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Structure–activity relationships in nitrothiophenes

John O. Morley* and Thomas P. Matthews†

Chemistry Department, University of Wales Swansea, Singleton Park, Swansea, Swansea SA2 8PP, UK

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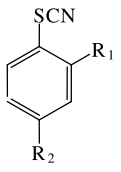
Abstract—The structure and electronic properties of a series of biologically active 2-nitrothiophenes (**1**) have been calculated using both semi-empirical and ab initio molecular orbital methods. Multi-linear regression analysis suggests that there is a reasonable correlation between the experimental activity of the derivatives against either *Escherichia coli* or *Micrococcus luteus* and calculated properties such as the HOMO energies, the total atomic charges and ring angles at the heterocyclic sulfur atom, but there is no correlation with the calculated solvation energies or dipole moments. The presence or absence of an additional nitro group at the 3-position of the ring also has a significant effect on the activity. From the derived QSAR equations, the 2-chloro- or 2-bromo-3,5-dinitrothiophenes (**1a** and **1c**) are predicted to show the highest activity against both bacteria, while 2-nitrothiophene (**1n**) is predicted to be the least active, in line with the experimental results.

1. Introduction

It is well established that many organosulfur compounds have a pronounced biological activity *inter alia* as antibiotics, analgesics, antidepressants, anti-inflammatory agents, fungicides and bactericides.[1–7] Their biological activity, however, depends not only on the presence of one or more sulfur atoms in the molecule but also on the presence of additional activating groups. For example, 2-nitrothiophene (**1s**) shows virtually no activity against either the bacteria *Escherichia coli* (*E. coli*) and *Micrococcus luteus* (*M. luteus*) or the fungus *Aspergillus niger* (*A. niger*), but 2-chloro-3,5-dinitrothiophene (**1a**) is three orders of magnitude more active.[3]

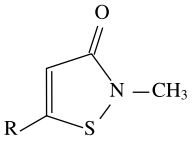
Similarly, the fungicidal activity of phenylthiocyanate (**2a**) against *A. niger* is substantially enhanced by the presence of electron withdrawing substituents in the aromatic ring so that the 2,4-dinitro derivative (**2b**) has been patented as a potent antifungal agent.[4] Antibacterials such as the 3-isothiazolones (**3**) are highly effective against *E. coli* and *Staphylococcus aureus* but again the relative efficacy depends on the presence of additional substituents attached to the heterocyclic ring.[5,6]. Thus, 5-chloro-*N*-methyl-3-isothiazolone (**3a**) is several orders of magnitude more active than the simpler *N*-methyl derivative (**3b**).[7]



(**2**)

a $R_1 = R_2 = R_3 = H$

b $R_1 = R_2 = NO_2$; $R_3 = H$



(**3**)

a R = Cl

b R =H

In all these cases, the biological activity of the molecules is thought to arise from their ability to initially diffuse through the membranes of bacteria or the fungal cell walls and then react with important intracellular sulfur containing proteins, or simpler thiols inside the cell, causing the cell function to be impaired.[8–11] As far as the simple thiophenes (**1**) are concerned, the most active molecules require electron attracting nitro groups to enhance activity not only against bacteria and fungi,[3] but also against other organisms.[12–18] For example, 2,4-dinitrothiophene (**1h**) and related derivatives are potent insecticides,[14] 2-methylamino-3,5-dinitrothiophene (**1t**) is an effective marine anti-fouling agent,[15] 2-acetyl-3,5-dinitrothiophene (**1u**) shows pronounced antibiotic properties[16,17] and 2,4-dinitro-5-methylsulfonylthiophene (**1v**) shows broad spectrum activity.[18]

Keywords: QSAR; Nitrothiophenes; *E. coli*; MO calculations.
* Corresponding author. Tel.: +1792 295861; fax: +1792 295747; e-mail: j.o.morley@swansea.ac.uk
† Present address: The Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK.

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We have recently synthesised and assessed the biological activity of nineteen diverse 2-nitrothiophenes (**1**), by identifying the minimum inhibitory concentrations required to inhibit actively growing *E. coli*, *M. luteus* and *A. niger* cultures using agar diffusion techniques.[3] The series displays a wide range of activities with 2-chloro-3,5-dinitrothiophene (**1a**) or 2-bromo-3,5-dinitrothiophene (**1c**) showing the highest activity against all three organisms, while the simplest compound of the series, 2-nitrothiophene (**1s**), shows the smallest activity in each case.[3] Their mode of action is thought to involve attack by intracellular thiols on the heterocyclic ring at the 2-position to form covalently bound cellular species but the precise mechanisms involved have not been established.[3] In these studies, we have calculated the structures and properties of all these molecules in an attempt to develop a quantitative structure–activity relationship (QSAR) which could be used to rationalise their behaviour against the two bacteria and would be useful for predictive purposes.

2. Methods of calculation

Molecular orbital calculations were carried out on empirical structures for the nitrothiophenes using the MNDO,[19] AM1[20] and PM3[21] methods of the MOPAC93 Program[22] with full optimisation of all bond lengths, angles and torsion angles in cartesian space (keywords: prec mndo xyz ef nomm). The effect of water on the structures and energies were assessed using the COSMO method[23] incorporated in the MOPAC 93 program (keywords: prec mndo xyz ef eps = 78.4). A series of reference calculations were carried out also at the ab initio RHF level using the 6-31G** basis set of the GAMESS program[24] to check the validity of the semi-empirical results (directives: runtype optxyz, scftype rhf). To ensure that the structures were properly optimised, the nitro groups present were twisted initially by 15° from the plane of the thiophene ring. Molecules and crystal structures were displayed and analysed using the SYBYL Molecular Modelling package.[25] Multi-linear regression analysis between the calculated properties and the experimental activity was carried out using the SPSS package.[26]

3. Results and discussion

The minimum inhibitory concentrations of all nineteen nitrothiophenes (**1a**–**s**) which we recently determined[3] against *E. coli* and *M. luteus* are shown in Scheme 1. Overall, we found that fifteen of the nitrothiophenes inhibited the growth of the Gram-negative bacteria, *E. coli*, and seventeen inhibited the growth of the Gram-positive bacteria *M. luteus*. An analysis of the results shows that 2-chloro-3,5-dinitrothiophene (**1a**) and 2-bromo-3,5-dinitrothiophene (**1c**) are the most active against *E. coli* and *M. luteus*, respectively, while the simplest compound of the series, 2-nitrothiophene (**1s**), is the least active against the two bacteria (Scheme 1). The high biological activity appears to be associated with the presence of two nitro groups in the heterocyclic ring, but there are notable exceptions such as 5-nitrothiophene-2-carbaldehyde (**1d**) and 5-nitrothiophene-2-acrylaldehyde (**1g**) which are both more active against *E. coli* than 2,4-dinitro-2-thiocyanatothiophene (**1k**) or 2-amino-3,5-dinitrothiophene (**1m**) (Table 1). Consequently, the activity of the nitrothiophenes does not appear to relate simply to the number of electron attracting groups present in the ring. Furthermore, there are also wide differences found between the activities of some of the derivatives against both *E. coli* and *M. luteus* suggesting that the mode of action may differ for Gram-negative and Gram-positive bacteria, respectively, perhaps because of differences in the structure of their cell walls. For example, 5-nitrothiophene-2-carbaldehyde (**1d**) is highly active against *E. coli* but an order of magnitude less active against *M. luteus*, while 2,4-dinitro-5-thiocyanatothiophene (**1k**) is highly active against *M. luteus* but an order of magnitude less active against*E. coli* (Scheme 1). There is no overall obvious pattern to the results and accordingly we have attempted to relate the biological activity of these molecules to a combination of calculated physical and/or chemical properties.

As we have observed previously,[7] most QSAR treatments relate the biological response of a molecule to its distribution or partition between the aqueous and membrane phases of the cell which is represented by water and octanol.[27] In the simplest case,[27] the biological activity of a series of compounds is related to a hydrophobic substituent coefficient π defined as:

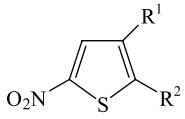
$$\pi = \log P_X - \log P_H \quad (1)$$

where P_H is the partition coefficient of the parent species and P_X is the partition coefficient of a derivative in the two solvents. For many aromatic systems, the overall biological response has additionally been attributed to electronic interactions[27] such as the Hammet substituent constant, σ,[28,29] and steric factors such as the Taft steric substituent, E_s,[29,30] so that the biological response is related to at least three parameters.[31–33] Here, the minimum inhibitory concentration, C, of an active aromatic molecule is related to experimental data by the equation[31,32]:

$$\log 1/C = a\pi + b\sigma + cE_s + \text{constant} \quad (2)$$

where π, σ and E_s are the hydrophobic, electronic and steric substituent parameters or properties respectively, and a, b and c are coefficients fitted by regression analysis.

There have been many attempts to relate the calculated structures and electronic properties of a range of organic molecules to their biological activity.[33–40] Errors arising from the approximate nature of semi-empirical methods and the absence of solvation effects in the calculations appear to be transferable within structurally related series so that the calculated properties can be meaningful.[34,35] Good correlations have been reported between calculated properties and biological properties such as enzyme inhibition activity and hallucinogenic activity among others.[36–40] Among the theoretical properties which have been described in the literature are the



1

Substituents	*E.coli*	*M.luteus*
3		
	log1/C	log1/C
a $R^1 = NO_2$; $R^2 = Cl$	3.97	3.94
b $R^1 = NO_2$; $R^2 = OPh$	3.78	3.80
c $R^1 = NO_2$; $R^2 = Br$	3.69	4.11
d $R^1 = H$; $R^2 = CHO$	3.54	2.59
e $R^1 = NO_2$; $R^2 = OMe$	3.47	2.77
f $R^1 = NO_2$; $R^2 = NHCOCH_3$	3.36	3.18
g $R^1 = H$; $R^2 =$ CH:CHCHO	3.22	2.87
h $R^1 = NO_2$; $R^2 = H$	3.20	2.58
i $R^1 = NO_2$; $R^2 = SPh$	2.90	3.09
j $R^1 = NO_2$; $R^2 = SC(CH_3)_3$	2.78	2.30
k $R^1 = NO_2$; $R^2 = SCN$	2.70	3.70
l $R^1 = NO_2$; $R^2 = SO_2C(CH_3)_3$	2.42	3.49
m $R^1 = NO_2$; $R^2 = NH_2$	2.36	2.65
n $R^1 = H$; $R^2 = Br$	1.82	1.77
o $R^1 = NO_2$; $R^2 = N{:}NC_6H_4N(C_2H_4CO_2CH_3)_2$	<2.00	2.64
p $R^1 = NO_2$; $R^2 = NHCOPh$	<1.00	-
q $R^1 = NO_2$; $R^2 = NHPh$	<1.00	<2.00
r $R^1 = H$; $R^2 = Cl$	<1.00	<1.00
s $R^1 = R^2 = H$	<1.00	<1.00
t $R^1 = NO_2$; $R^2 = NHMe$		
u $R^1 = NO_2$; $R^2 = COCH_3$		
v $R^1 = NO_2$; $R^2 = SO_2CH_3$		

Scheme 1. Structures of the thiophenes (**1**) and minimum inhibitory concentrations (Ref. 3) in the form $\log 1/C$ where C is the minimum inhibitory concentration (mol L^{-1}).

HOMO and LUMO energies, atomic charges, orbital electron densities, dipole moments, superdelocalizabilities, atom-atom and molecular polarizabilities, polarity indices and molecular energies including heats of formation, ionization potentials and electron affinities.[34] Recently, we have reported a good correlation between the biological activity of a series of structurally diverse 3-isothiazolones and their calculated solvation energies.[7]

4. Mode of action

It is not known how specific organosulfur compounds disable the cells of bacteria, but is well established that low molecular weight thiols, such as glutathione (GSH), and reactive protein thiols such as cysteine participate in cellular anti-oxidant processes.[41–44] While glutathione (GSH) is abundant in cytoplasm, nuclei and mitochondria (3–10 mM), reactive protein thiols are abundant in both soluble proteins and in membrane-bound proteins. There is little doubt that the sulfur atom in these species easily accommodates the loss of a single electron, and the thiol groups can also partially ionize to produce the more reactive and strongly nucleophilic thiolate anion, for example,

$$\text{G-SH} \rightleftharpoons \text{G-S}^- + \text{H}^+$$

This is true for cysteine which partially ionizes at neutral or cellular pH, but the high pKa of glutathione at 9.3 means there is very little of the anion at pH 7. Despite this, the thiolate anion appears to be responsible for the reactivity of cellular thiols during xenobiotic metabolism where, for example, glutathione transferases bind to glutathione in such a way that the sulfur is induced to ionize more completely and then react with xenobiotic materials, such as toxins and drugs.[41–44]

Table 1. Geometries, electronic properties and frontier orbital energies of the nitrothiophenes (**1**) and the Meisenheimer complex (**4h-2**) calculated at either the semi-empirical and/or 6-31G** levels versus X-ray data and experimental activity against *E. coli*[a]

	1a					**1h**	**4h-2**	**1m**	**1s**
	AM1	PM3	MNDO	6-31G**	X-ray[b]	6-31G**	6-31G**	6-31G**	6-31G**
S1–C2	1.660	1.719	1.673	1.719	1.708	1.703	1.843	1.737	1.718
C2–C3	1.404	1.393	1.387	1.359	1.366	1.350	1.504	1.386	1.350
C3–C4	1.428	1.429	1.445	1.428	1.409	1.422	1.379	1.423	1.417
C4–C5	1.392	1.380	1.387	1.339	1.362	1.343	1.365	1.339	1.347
C5–S1	1.682	1.739	1.686	1.727	1.710	1.729	1.776	1.749	1.725
C2–X6	1.662	1.635	1.716	1.701	1.683	1.070	1.851[c]	1.327	1.072
C3–N8	1.471	1.488	1.489	1.444	1.442	1.440	1.374	1.416	—
C5–N7	1.463	1.496	1.477	1.433	1.431	1.433	1.372	1.420	1.432
N8–O[d]	1.199	1.214	1.208	1.189	1.230	1.193	1.217	1.206	—
N7–O[d]	1.203	1.212	1.211	1.195	1.239	1.194	1.214	1.198	1.195
S1–C2–C3	111.6	111.7	112.0	111.9	111.7	111.7	104.2	110.4	113.2
C2–C3–C4	112.0	112.5	112.4	113.5	113.9	114.6	116.6	114.3	112.3
C3–C4–C5	110.4	112.2	109.3	110.3	109.8	109.3	113.6	111.1	111.2
C4–C5–S1	112.3	111.7	113.3	114.4	114.2	114.3	113.4	113.9	113.8
C5–S1–C2	93.7	92.0	92.8	89.89	90.37	90.06	91.91	90.38	89.59
S1–C2–X6	121.6	123.1	119.9	117.8	118.4	121.9	110.2	120.7	119.9
C2–C3–N8	127.0	125.5	125.8	126.0	125.6	122.6	121.5	123.7	—
C4–C5–N7	123.6	122.5	125.7	126.0	126.6	125.5	125.5	126.8	125.3
C2–C3–N8–O[d]	11.4	3.10	63.6	11.90	18.93	1.28	−2.08	0.56	—
S1–C5–N7–O[d]	0.90	4.50	12.2	0.61	−0.62	−0.21	−0.15	1.05	0.54
E_{HOMO}	−11.04	−10.69	−11.44	−10.85		−11.08	−3.635	−9.55	−10.06
E_{LUMO}	−2.359	−2.528	−2.634	0.297		0.517	5.145	1.211	1.312
Q_{S1}	0.816	0.484	0.542	0.504		0.461	0.220	0.412	0.398
Q_{C2}	−0.337	−0.250	−0.149	−0.325		−0.303	−0.440	0.174	−0.345
Q_{C5}	−0.502	−0.645	−0.326	−0.093		−0.089	−0.144	−0.114	−0.067
μ	4.238	4.313	2.544	2.372		2.965	5.401	5.501	5.333
log 1/*C*			3.97			3.20		2.36	<1.00

[a] Bond lengths are given in angstroms, angles in degrees; *C* is the minimum inhibitory concentration of the respective nitrothiophene (mol L^{-1}); E_{HOMO} and E_{LUMO} are the energies of the highest occupied and lowest unoccupied molecular orbitals, respectively, in eV; Q_{S1}, Q_{C2} and Q_{C5} are the total atomic charges at S1, C2 and C5, respectively (see Fig. 1 for the numbering convention); μ is the molecular dipole moment in D.

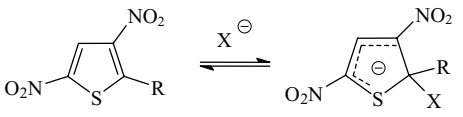
[b] Ref. 55.

[c] C2-SCH_3 bond length (see text).

[d] Oxygen atom which lies in a *cis* conformation to the three specified atoms.

It is well established that activated halothiophenes react with a variety of nucleophiles via a two-step S_NAr mechanism which involves the displacement of the halogen,[45–50] and it seems likely that the mode of action of (**1a**) and (**1c**) may involve nucleophilic attack by intracellular thiols (RSH) at the 2-position of the heterocyclic ring leading to the displacement of the substituent and the formation of covalently bound products, that is,

The higher activity of 2-phenoxy-3,5-dinitrothiophene (**1b**) over the 2-methoxy derivative (**1e**) is supportive of the S_NAr mechanism also as the phenoxy goup is a better leaving group than the methoxy group, but this mode of action cannot apply to 5-nitrothiophene-2-carbaldehyde (**1d**) nor to 2,4-dinitrothiophene (**1h**), which have good to moderate activity against *E. coli* (Scheme 1), because neither has a displaceable leaving group. However, it is well known that nitrothiophenes form brightly coloured solutions on the addition of alkali[45] due to the presence of Meisenheimer complexes (**4**) which are, in some cases, more stable in solution than the related 1,3,5-trinitrobenzene complexes.[51] In general, these complexes are formed by the attack of a nucleophile such as methoxide at the 2- (or 5) position of the thiophene ring with the negative charge thought to be delocalised both by the nitro group(s) and the vacant d orbitals on the sulfur atom, for example,



(**1**)

e R= OMe

h R = H

(**4**)

e R= OMe; X = OMe

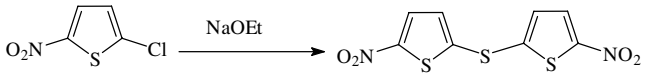
h-1 R = H; X = OMe

h-2 R = H; X = SMe (this work)

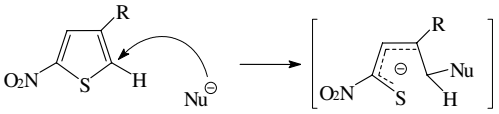
In principle, many of the nitrothiophenes could behave the same way in vivo and react with intracellular thiols to form Meisenheimer complexes

but there are no reports of this type of reaction occurring either in vivo or in vitro although related 1-chloro-2,4,6-trinitrobenzene and 1,3,5-trinitrobenzenes readily form Meisenheimer complexes with thiolate ions.[52,53]

However, a third possible reaction may occur also which involves the opening of the heterocyclic ring again by the initial attack of a nucleophile at the 2-position of the ring. There is little doubt that these reactions occur when nitrothiophenes are attacked by hard nucleophiles, but it is not known whether soft nucleophiles such as thiolates can act in the same way as the products have rarely been identified.[45,46] 2-Nitrothiophene (**1s**) and 3,4-dinitrothiophene readily react with secondary amines under mild conditions to yield nitrobutadienylthiols,[45] and 2-chloro-5-nitrothiophene (**1r**) is known to react with sodium allyloxide or ethoxide to form bis(5-nitro-2-thienyl)sulfide[54] probably by a similar ring opening process, for example,



In both these reactions, nucleophilic attack by a secondary amine or by ethoxide is thought to result in the breaking of the S1–C2 bond to generate intermediate thiolates, that is,



These in turn can either abstract hydrogen from the attached quaternary nitrogen at the sp^3 carbon in the first case to give nitrobutadienylthiols in good yield[45] or react with a further molecule of the thiophene in the latter case to eventually give bis(5-nitro-2-thienyl)sulfide.[54]

4.1. Calculated properties of the nitrothiophenes

It is possible that the mode of action of the nitrothiophenes may involve any one of these mechanisms and to investigate some aspects of these processes we have selected a number of calculated properties which we considered relevant, in an attempt to develop a model which would describe their biological activity. Initially it was necessary to find a suitable method to calculate the structures of all the derivatives discussed here before the properties were evaluated. Preliminary calculations were carried out using the MNDO, AM1 and PM3 methods of the MOPAC 93 package on 2-chloro-3,5-dinitrothiophene (**1a**) and the results compared with the well-resolved crystal structure of the same molecule[55] in the Cambridge Structural Database.[56] The atom numbering system used is shown in Figure 1 and the calculated bond lengths and angles are shown in Table 2.

None of the three methods give completely satisfactory results although the PM3 method gives the best S1–C2 and C5–S1 bond lengths of 1.719 and 1.739 Å versus the crystallographic values 1.708 and 1.710 Å, respectively (Table 1). This method also gives the best C2–S1–C5 and S1–C2–C3 angles of 92.0 and 111.7° versus the experimental values of 90.4 and 111.7°, respectively. Both the AM1 and MNDO methods appear to substantially underestimate the important S1–C2 and C5–S1 bond lengths and overestimate the C2–S1–C5 angle (Table 1). The MNDO method is also unsatisfactory because it twists the 3-nitro group 63.6° out of the molecular plane compared with the much smaller twist of 18.9° found in the crystal structure although the 5-nitro group is only twisted by 12.2° using this method. Because the PM3 method is superior to the AM1 method for the geometry around the C2–S1–C5 bond angle (subsequently used as a variable), it was decided to use this method to calculate the structures and electronic properties of all the other nitrothiophenes both in the gas phase and in water. A series of reference calculations were also carried out at the 6-31G** level (see later) using the same numbering convention (Fig. 1).

Ten properties were employed to model the activity of the nitrothiophenes. These were as follows:

(a) The energies of the frontier molecular orbitals (E_{LUMO} and E_{HOMO}). The precise interactions between the nitrothiophene and the intracellular species present in the bacterial cells are not known, but frontier orbital interactions between the HOMO of an intracellular thiol and the LUMO of the substrate may be important in the formation of a transition state.[57] In this mechanism, the lone pair of electrons at the sulfur atom of the thiol would be expected initially to donate into the LUMO of the nitrothiophene and it follows that the lower the energy of the LUMO (E_{LUMO}) the more readily it will be attacked. In contrast, the HOMO energy (E_{HOMO}) is the energy required to remove an electron from the molecule, and the higher the value, the less likely it is that the molecule will wish to acquire electrons from an attacking nucleophile. Both these quantities are directly related to the electron affinity and ionization potential of the molecules in question, though they have positive rather than negative values.

However, preliminary statistical analysis using both these parameters plotted against biological activity suggested a parabolic relationship. To cover this possibility, two additional terms (E^2_{HOMO}) and (E^2_{LUMO}) were included in the analysis in an analogous way to the addition of a π^2 term to Eq. 2 [27,31].

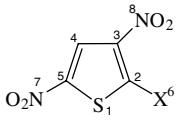


Figure 1. Atom numbering convention used for the nitrothiophenes.

Table 2. Energies, electronic and geometric properties of the nitrothiophenes (**1**) calculated at the PM3 level in the gas phase[a]

	E_{HOMO}	E_{LUMO}	μ	Q_S	q_S	q_C	θ_S	MV	MW	NO_2	$\delta H°_S$
1a	−10.69	−2.53	4.32	0.48	0.29	0.18	91.91	114.3	208	1	−42.30
1b	−10.68	−2.12	6.75	0.43	0.01	0.04	91.01	177.0	266	1	−43.94
1c	−11.18	−2.51	3.97	0.54	0.61	0.21	91.06	120.2	253	1	−42.24
1d	−10.58	−2.14	4.98	0.51	1.15	0.00	91.40	100.0	157	0	−28.85
1e	−10.85	−2.19	5.88	0.42	0.31	0.29	90.98	125.6	204	1	−44.85
1f	−10.12	−2.15	6.37	0.49	0.33	0.10	91.78	146.2	231	1	−48.53
1g	−10.30	−2.19	5.33	0.46	0.06	0.36	91.33	124.6	181	0	−30.44
1h	−11.33	−2.42	3.69	0.52	1.11	0.02	91.78	101.9	174	1	−43.93
1i	−9.83	−2.36	5.21	0.50	0.17	0.07	92.38	186.2	282	1	−39.23
1j	−10.17	−2.57	5.03	0.51	0.09	0.02	92.53	185.6	262	1	−41.06
1k	−10.43	−2.87	2.16	0.51	0.17	0.09	92.67	135.0	231	1	−45.88
1l	−11.29	−2.76	5.26	0.57	1.09	0.01	93.01	196.6	312	1	−55.76
1m	−9.95	−1.95	6.20	0.41	0.34	0.07	91.43	115.5	189	1	−52.45
1n	−10.50	−1.81	5.10	0.48	1.17	0.00	90.32	103.1	208	0	−22.71
1o	−9.12	−2.17	6.89	0.48	0.01	0.00	91.36	340.3	465	1	−62.10
1p	−10.04	−2.10	7.37	0.49	0.32	0.10	91.80	196.3	293	1	−47.59
1q	−9.36	−1.99	7.82	0.41	0.23	0.00	92.01	180.6	265	1	−43.98
1r	−10.19	−1.80	5.27	0.45	0.11	0.31	91.10	96.4	163	0	−21.82
1s	−10.38	−1.63	5.45	0.44	1.15	0.01	91.31	83.4	129	0	−22.60

[a] The results shown have been rounded up to a maximum of two decimal places or three significant figures; E_{HOMO} and E_{LUMO} are the energies of the highest occupied and lowest unoccupied molecular orbitals, respectively, in eV; μ is the molecular dipole moment in D; Q_S is the total atomic charge at sulfur; θ_S is the angle at the sulfur atom of the thiophene ring; MV is the molecular volume in $Å^3$; MW is the molecular weight; $\delta H°_S$ is the difference between the calculated heats of formation of the molecule in water and the gas phase (see text); q_S and q_c are the number of electrons at sulfur (S1) and adjacent carbon atom (C2) in the HOMO, and NO_2 is an indicator variable for the presence or absence of a nitro group at the 3-position of the heterocyclic ring (see Figure 1 for atom numbering sequence).

(b) Dipole moment (μ). The calculated dipole moment of the nitrothiophenes represents the vector sum of all the atomic charges at each centre. Molecules with large dipole moments are often soluble in polar solvents, such as water, and more likely to diffuse through the membranes of bacteria or fungal cell walls. In contrast, those with small dipole moments have a less polar character, are less soluble in water and less likely to penetrate the cell.

(c) Total atomic charge on the sulfur atom of the thiophene ring (Q_S). Semi-empirical calculations on the most reactive thiophene (**1a**) indicate that the atomic charges at the expected site of nucleophilic attack, C2, are strongly negative (Table 1). These results are confirmed by calculations at the 6-31G** level on derivatives which have high activity (**1a**), moderate activity (**1h**) and weak activity (**1s**) and imply that the transition state of the reaction does not resemble the reactants, but occurs much later along the reaction coordinate. In contrast, the adjacent sulfur atom, S1, is strongly positive at both the semi-empirical and ab initio levels (Table 1) suggesting that this centre may help to accommodate and dissipate the negative charge of the nucleophile as it approaches the C2 position. An optimisation at the 6-31G** level of a hypothetical structure formed by attack of methanethiol at the C2 position of the dinitrothiophene (**1h**) results in a stable Meisenheimer complex (**4h-2**) containing an sp^3carbon at C2, with S1–C2 and C2–C3 bond lengths of 1.843 and 1.504 Å, respectively, which are significantly longer than those found in the free thiophene (Table 1). Significantly, the atomic charge at sulfur falls from 0.461 in (**1h**) to 0.220 in (**4h-2**) with a concomitant increase in the negative charge at C2 which rises from −0.303 to −0.440 (Table 1); the bulk of the remaining negative charge is accommodated by the two nitro groups. As a significant proportion of the negative charge has been transferred to C2 and the adjacent sulfur atom, S1, we have assumed that the greater the positive charge at sulfur the more readily it will assist the nucleophilic reaction by accommodating the developing charge as the initial Meisenheimer complex forms.

(d) Electron density at the carbon atom (C2) and adjacent sulfur atom (S1) in the HOMO of the nitrothiophene (q_C and q_S). The HOMO of the nitrothiophene is a π-orbital containing two electrons which are distributed over the conjugated atoms of the ring system. In all the proposed mechanisms, initial nucleophilic attack will occur either above or below the molecular plane at the C2 position of the ring to form an initial Meisenheimer complex which may be stable, or which may react further to expel halogen or a substituent to form a substituted product, or ring open by a cleavage of the S1–C2 bond to form a substituted butadiene with the negative charge initially transferred to the sulfur atom. The electron density, or number of electrons, found in the HOMO at C2 and at S1 may therefore be an indication of the likely reactivity of the molecule.

(e) Angle at the sulfur atom (θ_S). In the third possible mode of action of the nitrothiophenes, attack by intracellular species may result in a ring opening reaction. The nature and the presence or absence of substituents will have an influence on the heterocyclic ring angles particularly at sulfur (C2–S1–C5) which might prove to be an indicator of the reactivity through the degree of ring strain. For example, while the unstrained sulfur bond angle C–S–C in dimethyl sulfide[58] is 99.0°, the value falls to 92.1° in thiophene,[59] and falls further to 90.4°

in 2-chloro-3,5-dinitrothiophene (**1a**),[55] though the calculated results are somewhat larger (Table 1). If the ring opening step is part of the mechanism of the mode of action, smaller angles at the sulfur atom will result in more strain and higher reactivity. The ring angle may be important also in the formation of the Meisenheimer complexes where the values at S1, C2 and C3 would be expected to show significant changes from their values in the free thiophenes. The calculated results at the 6-31G** level confirm these changes with the S1–C2–C3 angle falling from 111.7° in (**1h**) to 104.2° in (**4h-2**),and the corresponding angles C5–S1–C2 and C2–C3–C4 increasing from 90.06 and 114.6°, respectively, in the former to 91.91 and 116.6°, respectively, in the latter (Table 1).

(f) Molecular volume (MV) and molecular weight (MW) The molecular volume of the molecule ($Å^3$), based on the three-dimensional van der Waals surface area, is a direct measure of the overall dimensions of the reactive surface of the molecule. Size may play a key role in the way hydrophilic molecules gain entry into the cell, for example by diffusion through the cell wall of *M. luteus* or the outer membrane proteins of *E. coli*, with small molecules showing faster diffusion rates than large ones. However, for lipophilic molecules, size may prove to be an advantage as the size of the invading species may disrupt the ordered membrane, and allow quicker diffusion into the cell and increased cellular damage. The effect of molecular weight on activity would be expected to be similar to the role described for molecular volume but here the substitution of the thiophene ring with additional atoms such as halogens or sulfur with 'heavy' atomic masses might cause greater disruption of the cell membranes.

(g) Indicator variable (NO_2) The presence of a nitro group at the 3-position of the thiophene ring appears to be associated with enhanced activity. Consequently, an indicator variable (0 or 1) was used to denote its presence or absence in the molecule under evaluation.

(h) Solvation energy terms (δH_S) The Hansch partition coefficient is often critical in assessing the efficacy of a biocide as it mirrors the ability of the molecule to permeate into the cell. We have suggested previously,[7] as a theoretical alternative to measuring the Hansch partition coefficient, the hydrophilicity or hydrophobicity of a compound can be evaluated by assessing the degree to which it is stabilized in water. By calculating the difference in the heat of formation (ΔH°_f) of the nitrothiophene in the gas phase on the one hand, which represents the hydrophobic environment of the cell, and the modified value in water on the other, which represents the aqueous phase of the cell, a measure of the solvation energy, δH°_S, can be evaluated, that is,

$$\delta H^\circ_S = \Delta H^\circ_f(H_2O) - \Delta H^\circ_f(\text{gas}) \quad (3)$$

Nitrothiophenes with large solvation energies would be expected to be more soluble in the aqueous phase of the cell but less soluble in the hydrophobic phase and vice versa.

Two possible QSARs have been explored using these variables by calculating all the molecules in both the gas phase (Table 2) and in water (Table 3). However, as we have noted previously for isothiazolones,[7] the variations produced by changing the dielectric constant of the solvent from 1 (gas) to 78.4 (water) have relatively little effect on the geometry of the molecules, but other properties such as the frontier orbital energies, atomic charges and dipole moments show much larger differences (Tables 2 and 3).

4.2. Structure–activity relationships

As in our previous study,[7] the results obtained were subjected to multi-linear regression with the SPSS package[26] and analysed using (1) the *t*-statistic, which measures the significance of each individual independent variable in the regression equation, (2) the *F*-statistic, which assesses the overall significance of the model, (3)

Table 3. Energies, electronic and geometric properties of the nitrothiophenes (**1**) calculated at the PM3 level in water[a]

	E_{HOMO}	E_{LUMO}	μ	Q_S	q_S	q_C	θ_S	MV	MW	NO_2	δH°_S
1a	−9.65	−1.85	7.63	0.49	0.37	0.09	92.48	113.3	208	1	−42.30
1b	−10.19	−1.71	8.71	0.47	0.16	0.05	91.24	177.3	266	1	−43.94
1c	−10.72	−1.87	6.00	0.64	0.79	0.18	91.59	120.1	253	1	−42.24
1d	−10.09	−1.86	7.77	0.46	1.15	0.01	91.36	100.3	157	0	−28.85
1e	−10.19	−1.61	8.40	0.47	0.51	0.24	91.22	125.5	204	1	−44.85
1f	−9.51	−1.64	10.13	0.43	0.41	0.05	92.13	144.8	231	1	−48.53
1g	−9.91	−1.93	8.29	0.41	0.00	0.34	90.98	124.6	181	0	−30.43
1h	−10.43	−1.59	6.27	0.62	1.07	0.06	92.30	101.8	174	1	−43.93
1i	−9.53	−2.17	5.96	0.61	0.12	0.05	93.00	183.9	282	1	−39.23
1j	−9.67	−2.03	9.01	0.52	0.14	0.05	93.11	183.7	262	1	−41.06
1k	−9.71	−2.10	3.09	0.58	0.26	0.11	93.11	134.7	231	1	−45.88
1l	−10.51	−2.27	10.58	0.74	0.95	0.05	94.26	196.5	312	1	−55.76
1m	−9.02	−1.12	13.84	0.28	0.54	0.00	91.99	114.0	189	1	−52.45
1n	−10.17	−1.66	7.51	0.46	1.15	0.01	90.43	103.2	208	0	−22.71
1o	−9.05	−1.89	7.24	0.58	0.00	0.00	91.67	342.0	465	1	−62.10
1p	−9.57	−1.72	10.12	0.45	0.40	0.06	92.10	193.9	293	1	−47.59
1q	−8.93	−1.51	12.42	0.36	0.35	0.00	92.19	177.4	265	1	−43.98
1r	−9.77	−1.66	8.12	0.41	0.08	0.25	91.23	95.5	163	0	−21.82
1s	−10.04	−1.46	8.29	0.42	1.12	0.01	91.36	83.4	129	0	−22.60

[a] See Table 2 for definitions.

the square of the multiple correlation coefficient, R^2, which indicates how well the regression line fits the data and finally (4) the predictive residual sum of the squares, PRSS, which reflects the difference between actual activity and predicted activity.[32,33,40] Two methods were used in the SPSS regression analysis, including forward stepping, where each independent variable is added into the regression equation one at a time, and backward stepping, where all the independent variables are used to describe the dependent variable and are removed one at a time.[32,33,40]

A comparison of the experimental data (Scheme 1) with the calculated properties either in the gas phase (Table 2) or in water (Table 3) before analysis, however, shows no obvious correlations. For example, the LUMO energies are unsatisfactory as the most active derivative (**1a**) has a larger value than the less active derivatives (**1j–l**), whereas the reverse would be expected. The solvation energies present a confused picture with the active derivatives (**1a–c**) showing less stabilization in water than (**1k–m**) but a greater stabilization than (**1r–s**). The charge at sulfur varies considerably with (**1a**) showing a smaller positive value than (**1h**), while the electron density or number of electrons in the HOMO at C2 is larger for the former than that found in all other derivatives with the exception of the less active derivatives (**1b**, **1c**, **1g** and **1r**) suggesting that it is less likely to be attacked by nucleophiles. A similar pattern emerges for the C2–S1–C5 bond angle which is larger in (**1a**) than the less active derivatives mentioned suggesting that ring strain is also unimportant. The possibility that the PM3 results show the wrong trends because of the theoretical approximations adopted by the method was carefully checked by calculating four of the thiophenes, (**1a**), (**1h**), (**1m**), and (**1s**) which show high, moderate and low activity, at the ab initio 6-31G** level[24] and then comparing the ab initio and semi-empirical results (Table 1). It is clear that both sets of results show *exactly the same trends* for the LUMO energies (**1s > 1m > 1h > 1a**), HOMO energies (**1h > 1a > 1s > 1m**), the charge at sulfur (**1a > 1h > 1m > 1s**) and the dipole moments (**1m > 1s > 1a**) (Table 2) therefore justifying the use of the semi-empirical method in the QSAR studies.

QSAR analyses were next carried out for both *E. coli* and *M. luteus* using the $\log 1/C$ data shown in Scheme 1 together with the full calculated data obtained either in the gas phase or in water (rounded up values are shown in Tables 1 and 2). Generally, the models based on the gas-phase results displayed better predictive power than those based on the aqueous-phase results as the latter suffered from a small number of individual predictions that exhibited large errors. Thus, the QSAR for *E. coli* using the calculated gas-phase data proved to be more significant than that calculated using the aqueous-phase data though it was necessary to exclude three results (**1d**, **1g**, and **1p**) from the data set (see later) to achieve a statistically significant correlation, that is,

$$\log 1/C = -91.11 - 28.12E_{\mathrm{HOMO}} - 1.33E^2_{\mathrm{HOMO}} + 2.58\mathrm{NO}_2 - 0.65\theta_{\mathrm{S}} + 6.57Q_{\mathrm{S}} \quad (4)$$

The five term Eq. 4 based on 16 observations is significant at the 5% confidence level and it explains 95% of the variance in the activity of the nitrothiophenes. In this model, the square of the correlation coefficient, $R^2 = 0.95$, the F-statistic = 39.99 and the predictive residual sum of the squares, PRSS = 1.72, confirming that the equation gives a good account of the experimental activity with a mean error of prediction of only 10%. The QSAR determined using the corresponding aqueous-phase data contained the same variables excepting θ_S, but here $R^2 = 0.85$, the F-statistic = 15.35 and the predictive residual sum of the squares, PRSS = 6.09. The mean error of prediction in this aqueous model is unsatisfactory at 25%.

The QSAR found for M.luteus using the gas-phase data contain similar terms to those found for *E. coli* (Eq. 4) though again it was necessary to exclude three results (**1d**, **1g**, and **1j**) from the data set to achieve a statistically significant correlation, that is,

$$\log 1/C = -147.6 - 32.9E_{\mathrm{HOMO}} - 1.57E^2_{\mathrm{HOMO}} + 2.03NO_2 + 0.00694\mathrm{MW} - 0.319\theta_{\mathrm{S}} + 8.32Q_{\mathrm{S}} \quad (5)$$

In this model, which includes a molecular volume term, the square of the correlation coefficient, $R^2 = 0.95$, the F-statistic = 30.70 and the predictive residual sum of the squares, PRSS = 3.45 again suggesting that the equation gives a good account of the experimental activity though the mean error of prediction here is less satisfactory at 17%. As before, the QSAR found using the corresponding aqueous-phase data was again unsatisfactory with a mean error of prediction of 28%.

The absence of the solvation energy term, $\delta H^{\circ}_{\mathrm{S}}$, in either Eqs. 4 or 5 suggests that the activity of the nitrothiophenes (**1**) is not dependent on cell wall diffusion but probably on subsequent reactions within the cell environment. Their behaviour differs significantly therefore from that of isothiazolones (**3**) where we found that the solvation energy term, $\delta H^{\circ}_{\mathrm{S}}$, dominates the QSAR equation.[7] The necessary omission of thiophenes (**1d** and **1g**) from the data sets for both bacteria possibly reflects an additional or alternative mode of action for these molecules which involves the aldehyde group. Aldehydes are known to readily react with a variety of thiols to form thioacetals[29] and it is possible that the aldehydic thiophenes (**1d** and **1g**) react in vivo with intracellular thiols, such as cysteine or glutathione, at the carbonyl group in an additional or competing mechanism to the main attack at C2 discussed above. Indeed, this mode of action may explain the biological activity of a variety of aromatic aldehydes and ketones such as 4-nitrocinnamaldehyde which is known to be an effective biocide.[60] The anomalous result for the amide (**1p**) with *E. coli* may be due to a similar competing reaction, but this does not explain why the sulfide (**1j**) shows anomalous behaviour with *M. luteus*.

From both these QSAR analyses, it is apparent that the overall biological effect is associated with a large positive

contribution from the E_{HOMO} parameter and a moderating, negative effect produced by the second order contribution of E^2_{HOMO}. For example, taking the value of −10.69 eV for (**1a**) (Table 2), the relative contributions in Eq. 4 for the E_{HOMO} term = +300.6, while the E^2_{HOMO} term = −152.0. The appearance of these terms in the QSAR expression is unexpected as they imply that the ionization potential is an important factor in the biological activity, whereas usually it is the electron affinity or LUMO energy which is significant for molecules which undergo nucleophilic attack. It follows from the QSAR results that those nitrothiophenes with the highest stability appear to be the most active though the counteracting influence of the other parameters in the multi-regression analysis, such as θ_S and Q_S (see below), cannot be discounted.

The presence of the nitro group indicator variable in both equations confirms that the dinitrothiophene derivatives are generally much more active than their mononitro analogues as expected. The C2–S1–C5 bond angle, θ_S, also appears to be a significant factor in the equations where the smaller the angle the greater the activity presumably because during the formation of the Meisenheimer complex any ring strain is released. The charge at sulfur, Q_S, is another factor which exerts an influence on the biological activity possibly by dissipating the negative charge of the attacking nucleophile in line with the arguments given above. On balance however, both θ_S and Q_S carry less weight than expected originally. The main difference between the QSAR models for the Gram-negative (*E. coli*) and Gram-positive (*M. luteus*) bacteria is the presence of a molecular weight term in the latter (see Eqs. 4, 5). This may reflect the difference in structure between the two types of bacteria where the cell wall of the former provides a larger barrier to entry into the cell than the outer membrane of the latter, but the effect is smaller and therefore less significant than the effect of other variables, such as Q_S and θ_S, which also appear in Eq. 5.

Overall, the application of Eq. 4 leads to satisfactory results for *E. coli* in most cases with predicted values for log1/*C* of 3.76 and 3.97 for (**1b**) and (**1c**), which contain a displaceable group at C2, and 3.10 and 1.10 for (**1h**) and (**1s**) which do not, versus the experimental values of 3.78, 3.69, 3.20, and ⩽1.00, respectively. However, the application of Eq. 5 leads to less satisfactory results for *M. luteus* with predicted values for log1/*C* of 3.13 and 3.25 for (**1b**) and (**1c**) on the one hand, and 1.91 and 0.20 for (**1h**) and (**1s**) on the other, versus the experimental values of 3.80, 4.11, 2.58 and ⩽1.00, respectively, though the general trends over the 16 examples are well reproduced.

5. Conclusion

Multi-linear regression analysis shows that there is a significant correlation between the experimental activity of the 2-nitrothiophenes against either *E. coli* or *M. luteus* and calculated properties such as the HOMO energies, total atomic charges and ring angles at the heterocyclic sulfur atom, but there is no correlation with the calculated solvation energies or dipole moments. The presence or absence of an additional nitro group at C3 also has a significant effect on the activity. The derived QSAR Eqs. 4 and 5 appear to give a satisfactory account of the activity of most derivatives with the 2-chloro- or 2-bromo-3,5-dinitrothiophenes (**1a** and **1c**) predicted to be the most active against both bacteria and 2-nitrothiophene (**1n**) the least.

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