) to yield the corresponding 2,3-dihydroxythiophene (**24**) as its preferred thiolactone tautomer (**23**). A simple model to allow prediction of the structure of metabolites, formed from TDO-catalysed bacterial oxidation of thiophene substrates **1**, is presented.

Introduction

The monocyclic thiophene ring system is ubiquitous in nature and many examples can be easily found in foods and plants.[1] Thiophenes are liberated into the environment during cooking processes and the combustion of fossil fuels.[2] Examples of naturally occurring 2-substituted thiophenes include 2-methylthiophene **1B**, 2-ethylthiophene **1C** and 2-propylthiophene (from yeast extract).[3] Animal (eucaryotic) metabolic pathways, adopted for the monosubstituted thienyl moiety (Th), are exemplified by the drugs methapyrilene **2**, tenidap **3**, tienilic acid **4** and temocillin **5**.[4] The monooxygenase (cytochrome P-450) isozymes, present in animal liver systems, are considered to be mainly responsible for the different oxidation pathways of thiophene drugs **2** (methylene hydroxylation),[5] **3** (thiophene ring hydroxylation),[6] and **4** (sulfoxidation).[7] The transient thiophene oxide derivative of compound **4** was trapped as a Michael adduct using mercaptoethanol as nucleophile.[7]



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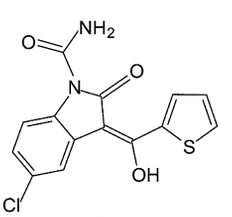

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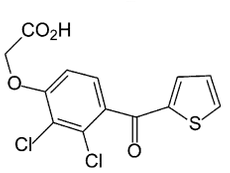

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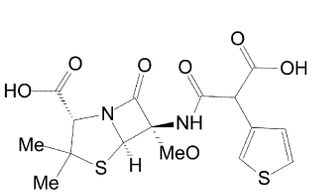

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The bacterial (procaryotic) *cis*-dihydroxylation of monosubstituted benzene ring systems is catalysed by ring hydroxylating dioxygenases, *e.g*. TDO present in *P. putida* UV4; to date more than fifty examples have been reported.[8,9] Since monosubstituted benzene and thiophene ring systems are both planar and aromatic, with large resonance energies (*ca.* 36 and 29 kcal mol^{-1} respectively), and show similar chemical reactivity (*e.g.* electrophilic substitution), it was anticipated that the ring systems would utilize similar procaryotic metabolic pathways. Studies from these and other laboratories have shown that the bacterial TDO enzyme is also able to catalyse monohydroxylation (benzylic and allylic hydroxylation)[10] and sulfoxidation of substituted arenes.[11–13] In view of the ability of dioxygenase enzymes to catalyse both mono- and poly-oxygenation reactions,[8,10] use of the term *Rieske-type non-heme iron oxygenases* has recently been recommended.[14] The term *dioxygenase* has been used in this report as an abbreviation of the latter definition. Preliminary studies on the dioxygenase-catalysed oxidation of mono- and bi-cyclic thiophenes, using *P. putida* UV 4,[12,13] have indicated that the metabolic profile is dependent upon the nature and position of substituents. The results of biotransformations using the parent thiophene **1A**, and its 2- and 3-substituted derivatives **1B**–**1N** as substrates, with *P. putida* UV4 are presented herein.

Sulfoxidation of organic sulfides is a relatively fast chemical oxidation reaction. Although found using enzyme-catalysed[7,11,15] and chemical methods, sulfoxidation of a thiophene ring is generally slower, since one of the lone pairs on the sulfur atom is required to make up the aromatic sextet and formation of a thiophene oxide would involve a considerable loss of resonance energy.[16,17] The sulfoxide derivative **6A** of thiophene **1A** (Scheme 1) was thus found to be formed relatively slowly *via* peroxyacid oxidation, but once formed, was then rapidly oxidized further to the corresponding sulfone. Both the thiophene sulfoxide and sulfone oxidation products were

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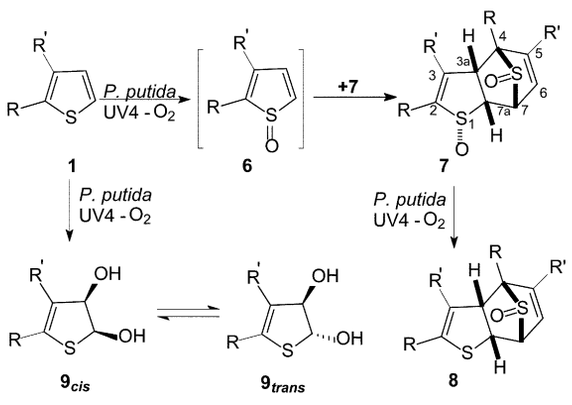


Scheme 1

found to form cycloadducts (disulfoxides and sesquioxides) spontaneously.[18,19] The use of peroxyacids, *e.g*. *meta*-chloroperoxybenzoic acid (MCPBA) in the presence of boron trifluoride, has been shown to slow down further oxidation of the initial thiophene oxide to the corresponding sulfone and thus results in the formation of a disulfoxide cycloadduct.[20–22] The first example of an isolable thiophene oxide (2,5-di-*tert*-butylthiophene oxide) was observed because the bulky *tert*-butyl groups at the 2- and 5-positions were able to prevent spontaneous cycloaddition.[23] The bulky *tert*-octyl groups at these positions, similarly, allowed 2,5-di-*tert*-octylthiophene oxide to be isolated and the barrier to pyramidal sulfoxide inversion to be determined ($\Delta G^{\neq}$ 14.8 kcal mol^{-1}) by low temperature ^{1}H-NMR analysis.[23] This, and later studies,[20,21] of the more stable thiophene sulfoxides, have indicated that a single enantiomer of a substituted monocyclic thiophene cannot exist at ambient temperature without undergoing spontaneous racemization. The pyramidal shape of the thiophene sulfoxide centre was shown by X-ray crystallographic studies of 2,5-diphenylthiophene 1-oxide[20] and 2-methylbenzo[*b*]thiophene 1-oxide.[13]

Results and discussion

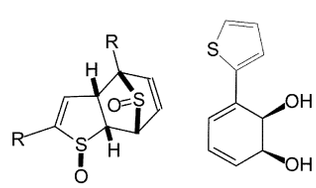
(a) TDO-catalysed monooxygenation of 2-substituted thiophenes 1A–1G to yield unstable thiophene oxides 6A–6G and their stable disulfoxide derivatives 7A–7G and 10G

Addition of thiophene **1A**, as a substrate to growing cultures of *P. putida* UV4, containing TDO, yielded the disulfoxide cycloadduct **7A**. This major bioproduct (45% yield) was separated by chromatography and characterised by two strong sulfoxide absorption bands in the IR spectrum (*ca*. 1031 and 1043 cm^{-1}) and by NMR spectral analysis. The structure of disulfoxide **7A** had earlier been established, unequivocally, by X-ray crystallography.[24] A monosulfoxide **8A** (IR absorption band at 1066 cm^{-1}) and a dihydrodiol **9A** were also present as minor bioproducts. While the disulfoxide epimer **10A** was also obtained from monooxygenase-catalysed oxidation of thiophene **1A** (rat liver microsomes)[24] and chemical oxidation (H_2O_2–CF_3CO_2H),[24] it was not isolated from *P. putida* UV4 biotransformation. Disulfoxide **7A**, with five stereogenic centres, was found to be racemic based upon ^{1}H-NMR analysis in the presence of the chiral solvating agent (+)-1-(9-anthryl)-2,2,2-trifluoroethanol (Pirkle solvent).

The possibility of bioproduct **7A** being formed from the thiophene oxide **6A**, by an enzyme-catalysed cycloaddition process (using a Diels–Alderase enzyme), could not be excluded at this stage. However, the isolation of cycloadduct **7A**, in racemic form, was more consistent with a biocatalytic sulfoxidation of substrate **1A** and release of the transient thiophene oxide **6A** from the TDO active site, followed by a spontaneous cycloaddition reaction.

Chromatographic separation of the crude mixture of bioproducts from *P. putida* UV4 and thiophene substrate **1A** yielded disulfoxide **7A** (45% yield) and a less polar product (12% yield) that was identified as the monosulfoxide **8A** by NMR analysis and comparison with literature data.[24] It is noteworthy that the biotransformation yielded only one of the possible monosulfoxide regio- and diastereo-isomers (**8A**). Chemical oxidation ($NaIO_4$) of the monosulfoxide **8A** gave a separable mixture of disulfoxide **7A** and its epimer, **10A**. In a preliminary report of this work[12] it was postulated that the monosulfoxide bioproduct **8A** could have arisen from a cycloaddition reaction between thiophene **1A** and thiophene oxide **6A**. However, our attempts to reproduce this cycloaddition by chemical sulfoxidation, using an excess of thiophene **1A**, have been unsuccessful. In addition, no literature precedent for this type of cycloaddition could be found. Hence an alternative explanation for the formation of monosulfoxide **8A** was sought.

Enzyme-catalysed deoxygenation of disulfoxide **7A** could, in principle, account for the isolation of enantiomerically enriched monosulfoxide bioproduct **8A**. A sulfoxide deoxygenation process, catalysed by a dimethyl sulfoxide reductase, has recently been reported to yield enantiopure sulfoxides *via* kinetic resolution.[25,26] A precedent for deoxygenation reactions of cyclic racemic sulfoxides, using *P. putida* UV4, has also recently been observed during unrelated biotransformation studies in our laboratories (unpublished results). The stereochemistry of the monosulfoxide metabolite **8A**, obtained from substrate **1A** using *P. putida* UV4, was analysed using chiral stationary phase (CSP) HPLC. Metabolite **8A** was isolated with variable (3–77%) enantiopurity values from repeated biotransformations having an excess of the same (−) enantiomer (of undetermined absolute configuration). This observation is consistent with the involvement of a sulfoxide reductase enzyme and a partial kinetic resolution process. Further evidence for enzyme-catalysed deoxygenation was obtained from the isolation of monosulfoxide product **8A**, with a modest excess (15% ee) of the (−) enantiomer, using a racemic sample of the disulfoxide **7A** as substrate with *P. putida* UV4. It is noteworthy that the more accessible oxygen atom at C-1 is exclusively removed from the disulfoxide both chemically (Me_3SiCl/NaI)[24] and enzymatically (*P. putida* UV4). The enzyme-catalysed mono-and dideoxygenation of disulfoxides has previously been reported in the fungus *Mortierella isabellina*.[27] A trace metabolite (0.1% yield) was identified as dihydrodiol **9A**$_{cis}$/**9A**$_{trans}$; its structure and stereochemistry are presented in section (**d**).



Biotransformation of 2-methylthiophene **1B** yielded the expected racemic disulfoxide cycloadduct **7B** (4% yield) and the corresponding monosulfoxide **8B** (12% yield) but no trace of dihydrodiol product **9B**$_{cis}$/**9B**$_{trans}$ was found. Cycloadduct **7B** has also been reported as a result of the chemical oxidation (MCPBA–BF_3) of 2-methylthiophene **1B**.[28] Earlier studies of toluene dioxygenase-catalysed *cis*-dihydroxylation of arenes,[8–10] alkenes,[8–10] sulfides[11,29] and polycyclic thiophenes[12,13] in *P. putida* UV4 have generally yielded metabolites having high ee values; it is probable that the thiophene oxide **6B** initially formed within the toluene dioxygenase active site was enantioenriched. However, in view of the low barriers to pyramidal

inversion in monocyclic thiophene oxides ($\Delta G^{\neq}$ *ca.* 13–15 kcal mol^{-1}) obtained both by experiment[23] and calculation,[30] it is also probable that the racemization occurred after bioproduct release and before dimerisation. The monosulfoxide metabolite **8B**, in common with monosulfoxide **8A**, was enantioenriched (8% ee, CSP HPLC analysis) and of unknown absolute configuration. This is again consistent with a sulfoxide reductase-catalysed deoxygenation of a racemic disulfoxide adduct **7B** occurring when whole cells of *P. putida* UV4 were used.

The biotransformations of thiophene **1A** and 2-methylthiophene **1B** were carried out on a relatively large scale (5–25 g) to facilitate the detection of minor bioproducts and to establish the major metabolic pathways. However, in order to confirm the general applicability of the dioxygenase-catalysed sulfoxidation reactions to the thiophene ring system, a wider range of 2-substituted thiophene substrates **1C**–**1G**, were added to *P. putida* UV4 in smaller quantities. Small-scale biotransformations (0.1–0.5 g) were carried out with 2-substituted thiophene substrates **1C**–**1G** and the corresponding disulfoxide adducts **7C**–**7G** were isolated (2–17%). As expected, yields were generally higher for thiophenes bearing smaller substituents at C-2. A similar trend was noticed during dioxygenase-catalysed sulfoxidation of a series of alkyl phenyl sulfides with *P. putida* UV4.[29] The structures of metabolites **7C**–**7G** were established by spectral methods (^{1}H-NMR, MS) and comparison with a typical disulfoxide **7D** whose structure was confirmed by X-ray crystallography (Fig. 1).

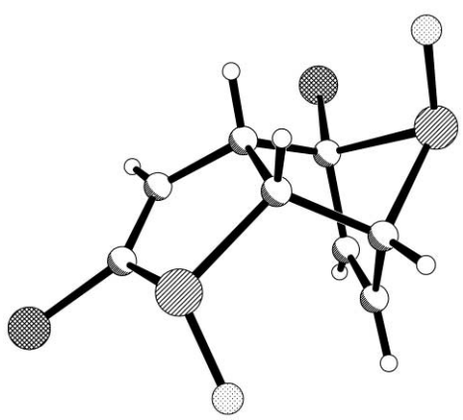


Fig. 1 X-Ray structure of **7D**.

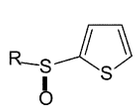
The *anti* relative positions of the two sulfoxide groups to the bridgehead hydrogen atoms are identical to those found earlier by X-ray analysis of disulfoxide **7A**[24] and **7B**.[28] The Diels–Alder cycloaddition reaction resulting from this biotransformation places a chlorine atom at C-4, *i.e.* as far away as possible from the oxygen atom at S-1. From the similar spectral characteristics of the other cycloadducts **7C**, **7E**–**7G**, it is assumed that all have similar geometries to **7B** and **7D**. Disulfoxide bioproducts **7A**–**7G** were all found to have no detectable optical rotations and were assumed to be racemates. None of the possible monosulfoxide metabolites (**8C**–**8G**) were detected during these relatively small-scale biotransformations.

Addition of 2-phenylthiophene **1G**, as substrate to *P. putida* UV4, provided the TDO enzyme with the choice of catalysing the oxidation of either a thiophene or a benzene ring; the major metabolite again proved to be dimer **7G** (33% yield). Two minor metabolites, **10G** (3% yield) and **11** (3% yield) along with phenols, 2-(2′-hydroxyphenyl)- and 2-(3′-hydroxyphenyl)thiophene (decomposition products of metabolite **11**, 10% yield), were isolated by a combination of column chromatography and PLC of the crude mixture of bioproducts. The structure of the major cycloadduct **7G** was similar to the other disulfoxide bioproducts **7A**–**7E**. The minor cycloadduct **10G** was identified as the epimer of compound **7G**, by comparison of its spectral data (^{1}H-NMR, MS and IR) with a sample of disulfoxide **10A** obtained by chemical oxidation. The relative *syn* geometry of the two sulfoxide groups in cycloadduct **10G** was evident from the upfield position of H-7_a (δ_H 4.69) as compared to H-3_a (δ_H 5.40).

The third bioproduct, a carbocyclic *cis*-dihydrodiol, was assigned the structure **11** from its spectral data (^{1}H-NMR and MS). It was found to be a single enantiomer ($[\alpha]_D$ +231; ≥98% ee) of (1*S*,2*R*) configuration from ^{1}H-NMR spectral analysis of its diastereoisomeric boronate esters formed with (−)-(*S*)- and (+)-(*R*)- 2-(1-methoxyethyl)benzeneboronic acid (MEBBA). A similar method had earlier been used for stereochemical assignments of *cis*-dihydrodiol metabolites from other benzene substrates containing sulfur heterocyclic substituents.[11] *cis*-Dihydrodiol **11** was of an identical absolute configuration to those obtained from monosubstituted benzene substrates with *P. putida* UV4.[8,9] The formation of *cis*-dihydrodiol **11** indicated a preference for dihydroxylation of the monosubstituted benzene ring compared with the 2-substituted thiophene ring. *cis*-Dihydrodiol **11**, containing an electron-rich thiophene ring, turned out to be more unstable relative to the other monosubstituted benzene *cis*-dihydrodiol metabolites.[31] Dehydration/aromatisation of diol **11**, either by leaving it at room temperature in the solid state for a short time (*ca.* 2 h) or by treating in solution (MeOH) with a few drops of TFA, gave mainly 2-(2′-hydroxyphenyl)thiophene (*ca.* 90%), with a small amount (*ca.*10%) of the *meta* isomer 2-(3′-hydroxyphenyl)thiophene.

(b) TDO-catalysed monooxygenation of 2-substituted thiophenes 1H–1J to yield the corresponding exocyclic monosulfoxides 12H, 12I and 12J

Thiophenes **1H**–**1J** were selected as substrates to provide the TDO enzyme system with an exocyclic sulfur atom as an alternative site for heteroatom oxidation. The acyclic sulfur atom proved to be a much more attractive target and sulfoxidation occurred exclusively at this position to give sulfoxides **12H**, **12I** and **12J** respectively (18–52% yield).



12H (R = Me)
12I (R = Ph)
12J (R = 2-Th)

The biotransformation of alkylaryl sulfide **1H** with *P. putida* UV4 gave the (*R*) sulfoxide **12H** as the sole metabolite (41% yield, ≥98% ee).[29] Under similar conditions, the sulfides **1I** and **1J** yielded the corresponding diaryl sulfoxides **12I** (18% yield, 58% ee) and **12J** (52% yield) as metabolites. The absolute configuration of (+)-sulfoxide **12I** remains to be determined. It is significant that when an alternative exocyclic sulfur atom is available on substrates containing either one (**1H**) or even two (**1J**) 2-thienyl groups, no evidence of oxidation of a heterocyclic sulfur atom was observed. Based upon the biotransformation results from 2-substituted thiophenes **1A**–**1J**, TDO-catalysed oxidation would appear to occur preferentially, in the sequence: acyclic sulfoxidation (**1H**–**1J**) > thiophene ring sulfoxidation (**1A**–**1F**) > phenyl ring *cis*-dihydroxylation (**1G**) >2-substitututed thiophene ring dihydroxylation.

(c) Trapping of transient 2-substituted thiophene oxides 6 formed during TDO-catalysed sulfoxidation

Despite the assumption of enzyme-catalysed Diels–Alder type cycloaddition reactions occurring during the biosynthesis of natural products, *e.g.* polyketides, terpenoids and alkaloids,[32,33] relatively few examples of Diels–Alderase enzymes have been

found.[34] From the results obtained in the current study, no evidence was provided for the involvement of a similar type of enzyme in the intermolecular [4+2] cycloaddition reaction between two thiophene sulfoxide molecules to yield the racemic disulfoxides **7A**–**7G**. The transient thiophene oxide metabolites **6A**–**6G**, formed in *P. putida* UV4, exhibit both diene and dienophile character during the dimerisation reactions. The possibilities of cycloaddition occurring between two different molecules of transient thiophene sulfoxide metabolites **6**, and of trapping these intermediates with stable dienes and dienophiles, were also examined.

Biotransformation of a mixture containing equimolar quantities of two thiophene substrates, **1A** and **1D**, was carried out to determine if both dimeric disulfoxides **7A**, **7D** and crossed cycloadducts **13** and **14** were formed. Only cycloadducts **7A** (26% yield), **7D** (33% yield) and **14** (9% yield) were detected, separated and characterised. From the relative adduct yields, the cycloaddition reactions of thiophene oxide **6A** and 2-chlorothiophene oxide **6D** appear to show a slight preference for **6D** acting as a diene and for dimerisation. When the biotransformation of a similar (1 : 1) mixture of thiophenes **1B** and **1D** was carried out, a slight excess of dimeric cycloadducts **7B** (5% yield) and **7D** (6% yield) over crossed cycloadducts **15** (4% yield) and **16** (3% yield) was again observed. The crossed cycloadducts **15** and **16** were separated by semi-preparative HPLC and characterised by spectral methods.

Thiophene oxide metabolite **6B**, formed from thiophene **1B**, was also intercepted as the corresponding cycloadducts **17** and **18** with the help of an excess of the stable dienophiles, methyl vinyl ketone and *N*-methylmaleimide respectively. No evidence of dimers of sulfoxide **6B** was obtained. The structure of cycloadduct **17**, having acetyl and methyl groups proximate and *cis*, and the sulfoxide oxygen atom *trans* to the acetyl group, was confirmed by X-ray crystallographic analysis (Fig. 2). The

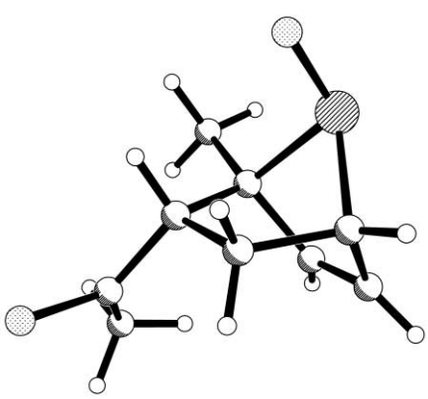
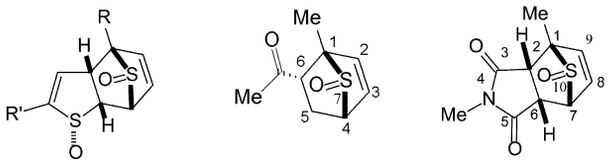


Fig. 2 X-Ray structure of **17**.

formation of the cycloadducts **17** and **18** show that it is also possible to trap transient thiophene oxide metabolites *in situ*, as found earlier during chemical oxidations.[28]

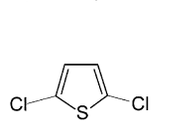
Attempts to intercept the transient thiophene oxide metabolites **6** as dienophiles by forming cycloadducts with cyclic dienes, *e.g.* 2,6-dimethylfuran or 1,2-dihydroxy-1,2-dihydrobenzene, were unsuccessful. It appears, from these enzyme-catalysed oxidation experiments, and chemical oxidations of substituted thiophenes **1**,[28] that thiophene oxide metabolites **6** prefer to behave as dienes rather than dienophiles.

Biotransformation of the 2,5-disubstituted thiophene **19** using *P. putida* UV4, did not yield a thiophene oxide metabolite of comparable stability to 2,5-di-*tert*-butylthiophene oxide.[23] Instead, the racemic disulfoxide cycloadduct **20**, whose structure was deduced from spectral comparison with other cycloadducts (**7A**–**7G**, **13**–**16**), was isolated in low yield (3%) as the sole bioproduct.



13 (R = Cl, R' =H)
14 (R H, R' = Cl)
15 (R = Cl, R' = Me)
16 (R = Me, R' = Cl)

17 **18**



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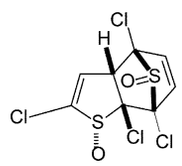

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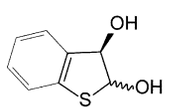
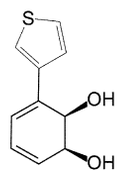


21_{cis}/21_{trans}



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(d) TDO-catalysed formation of monosulfoxide 8K, disulfoxides 7L–7N and the dihydrodiols $9K_{cis}$/$9K_{trans}$–$9N_{cis}$/$9N_{trans}$ from the 3-substituted thiophenes 1K–1N

Addition of the 3-substituted thiophenes **1K**–**1N** to *P. putida* UV4 yielded the corresponding racemic disulfoxide cycloadducts (**7L**–**7N**, *ca.* 13% yield). It is assumed that these adducts were formed *via* the transient thiophene oxides **6K**–**6N** in a similar manner to the disulfoxides **7A**–**7G** derived from the corresponding parent **1A** and the 2-substituted thiophenes **1B**–**1G**. Although disulfoxide **7K** also appears to have been formed from thiophene **1K** (*via* thiophene oxide **6K**), only the deoxygenation product monosulfoxide **8K**, was isolated (4% yield, 51% ee).

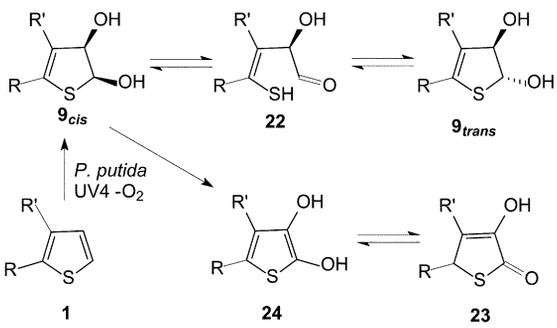
Similar metabolic pathways involving sulfoxidation were followed by both the parent thiophene **1A** and the 2- (**1B**–**1G**) and 3-substituted-thiophenes (**1K**–**1N**). However, an important difference, involving formation of heterocyclic dihydrodiol metabolites **9** in the parent substrate **1A** and the 3-substituted thiophene substrates (**1K**–**1N**), was observed. Biotransformation studies, of benzo[*b*]thiophenes, using *P. putida* UV4 as a source of TDO, had earlier shown the concomitant formation of disulfoxide cycloadducts and dihydrodiols resulting from dihydroxylation of carbocyclic and heterocyclic rings.[12] The heterocyclic ring dihydrodiol metabolites of benzo[*b*]thiophene were found to equilibrate, spontaneously, between *cis*- (*e.g.* $\mathbf{21}_{cis}$) and *trans*-dihydrodiol isomers (*e.g.* $\mathbf{21}_{trans}$).[12] X-Ray crystallography and ^{1}H-NMR spectroscopy were used to determine the configurations of the heterocyclic *cis*-and *trans*-dihydrodiol metabolites of benzo[*b*]thiophenes.[12] Thiophene **1A** and 3-substituted thiophenes **1K**–**1N** also gave equilibrating mixtures of the corresponding dihydrodiols $\mathbf{9A}_{cis}$/$\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}$/$\mathbf{9K}_{trans}$–$\mathbf{9N}_{cis}$/$\mathbf{N}_{trans}$ (Scheme 2); the isolated yields and *cis*/*trans* ratios are shown in Table 1.

With the exception of parent thiophene **1A**, where the dihydrodiol $\mathbf{9A}_{cis}$/$\mathbf{9A}_{trans}$ was only observed in trace quantities (0.1% yield) relative to the sulfoxidation products, the other dihydrodiols $\mathbf{9K}_{cis}$/$\mathbf{9K}_{trans}$–$\mathbf{9N}_{cis}$/$\mathbf{9N}_{trans}$ were generally found in similar or slightly greater quantities (6–14% yield) than the corresponding disulfoxide cycloadducts **7L**–**7N** (4–13% yield). As observed earlier for the heterocyclic dihydrodiol metabolites of benzo[*b*]thiophene,[12] the ratio of *cis*- and *trans*- isomers present at equilibrium was solvent-dependent with a higher proportion (55–65% yield) of the *cis*-isomer in the less polar solvent $CDCl_3$ and of the *trans*-isomer (80–92% yield) in the more polar

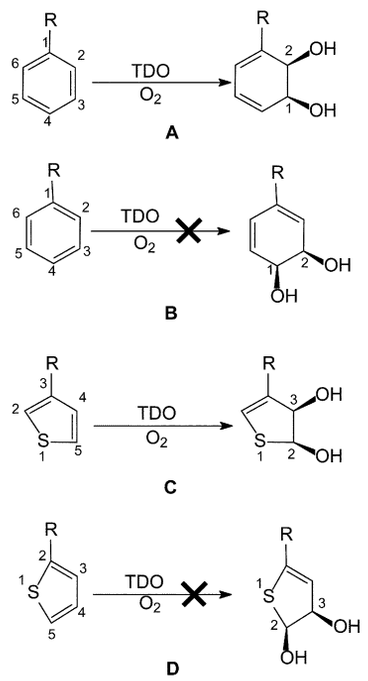
Table 1 Isolated yield, *cis*/*trans* ratio, enantiopurity and absolute configuration of dihydrodiol metabolites $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$–$\mathbf{9N}_{cis}/\mathbf{N}_{trans}$

Substrate	Dihydrodiol metabolite	Isolated yield (%)	*cis* : *trans* ratio (%) of dihydrodiol	Enantiomeric excess (%)	Absolute configuration
1A	$\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$	0.1	60 : 40 [a] 10 : 90 [b]	43,[c] 45 [d]	3*R*
1K	$\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$	11	60 : 40 [a] 20 : 80 [b]	48,[c] 40 [d]	3*S*
1L	$\mathbf{9L}_{cis}/\mathbf{9L}_{trans}$	9	65 : 35 [a] 10 : 90 [b]	49 [c]	3*S* [e]
1M	$\mathbf{9M}_{cis}/\mathbf{9M}_{trans}$	14	63 : 37 [a] 17 : 83 [b]	44 [c]	3*S* [e]
1N	$\mathbf{9N}_{cis}/\mathbf{9N}_{trans}$	18	55 : 45 [a] 8 : 92 [b]	>98 [c]	3*R*

[a] ^{1}H-NMR analysis in $CDCl_3$. [b] ^{1}H-NMR analysis in CD_3OD. [c] ^{1}H-NMR analysis of the MEBBA derivative. [d] ^{1}H-NMR analysis of the diMTPA derivative. [e] Change in absolute configuration due to Sequence Rule priority change.



Scheme 2



Scheme 3

CD_3OD solvent (Table 1). The relative stereochemistry was assigned on the basis of the coupling constants $J_{2,3}$ being larger for the *cis* isomers ($\mathbf{9A}_{cis}$, $\mathbf{9K}_{cis}$–$\mathbf{9N}_{cis}$, *ca.* 5.4–5.7 Hz) compared with the *trans* isomers ($\mathbf{9A}_{trans}$, $\mathbf{9K}_{trans}$–$\mathbf{9N}_{trans}$, *ca.* 1 Hz). Similar ^{1}H-NMR trends have been observed for the corresponding heterocyclic dihydrodiols of benzo[*b*]thiophenes whose relative stereochemistry was confirmed by X-ray crystallography.[12] The mechanism for *cis*–*trans* diol isomerization was assumed to involve spontaneous ring opening *via* an undetected aldehyde intermediate **22** (Scheme 2). This process is similar to the spontaneous epimerisation of the α-and β-anomers of glucose *via* a small proportion (<1%) of the acyclic aldehyde. It is interesting to note that the predominant isomer in hydroxylic solvents is the *trans*-dihydrodiol $\mathbf{9}_{trans}$, although it is assumed that the *cis* isomer $\mathbf{9}_{cis}$ is formed initially at the dioxygenase active site *in vivo*, in common with dihydroxylations of carbocyclic arenes (Scheme 3).

The enantiopurity values of the *cis*–*trans* dihydrodiols **9A**, **9K**–**9N** were assigned by exclusive reaction of the *cis*-isomers $\mathbf{9A}_{cis}$ and $\mathbf{9K}_{cis}$–$\mathbf{9N}_{cis}$ with (−)-(*S*)-[2-(1-methoxyethyl) benzene]-boronic acid) to yield the corresponding diastereoisomeric MEBBA esters. The advantage of this approach was that MEBBA derivatives showed diagnostic MeO signals in the ^{1}H-NMR spectra. With the exception of the enantiopure dihydrodiol $\mathbf{9N}_{cis}/\mathbf{9N}_{trans}$, the other diols $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$–$\mathbf{9M}_{cis}/\mathbf{M}_{trans}$ were found to have lower ee values (43–49%). Independent confirmation of the ee values for metabolites $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$ was obtained by formation and ^{1}H-NMR analysis of the corresponding exclusively *trans*-diMTPA diesters (45 and 40% ee) by using (+)-(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA). Although not observed during the course of $[\alpha]_D$ measurements or ^{1}H-NMR spectral analysis in $CDCl_3$ or CD_3OD solution, the possibility of partial racemization of dihydrodiols $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$–$\mathbf{9M}_{cis}/\mathbf{9M}_{trans}$ occurring during the biotransformation *via* the corresponding acyclic aldehyde intermediates **22A**,**22K**–**22M** containing a single stereogenic centre, cannot be totally excluded. However, the earlier isolation of enantiopure heterocyclic dihydrodiol metabolites of benzo[*b*]thiophene $\mathbf{21}_{cis}/\mathbf{21}_{trans}$[12] and the formation of 3-phenylthiophene dihydrodiols $\mathbf{9N}_{cis}/\mathbf{9N}_{trans}$ as single enantiomers (>98% ee) in this study, suggest that the larger sizes of benzo[*b*]thiophene and 3-phenylthiophene **1N**, and their reduced flexibility at the active site compared with smaller thiophenes **1A**,**1K**–**1M** may be a more important factor. Earlier work has shown that all *cis*-dihydrodiol metabolites from TDO-catalysed mono- and 1,2-di-substituted benzene substrates were enantiopure with exceptions of those bearing the smallest substituents *e.g.* fluorine atoms.[8] The carbocyclic *cis*-dihydrodiol **25** was also isolated as a metabolite from 3-phenylthiophene **1N**. In common with carbocyclic *cis*-dihydrodiol metabolite **11**, derived from 2-phenylthiophene **1G** and other monosubstituted benzene substrates, *cis*-dihydrodiol **25** was enantiopure (>98% ee) and of the (1*S*, 2*R*) configuration.

The absolute configurations of thiophene ring *cis*/*trans*-dihydrodiol metabolites of several benzo[*b*]thiophene substrates have been determined by X-ray crystallography and ^{1}H-NMR spectroscopy.[12] Based upon these, and earlier results for *cis*-dihydrodiol metabolites of monosubstituted benzene substrates,[35] a trend in the ^{1}H-NMR spectra had been observed for the corresponding MEBBA derivatives. This method was extended to assign absolute configurations of heterocyclic dihydrodiols $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$ and $\mathbf{9K}_{cis}/\mathbf{9K}_{trans}$–$\mathbf{9N}_{cis}/\mathbf{9N}_{trans}$. Thus, when (−)-(*S*)-[2-(1-methoxyethyl)benzene]boronic acid) was used, the methoxyl signal for the corresponding MEBBA derivative was found to be upfield for the 3*R* configuration in the dihydrodiol $\mathbf{9A}_{cis}/\mathbf{9A}_{trans}$.

The results shown in Table 1 indicate that, when a change in Sequence Rule priorities for the dihydrodiols **9L**$_{cis}$/**9L**$_{trans}$ and **9M**$_{cis}$/**9M**$_{trans}$ are taken in account, the major enantiomer (except **9K**$_{cis}$/**9K**$_{trans}$) was found to have the same absolute configuration at C-3. The absolute configurations of the preferred diol enantiomers **9A**$_{cis}$/**9A**$_{trans}$ and **9L**$_{cis}$/**9L**$_{trans}$–**9N**$_{cis}$/**9N**$_{trans}$ are identical to those found at the equivalent C-2 in the *cis*-dihydrodiol metabolites of monosubstituted benzene substrates (Scheme 3A). Similarly, all *cis*/*trans* diol mixtures in Table 1 were laevorotatory in $CDCl_3$ and CD_3OD solutions except for the diols **9K**$_{cis}$/**9K**$_{trans}$. In view of the apparently opposite stereochemistry of metabolite **9K**$_{cis}$/**9K**$_{trans}$, the absolute configurations of the diols **9A**$_{cis}$/**9A**$_{trans}$ and **9K**$_{cis}$/**9K**$_{trans}$ were determined by ^{1}H-NMR analysis of the diagnostic H-3 protons from the corresponding *trans*-diMTPA esters; the absolute configurations of dihydrodiols **9A**$_{cis}$/**9A**$_{trans}$ and **9K**$_{cis}$/**9K**$_{trans}$ were again found to be opposite. The diMTPA method for absolute configuration assignment had earlier been successfully applied to a series of dihydrodiol metabolites.[36]

The opposite absolute configuration of the dihydrodiol metabolite **9K**$_{cis}$/**9K**$_{trans}$, derived from 3-methylthiophene **1K**, is difficult to rationalize on the basis of a direct dioxygenase-catalysed asymmetric dihydroxylation process. A more plausible explanation involves the formation of both enantiomers and selective removal of one by kinetic resolution. The earlier isolation of monosulfoxide **8K** (51% ee) *via* enzyme-catalysed deoxygenation was consistent with one type of kinetic resolution happening during biotransformations with *P. putida* UV4. An alternative type of kinetic resolution could, in principle, also occur during the dehydrogenase-catalysed desaturation of the dihydrodiol **9K**$_{cis}$/**9K**$_{trans}$ to yield the 2,3-dihydroxythiophene **24**. Although this metabolite was not found, the tautomeric thiolactone **23** (R = H, R′ = Me) was isolated as a minor (2%) metabolite of 3-methylthiophene **1K** (Scheme 2). 3-Hydroxy-4-methyl-5*H*-thiophen-2-one **23** (R = H, R′ = Me), synthesised earlier by dealkylation of the di-*tert*-butyl ether of the 2,3-dihydroxythiophene **24**,[37] was found to be the preferred tautomeric form of the 1,2-dihydroxythiophene **24**. It is thus proposed that the latter is the initial product of a dihydrodiol dehydrogenase-catalysed desaturation of the *cis*/*trans* dihydrodiol **9K** (Scheme 2). The desaturation of benzene *cis*-dihydrodiol metabolites to catechols is part of the normal metabolic pathway for arenes in wild-type strains of *P. putida* and other bacteria.[38,39] The toluene *cis*-diol dehydrogenase (TDD) enzyme is, however, absent from the UV4 mutant strain of *P. putida*. The formation of the thiolactone metabolite **23** suggests that an alternative heterocyclic diol dehydrogenase enzyme, present in the UV4 mutant strain, can catalyse the desaturation of the dihydrodiol **9K**$_{cis}$/**9K**$_{trans}$ of 3-substituted thiophene **1K**. It has been shown that the wild-type precursor of *P. putida* UV 4 (*P. putida* NCIMB 11767) contains TDD that can accept only one *cis*-dihydrodiol enantiomer produced by the initial TDO-catalysed dihydroxylation step in monosubstituted benzene substrates.[39] If it is assumed that a heterocyclic diol dehydrogenase enzyme is present in *P. putida* UV4 that catalyses the conversion of the diol **9K**$_{cis}$/**9K**$_{trans}$ to the 2,3-dihydroxythiophene **24** and its preferred tautomer **23**, then it could be postulated that the major 3*R* enantiomer formed would be removed selectively leaving an excess of the 3*S* enantiomer. No evidence was obtained for the formation of the corresponding thiolactones **23** from the other thiophene substrates **1A**,**1L**–**1N**, suggesting that the diol **9K**$_{cis}$/**9K**$_{trans}$ was a particularly good substrate for this enzyme.

(e) Model system for predicting the structure and regiochemistry of TDO-catalysed mono- and di-oxygenation of monosubstituted thiophenes

The similarities in shape, aromaticity and chemical reactivity between monosubstituted benzene and thiophene ring systems are important factors in predicting the structure and type of biotransformation products obtained using dioxygenase-containing bacterial strains. TDO-catalysed *cis*-dihydroxylation of monosubstituted benzene substrates has been found to occur exclusively at a 2,3-bond (Scheme 3, **A**). However, TDO was not found to catalyse the formation of heterocyclic *cis*/*trans*-dihydrodiols *e.g.* **9B**–**9G** from the corresponding 2-substituted thiophene substrates **1B**–**1G** and only traces of dihydrodiol **9A**$_{cis}$/**9A**$_{trans}$ was found when the parent thiophene **1A** was metabolised.

Based on the metabolic profile, obtained from TDO-catalysed oxidation of the monosubstituted thiophenes **1B**–**1N** discussed herein, it is possible to construct a simple model that allows prediction of the product distribution from monosubstituted thiophenes (Scheme 3).

Earlier studies[9,10] have shown that the TDO enzyme in *P. putida* UV4 catalyses *cis*-dihydroxylation of monosubstituted benzene substrates in a highly regioselective manner *i.e.* exclusive attack at the 2,3- rather than 3,4-bond (Scheme 3, **A** and **B**). The 3,4-bond in monosubstituted benzene substrates (Scheme 3, **B**) is equivalent to the 4,5-bond in 2-substituted thiophenes (Scheme 3, **D**) and thus a similar lack of *cis*-dihydroxylation in the latter heterocyclic arene system would be expected. This results in heteroatom oxidation, exclusively, yielding the corresponding thiophene sulfoxides (*e.g.* **6B**–**6G**) and dimers (*e.g.* **7B**–**7G**). Conversely the 4,5-bond of 3-substituted thiophenes (Scheme 3, **C**) is equivalent to the 2,3-bond in monosubstituted benzenes and *cis*-dihydroxylation of 3-substituted thiophenes would be expected. The formation of *cis*/*trans*-dihydrodiols from thiophene **1A** and 3-substituted thiophenes **1K**–**1N** provides support for the validity of the simple model shown in Scheme 3.

Conclusion

The following conclusions can be drawn from this study into the dioxygenase-catalysed oxidations of thiophene substrates **1A**–**1N** using *P. putida* UV4.

(i) The heterocyclic sulfur atom in 2-substituted thiophenes **1A**–**1G** is the preferred oxidation site.

(ii) Dioxygenase-catalysed sulfoxidation of the acyclic sulfur centre occurred more readily than at the alternative heterocyclic sulfur in thiophenes **1H**,**1I** and **1J**.

(iii) The initially formed transient thiophene oxide intermediates **6** were trapped by dimerisation, cycloaddition to a different thiophene oxide metabolite and by formation of Diels–Alder cycloadducts with stable dienophiles.

(iv) The first evidence of an enzyme-catalysed deoxygenation using *P. putida* UV4 was found on the disulfoxide cycloadducts **7A**,**7B** and **7K** to yield the corresponding monosulfoxides **8A**,**8B** and **8K**.

(v) Biotransformation of 3-substituted thiophenes **1K**–**1N** was found to yield both sulfoxidation products **7L**–**7N**,**8K** and dihydrodiols **9K**$_{cis}$/**9K**$_{trans}$–**9N**$_{cis}$/**9N**$_{trans}$.

(vi) An unprecedented diol dehydrogenase-catalysed desaturation of a heterocyclic ring, to give diol **9K**$_{cis}$/**9K**$_{trans}$, was observed.

(vii) The validity of a new model, that allows the TDO-catalysed oxidation products from monosubstituted thiophene substrates to be predicted, was confirmed.

Experimental

^{1}H-NMR spectra of compounds were recorded on Bruker Avance DPX-300 and DPX-500 instruments. High-resolution mass spectra were recorded at 70 eV on a VG Autospec mass spectrometer using a heated inlet system. Accurate MW values were determined by the peak matching method with perfluorokerosene as standard. Flash chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and

$PF_{254/366}$ respectively. Merck Kieselgel $60F_{254}$ analytical plates were used for TLC. Optical rotation ($[\alpha]_D$) measurements were carried out with a Perkin-Elmer 214 polarimeter at ambient temperature (*ca*. 20 °C) and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded using a JASCO J-720 instrument and spectroscopic grade methanol as solvent. Enantiopurity values of sulfoxides were determined by CSP HPLC using specified Chiralcel columns.

Substrates were metabolized with growing cultures of the UV4 mutant strain of *Pseudomonas putida* according to the method described earlier.[11,29,40] The bioproducts were harvested, after *ca*. 18 h, unless mentioned otherwise, by repeated extraction (EtOAc) of the sodium chloride-saturated aqueous solution containing the biotransformed material, and concentration of the combined organic extracts under reduced pressure. ^{1}H-NMR spectra of the crude mixture of bioproducts, obtained from each biotransformation, were routinely examined prior to using any purification procedure. None of the cycloadduct metabolites **7A**–**7G**, **7N**, **10G**, **14**–**18** and **20** showed any optical rotation ($[\alpha]_D$ zero) and were found to be racemic by ^{1}H-NMR analysis in the presence of Pirkle solvent.

Biotransformation of thiophenes 1A–1N with *P. putida* UV4

Thiophene 1A. Substrate **1A** (7.36 g, 87.6 mmol, 4 h) gave a crude mixture of three compounds which, on separation by flash chromatography (5% MeOH in $CHCl_3$), yielded *cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide **8A** (0.92 g, 12%), *cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1, *syn*-8-dioxide **7A** (3.9 g, 45%) and the inseparable isomeric mixture *cis*/*trans*-2,3-dihydroxy-2,3-dihydrothiophene **9A** (0.008 g, 0.1%).

(−)-*cis*-3a,4,7,7a-Tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide 8A

Mp 128–130 °C (from $CHCl_3$–hexane) (lit.,[24] mp 123–124 °C); $[\alpha]_D$ −30.5 (*c* 0.55, $CHCl_3$); R_f 0.54 (5% MeOH in $CHCl_3$) (Found: C, 52.4; H, 4.6; S, 34.9; $C_8H_8OS_2$ requires C, 52.2; H, 4.4; S, 34.8%); δ_H (500 MHz, $CDCl_3$) 4.06–4.08 (1 H, m, H-4), 4.13–4.15 (1 H, m, H-7), 4.42–4.46 (1 H, m, H-3a), 4.82 (1 H, dd, $J_{7a,7}$ 3.8, $J_{7a,3a}$ 10.1, H-7a), 5.49 (1 H, dd, $J_{3,3a}$ 2.6, $J_{3,2}$ 6.0, H-3), 6.00 (1 H, dd, $J_{2,3a}$ 1.8, $J_{2,3}$ 6.0, H-2), 6.10–6.16 (2 H, m, H-5 and H-6); *m*/*z* 184 (M^+, 29%), 136 (66) and 135 (100). The enantiopurity (77% ee), of metabolite **8A** was determined by CSP HPLC (Chiralpak AD, 25cm × 4.6mm, 10% propan-2-ol in hexane, 0.6 cm^3 min^{-1}, α 1.15).

(±)-*cis*-3a,4,7,7a-Tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7A

Mp 144–147 °C decomp. (from $CHCl_3$–hexane); R_f 0.32 (5% MeOH in $CHCl_3$) (Found: C, 47.6; H, 3.8; S, 32.4; $C_8H_8O_2S_2$ requires C, 48.0; H, 4.0; S, 32.0%); δ_H (300 MHz, $CDCl_3$) 4.02–4.05 (1 H, m, H-4), 4.12–4.14 (1 H, m, H-7), 4.45–4.49 (1 H, m, H-3a), 4.80 (1 H, dd, $J_{7a,7}$ 4.2, $J_{7a,3a}$ 7.7, H-7a), 5.92–5.96 (1 H, m, H-5), 6.34 (1 H, dd, $J_{3,3a}$ 2.6, $J_{3,2}$ 6.1, H-3), 6.34 (1 H, m, H-6) 6.54 (1 H, dd, $J_{2,3a}$ 1.7, $J_{2,3}$ 5.9, H-2); *m*/*z* 200 (M^+, 26%), 152 (83) and 135 (100).

(−)-2,3-Dihydroxy-2,3-dihydrothiophene 9A$_{cis}$/9A$_{trans}$

R_f 0.23 (5% MeOH in $CHCl_3$) (Found M^+ 118.0092; $C_4H_6SO_2$ requires 118.0092); *m*/*z* 118 (M^+, 62%) and 57 (100); $[\alpha]_D$ −83 (*c* 0.7,$CHCl_3$, 60% *cis* isomer) and $[\alpha]_D$ −133 (*c* 0.7, MeOH, 90% *trans* isomer).

(2*R*,3*R*)-2,3-Dihydroxy-2,3-dihydrothiophene 9A$_{cis}$

δ_H (500 MHz, $CDCl_3$) 4.74 (1 H, m, H-3), 5.68 (1 H, d, $J_{2,3}$ 5.4, H-2), 5.74 (1 H, dd, $J_{4,3}$ 2.5, $J_{4,5}$ 6.0, H-4), 6.41 (1 H, d, $J_{5,4}$ 6.0, H-5).

(2*S*,3*R*)-2,3-Dihydroxy-2,3-dihydrothiophene (9A$_{trans}$)

δ_H (300 MHz, CD_3OD) 4.71 (1 H, d, $J_{3,2}$ 1.8, H-3), 5.36 (1 H, s, H-2), 5.65 (1 H, dd, $J_{4,3}$ 2.9, $J_{4,5}$ 6.0, H-4), 6.49 (1 H, d, $J_{5,4}$ 6.0, H-5).

2-Methylthiophene 1B. Substrate **1B** (23.8 g, 0.24 mol, 7 h) furnished a mixture of two compounds; separation, by flash chromatography ($CHCl_3$ → 5% MeOH in $CHCl_3$), afforded 2,4-dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide **8B** (3.0 g, 12%) and 2,4-dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1, *syn*-8-dioxide **7B** (0.9 g, 4%).

2,4-Dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide 8B

A viscous oil; R_f 0.42 (5% MeOH in $CHCl_3$) (Found M^+ 212.0333. $C_{10}H_{12}OS_2$ requires 212.0330); δ_H (300 MHz, $CDCl_3$) 1.62 (3 H, s, Me), 1.86 (3 H, s, Me), 3.98–4.03 (2 H, d, m, H-3a, H-7), 4.85 (1 H, dd, $J_{7a,3a}$ 10, $J_{7a,7}$ 4.4, H-7a); 5.17 (1 H, d, $J_{3,3a}$ 1.9, H-3), 5.98 (1 H, d, $J_{5,6}$ 4.6, H-5), (1 H, m, H-6); *m*/*z* 212 (M^+, 11%), 162 (23), 149 (51) and 105 (100). The enantiopurity (8% ee) of metabolite **9B** was determined by CSP HPLC (Chiralpak AD, 10% propan-2-ol in hexane, 0.6 cm^3 min^{-1}, α 1.34).

(±)-2,4-Dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7B.

Mp 133–135 °C decomp. (from $CHCl_3$–hexane) (lit.[28] mp 148–149, decomp.); R_f 0.32 (5% MeOH in $CHCl_3$) (Found: C, 52.5; H, 5.1; S, 27.8; $C_{10}H_{12}O_2S_2$ requires C, 52.6; H, 5.3; S, 28.0%); δ_H (500 MHz, $CDCl_3$) 1.63 (3 H, s, Me), 2.10 (3 H, s, Me) 3.94–3.97 (1 H, m, H-3a), 4.08 (1 H, dd, $J_{7,7a}$ 4.2Hz, $J_{7,6}$ 4.2, H-7), 4.82 (1 H, dd, $J_{7a,3a}$ 7.7, $J_{7a,7}$ 3.9, H-7a), 5.81 (1 H, d, $J_{5,6}$ 7.0, H-5), 5.85 (1 H, s, H-3), 6.37 (1 H, dd, $J_{6,5}$ 6.8, $J_{6,7}$ 4.9, H-6); *m*/*z* 228 (M^+, 5%), 180 (53) and 163 (100).

2-Ethylthiophene 1C. Substrate **1C** (0.25 g, 2.2 mmol) produced the disulfoxide cycloadduct, (±)-2,4-diethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7C**; purification by PLC ($CHCl_3$) furnished a pure sample of metabolite **7C** (0.022 g, 8%), mp 133–134 °C decomp. (from $CHCl_3$–hexane); R_f 0.3 (5% MeOH in $CHCl_3$) (Found M^+ 256.0591. $C_{12}H_{16}O_2S_2$ requires 256.0592); δ_H (500 MHz, $CDCl_3$) 0.99 (3 H, t, *J* 7.3, -CH_2*Me*), 1.13 (3 H, t, *J* 7.4, -CH_2*Me*), 1.95 (2 H, m, -C*H*$_2$Me), 2.37 (2 H, m, -C*H*$_2$Me), 3.90 (1 H, dd, $J_{3a,7a}$ 7.8, $J_{3a,3}$ 2.1, H-3a), 4.03 (1 H, m, H-7), 4.75 (1 H, dd, $J_{7a,3a}$ 7.8, $J_{7a,7}$ 3.9, H-7a), 5.70 (1 H, d, $J_{3,3a}$ 2.0, H-3), 5.78 (1 H, d, $J_{5,6}$ 6.9, H-5), 6.29 (1 H, dd, $J_{6,5}$ 6.9, $J_{6,7}$ 4.7, H-6); *m*/*z* 256 (M^+, 26%), 190 (50), 175 (48), 117 (100).

2-Chlorothiophene 1D. Substrate **1D** (0.4 g, 3.4 mmol) yielded bioproduct (±)-2,4-dichloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7D**; purification by PLC ($CHCl_3$) furnished cycloadduct **7D** (0.076 g, 17%), mp 129–131 °C decomp. (from $CHCl_3$–hexane); R_f 0.3 (5% MeOH in $CHCl_3$) (Found: C 35.3, H 2.3; $C_8H_6O_2S_2Cl_2$ requires C 35.7, H 2.2); δ_H (500 MHz, $CDCl_3$) 4.15 (1 H, ddd, $J_{7,6}$ 5.2, $J_{7,7a}$ 3.9, $J_{7,5}$ 1.3, H-7), 4.30 (1 H, dd, $J_{3a,7a}$ 8.0, $J_{3a,3}$ 2.6, H-3a), 4.92 (1 H, dd, $J_{7a,3a}$ 8.1, $J_{7a,7}$ 4.0, H-7a), 6.02 (1 H, ddd, $J_{5,6}$ 7.0, $J_{5,7}$ 1.4, *J* 0.6, H-5), 6.39 (1 H, dd, $J_{3,3a}$ 2.9, *J* 0.4, H-3), 6.48 (1 H, dd, $J_{6,5}$ 7.0, $J_{6,7}$ 5.1, H-6); *m*/*z* 220 (M^+ −SO, 14%), 204 (12), 168 (55), 103 (88), 75 (100).

Crystal data for 7D. $C_8H_6Cl_2O_2S_2$, M_r = 269.2, monoclinic, a = 8.283(3), b = 12.655(4), c = 9.554(2) Å, β = 90.09(2), V = 1001.4(5) Å^3, T = 293 K, Cu–Kα radiation, λ = 1.54178 Å, space group $P2_1/n$, Z = 4, $F(000)$ = 544, D_x = 1.79 g cm^{-3}, μ = 9.49 mm^{-1}, blocks 0.63 × 0.45 × 0.43 mm, Siemens P3 diffractometer, ω scan, 11.6 <2θ <110°, measured/independent

reflections: 1409/724, direct methods solution, full matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms, hydrogens located in difference Fourier but included at positions determined by the geometry of the molecule using the riding model with isotropic vibration parameters, R_1 = 0.068 for 600 data with $F_o > 4\sigma(F_o)$, 128 parameters, wR_2 = 0.182 (all data), GoF = 1.06, $\Delta\rho_{min,max}$ = −0.45/0.55 e Å^{-3}. CCDC reference number 192255. See http://www.rsc.org/suppdata/ob/b3/b300867n/ for crystallographic files in .cif or other electronic format.

2-Bromothiophene. Substrate **1E** (0.5 g, 3.1 mmol) yielded the cycloadduct metabolite, (±)-2,4-dibromo-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7E**; purification by PLC ($CHCl_3$) gave compound **7E** (0.080 g, 15%); mp 119–120 °C decomp. (from $CHCl_3$–hexane); R_f 0.35 (5% MeOH in $CHCl_3$) (Found: C 26.8; H 1.8; $C_8H_6S_2O_2Br_2$ requires C 26.8; H 1.7%); δ_H (500 MHz, $CDCl_3$) 4.09 (1 H, ddd, $J_{7,6}$ 5.1, $J_{7,7a}$ 3.9, $J_{7,5}$ 1.1, H-7), 4.31 (1 H, dd, $J_{3a,7a}$ 8.1, $J_{3a,3}$ 2.8, H-3a), 4.88 (1 H, dd, $J_{7a,7}$ 4.1, $J_{7a,3a}$ 8.1, H-7a), 6.07 (1 H, dd, $J_{5,6}$ 6.9, $J_{5,7}$ 1.0, H-5), 6.46 (1 H, dd, $J_{6,5}$ 6.9, $J_{6,7}$ 5.1, H-6), 6.56 (1 H, d, $J_{3,3a}$ 2.9, H-3); *m*/*z* 310 (M^+ −SO, 5%), 231 (100), 229 (90), 150 (98), 77 (96).

2-Iodothiophene 1F. Substrate **1F** (0.25 g, 1.2 mmol) gave bioproduct (±)-2,4-diiodo-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7F**; purification by PLC ($CHCl_3$) furnished compound **7F** (0.033 g, 12%) (Found M^+ 451.7882. $C_8H_6O_2S_2I_2$ requires 451.7899); mp 91–93 °C decomp. (from $CHCl_3$–hexane); R_f 0.35 (5% MeOH in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.8 (1 H, m, H-7), 4.3 (1 H, dd, $J_{3a,3}$ 2.7, $J_{3a,7a}$ 7.9, H-3a), 4.7 (1 H, dd, $J_{7a,3a}$ 8.0, $J_{7a,7}$ 4.0, H-7a), 6.0 (1 H, d, $J_{5,6}$ 6.0, H-5), 6.3 (1 H, m, H-6), 6.6 (1 H, d, $J_{3,3a}$ 2.7, H-3); *m*/*z* 452 (M^+, 4%), 227 (30), 260 (54), 254 (100).

2-Phenylthiophene 1G. Substrate **1G** (3.0 g, 18.8 mmol) gave a crude mixture of bioproducts that was separated by flash column chromatography (hexane ⟶ EtOAc) followed by PLC (50% EtOAc in hexane). This yielded compounds **7G**, **10G**, **11** and a mixture of two phenols, which on further separation by PLC (25% Et_2O in hexane), gave 2-(2′-hydroxyphenyl)-thiophene (0.30 g, 9%); mp 41–42 °C (sublimation at reduced pressure) [lit.,[41] 40–42 °C] and 2-(3′-hydroxyphenyl)thiophene (0.03 g, 1%); mp 81–82 °C (Et_2O–hexane) [lit.,[41] 80–81 °C]. The spectral data of the two phenols were identical to that reported in the literature.

(±)-2,4-Diphenyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7G. A viscous gum (1.10 g, 33%) (Found M^+ 352.0573. $C_{20}H_{16}O_2S_2$ requires 352.0592); R_f 0.33 (EtOAc); δ_H (500 MHz, $CDCl_3$) 4.31 (1 H, m, H-7), 4.89 (1 H, dd, $J_{3a,3}$ 2.8, $J_{3a,7a}$ 7.9, H-3a), 5.17 (1 H, dd, $J_{3a,7a}$ 7.8, $J_{7a,7}$ 4.0, H-7a), 6.30 (1 H, d, $J_{5,6}$ 6.0, H-5), 6.31 (1 H, d, $J_{3,3a}$ 2.8, H-3), 6.63 (1 H, dd, $J_{6,7}$ 2.7, $J_{6,5}$ 7.0 H-6), 7.36–7.64 (10 H, m, ArH); *m*/*z* 352 (M^+, 4%), 286 (50), 255 (43), 84 (90), 43 (100).

(±)-2,4-Diphenyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *cis*-1,*syn*-8-dioxide 10G. A viscous gum (0.1 g, 3%); (Found $M^+ - SOH_2$ 302.0772. $C_{20}H_{14}OS$ requires 302.0765); R_f 0.28 (EtOAc); δ_H (500 MHz, $CDCl_3$) 4.49 (1 H, m, $J_{7,6}$ 4.2, H-7), 4.69 (1 H, dd, $J_{7a,3a}$ 7.6, $J_{7a,7}$ 4.2, H-7a), 5.40 (1 H, dd, $J_{3a,7a}$ 7.6, $J_{3a,3}$ 2.8, H-3a), 6.45 (2 H, m, $J_{6,7}$ 4.2, H-5 and H-6), 6.51 (1 H, d, $J_{3,3a}$ 2.8, H-3), 7.36–7.59 (10 H, m, ArH); *m*/*z* 352 (M^+, 0.1%), 302 (35), 286 (100), 105 (51).

(+)-(1S,2R)-1,2-Dihydroxy-3-(2-thienyl)-cyclohexa-3,5-diene 11. Colourless crystals (0.11 g, 3%), mp 74–76 °C (EtOAc); R_f 0.47 (50% EtOAc in hexane) (Found M^+ 194.0408. $C_{10}H_{10}SO_2$ requires 194.0402); $[\alpha]_D$ +231 (*c* 1.0, MeOH); δ_H (500 MHz, $CDCl_3$) 4.52 (1 H, d $J_{1,2}$ 5.9, H-2), 4.59 (1 H, m, H-1), 5.87 (1 H, dd, $J_{6,5}$ 9.6, $J_{6,1}$,2.2, H-6), 6.05 (1 H, m, H-5), 6.35 (1 H, d, $J_{4,5}$ 5.6, H-4), 7.02–7.24 (3 H, m, 2-Th–H); *m*/*z* 194 (M^+, 4%), 176 (80); electronic CD data (MeOH) λ/nm 236 ($\Delta\varepsilon$ − 5.16) and 358 ($\Delta\varepsilon$ 2.35); >98% ee [MEBBA derivative].

2-Thienyl methyl sulfide 1H. Substrate **1H** (0.17 g, 1.3 mmol) gave sulfoxide metabolite **12H**; purification by PLC (5% MeOH in $CHCl_3$) afforded (+)-(*R*)-2-thienyl methyl sulfoxide **12H** as an oil (0.078 g, 41%) (lit.,[42] bp 87–89 °C at 0.1 mmHg); $[\alpha]_D$ +72 (*c* 1.3, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 2.94 (3 H, s, Me), 7.13 (1 H, dd, $J_{4,3}$ 4.9, $J_{4,5}$ 3.7, H-4), 7.49 (1 H, dd, $J_{5,4}$ 3.6, $J_{5,3}$ 1.3, H-5), 7.65 (1 H, dd, $J_{3,4}$ 4.9, $J_{3,5}$ 1.2, H-3); *m*/*z* 146 (M^+, 33%), 131 (100); electronic CD data (MeCN) λ/nm 209 ($\Delta\varepsilon$ −8.54), 222 ($\Delta\varepsilon$ −8.84) and 248 ($\Delta\varepsilon$ 4.37). The enantiopurity (≥98%) of metabolite **12H**, with an excess of the (+)-enantiomer having a shorter retention time, was determined by CSP HPLC (Chiralcel OD, 25cm × 4.6mm, 10% propan-2-ol in hexane, 0.5 cm^3 min^{-1}, α 1.06).

2-Phenylthiothiophene 1I. Substrate **1I** (0.2 g, 1 mmol) gave metabolite (+)-2-phenylsulfinylthiophene **12I**. The crude sample was purified by PLC (1% MeOH in $CHCl_3$) to yield sulfoxide **12I** (0.038 g, 18%), R_f 0.3; $[\alpha]_D$ +25 (*c* 1.8, $CHCl_3$) (Found M^+ 208.0026. $C_{10}H_8S_2O$ requires 208.0017); δ_H (500 MHz, $CDCl_3$) 7.06 (1 H, dd, *J* 3.6, *J* 5.0, Ar–H), 7.51 (3 H, m, Ar–H), 7.58 (2 H, m, Ar–H), 7.70 (2 H, m, Ar–H) δ_C (125 MHz, $CDCl_3$) 124.25, 126.99, 127.18, 128.89, 129.15, 130.82, 131.09, 132.31, 145.01, 147.97; *m*/*z* 208 (M^+, 16%), 160 (100). The ee (58%) of sulfoxide **12I** was determined by CSP HPLC (Chiralcel OD, 25 cm × 4.6 mm, 10% propan-2-ol in hexane, 0.5 cm^3 min^{-1}, α 1.14).

2-(2-Thienylthio)thiophene 1J. Substrate **1J** (0.1 g, 0.47 mmol) gave metabolite, 2-(2-thienylsulfinyl)thiophene **12J** as a crude product, which was purified by PLC ($CHCl_3$) to yield purified sulfoxide **12J** as an oil (0.056 g, 52%), R_f 0.25 (3% MeOH in $CHCl_3$) (Found M^+ 213.9593. $C_8H_6S_3O$ requires 213.9581); δ_H (500 MHz, $CDCl_3$) 7.08–7.16 (2 H, m, Ar–H), 7.44 (1 H, dd, *J* 1.3, *J* 3.8, Ar–H), 7.55 (1 H, dd, *J* 1.2, *J* 3.7, Ar–H), 7.62 (1 H, dd, *J* 1.2, *J* 5.0, Ar–H), 7.87 (1 H, dd, *J* 1.2, *J* 3.0, Ar–H); δ_C (125 MHz, $CDCl_3$) 123.75, 126.40, 127.38, 128.34, 131.10, 132.26, 144.45, 147.29; *m*/*z* 214 (M^+, 8%), 198 (12), 166 (100).

Thiophene 1A and 2-chlorothiophene 1D. A mixture of thiophene **1A** (0.10 g, 1.2 mmol) and 2-chlorothiophene **1D** (0.14 g, 1.0 mmol) as substrate yielded a mixture of bioproducts, (±)-2,4-dichloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7D** (0.045 g, 33%), (±)-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7A** (0.031 g, 26%) and (±)-4-chloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **14** (0.023 g, 9%) which were separated by PLC ($CHCl_3$). Disulfoxides **7A** and **7D** were spectrally identical to the metabolite samples isolated earlier from the biotransformation of thiophenes **1A** and **1D** respectively as individual substrate.

(±)-4-Chloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 14. Mp110–112 °C decomp. (from $CHCl_3$–hexane); R_f 0.38 (5% MeOH in $CHCl_3$) (Found: C, 40.8; H, 3.2. $C_8H_7O_2S_2Cl$ requires C, 41.0; H, 3.0%); δ_H (500 MHz, $CDCl_3$) 4.07 (1 H, ddd, $J_{7,6}$ 5.1, $J_{7,7a}$ 3.9, $J_{7,5}$ 1.3, H-7), 4.38 (1 H, dd, $J_{3a,7a}$ 7.9, $J_{3a,3}$ 2.7, H-3a), 4.87 (1 H, dd, $J_{7a,3a}$ 8.0, $J_{7a,7}$ 4.1, H-7a), 5.98 (1 H, dd, $J_{5,6}$ 6.2, $J_{5,7}$ 0.9, H-5), 6.45–6.50 (2 H, m, H-3 and H-6), 6.65 (1 H, dd, $J_{2,3}$ 6.1, $J_{2,3a}$ 1.8, H-2); δ_C (125MHz, $CDCl_3$) 60.7, 61.9, 62.1, 89.6, 127, 134.5, 128.5, 138.6; *m*/*z* 234 (M^+, 4%), 151 (50), 134 (100).

2-Methylthiophene 1B and 2-chlorothiophene 1D. A mixture of thiophenes **1B** (0.1 g, 1 mmol) and **1D** (0.140 g, 1 mmol) as substrate yielded cycloadduct metabolites, **7B**, **7D** along with cross cycloadducts **15** and **16**. Disulfoxides **7B** (0.011 g, 5%) and **7D** (0.016 g, 6%), separated from the crude mixture by flash column chromatography ($CHCl_3 \longrightarrow$ 10% MeOH in $CHCl_3$) followed by PLC (2% MeOH in $CHCl_3$), were spectrally identical to the disulfoxides, of thiophenes **1B** and **1D** respectively, isolated earlier from the biotransformation of the individual substrate. The mixture of cross cycloadducts **15** and **16** was separated by HPLC (Phenomenex C-18 column, 25 cm × 10 mm; MeOH : H_2O 1 : 1; 2 cm^3 min^{-1}, α 1.4).

(±)-2-Chloro-4-methyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 15. Cycloadduct **15** (0.010 g, 4%) was collected, as an early eluting fraction, from HPLC, mp 135 °C (Found M^+ 247.9728. $C_9H_9O_2S_2Cl$ requires 247.9732); δ_H (500 MHz, $CDCl_3$) 1.65 (3 H, s, Me), 3.99 (1 H, dd, $J_{3a,3}$ 2.9, $J_{3a,7a}$ 7.8, H-3a), 4.12 (1 H, m, H-7), 4.87 (1 H, dd, $J_{7a,7}$ 3.9, $J_{7a,3a}$ 7.8, H-7a), 5.85 (1 H, d, $J_{5,6}$ 6. 8, H-5), 6.23 (1 H, d, $J_{3,3a}$ 2.9, H-3), 6.41 (1 H, dd, $J_{6,7}$ 4.7, $J_{6,5}$ 6.8, H-6); *m*/*z* 249 (M^+, 25%), 248 (40), 184 (54), 183 (74), 182 (86), 165(86), 149 (65), 147 (92), 117 (64), 116 (87), 115(54).

(±)-2-Methyl-4-chloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 16. Cycloadduct **16** (0.008 g, 3%) was collected as a late eluting fraction from HPLC, mp >250 °C decomp. (Found M^+ 247.9727. $C_9H_9O_2S_2Cl$ requires 247.9732); δ_H (500 MHz, $CDCl_3$) 2.13 (3 H, s, Me), 4.08 (1 H, m, $J_{3a,7a}$ 8.1, H-3a), 4.27 (1 H, m, $J_{7,7a}$ 4.1, H-7), 4.85 (1 H, dd, $J_{7a,7}$ 4.1, $J_{7a,3a}$ 8.1, H-7a), 5.97 (1 H, d, $J_{5,6}$ 7.0, H-5), 6.01 (1 H, br s, H-3), 6.40 (1 H, dd, $J_{6,7}$ 5.2, $J_{6,5}$ 7.0, H-6); *m*/*z* 249 (M^+, 3%), 200 (30), 199 (46), 184 (41), 182 (69), 149 (74), 147 (76), 117 (72), 115 (54), 89 (47), 77 (100), 75 (37).

2-Methylthiophene 1B in the presence of methylvinyl ketone. Substrate 2-methylthiophene **1B** (0.2 g, 2.0 mmol), in the presence of methyl vinyl ketone (0.28 g, 4 mmol), yielded a crude sample of cycloadduct (±)-6-acetyl-1-methyl-7λ^4-thiabicyclo[2.2.1]hepta-2-en-7-one **17**, (0.015 g, 4%). Purification by PLC, R_f 0.4, (2% MeOH in $CHCl_3$) gave a purified sample of cycloadduct **17**, mp 88–90 °C (from Et_2O) (Found: C, 58.5; H, 6.6. $C_9H_{12}O_2S$ requires C, 58.7; H, 6.5%) (Found M^+ 184.0551. $C_9H_{12}O_2S$ requires 184.0558); δ_H (500 MHz, $CDCl_3$) 1.60 (3 H, s, Me), 1.68 (1 H, dd, $J_{6,5}$ 6.1, $J_{6,5'}$ 4.9, H-6), 1.82 (3 H, s, COMe), 2.93 (1 H, ddd, $J_{5,6}$ 6.1, $J_{5,5'}$ 10.3, $J_{5,4}$ 3.6, H-5), 3.58 (1 H, dd, $J_{5',5}$ 10.3, $J_{5',6}$ 4.9 H-5′), 3.61 (1 H, dd, $J_{4,5}$ 3.6, $J_{4,3}$ 6.5, H-4), 6.13 (1 H, dd, $J_{3,2}$ 3.6, $J_{3,4}$ 6.5, H-3), 6.14 (1 H, d, $J_{2,3}$ 3.6, H-2); *m*/*z* 184 (M^+, 9%), 136 (82), 121 (40), 92 (100), 43 (83).

Crystal data for 17. $C_9H_{12}O_2S$, M_r = 184.3, monoclinic, a = 11.1658(9), b = 6.6249(5), c = 13.2296(10) Å, β = 111.526(1), V = 910.37(12) Å^3, T = 150 K, Mo–Kα radiation, λ = 0.71073 Å, space group $P2_1/n$, Z = 4, $F(000)$ = 392, D_x = 1.34 g cm^{-3}, μ = 0.31 mm^{-1}, colourless prisms 0.25 × 0.20 × 0.20 mm, Bruker SMART CCD diffractometer, ϕ/ω scan, 4.1 <2θ <56.65°, measured/independent reflections: 9434/2063, semi-empirical absorption correction (SADABS), direct methods solution, full matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms, hydrogens located in difference Fourier but included at positions determined by the geometry of the molecule using the riding model with isotropic vibration parameters, R_1 = 0.091 for 1794 data with F_o >4$\sigma(F_o)$, 111 parameters, wR_2 = 0.276 (all data), GoF = 0.95, $\Delta\rho_{min,max}$ = −0.89/1.61 e Å^{-3}, CCDC reference number 192256, see http://www.rsc.org/suppdata/ob/b3/b300867n/ for crystallographic files in .cif or other electronic format.

2-Methylthiophene 1B in presence of *N*-methylmaleimide. Substrate 2-methylthiophene **1B** (0.2 g, 2 mmol) in the presence of *N*-methylmaleimide (0.3 g, 2.7 mmol) gave cycloadduct, (±)-1,4-dimethyl-10-oxo-10λ^4-thia-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione **18**, (0.013 g, 3%). The crude product was purified by PLC, R_f 0.8 (5% MeOH in $CHCl_3$) to yield cycloadduct **18** as a semisolid (Found M^+ 225.0453. $C_{10}H_{11}NO_3S$ requires 225.0459); δ_H (500 MHz, $CHCl_3$) 1.78 (3 H, s, Me), 2.92 (3 H, s, NMe), 3.65 (1 H, d, $J_{2a,6a}$ 8.1, H-2a), 4.10 (2 H, m, H-6 and H-6a), 6.12 (1 H, d, $J_{4,3}$ 7.0, H-4), 6.20 (1 H, dd, $J_{3,4}$ 7.0 $J_{3,6}$ 4.8, H-5); *m*/*z* 225 (M^+, 20%), 177 (93), 156 (43), 113 (35).

2,5-Dichlorothiophene 19. Substrate **19** (0.3 g, 2.0 mmol) yielded disulfoxide cycloadduct, (±)-2,4,7,7a-tetrachloro-*cis*-3a-hydro-4,7,7a-trichloro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **20**; purification by PLC ($CHCl_3$) gave the metabolite (0.009 g, 3%), mp 115–118 °C decomp. (from $CHCl_3$–hexane); R_f 0.42 (10% MeOH in $CHCl_3$) (Found: C 28.0, H, 1.0. $C_8H_4O_2S_2Cl_4$ requires C, 28.4; H, 1.2%); δ_H (500 MHz, $CDCl_3$) 4.88 (1 H, d, $J_{3a,3}$ 3.3, H-3a), 6.49 (2 H, s, H-5 and H-6), 6.55 (1 H, d, $J_{3,3a}$ 3.4, H-3); *m*/*z* 338 (M^+, 1%), 290 (4).

3-Methylthiophene 1K. Substrate **1K** (24.4 g, 0.25mol, 7h) yielded a mixture of three compounds. Purification by flash chromatography ($CHCl_3 \longrightarrow$ 5% MeOH in $CHCl_3$), afforded an inseparable *cis*/*trans* mixture of 4-methyl-2,3-dihydroxy-2,3-dihydrothiophenes **9K**$_{cis}$/**9K**$_{trans}$ (3.6 g, 11%) and a mixture of two other metabolites. Further PLC (Et_2O) separation of the latter mixture afforded pure samples of the monosulfoxide cycloadduct, 3,5-dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide **8K** (0.7 g, 4%), and the thiolactone, 3-hydroxy-4-methyl-5*H*-thiophen-2-one **23** (R = H, R′ = Me) (0.6 g, 2%).

4-Methyl-2,3-dihydroxy-2,3-dihydrothiophene 9K$_{cis}$/9K$_{trans}$. R_f 0.25 (5% MeOH in $CHCl_3$) (Found M^+ 132.0249. $C_5H_8SO_2$ requires 132.0245); *m*/*z* 132 (M^+, 72), 114 (10) and 71 (100); $[\alpha]_D$ + 18.6 (*c* 1.85, $CHCl_3$, 60% *cis* isomer) and $[\alpha]_D$ +44.8 (*c* 1.8, MeOH, 80% *trans* isomer).

(2*R*,3*S*)-4-Methyl-2,3-dihydroxy-2,3-dihydrothiophene 9K$_{cis}$. δ_H (500 MHz, $CDCl_3$) 1.84 (3 H, s, Me), 4.54 (1 H, d, $J_{3,2}$ 5.6, H-3), 5.55 (1 H, d, $J_{2,3}$ 5.6, H-2), 5.85 (1 H, s, H-5).

(2*S*,3*S*)-4-Methyl-2,3-dihydroxy-2,3-dihydrothiophene 9K$_{trans}$. δ_H (500 MHz, CD_3OD) 1.89 (3 H, s, Me), 4.52 (1 H, s, H-3), 5.41 (1 H, s, H-2), 5.96 (1 H, s, H-5).

3-Hydroxy-4-methyl-5*H*-thiophen-2-one 23(R = H, R′ = Me). Mp 32–34 °C (from CH_2Cl_2–hexane) (lit.[37] 32 °C); R_f 0.67 (Et_2O); δ_H (300 MHz, $CDCl_3$) 2.00 (H, d, $J_{Me,5}$ 1.0, Me), 3.65 (2 H, d, $J_{5,Me}$ 1.0, H-5), 6.28 (1 H, br s, OH); *m*/*z* 130 (M^+, 100%).

(−)-3,5-Dimethyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *syn*-8-oxide 8K. Mp 114–116 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ −19.1 (*c* 0.56, $CHCl_3$); R_f 0.37 (Et_2O) (Found: C, 56.7; H, 5.6; $C_{10}H_{12}OS_2$ requires C, 56.6; H, 5.7); δ_H (500 MHz, $CDCl_3$) 1.80 (6 H, s, 2Me), 3.92 (1 H, d, $J_{4,3a}$ 3.6, H-4), 4.02–4.04 (1 H, m, H-7); 4.17–4.21 (1 H, m, H-3a), 4.76 (1 H, dd, $J_{7a,7}$ 3.9, $J_{7a,3a}$ 10.0, H-7a); 5.56 (1 H, d, $J_{2,3a}$ 1.4, H-2), 5.72 (1 H, d, $J_{6,7}$ 3.9, H-6); 51% ee, by CSP HPLC using a Chiralpak AD column (10% propan-2-ol–hexane, 0.6 cm^3 min^{-1}, α 1.12).

3-Chlorothiophene 1L. Substrate **1L** (0.5g, 4.2 mmol) yielded a mixture of two compounds. Purification by flash chromatography, initially eluting with $CHCl_3$, afforded a pure sample of the isomeric mixture of 4-chloro-2,3-dihydroxy-2,3-dihydrothiophene **9L**$_{cis}$/**9L**$_{trans}$ (0.082 g, 9%), followed by elution with 5% MeOH in $CHCl_3$ yielded (±) 3,5-dichloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7L** (0.074 g, 13%).

4-Chloro-2,3-dihydroxy-2,3-dihydrothiophene $9L_{cis}/9L_{trans}$. A viscous oil, R_f 0.25 (5% MeOH in $CHCl_3$) (Found M^+ 151.970072, $C_4H_5SO_2Cl$ requires 151.969879); *m*/*z* 152 (M^+ (^{35}Cl), 90%), 123 (70) and 91 (100); $[\alpha]_D$ −55 (*c* 1.8, $CHCl_3$, 65% *cis* isomer) and $[\alpha]_D$ −209 (*c* 1.5 MeOH, 83% *trans* isomer).

(2*S*,3*S*)-4-Chloro-2,3-dihydroxy-2,3-dihydrothiophene $9L_{cis}$. δ_H (500 MHz, $CDCl_3$) 4.64 (1 H, d, $J_{3,2}$ 5.7, H-3), 5.66 (1 H, d, $J_{2,3}$ 5.7, H-2), 6.26 (1 H, s, H-5).

(2*R*,3*S*)-4-Chloro-2,3-dihydroxy-2,3-dihydrothiophene $9L_{trans}$. δ_H (500 MHz, CD_3OD) 4.46 (1H, s, H-3), 4.84 (2H, br s, 2 × –OH), 5.36 (1H, s, H-2), 6.39 (1H, s, H-5).

(±)-3,5-Dichloro-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7,epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7L. Mp 149–150 °C decomp. (from $CHCl_3$–hexane); R_f 0.4 (5% MeOH in $CHCl_3$) (Found: C 35.3, H 2.3, $C_8H_6S_2O_2Cl_2$ requires C 35.7, H 2.2); δ_H (500 MHz, $CDCl_3$) 4.19 (1 H, m, H-7), 4.26 (1 H, m, H-4), 4.48 (1 H, dd, $J_{3a,4}$ 3.9, $J_{3a,7a}$ 7.8, H-3a), 4.84 (1 H, dd, $J_{7a,3a}$ 7.8, $J_{7a,7}$ 3.8, H-7a), 6.41 (1 H, dd, $J_{6,7}$ 4.9, $J_{6,7a}$ 1.7, H-6), 6.61 (1 H, d, $J_{2,3a}$ 1.0, H-2); *m*/*z* 268 (M^+, 40%) 220 (82), 168 (95), 136 (100).

3-Bromothiophene substrate 1M. (0.5 g, 3.1 mmol) yielded a mixture of two compounds. Purification by PLC (5% MeOH in $CHCl_3$) afforded samples of 4-bromo-2,3-dihydroxy-2,3-dihydrothiophene $9M_{cis}/9M_{trans}$ (0.086 g, 14%) and 3,5-dibromo-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7M** (0.069 g, 12%).

4-Bromo-2,3-dihydroxy-2,3-dihydrothiophene $9M_{cis}/9M_{trans}$. R_f 0.22 (5% MeOH in $CHCl_3$) (Found M^+ 195.919306, $C_4H_5SO_2Br$ requires 195.919362); *m*/*z* 198 (M^+ 38%), 169 (30), 137 (42) and 84 (100).

(−)-(2*S*,3*S*)-4-Bromo-2,3-dihydroxy-2,3-dihydrothiophene $9M_{cis}$. $[\alpha]_D$ −36 (*c* 1.9, $CHCl_3$, 63% *cis* isomer); δ_H (500 MHz, $CDCl_3$) 3.3 (2 H, br s, 2 OH), 4.6 (1 H, d, $J_{3,2}$ 5.6, H-3), 5.6 (1 H, d, $J_{2,3}$ 5.7, H-2), 6.3 (1 H, s, H-5).

(−)-(2*R*,3*S*)-4-Bromo-2,3-dihydroxy-2,3-dihydrothiophene $9M_{trans}$. $[\alpha]_D$ −146 (*c* 1.9, MeOH, 83% *trans* isomer); δ_H (500 MHz, CD_3OD) 4.48 (1H, s, H-3), 4.81 (2H, br s, 2 × OH), 5.36 (1H, s, H-2), 6.52 (1H, s, H-5).

(±)-3,5-Dibromo-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7M. Mp 135–137 °C decomp. (from $CHCl_3$–hexane); R_f 0.35 (5% MeOH in $CHCl_3$) (Found M^+ 357.814265, $C_8H_6S_2O_2Br_2$ requires 357.8155490); δ_H (500 MHz, $CDCl_3$) 4.1 (1 H, m, H-7), 4.2 (1 H, m, H-4), 4.5 (1 H, ddd, $J_{3a,7a}$ 7.8, $J_{3a,4}$ 3.8, $J_{3a,2}$ 1.5, H-3a), 4.7 (1 H, dd, $J_{7a,3a}$ 7.8, $J_{7a,7}$ 3.9, H-7a), 6.5 (1 H, dd, $J_{6,7}$ 4.9, $J_{6,4}$ 1.8, H-6), 6.7 (1 H, d, $J_{2,3a}$ 1.5, H-2); *m*/*z* 358 (M^+, 24%) 310 (36), 212 (47), 150 (72), 102 (100).

3-Phenylthiophene 1N. Substrate **1N** (5.0 g, 31.3 mmol) yielded a mixture of three metabolites. Purification by flash chromatography (25% EtOAc in hexane ⟶ 100% EtOAc), afforded an inseparable *cis*/*trans* mixture of 4-phenyl-2,3-dihydroxy-2,3-dihydrothiophenes $9N_{cis}/9N_{trans}$ (1.1 g, 18 %), 3,5-diphenyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide **7N** (0.7 g, 13%) and 1,2-dihydroxy-3-(3-thienyl)-cyclohexa-3,5-diene **25** (0.4 g, 6.5%).

4-Phenyl-2,3-dihydroxy-2,3-dihydrothiophene $9N_{cis}/9N_{trans}$. Viscous oil, R_f 0.50 (50% EtOAc in hexane) (Found M^+ 194.0408. $C_{10}H_{10}SO_2$ requires 194.0402); *m*/*z* 194 (M^+, 72), 176 (93), 147 (100), 102 (93) and 86 (84); $[\alpha]_D$ −78 (*c* 1.1, $CHCl_3$, 55% *cis* isomer) and $[\alpha]_D$ −277 (*c* 0.9, MeOH, 92 % *trans* isomer).

(2*S*,3*R*)-4-Phenyl-2,3-dihydroxy-2,3-dihydrothiophene $9N_{cis}$. δ_H (500 MHz, $CDCl_3$) 5.15 (1 H, s, H-3), 5.46 (1 H, s, H-2), 6.77 (1 H, s, H-5), 7.24–7.49 (5H, m, Ar–H).

(2*R*,3*R*)-4-Phenyl-2,3-dihydroxy-2,3-dihydrothiophene $9N_{trans}$. δ_H (500 MHz, $CDCl_3$) 4.95 (1 H, d, $J_{3,2}$ 5.3, H-3), 5.41 (1 H, s, H-2), 5.88 (1 H, d, $J_{2,3}$ 5.3, H-2) 6.65 (1H, s, H-5) 7.24–7.49 (5H, m, Ar–H).

(±)-3,5-Diphenyl-*cis*-3a,4,7,7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene *trans*-1,*syn*-8-dioxide 7N. White crystals, mp 168–170 °C (from $CHCl_3$) (Found M^+ 352.0573. $C_{20}H_{16}O_2S_2$ requires 352.0592); R_f 0.15 (EtOAc); δ_H (500 MHz, $CDCl_3$) 4.33 (1 H, d, $J_{7,6}$ 2.3, H-7), 4.53 (1 H, s, H-3a), 5.09 (2 H, m, H-4 and H-7a), 6.61 (1 H, s, H-2), 6.69 (1 H, dd, $J_{6,7}$ 2.3, $J_{6,4}$ 0.9, H-6), 7.00–7.40 (10 H, m, Ar–H); *m*/*z* 352 (M^+, 4%), 304 (36), 286 (24), 254 (10), 154 (8), 44 (100).

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(3-thienyl)-cyclohexa-3,5-diene 25. Colourless crystals, mp 112–113 °C (from EtOAc–hexane); R_f 0.2 (75% EtOAc in hexane) (Found M^+ 194.0403. $C_{10}H_{10}SO_2$ requires 194.0402); $[\alpha]_D$ +209 (*c* 0.7, MeOH); δ_H (500 MHz, $CDCl_3$) 1.85 (1H, br s, OH), 2.56 (1H, br s, OH), 4.48 (1 H, m, $J_{1,2}$ 5.6, H-2), 4.59 (1 H, dd, $J_{2,1}$ 5.6, $J_{2,6}$ 2.5 H-1), 5.86 (1 H, dd, $J_{6,5}$ 9.6, $J_{6,1}$ 2.5, H-6), 6.06 (1 H, m, $J_{5,6}$ 9.6, $J_{5,4}$ 5.6, H-5), 6.34 (1 H, d, $J_{4,5}$ 5.6, H-4), 7.26–7.42 (3 H, m, Ar–H); *m*/*z* 194 (M^+, 37%), 176 (94),147 (97) 131(96), 115 (100); electronic CD data (MeOH) λ/nm 223 ($\Delta\varepsilon$ −3.71) and 315 ($\Delta\varepsilon$ 2.49); >98% ee [MEBBA derivative].

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