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Quantitative structure–activity relationship (QSAR) analysis of aromatic effector specificity in NtrC-like transcriptional activators from aromatic oxidizing bacteria

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Abstract

A quantitative structure–activity relationship (QSAR) approach was taken to provide mechanistic insights into the interaction between the chemical structure of inducing compounds and the transcriptional activation of aromatic monooxygenase operons among the XylR/DmpR subclass of bacterial NtrC-like transcriptional regulators. Compared to XylR and DmpR, a broader spectrum of effector compounds was observed for the TbuT system from *Ralstonia pickettii* PKO1. The results of QSAR analysis for TbuT suggested that a steric effect, rather than hydrophobic or electronic effects, may be the predominant factor in determining aromatic effector specificity, and the active site of the regulator may positively interact not only with the methyl moiety but also with the most electron-rich aryl side of an aromatic effector.

Keywords: Bacterial NtrC-like transcriptional activator; Aromatic monooxygenase pathway; Quantitative structure–activity relationship; Trichloroethylene cometabolism; Toluene oxidizing bacterium

1. Introduction

In natural environments, aromatic oxygenase pathways in bacteria are responsible for the biodegradation of many organic pollutants [1–4]. Bacterial aromatic monooxygenase pathways have been of particular interest in biodegradation research because trichloroethylene (TCE) has been shown to be co-oxidized by soil bacteria whose toluene monooxygenase pathways are induced in the presence of aromatic substrates as well as TCE [5–8].

Among bacterial aromatic monooxygenase pathways, two of the best-studied transcriptional regulators are XylR from *Pseudomonas putida* mt-2 [9–12] and DmpR from *Pseudomonas* sp. strain CF600 [13–16]. XylR and DmpR are members of a subclass of the NtrC family of prokaryotic transcriptional regulators, which activate gene expression in concert with the RNA polymerase holoenzyme containing the σ^{54} subunit [17–19]. Unlike other regulators in the NtrC family, members of the XylR/DmpR subclass sense their respective signals by direct binding of the aromatic effector to their amino-terminal 'A' domains [19]. Most of the currently identified XylR/DmpR regulators control expression of aromatic monooxygenase pathways [20].

Quantifying the biological, environmental, and structural factors that control the induction of biodegradation is crucial in predicting biodegradation rates [21–24]. However, the tremendous diversity of biological systems complicates biodegradation prediction, and the sheer number of chemicals hampers the progress. Particularly, the extent of transcriptional activation of aromatic monooxygenase

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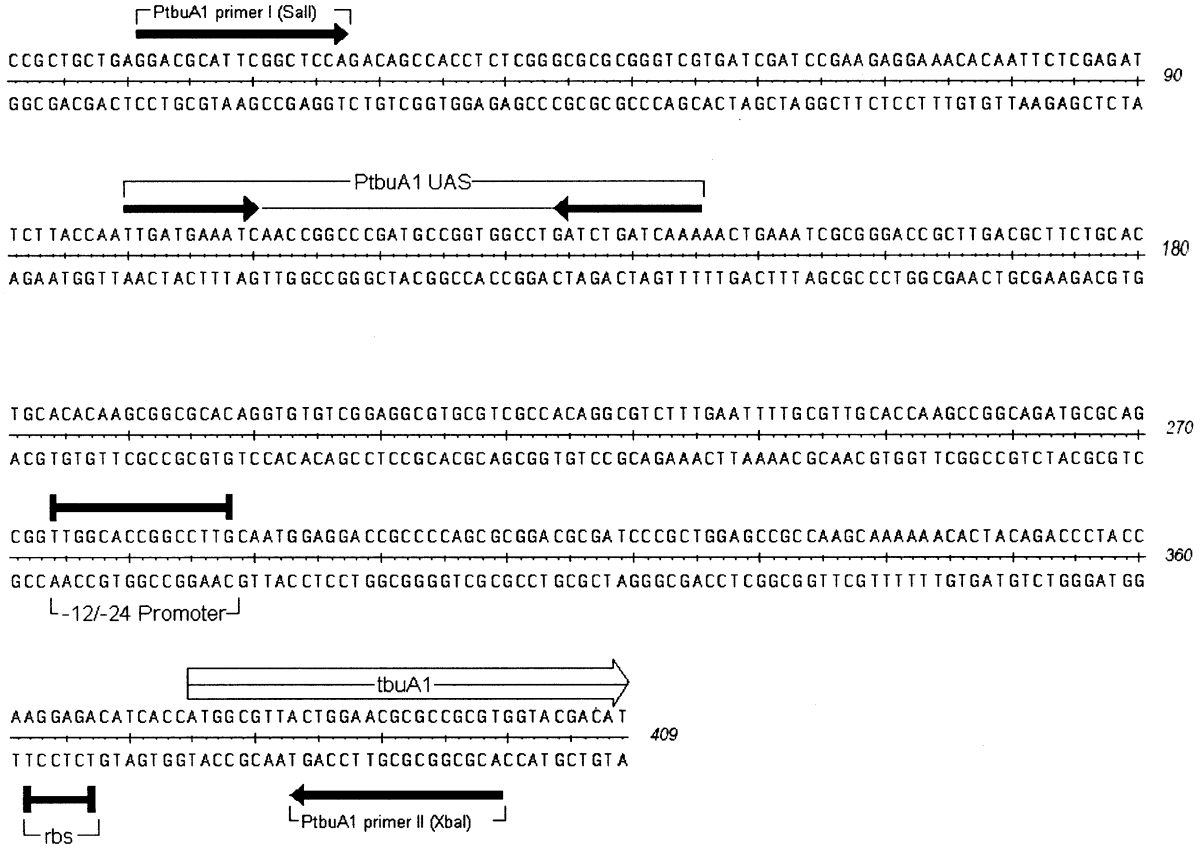

Fig. 1. The P*tbuA1* region includes an upstream activating sequence (UAS) where each dimer of TbuT binds with each half site of the UAS (indicated by a pair of inverted arrows). Once activated with an inducing molecule, TbuT is able to confer the necessary energy to the RNA–polymerase holoenzyme complex bound at the −12/−24 promoter for unwinding of the double stranded helix and subsequent initiation of transcription.

operons by the XylR/DmpR regulators has been shown to vary in response to various aromatic compounds [5,9,15,25] as well as to different regulators [20]. Addressing these complex issues requires methods that enable the prediction of fate and effect parameters based on chemical structure or physicochemical parameters via quantitative structure–activity relationships (QSAR) [22]. In this study, a QSAR approach was taken to quantitatively describe and provide mechanistic insights into interactions between the chemical structure of aromatic compounds and transcriptional activation by representative XylR/DmpR regulators from aromatic oxidizing bacteria.

2. Materials and methods

2.1. Bacterial strains, plasmids, and media

The bacterial strains and plasmids used in this study are listed in Table 1. All bacteria were grown in a Luria–Bertani (LB) medium, supplemented with the appropriate antibiotics [26]. The promoter region, P*tbuA1*, for the initial monooxygenase enzyme of the *tbu* pathway, TbuA1, was amplified by polymerase chain reaction (PCR) from a *Ralstonia pickettii* PKO1 derived subclone pRO1966 [28] using the following primers synthesized by Invitrogen: 5′-TTTTGTCGACGGACGCATTCGGCTCCA (nucleotides 10–27, Fig. 1), and 5′-GATTTCTAGAACGCGGCGCGTTCCAGT (nucleotides 383–399, Fig. 1). Primers were designed with *Sal*I and *Xba*I restriction endonuclease sites (underlined) at their 5′ ends to allow for directional cloning of the PCR product into *Sal*I-*Xba*I digested vector pKRZ1 [30]. The resulting construct, pKRZ1:P*tbuA1*, was initially transformed into *Escherichia coli* DH5α for restriction digest analysis and sequencing verification. Transformants were selected on solid LB amended with 35 μg ml^{-1} kanamycin A. A second plasmid containing a constitutively transcribed *tbuT* gene carried on pRO1614:: 3.1-kb *tbuT*Δ0.5kb *Sst*II was electroporated into *Pseudomonas aeruginosa* PAO1c, and transformants were selected on LB amended with 50 μg ml^{-1} tetracycline [5]. A single PAO1c colony containing the second construct was again electroporated with the first construct plasmid (pKRZ:: P*tbuA1*), and transformants containing the dual plasmid system were selected on LB amended with both tetracycline at 50 μg ml^{-1} and kanamycin A at 600 μg ml^{-1}.

2.2. Promoter activity in response to hydrocarbons

The activity of P*tbuA1* in PAO1c (Fig. 2) in response to

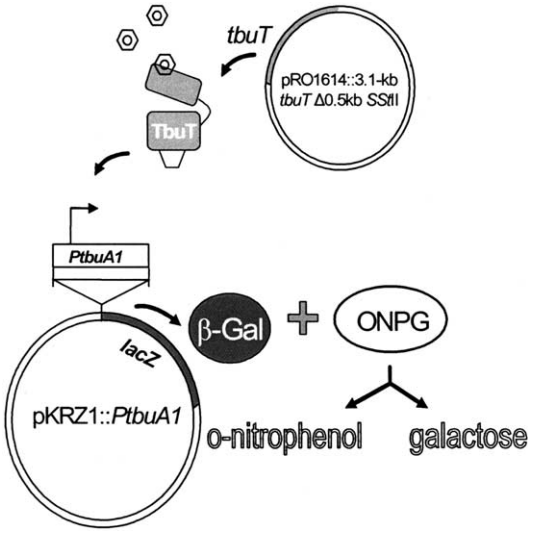

Fig. 2. Monitoring the activation of P*tbuA1* promoter by TbuT. *tbuT* is constitutively transcribed from the pRO1614 promoter, and the resulting TbuT protein lies dormant until it binds to an inducing molecule. Once active, the TbuT–inducer complex can initiate transcription at the P*tbuA1* promoter, causing transcription of the reporter *lacZ* gene and the subsequent translation of β-galactosidase. This enzyme cleaves the synthetic ONPG (*o*-nitrophenol galactose) substrate into galactose and *o*-nitrophenol – a colored compound that can be measured photometrically and used to quantify promoter strength.

various hydrocarbons was quantified by measuring *lacZ* reporter activity (i.e., β-galactosidase activity) as described by Miller [31]. The cells were grown overnight (18 h) in 3 ml of LB containing the appropriate antibiotics in a 37°C incubator/shaker set to 180 rpm, and the presence of both plasmids was confirmed by restriction digest analysis. For each hydrocarbon tested, three 27-ml sterile serum bottles (crimp-sealed with Teflon-coated septa) containing 2.94 ml fresh LB plus antibiotics were prepared, and subsequently inoculated with 0.06 ml of the overnight culture. To determine whether there was a toxic effect for each compound, the growth rate was quantified by optical density at 600 nm, and was compared to a no-effector control. Most chemical inducers were added to a final aqueous concentration of 2.5 mM. The concentrations of 2-ethylphenol, 4-chlorophenol, 3- and 4-chlorotoluene were reduced to an aqueous concentration of 1.5 mM due to the increased toxicity of those compounds on strain PAO1c. Because of their low aqueous solubility, monochloroethene and 1,1-dichloroethene (1,1-DCE) were added at 0.78 mM from a methanol phase stock to facilitate dissolution. After 8 h of incubation at 37°C on a shaker at 180 rpm, each bottle had either duplicate or triplicate samples (500 μl) removed for promoter activity measurement. The cell samples were centrifuged at $12\,000\times g$ for 1 min, and the cell pellet was stored at −80°C prior to the measurement of β-galactosidase activity. To examine the variations in the promoter activity measurements, standard deviations were calculated based upon the promoter activity data from the three independent experiments for each hydrocarbon (*y*-axis error bars in Fig. 3).

2.3. QSAR analysis for aromatic effector specificity

QSAR analyses were performed with the aromatic effector specificity data for the XylR/DmpR transcriptional regulators. The general form of a QSAR equation is:

$$\mathrm{Log}(A/Ao) = \mathrm{Intercept} + \sum_{i=1}^{n} \mathrm{Coefficient}_i \times \mathrm{Parameter}_i$$

n = number of parameters in one QSAR equation

The dependent variable used in the QSAR equations was the logarithm of the induction ratio of β-galactosidase activity for an aromatic compound relative, *A*, to β-galactosidase activity for no-effector control, *Ao*. The independent variables were Hammett substituent descriptors, i.e., the hydrophobic, steric, and electronic parameters of each compound [32]. To calculate the hydrophobic and global-steric parameters, benzene was used as the reference compounds in the QSAR analyses for TbuT because the tested aromatic compounds did not have a common substituent. Toluene and phenol were used for XylR and DmpR, respectively, as the tested aromatic compounds for each regulator had either a methyl moiety or a hydroxyl moiety as

Table 1
Bacterial strains and plasmids

Strain or plasmid	Characteristics[a]	Reference
E. coli DH5α	F$^-$ ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 phoA hsdR17* (r_K^- m_K^+) *supE44* λ$^-$ thi-1 gyrA96 relA1	[26]
P. aeruginosa PAO1c	Prototroph	[27]
pRO1966	Tc^s Cb^r; pRO1727::*Cla*I-*Bam*HI (10-kb) fragment of pRO1959 containing *tbuA1UBVA2C*, *tbuT*, and *tbuX*	[28]
pRO1959	Tc^s Cb^r; pRO1727::*Hin*dIII-*Bam*HI (15.1-kb) fragment of *R. pickettii* PKO1 containing *tbuD*, *tbuA1UBVA2C*, *tbuT*, and *tbuX*	[29]
pRO1614::3.1-kb Δ0.5kb *Sst*II	contains *tbuT* gene under control of a pRO1614 constitutive promoter	[5]
pKRZ1	Ap^r Km^r; broad-host-range promoter probe vector containing a promoterless *lacZ* reporter gene	[30]
pKRZ1::P*tbuA1*	Ap^r Km^r; pKRZ1::389-bp fragment; P*tbuA1-lacZ* fusion	This study

[a]Tc, tetracycline; Cb, carbenicillin; Ap, ampicillin; Km, kanamycin A.

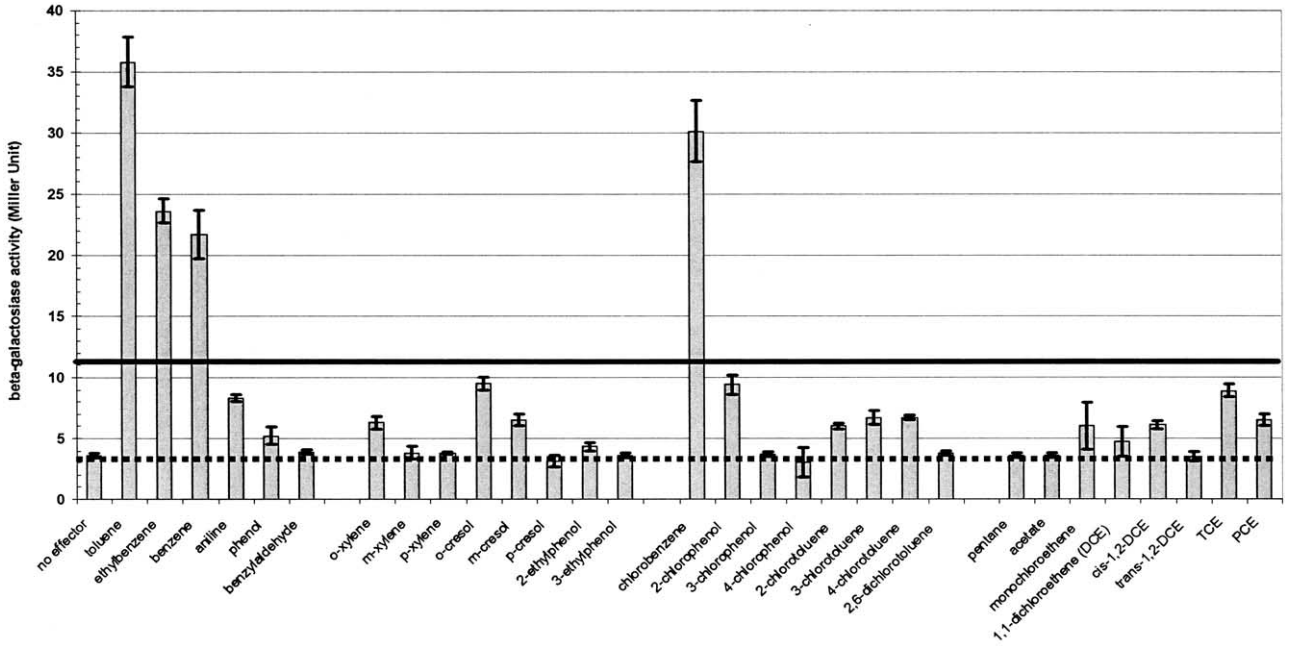

Fig. 3. Activation of P*tbuA1* by TbuT in response to various hydrocarbon compounds. The extent of activation of the P*tbuA1-lacZ* fusion report system by TbuT was measured by β-galactosidase assay. The dotted line indicates the promoter activity for no-effector control (background). The promoter activity above the solid line is at least three times greater than that for the no-effector control. The reported value for each hydrocarbon compound is the mean β-galactosidase activity value from three independent experiments (gray bars). The *y*-axis error bars indicate the standard deviations of the triplicate results.

a common substituent. To calculate the electronic parameters, different reference compounds were used depending upon the tested reference moiety in each QSAR analysis. Methyl, hydroxy, and the most electron-rich aryl moieties were tested. The unsubstituted aryl moiety of the minimal $\Sigma\sigma$ value was determined as the most electron-rich aryl moiety in each aromatic compound. The hydrophobic parameter (π) was calculated by subtracting the log K_{ow} (octanol–water partition coefficient) value for a reference compound from the log K_{ow} value for each aromatic compound [33,34]. The global steric parameter (ΣE_S) value was calculated by summing the Taft steric parameters for all the substituents in an aromatic compound [32,33]. Local steric parameters ($E_{S-LOCAL}$) were used for the independent variables only when the most electron-rich aryl side was considered as the reference moiety in each aromatic compound. The local steric parameter for each compound was estimated by summing the Hammett E_S values for the substituents that are expected to provide steric hindrance in the binding between the aryl side and the transcriptional regulator. Since one unsubstituted position is involved in two adjacent aryl sides of an aromatic compound, the local steric parameter value that was used was the average of the values for the two possibilities. The electronic parameter ($\Sigma\sigma$) was calculated by summing the Hammett σ descriptors for all the substituents in an aromatic compound [33,35]. Multiple linear regression was performed using SYSTAT8.0 [36] to estimate QSAR parameter coefficient values, their corresponding standard errors, R^2 and P values from each QSAR analysis.

3. Results

3.1. Activation of the PtbuA1 promoter by TbuT in response to various hydrocarbon compounds

Because Byrne and Olsen [5] previously suggested a broad effector specificity for TbuT, a member of the XylR/DmpR subclass from a TCE co-oxidizing toluene oxidizer *R. pickettii* PKO1, we extended the determination of effector specificity for this regulator by using multiple classes of aromatic and aliphatic compounds (Fig. 3). Toluene, ethylbenzene, benzene, and chlorobenzene were relatively strong inducers since the promoter activity was at least 3.0 times greater than that for the no-effector control (see the solid line in Fig. 3). While the promoter activity values were statistically greater than that for the no-effector control (the dotted line in Fig. 3), aniline, phenol, *o*-xylene, *o*-cresol, *m*-cresol, 2-chlorophenol, all monochlorotoluenes, *cis*-1,2-DCE, tetrachloroethene, and pentachloroethene (PCE) were relatively weak inducers since the induction ratios were less than 3.

The broad effector spectrum that was observed in this study is consistent with the reports for the same regulator by Byrne and Olsen [5] and Stiner and Halverson [37]. In addition, the extent of transcriptional activation by the TbuT regulator in response to various aromatic compounds shows a consistent trend with that reported by other investigators [5,7]. This latter point is supported by the following observations: (i) toluene, benzene, ethylbenzene, and chlorobenzene are strong inducers, and their

induction ratios are comparable to the previously reported values; (ii) TCE was the strongest inducer among chlorinated ethenes; and (iii) benzaldehyde, *m*-xylene, *p*-xylene, *p*-cresol, and *trans*-1,2-DCE are not inducers. This consistency reinforces the accuracy in measurement of effector specificity in this study. The results reveal that aniline, 2-chlorophenol, monochlorotoluenes, and PCE are also inducers for the TbuT regulator.

3.2. QSAR analysis for aromatic effector specificity in the TbuT activation system

Since the structural moiety of an aromatic compound involved in determining the regulator effector specificity (influential moiety) for the TbuT system is not known, an inductive approach was required. Such inductive QSAR approach enables us to attain the optimized QSAR result when the 'true' influential moiety is correctly used as the reference moiety. First, in order to identify the 'true' influential moiety, it is necessary to test and compare possible reference moiety scenarios. In this study, the methyl moiety, the hydroxyl moiety, and the most electron-rich aryl side were tested as possible reference moieties. The methyl and hydroxyl groups were identified as possible influential moieties because both hydroxylated and methylated aromatic compounds are inducers for the TbuT regulator. The most electron-rich aryl side was also tested based upon our hypothesis that an electron-rich unsubstituted aryl side of an aromatic compound where the toluene 3-monooxygenase reaction occurs [28,37,38] may influence the effector specificity of TbuT. This hypothesis was based upon the fact that the effector spectra for regulators in the XylR/DmpR subclass of NtrC regulators often overlap with the substrate spectra for the corresponding toluene monooxygenases [9,15,20,25]. Depending upon the reference moiety selected, different training sets were used in QSAR analysis. All the methylated aromatics in Fig. 3 were included in the training set for the methyl moiety scenario. All the hydroxylated aromatics were included for the hydroxyl moiety scenario. All of

Table 2
Input parameters used in QSAR analysis for the TbuT system

Compound	P*tbuA1* activity (β-galactosidase activity, Miller units)	Logarithmic induction ratio Log(*A*/*Ao*)[a]	Hydrophobic parameter π[b]	Global steric parameter ΣE_S[c]	Local steric parameter $E_{S-LOCAL}$[d]	Electronic parameter (the most electron-rich aryl side) $\Sigma\sigma$[e]	Electronic parameter (CH_3 moiety) $\Sigma\sigma$[f]	Electronic parameter (OH moiety) $\Sigma\sigma$[g]
Toluene	35.8	0.98	0.6	−1.12	0	−0.16	0	n.a.[h]
Ethylbenzene	23.6	0.81	1.02	−1.31	0	−0.15	n.a.	n.a.
Benzene	21.7	0.77	0	0	0	0	n.a.	n.a.
Aniline	8.3	0.35	−1.23	−1.8	0	−0.66	n.a.	n.a.
Phenol	5.2	0.15	−0.67	−0.55	0	−0.37	n.a.	0
Benzylaldehyde	3.9	0.02	−0.65	−1.06	−0.53	0.35	n.a.	n.a.
o-Xylene	6.3	0.23	0.99	−2.24	0	−0.23	−0.13	n.a.
m-Xylene	3.8	0.014	1.07	−2.24	−1.12	−0.29	−0.07	n.a.
p-Xylene	3.8	0.013	1.02	−2.24	−2.24	−0.29	−0.16	n.a.
o-Cresol	9.5	0.41	−0.18	−1.67	−0.56	−0.44	0.04	−0.13
m-Cresol	6.5	0.25	−0.17	−1.67	−1.12	−0.50	0.12	−0.07
p-Cresol	3.1	−0.071	−0.19	−1.67	−1.67	−0.03	−0.37	−0.16
2-Ethylphenol	4.3	0.061	0.34	−1.86	−0.66	−0.47	n.a.	−0.09
3-Ethylphenol	3.6	−0.011	0.27	−1.86	−1.31	−0.46	n.a.	−0.15
Chlorobenzene	30.1	0.91	0.76	−0.97	0	0.23	n.a.	n.a.
2-Chlorophenol	9.4	0.40	0.02	−1.52	−0.485	0	n.a.	0.68
3-Chlorophenol	3.7	−0.024	0.37	−1.52	−0.76	0.31	n.a.	0.37
4-Chlorophenol	3.0	−0.089	0.26	−1.52	−1.52	0.41	n.a.	0.23
2-Chlorotoluene	6.0	0.21	1.29	−2.09	−0.56	0.16	0.68	n.a.
3-Chlorotoluene	6.7	0.26	1.15	−2.09	−1.12	0.1	0.37	n.a.
4-Chlorotoluene	6.7	0.26	1.2	−2.09	−2.09	0.24	0.23	n.a.
2,6-Dichlorotoluene	3.8	0.015	2.16	−3.06	−0.97	0.58	1.36	n.a.

[a]The induction ratio (*A*/*Ao*) was calculated by dividing the promoter activity for each aromatic compound (*A*) by the promoter activity for the no-effector control (*Ao*).
[b]π = log (octanol–water partition coefficient for each compound) − log (octanol–water partition coefficient for benzene).
[c]Calculated by summing the steric parameter (E_S) values of all the substituents [33] of an aromatic compound. Benzene was used as the reference compound.
[d]Reflects a local steric effect only if TbuT binds to the most electron-rich aryl side in each aromatic compound. The parameter values were calculated as described in Section 2.
[e]The total electronic effect of substituents on the most electron-rich aryl side of each aromatic compound.
[f]The total electronic effect of substituents on methyl moiety in each toluene compound.
[g]Reflects the total electronic effect of substituents on methyl moiety in each phenol compound.
[h]Not applicable.

the aromatics were included for the most electron-rich moiety scenario. The QSAR parameter values used in the TbuT QSAR analysis are presented in Table 2.

According to the *P* values from the QSAR analysis for TbuT (Table 3), the most electron-rich aryl side and methyl moiety scenarios explain equally well the effector specificity data, but the hydroxyl moiety does not. This suggests that the methyl moiety and the most electron-rich aryl side are more involved in determining aromatic effector specificity than the hydroxyl moiety of an aromatic compound. The physiochemical effects of inducing compounds were also characterized. The positive coefficients for the hydrophobic descriptors (Table 3) indicate that the hydrophobicity of aromatics increases transcriptional activation by TbuT [32,33]. The positive coefficients for the steric descriptors indicate a negative effect of steric hindrance [32,33]. The effect of the electron donating nature of the substituent was either negative or positive, which can be explained by the electronic properties of the reference moiety chosen for the model (electron donating aryl vs. electron withdrawing methyl).

The magnitudes of the coefficients in Table 3 indicate which physicochemical factor contributes most to the determination of the extent of activation by the TbuT regulator. For the most electron-rich aryl side scenario, the similar magnitudes of all the coefficients suggest that the effects of hydrophobicity, global steric hindrance, local steric hindrance, and electron donating nature are competing in determining transcriptional activity. When the coefficients of the global and local steric parameters are summed, the effect of the combined steric hindrance may be the greatest. For the methyl moiety scenario, the magnitudes of the parameter coefficients suggest that the steric hindrance of an aromatic compound is the predominant factor in determining effector specificity in the TbuT system.

3.3. QSAR comparison of aromatic effector specificity among different XylR/DmpR transcriptional regulators

Unlike other XylR/DmpR regulators for aromatic monooxygenase pathways [9,15,25], both methylated and hydroxylated aromatics were inducers for the TbuT regulator. To explain the broad effector spectrum observed in the TbuT system, it would be informative to compare the TbuT QSAR results with those for other XylR/DmpR regulators that control aromatic monooxygenase operons. For this purpose, another QSAR analysis was performed for the XylR (from *P. putida* mt-2 [9]) and DmpR (from *Pseudomonas* sp. CF600 [15]) regulators. Because of its extremely narrow effector spectrum [25], the MopR regulator was not considered in the additional QSAR analysis. For the XylR QSAR analysis, the training set included the transcriptional activation data for toluene and substituted toluenes reported by Abril and coworkers [9], which were determined using *E. coli* 5K (pRD579, pTS174). For the DmpR QSAR analysis, the training set included the transcriptional activation data for hydroxyaromatic compounds reported by Shingler and Moore [15], which were determined using *P. putida* KT2440::DmpR (pVI360).

Unlike the TbuT QSAR analysis, the most electron-rich aryl side scenario did not show good correlations with the transcriptional activity data for the XylR (*P* value = 0.234) and DmpR (*P* value = 0.124) regulators, suggesting that the most electron-rich aryl side is not involved in determining effector specificity for the XylR and DmpR systems. Table 3 also presents the results from the XylR QSAR analysis for the methyl moiety scenario, and results from the DmpR QSAR analysis for the hydroxyl moiety scenario. Among the transcriptional activity data in the training set, the data for *o*-ethylphenol were identified as statistical outliers (beyond the 95% prediction level) for the XylR QSAR equation. Also the statistical outliers for the DmpR QSAR equation were the data for 4-ethylphenol, hydroxybenzoates, and 2,3-dimethylphenol. Since the elimination of these compounds from the training set resulted in less than 10% differences in coefficient values for the hydrophobic, steric, and electronic parameters, the outlier data were not considered in the QSAR analysis. The small *P* values indicate that the methyl moiety and hydroxyl moiety scenarios explain

Table 3
QSAR analysis results for TbuT, XylR, and DmpR

Transcriptional regulator (reference)	Reference moiety	Number of aromatic compounds	Coefficients for QSAR parameters				Intercept	Standard error of estimate	R^2	*P* value
			π	ΣE_S	$E_{S-LOCAL}$	$\Sigma\sigma$				
TbuT [This study]	The most electron-rich aryl side	22	0.26 (0.09)[a]	0.32 (0.10)	0.20 (0.08)	−0.25 (0.16)	0.82 (0.14)	0.22	0.63	0.001
	CH_3 moiety	13	0.24 (0.07)	0.77 (0.12)	n.a.[b]	0.16 (0.14)	1.58 (0.24)	0.15	0.83	0.001
	OH moiety	12	0.17 (0.15)	0.22 (0.17)	n.a.	0.08 (0.30)	0.43 (0.30)	0.23	0.29	0.234
XylR [9]	CH_3 moiety	20	0.66 (0.07)	0.43 (0.07)	n.a.	−0.50 (0.07)	1.20 (0.09)	0.15	0.85	<0.001
DmpR [15]	OH moiety	21	−0.11 (0.20)	0.64 (0.15)	n.a.	−0.47 (0.18)	2.16 (0.18)	0.32	0.79	<0.001

[a]The value in parentheses represents one standard error of each coefficient determined from multiple linear regression analysis.
[b]Not applicable.

well the observations for the XylR and DmpR systems, respectively.

According to the results in Table 3, the coefficients for steric descriptors are positive, and their magnitudes are comparable for all three regulators. This suggests that the steric hindrance of an aromatic compound has a universal impact on the different members of the XylR/DmpR subclass of NtrC regulators. Unlike steric hindrance, the hydrophobicity and electron donating nature of an aromatic compound have dissimilar effects in the different regulatory systems. The hydrophobicity of an aromatic compound has a positive effect on transcriptional activation in the TbuT and XylR systems, but has a slight and negative effect on transcriptional activation in the DmpR system. In the case of electronic effect, the coefficient from the TbuT QSAR analysis with the most electron-rich aryl side is statistically negative (-0.25 ± 0.16), and this suggests a mechanistic difference between TbuT and the other regulators.

4. Discussion

Among the tested chloroethenes (Fig. 3), *cis*-1,2-DCE, TCE, and PCE are inducers for TbuT, and TCE is the strongest inducer. *Trans*-1,2-DCE is a non-inducer. Monochloroethene and 1,1-DCE may or may not be inducers due to experimental variations. Although a QSAR result for a backbone structure cannot be used in describing the activity of a specific reaction in response to another chemical structure [32], the TbuT QSAR analysis for aromatic effector specificity data provides insight into chloroethene effector specificity in TbuT. Since the QSAR analysis for aromatic compounds showed that an electron-rich aryl side seems to positively interact with the active site of TbuT, the double bond of an ethene compound might positively influence the transcriptional activation in TbuT. In addition, the transcriptional activation in TbuT seems to increase with an increase of hydrophobicity but seems to decrease with a decrease of steric hindrance. Since the hydrophobicity (log K_{ow}) and steric hindrance (LeBas molecular volume) of chloroethenes increase with the increase of the degree of chlorination [39], it is possible that the transcriptional activation in TbuT is maximized when applying a chlorinated ethene that exhibits a minimal negating effect between hydrophobicity and steric hindrance. Although this does not explain the different promoter activities of the DCEs where log K_{ow} and LeBas value are similar, this possibility offers a plausible explanation for why TCE is the stronger inducer.

In this study, we showed that multiple linear QSAR equations could comprehensibly and quantitatively describe the combined effects of multiple physicochemical properties on effector specificity in NtrC-like transcriptional regulators from aromatic oxidizing bacteria. The results from the inductive QSAR analysis suggest two characteristics of the aromatic effector specificity in TbuT: (i) a steric effect, rather than hydrophobic or electronic effects, may be the predominant factor in determining the effector specificity; and (ii) the TbuT regulator may positively interact not only with the methyl moiety but also with the most electron-rich aryl side of an aromatic effector. The latter may be a unique feature of TbuT because, in the XylR and DmpR systems, only one moiety of an aromatic compound seems to positively interact with the active site of each regulator. This may explain why benzene, toluene and other mono-substituted aromatics are inducers for TbuT. In addition, the QSAR comparison of the three regulators raises an interesting question of why the activation site of XylR seems more similar to that of TbuT despite the fact that the sequence of XylR is close to that of DmpR [20].

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