First publ. in: Environmental Science & Technology 41 (2007), 4, pp. 1390-1398

Biochemical Interpretation of Quantitative Structure−Activity Relationships (QSAR) for Biodegradation of N-Heterocycles: A Complementary Approach to Predict Biodegradability

BODO PHILIPP,*,† MALTE HOFF,‡ FLORENCE GERMA,§ BERNHARD SCHINK,† DIETER BEIMBORN,§ AND VOLKER MERSCH-SUNDERMANN‡

Fakultaet fuer Biologie, Universitaet Konstanz, Fach M654, 78457 Konstanz, Germany, Institute of Indoor and Environmental Toxicology, University of Giessen−University Hospital, Aulweg 123, 35385 Gießen, Germany, and BASF-AG, ZH/TC, Z570, 67056 Ludwigshafen, Germany

Prediction of the biodegradability of organic compounds is an ecologically desirable and economically feasible tool for estimating the environmental fate of chemicals. We combined quantitative structure−activity relationships (QSAR) with the systematic collection of biochemical knowledge to establish rules for the prediction of aerobic biodegradation of N-heterocycles. Validated biodegradation data of 194 N-heterocyclic compounds were analyzed using the MULTICASE-method which delivered two QSAR models based on 17 activating (QSAR 1) and on 16 inactivating molecular fragments (QSAR 2), which were statistically significantly linked to efficient or poor biodegradability, respectively. The percentages of correct classifications were over 99% for both models, and cross-validation resulted in 67.9% (QSAR 1) and 70.4% (QSAR 2) correct predictions. Biochemical interpretation of the activating and inactivating characteristics of the molecular fragments delivered plausible mechanistic interpretations and enabled us to establish the following biodegradation rules: 1. Target sites for amidohydrolases and for cytochrome P450 monooxygenases enhance biodegradation of nonaromatic N-heterocycles. 2. Target sites for molybdenum hydroxylases enhance biodegradation of aromatic N-heterocycles. 3. Target sites for hydratation by an urocanase-like mechanism enhance biodegradation of imidazoles. Our complementary approach represents a feasible strategy for generating concrete rules for the prediction of biodegradability of organic compounds.

Introduction

Biodegradation of man-made compounds by bacteria and fungi is the basis for wastewater purification and remediation of contaminated soils (*1*). Knowledge of the biodegradability of a chemical compound should, therefore, be a prerequisite for its commercial use. Industry and public institutions invest a substantial amount of money and effort to assess the biodegradability of chemical products experimentally according to international standards, e.g., OECD standards. However, not all compounds already on the market have been subjected to such standardized biodegradation tests. It is desirable to fill this knowledge gap for obtaining homogeneous information about biodegradability but, regarding the enormous number of untested compounds, this puts a large financial burden on the chemical industry. The situation has been exacerbated by the "White Paper" of the European Commission for the future chemicals policy that actually demands a unified classification of all chemicals above a certain production volume on the EU-market (*2*). As a consequence of the new regulatory system for chemicals called REACH-program (Registration, Evaluation and Authorization for Chemicals), it is expected that 30 000 compounds have to be tested for their hazardous potential for the environment.

It is apparent that a chemical product prediction tool, which is based on models and existing information rather than on expensive, time-consuming experiments, would be of great benefit for ecological and economical reasons. Biodegradability prediction could not only complement and substitute in part for some of the costly experimental evaluation of biodegradability but it could also help to identify and potentially avoid the production of new chemical compounds which are not biodegradable. Thus, prediction of biodegradability would support the development of environmentally sustainable new products and the design of synthesis strategies that avoid poorly degradable intermediates and waste products. Finally, prediction of biodegradability would also be valuable for risk assessment of chemical compounds, for handling accidental situations, and for predicting the fate and behavior of degradates in the environment (*3*).

There are two main strategies used to establish models for prediction of biodegradation: Quantitative structure−activity relationships (QSAR) models and predictions of plausible metabolic pathways. While the first approach is based on statistics, the latter relies on the transfer of existing biochemical knowledge.

QSAR models for biodegradation are created with experimental biodegradation data and structures of the corresponding chemicals (for examples, see refs *4* and *5*). The advantages of QSAR models are that they provide statistically significant predictions and imply physicochemical properties of the respective chemicals. However, these models lack information about metabolic pathways, and, from a biochemical viewpoint, these models remain abstract.

The other strategy toward prediction of biodegradation is to collect and systematize knowledge about metabolic pathways. An outstanding example of this concept is the highly linked and interactive biocatalysis/biodegradation database developed by the University of Minnesota (UM−BBD; http://umbbd.ahc.umn.edu/; *6*). From this database, rules of biodegradation were extracted which can then be used for computer programs that create pathway-based models. Recently, a pathway-based prediction function was added to the UM−BBD (http://umbbd.msi.umn.edu/predict/; *7*). Further examples for such computer programs are META (*8*) and CATABOL (*9*). These programs predict plausible metabolic pathways based on SARs which, in contrast to QSAR models, do not deliver quantitative values for biodegradability. The pathway-based approach is always limited

* Corresponding author phone: +49 7531-884541; fax: +49 7531-884047; e-mail: bodo.philipp@uni-konstanz.de.
† Universitaet Konstanz.
‡ University of Giessen.
§ BASF-AG.

by the current status of research. Knowledge about metabolic pathways is far from being complete, and only a small part of it has been systematized in an appropriate way. For example, most of the compounds used in our study are not included in the UM−BBD. Degradation pathways were mainly elucidated with selected bacterial strains or purified enzymes. Extrapolations of such studies to the environment are certainly not sufficient to predict the biodegradability of untested compounds. Therefore, the pathway-based approach gives plausible mechanistical information but it lacks general transferability and requires proof of statistical significance.

Obviously, the two approaches to predict biodegradability are complementary. Computer-based QSAR-models would be rendered more conceivable by the addition of biochemical background information and, in turn, pathway-based models could be checked for their transferability and statistical significance.

In this study, we used such a complementary approach to predict aerobic biodegradation of N-heterocycles. Our aim was to establish a framework for deriving concrete biodegradation rules that could be applied to categorize chemical compounds without experimental biodegradation tests. We created and validated a database containing the results of experimental biodegradation tests for a number of N-heterocycles. This database was used as a learning set to create QSAR-models using the method MULTICASE (*8*). In parallel, we searched the literature for biochemical mechanisms involved in biodegradation of the compounds found in our database. There are several biochemical mechanisms known to initiate degradation of N-heterocycles (*10*−*14*). Based on this knowledge, we interpreted our QSAR models from a biochemical viewpoint.

Methods

Statistical Analysis. The MULTICASE method has been described in detail elsewhere (*15*). This software is designed to automatically perform statistical analysis of learning sets containing structures and biological activity data of organic compounds, detect the molecular fragments significantly linked to a biological endpoint, and derive predictive QSAR-models there from. In order to run statistical algorithms on the learning set, the program calculates all the possible fragments for each molecule, ranging from 2 to 10 non-hydrogen atoms along with their associated hydrogen atoms. Each of these fragments as well as automatically calculated 2-dimensional distance descriptors (based upon the presence of lipophilic centers and heteroatoms in the molecule) is associated with a confidence level and a probability of activity that is derived from its distribution among biological active and inactive molecules. Molecules containing the same activating fragments but having slightly different activity values are then searched for modulators of activity. These modulators may be chemical properties (eg. structural fragments), physicochemical properties (eg. log P_{ow}), or quantum chemical parameters (eg. HOMO and LUMO energies), which are calculated by the program. Provided that the learning set contained sufficient data on potential SAR, a QSAR-model is established which enables the program to run in a predictive mode and evaluate untested molecules.

Origin, Evaluation, and Use of the Data. Experimental biodegradation data were taken from a BASF database and from three public databases: the MITI-Database from Japan, the EFDB (Environmental Fate Database) from Syracuse Research Corporations in the U.S., and the IUCLID database from the EU. We extracted experimental data for ultimate aerobic biodegradation in water for 194 compounds containing at least one N-heterocycle. For most compounds, experimental data from different biodegradation test were available (e.g., for 1-methyl-pyrrolidine-2-one (compound 10 in Table 1) we had results from 29 degradation tests including modified and original MITI tests, OECD 302B test, Zahn/Wellens test). To define clear biological endpoints, we manually checked these experimental data for consistency. On the basis of this evaluation, we classified the N-heterocycles into three categories, namely readily/inherently, moderately, and poorly degradable, according to OECD standards (*16*). We obtained the permission from BASF to publish the structures and biodegradability for 134 compounds of this database. These 134 N-heterocycles (Table 1) comprised 58 biodegradable (readily or inherently), 21 moderately biodegradable, and 55 poorly biodegradable compounds.

MULTICASE analysis was performed with the 194 data set to determine fragments that activate (QSAR 1) or inactivate (QSAR 2) the aerobic biodegradation of N-heterocycles. In QSAR 1, biodegradable compounds were considered as active and were assumed to contain biodegradation-related structural fragments (activating fragments). Poorly biodegradable compounds were considered as inactive and were assumed not to contain structural fragments activating biodegradation or containing inactivating fragments. To identify inactivating fragments (QSAR 2), the scale of activity was inversed, labeling biodegradable molecules as inactive and poorly biodegradable molecules as active before analysis with MULTICASE.

Literature Analysis. For literature analysis of biodegradation of N-heterocycles, freely accessible Internet databases (UM−BBD, Pubmed, Agricola) and standard textbooks were used. Literature analysis was focused mainly on the primary enzymatic attack on a specific molecule and on mechanistic aspects of the respective enzymes.

Results and Discussion

Statistical Analysis with MULTICASE. Seventeen fragments were found to activate (Table 2a) and 16 fragments were found to inactivate (Table 2b) aerobic biodegradation of N-heterocycles. The last two columns in Table 1 indicate which fragments occur in the respective molecules.

For validation of the QSAR models, we conducted two test procedures using the MULTICASE method's predictive mode. The internal consistency was checked by submitting every single molecule of the learning set to the QSAR model that has been generated with this learning set. This auto-validation resulted in over 99% correct predictions for both models. Two compounds were not correctly predicted: the poorly degradable 3-hydroxy-6-methylpyridine (compound 116 in Table 1) was predicted as degradable by both QSAR models, and the degradable vitamine B1 hydrochloride (compound 58 in Table 1) was predicted poorly degradable by QSAR 2. Possible explanations for these wrong predictions are given below.

Predictive performance was assessed by the so-called "leave 10% out" method. For that, each model was re-established using a learning set reduced by 10% of its data. The activities of these withdrawn 10% molecules were then predicted by QSAR models created with the remaining 90% of the dataset. This procedure was repeated 10 times in a way that every molecule was left out of the learning set and tested by the respective model once. If MULTICASE detects unknown fragments, or fragments with a low confidence level or with a low probability, the respective molecules are rejected as inconclusive and no prediction is made. To optimize the validation we used an unequivocal validation set by leaving out the moderately biodegradable compounds. Nicotinic acid (compound 40 in Table 1) was the only readily degradable compound that was predicted as moderately degradable in both QSAR models, and was counted as false. In total, 84 and 81 predictions were obtained by the models of which 67.9 and 70.4% were correct in QSAR 1 and QSAR 2, respectively (Figure 1).

TABLE 1. List of 134 N-heterocyclic Compounds[a]

no	CAS	name	experim. biodeg.	pred. biodeg. (QSAR 1)	pred. biodeg. (QSAR 2)	activating fragments present in analysis	inactivating fragments present in analysis
1	3699−54−5	1-(2-HYDROXY-ETHYL)-IMIDAZOLIDINE−2-ONE	+	+	+	2, 15	4
2	3445−11−2	1-(2-HYDROXY-ETHYL)-PYRROLIDINE−2-ONE	+	+	+	1	4
3	123−33−1	1,2-DIHYDRO-PYRIDAZINE−3,6-DIONE	+	+	−	2	
4	5395−50−6	1,3,4,6-TETRAKIS-HYDROXYMETHYL-TETRAHYDRO-IMIDAZO[4,5-D]IMIDAZOLE−2,5-DIONE	+			3	
5	121−82−4	1,3,5-TRINITRO-[1,3,5]TRIAZINANE	+	+		3	
6	13811−50−2	1,3-DIVINYL-IMIDAZOLIDINE−2-ONE	+	−		17	
7	106−58−1	1,4-DIMETHYL-PIPERAZINE	+	−	−	16	4
8	6837−24−7	1-CYCLOHEXYL-PYRROLIDINE−2-ONE	+	+	−	1	
9	2687−91−4	1-ETHYL-PYRROLIDINE−2-ONE	+	+	+	1	4
10	872−50−4	1-METHYL-PYRROLIDINE−2-ONE	+	+		1	4
11	2687−94−7	1-OCTYL-PYRROLIDINE−2-ONE	+	+	+	1	4
12	4370−23−4	1-VINYL-PIPERIDINE−2-ONE	+	+	+	1, 17	
13	931−36−2	2-ETHYL−5-METHYL-1H-IMIDAZOLE	+	−	+	6	
14	1072−62−4	2-ETHYL-IMIDAZOLE	+		+	6	
15	36947−68−9	2-ISOPROPYL-IMIDAZOLE	+	−	+	6	
16	693−98−1	2-METHYL-IMIDAZOLE	+	−	+	6	
17	109−07−9	2-METHYL-PIPERAZINE	+	+	+	7	4
18	103−76−4	2-PIPERAZINE−1-YL-ETHANOL	+	−	−	15	4
19	113−98−4	3,3-DIMETHYL−7-OXO−6-PHENYLACETYLAMINO−4-THIA−1-AZA-BICYCLO[3.2.0]HEPTANE−2-CARBOXYLIC ACID	+	+		1, 2, 9	
20	101−25−7	3,7-DINITROSO−1,3,5,7-TETRAAZA-BICYCLO[3.3.1]NONANE	+	+		3	
21	66−72−8	3-HYDROXY−5-HYDROXYMETHYL−2-METHYL-PYRIDINE−4-CARBALDEHYDE	+	+		5	
22	626−56−2	3-METHYL-PIPERIDINE	+		+	7	4
23	108−99−6	3-METHYL-PYRIDINE	+	+	+	11	
24	2555−05−7	3-METHYL-PYRROLIDINE−2-ONE	+	+		2	
25	810−16−2	4,4′-[METHYLENEBIS(METHYLIMINO)]BIS[1,2-DIHYDRO−1,5-DIMETHYL−2-PHENYL-3H-PYRAZOL−3-ONE]	+	+		3	
26	58−56−0	4,5-BIS-HYDROXYMETHYL−2-METHYL-PYRIDINE−3-OL; HYDROCHLORIDE	+			5	
27	96−45−7	4,5-DIHYDRO-IMIDAZOLE−2-THIOL	+			8	
28	1854−26−8	4,5-DIHYDROXY−1,3-DIMETHYL-IMIDAZOLIDINE−2-ONE	+		+		
29	51−35−4	4-HYDROXY-PYRROLIDINE−2-CARBOXYLIC ACID	+	+	+	1, 9	
30	108−89−4	4-METHYL-PYRIDINE	+	+	+	11	
31	822−36−6	5-METHYL-IMIDAZOLE	+	−	+	6	
32	120081−44−9	ACETIC ACID 2-(5-OXO-PYRROLIDINE−2-YL)-ETHYL ESTER	+	+		1, 2, 9	
33	947−04−6	AZACYCLOTRIDECANE−2-ONE	+	+	+	1, 2	
34	95−16−9	BENZOTHIAZOLE	+	−	+	9	
35	59−30−3	FOLIC ACID	+	+		1, 2, 4, 9	
36	288−32−4	IMIDAZOLE	+	−	+	6	6
37	120−93−4	IMIDAZOLIDINE−2-ONE	+		+	2, 7	
38	120−72−9	INDOLE	+	−	+	13	
39	98−92−0	NICOTINAMIDE	+	+		4	
40	59−67−6	NICOTINIC ACID	+	o	o	4, 9	
41	85−41−6	PHTHALIMIDE	+	−	+	2, 4	7
42	98−98−6	PICOLINIC ACID	+	+	+	4, 9	
43	110−85−0	PIPERAZINE	+	−	+	7	4
44	675−20−7	PIPERIDINE−2-ONE	+	+	+	1, 2	
45	72762−00−6	PYRIDINE−2-OL	+	−	−	10	
46	109−00−2	PYRIDINE−3-OL	+	−	+	12	
47	626−64−2	PYRIDINE−4-OL	+	−	+	12	
48	89−00−9	PYRIDINE−2,3-DICARBOXYLIC ACID	+			4, 9	
49	499−80−9	PYRIDINE−2,4-DICARBOXYLIC ACID	+	+	+	4, 9	
50	100−26−5	PYRIDINE−2,5-DICARBOXYLIC ACID	+	+	+	4, 9	
51	499−83−2	PYRIDINE−2,6-DICARBOXYLIC ACID	+		−	4, 9	
52	499−81−0	PYRIDINE−3,5-DICARBOXYLIC ACID	+	+	+	4, 9	
53	147−85−3	PYRROLIDINE−2-CARBOXYLIC ACID	+	+	+	1, 9	
54	91−22−5	QUINOLINE	+	−	+	14	
55	93−10−7	QUINOLINE−2-CARBOXYLIC ACID	+	+	+	4, 9	
56	486−74−8	QUINOLINE−4-CARBOXYLIC ACID	+		+	4, 9	
57	496−46−8	TETRAHYDRO-IMIDAZO[4,5-D]IMIDAZOLE−2,5-DIONE	+			2, 3	
58	67−03−8	THIAMINE HYDROCHLORIDE (VITAMINE B1)	+		−	5, 8	5
59	61−82−5	1,2,4-TRIAZOL−3-YLAMINE	o				3
60	3270−74−4	1,3-BIS-HYDROXYMETHYL-TETRAHYDRO-PYRIMIDIN−2-ONE	o	−	+		4
61	1072−68−0	1,4-DIMETHYL-1H-PYRAZOLE	o	+			
62	108−47−4	2,4-DIMETHYL-PYRIDINE	o	o	o	5, 11	
63	108−48−5	2,6-DIMETHYL-PYRIDINE	o	o	o		
64	931−35−1	2-ETHYL−4-METHYL−4,5-DIHYDRO-IMIDAZOLE	o		+	7	
65	36734−19−7	3-(3,5-DICHLORO-PHENYL)−2,4-DIOXO-IMIDAZOLIDINE−1-CARBOXYLIC ACID ISOPROPYLAMIDE	o	+			7
66	626−61−9	4-CHLORO-PYRIDINE	o	−	−		10
67	72−40−2	5-AMINO-3H-IMIDAZOLE−4-CARBOXYLIC ACID AMIDE; HYDROCHLORIDE	o				
68	00−00−0	5-CHLORO-INDOLE−2,3-DIONE 3-OXIME	o			2	10
69	86−50−0	O,O-DIMETHYL-S-(1,2,3-BENZOTRIAZINYL−4-KETO)-METHYL PHOSPHORODITHIOATE	o			4	11

TABLE 1 (Continued)

no.	CAS	name	experim. biodeg.	pred. biodeg. (QSAR 1)	pred. biodeg. (QSAR 2)	activating fragments present in analysis	inactivating fragments present in analysis
70	67−52−7	BARBITURIC ACID	o	+	+	10	
71	149−30−4	BENZOTHIAZOLE−2-THIOL	o				
72	133−06−2	1,2,3,6-TETRAHYDRO-N-(TRICHLOROMETHYLTHIO)-PHTHALIMIDE	o				7, 13
73	64902−72−3	CHLORSULFURON	o	+	−	2	1
74	108−80−5	CYANURIC ACID	o	+	+	2	7
75	55−22−1	ISONICOTINIC ACID	o	+	+	4, 9	
76	00−00−0	{[4-(BIS-METHOXYMETHYL-AMINO)−6-(METHOXYMETHYL-AMINO)-[1,3,5]TRIAZINE−2-YL]-METHOXYMETHYL-AMINO}-METHANO	o				
77	100−97−0	METHENAMINE	o	+	+	3	
78	16867−04−2	PYRIDINE−2,3-DIOL	o	−	−	10	
79	136−45−8	PYRIDINE−2,5-DICARBOXYLIC ACID DIPROPYL ESTER	o	+		4, 9	
80	119−17−5	1-(3′-SULFOPHENYL)−3-METHYL−5-PYRAZOLON	−	−	−		1, 11
81	288−88−0	1,2,4-TRIAZOLE	−			8	
82	551−92−8	1,2-DIMETHYL−5-NITRO-1H-IMIDAZOLE	−		−		1
83	1739−84−0	1,2-DIMETHYL-IMIDAZOLE	−	−	+		
84	839−90−7	1,3,5-TRIS-(2-HYDROXY-ETHYL)-[1,3,5]TRIAZINANE−2,4,6-TRIONE	−	−	+		7
85	80−73−9	1,3-DIMETHYL-IMIDAZOLIDINE−2-ONE	−	+	+		4, 9
86	7226−23−5	1,3-DIMETHYL-TETRAHYDRO-PYRIMIDIN−2-ONE	−	−	−		4, 9
87	6674−22−2	1,8-DIAZABICYCLO[5.4.0]UNDEC−7-EN	−	−	+	8	4, 9
88	5308−25−8	1-ETHYL-PIPERAZINE	−	−	−		4
89	616−47−7	1-METHYL-IMIDAZOLE	−	−			6
90	109−01−3	1-METHYL-PIPERAZINE	−	−	+		4
91	1462−84−6	2,3,6-TRIMETHYL-PYRIDINE	−		+		
92	2402−77−9	2,3-DICHLORO-PYRIDINE	−	−	−		2
93	108−75−8	2,4,6-TRIMETHYL-PYRIDINE	−	−	+		
94	16110−09−1	2,5-DICHLORO-PYRIDINE	−	−	−		2, 10
95	2402−78−0	2,6-DICHLORO-PYRIDINE	−	−	−		2
96	122−96−3	2-[4-(2-HYDROXY-ETHYL)-PIPERAZINE−1-YL]-ETHANOL	−	+	+		4
97	5407−87−4	2-AMINO−4,6-DIMETHYLPYRIDINE	−	−	−		5
98	16867−03−1	2-AMINO-PYRIDINE−3-OL	−	+	+		5
99	504−29−0	2-AMINO-PYRIDINE	−	−			5
100	109−09−1	2-CHLORO-PYRIDINE	−	−			2
101	89−25−8	2-CYCLOHEXYL−5-METHYL−2,4-DIHYDRO-PYRAZOL−3-ONE	−				11
102	13373−32−5	2-ISOPROPYL−4-NITRO-IMIDAZOLE	−	−	−		1
103	696−23−1	2-METHYL−4-NITRO-IMIDAZOLE	−	−	−		1
104	670−96−2	2-PHENYL-IMIDAZOLE	−	+		6	
105	140−31−8	2-PIPERAZINE−1-YL-ETHYLAMINE	−	−			4, 12
106	21564−17−0	2-THIOCYANATOMETHYLSULFANYL-BENZOTHIAZOLE	−				14
107	100−69−6	2-VINYL-PYRIDINE	−				15
108	462−08−8	3-AMINO-PYRIDINE	−	−	−		5
109	626−60−8	3-CHLORO-PYRIDINE	−	−	−		10
110	887−76−3	3-HYDROXY-BENZO[CD]INDAZOLE−5-SULFONIC ACID	−		−		1
111	2859−67−8	3-PYRIDINE−3-YL-PROPAN−1-OL	−	−	+		16
112	3034−38−6	4-NITROIMIDAZOLE	−	+			1, 6, 14
113	92−53−5	4-PHENYL-MORPHOLINE	−	−	+		4
114	551−16−6	6-AMINO−3,3-DIMETHYL−7-OXO−4-THIA−1-AZA-BICYCLO[3.2.0]HEPTANE−2-CARBOXYLIC ACID	−			1, 9	12
115	4684−94−0	6-CHLORO-PYRIDINE−2-CARBOXYLIC ACID	−	+	−	4, 9	2
116	1121−78−4	6-METHYL-PYRIDINE−3-OL	−	+	+	5, 12	
117	85−02−9	BENZO[F]QUINOLINE	−	+	+		
118	95−14−7	BENZO-TRIAZOLE	−		−		3
119	59756−60−4	1-METHYL−3-PHENYL−5-(3-(TRIFLUOROMETHYL)PHENYL)−4(1H)-PYRIDINONE	−		−		6
120	108−78−1	MELAMINE	−	−	+		5
121	13358−11−7	N-(2-ETHYLHEXYL)−1-ISOPROPYL−4-METHYLBICYCLO[2,2,2]-OCTA−5-ENE−2,3-DICARBOXYIMIDE	−				7
122	5036−48−6	N-(3-AMINOPROPYL)-IMIDAZOLE	−				6, 12
123	104−19−8	N-DIMETHYLAMINOETHYL-N-METHYL-PIPERAZINE	−	+			4
124	1929−82−4	2-CHLORO−6-(TRICHLOROMETHYL)-PYRIDINE	−		−		2
125	92−84−2	PHENOTHIAZINE	−	−	+		
126	139−40−2	2-CHLORO−4,6-BIS(ISOPROPYLAMINO)-s-TRIAZINE	−	−	−		2
127	1970−40−7	2,3,5-TRICHLORO-PYRIDINE−4-OL	−	−	−		2
128	504−24−5	PYRIDINE−4-YLAMINE	−	−	−		5
129	141−86−6	PYRIDINE−2,6-DIAMINE	−	−	−		5
130	452−58−4	PYRIDINE−3,4-DIAMINE	−		−		5
131	886−50−0	2-TERT-BUTYLAMINO−6-METHYLTHIO−1,3,5-TRIAZINE	−				
132	27813−21−4	TETRAHYDROPHTHALIMIDE	−	+	+	2	7, 13
133	2921−88−2	THIOPHOSPHORIC ACID O,O′-DIETHYL ESTER O″-(3,5,6-TRICHLORO-PYRIDIN−2−YL) ESTER	−		−		2
134	124172−53−8	N,N′-BISFORMYL-(2,2,6,6-TETRAMETHYL−4-PIPERIDINYL)-HEXAMETHYLENDIAMINE	−				

[a] Compounds are grouped according to their experimental degradation rates and listed alphabetically within this category. Experimental biodegradation (experim. biodeg.) rates and predicted biodegradation (pred. biodeg.) rates are indicated as readily/inherently (+), moderately (○), or poorly (−). The predicted biodegradation rates refer to the result of the "leave 10% out" cross-validation of the QSAR models. The numbers for activating and inactivating fragments refer to the molecular fragments depicted in Table 2a and b.

TABLE 2. Molecular Fragments Identified by MULTICASE Analysis that are Either (a) Activating or (b) Inactivating Aerobic Biodegradation of N-heterocycles

fragment number	activating fragments[a]	expanded fragments[a,b]
	a	
1	CO −CH2−CH2-	CO−CH−NH
2	CO −NH−	
3	N −CH2−N −	N−CH−N−
4	CO −c =cH −	
5	CH3−c =n −cH =	
6	NH −C=N −CH =	
7	NH −CH2−CH −CH3	NH−CH2−CH−NH−
8	CH2−N=C −	
9	O −C =	S−CH=
10	OH −c =n −	
11	cH =cH −c =cH − <3−CH3>	
12	cH =cH −c =cH − <3−OH>	
13	CH″−NH −c. =	
14	cH =cH −cH =c. −cH =cH −cH =	
15	OH −CH2−CH2−N −CH2−CH2−NH −	
16	CH3−N −CH2−CH2−N −CH2− <5CH3>	
17	CH2−N −CH = <2−CO>	
	b	
1	SO2−c =	NO2−c=
2	Cl −c =n −	
3	NH −N =	
4	CH2−N −CH2−CH2−	
5	NH2−c =	
6	CH″−N −CH =	
7	CO −N −CO −	
8	N =C. −CH2−	
9	N −CO −N −CH2− <3−CH3>	
10	cH =cH −c =cH − <3−Cl>	
11	CO −N −N =	
12	NH2−CH −	
13	CO −CH −CH2−CH =	
14	NO2−CH −	CN−S−
15	n =c −CH =	
16	OH −CH2−CH2−CH2−	

[a] Legend for atoms in fragments: C: aliphatic atom (carbon); c: aromatic atom (carbon); <5-CH3>: side chain (CH3) with location (*5*); N″: atom (nitrogen) linked by a double-bond to a group not shown in the fragment; .: atom common between two rings. [b] Expanded fragments are found in insufficient number of molecules to warrant their selection as independent fragments, but selected anyhow because they only exist in active molecules and their structures are similar to bona fide fragments. Expanded fragments are listed right after the independent fragment they are emulating.

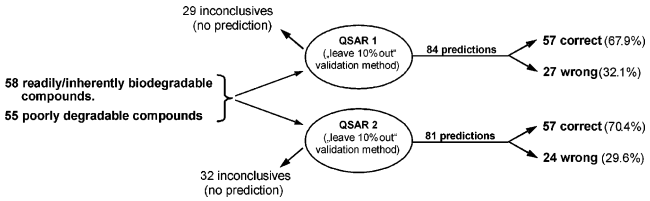

FIGURE 1. Flowchart of the validation procedure for QSAR-models 1 and 2 by the "leave 10 % out" method using the predictive mode of the MULTICASE software. We considered only those compounds that were biodegradable (readily or inherently, 58 compounds) or poorly biodegrable (55 compounds). Twenty One compounds with a moderate biodegradability were not included in the validation. Of the remaining 113 molecules, 29 and 32 were inconclusive and could not be predicted by QSAR models 1 and 2, respectively.

Key Reactions Initiating Aerobic Degradation of N-**Heterocyclic Compounds.** Analysis of the literature data led to the identification of four key reactions for primary enzymatic attack on most N-heterocycles in our database. Examples of each key reaction and the corresponding type of enzyme are depicted in Figure 2. All key reactions involve the introduction of an oxygen atom either from water or from dioxygen into the molecule leading either to hydrolysis or hydroxylation of N-heterocycles. The site of hydroxylation is the later target site for ring cleavage.

Nonaromatic Heterocycles. Hydroxylation of the *C*2-atom in nonaromatic heterocycles is catalyzed by cytochrome P450 monooxygenases and is found to initiate degradation of piperidines, pyrrolidines, and morpholines (*12*, *17*−*19*). This reaction is analogous to N-dealkylations and proceeds most probably via a radical mechanism which involves the intermediate formation of an aminium-radical (*20*; Figure 3). N-heterocycles with an oxo group adjacent to the N-heteroatom can be cleaved directly by amidohydrolases, an important group of enzymes in the metabolism of N-heterocycles (*21*).

*Aromatic Heterocycles***.** The most common initial attack is a hydroxylation at *C*2-atom adjacent to the N-atom in the ring. In the case of quinolines and substituted pyridines, a hydroxyl group derived from water is introduced by a nucleophilic attack catalyzed by molybdenum-containing enzymes (*13*, *14*). Quinoline hydroxylases are also capable of hydroxylating pyrimidines as a cometabolic reaction (*22*). Pyrimidines can also be hydroxylated by a specific enzyme, which is most probably a molybdoenzyme (*23*). Another way

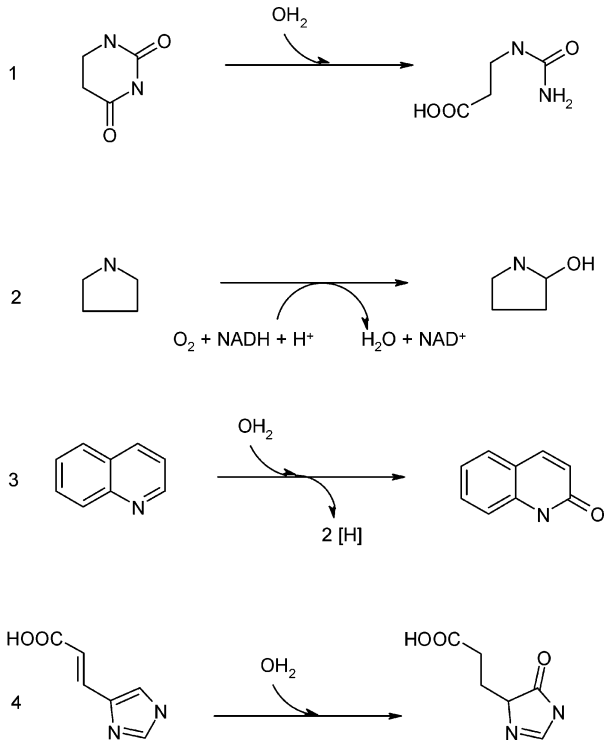

FIGURE 2. Examples for key reactions initiating aerobic bacterial degradation of N-heterocyclic compounds: (1) Hydrolysis of dihydrouracil by an amidohydrolase (see ref *23*); (2) Monohydroxylation of pyrrolidine catalyzed by a cytochrome P450 monooxygenase (*18*); (3) Hydroxylation of quinoline catalyzed by a molybdenum hydroxlase (*14*); (4) Hydratation of the imidazole moiety of urocanate catalyzed by urocanase (*24*).

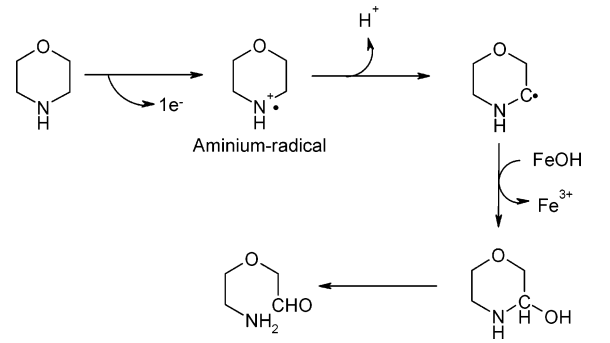

FIGURE 3. Hypothetical radical mechanism for ring cleavage of morpholine catalyzed by a cytochrome P450 monooxygenase in analogy to N-dealkylation (see ref *20*).

of introducing a hydroxyl group is the addition of water to a double bound. Such a hydration occurs in degradation of the imidazole moiety of urocanate, the first intermediate in degradation of histidine. This reaction is catalyzed by urocanase, an enzyme containing a tightly bound NAD^+-cofactor (*24*−*25*). Unsubstituted pyridine and pyrimidine bases like uracil or thymine are generally, although not exclusively, reduced prior to hydroxylation (*26*). The resulting dihydropyrimidines are cleaved by amidohydrolases. In degradation of *s*-triazine derivatives, cyanuric acid is the central intermediate (*27*). Its aromatic character is so much diminished that it can be cleaved directly by an amidohydrolase.

Biochemical Interpretation of QSAR Models. We compared the results of both our statistical and our literature analysis in order to explore plausible biochemical explanations for the activating or inactivating character of the molecular fragments. Only fragments occurring in at least

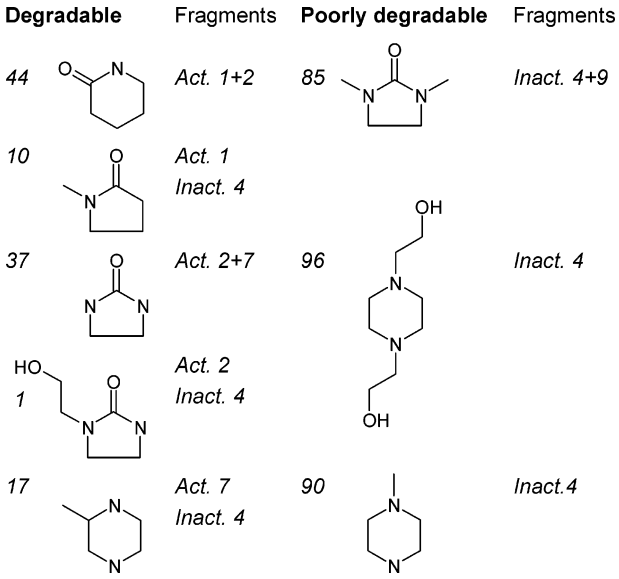

FIGURE 4. Examples for degradable and poorly degradable nonaromatic N-heterocycles containing selected activating (act.) or inactivating (inact.) fragments. Numbers left to the structural formulas indicate their position in Table 1 (*44*). Piperidine-2-one, (*10*) 1-methyl-pyrrolidine-2-one, (37) imidazolidin-2-one, (*1*) 1-(2-hydroxy-ethyl)-imidazolidine-2-one, (*17*) 2-methyl-piperazine, (85) 1,3-dimethyl-imidazolidine-2-one, (96) 2-[4-(2-hydroxy-ethyl)-piperazine-1yl]-ethanol, (90) 1-methyl-piperazine. Molecules 1, 10, and 17 are readily degradable despite the presence of inactivating fragment 4. The presence of inactivating fragment 4 does not lead to poor biodegradation if target sites for amidohydrolases (activating fragments 1 or 2) are present or if the N-atoms in the ring are not alkylated (molecule 17). N-alkylated molecules without activating fragments 1 or 2 are mainly poorly biodegradable (molecules 90 and 96).

three molecules were considered (with the exception of inactivating fragments 3 and 9):

Nonaromatic Heterocycles. Activating fragments 1 and 2 are both found mainly within nonaromatic heterocycles (92% for fragment 1 and 73% for fragment 2) carrying an oxo group, e.g., pyrrolidones and piperidones (as examples see molecules 1, 10, 37, and 44 in Figure 4). In five molecules, both fragments are found. The oxo group of activating fragment 1 is mostly located adjacent to the N-heteroatom like in activating fragment 2, and this substructure represents a target site for the cleavage of the C−N bond by an amidohydrolase. All readily degradable molecules with activating fragment 2 and 84% of readily degradable molecules with activating fragment 1 had such target sites. Thus, the biochemical interpretation of the biodegradation-enhancing character of activating fragments 1 and 2 is that they are target sites for amidohydrolases.

Activating fragment 7 is mainly found in derivatives of piperidine and piperazine (as an example, see molecule 17 in Figure 4) that do not carry an oxo group attached to the ring. Literature analysis suggests a hydroxylation of the *C*2-atom as the initiating reaction in degradation of piperazines and piperidines, catalyzed by a cytochrome P450 monooxygenase. All readily degradable molecules carrying activating fragment 7 have at least one secondary C-atom adjacent to the N-heteroatom which might be a structural prerequisite for hydroxylation at this position. Inactivating fragment 4 is structurally related to activating fragment 7 and was found in 9 poorly, in 1 moderately, and in 10 readily degradable nonaromatic N-heterocycles. In 14 of these molecules, an alkyl side chain is attached to at least one N-heteroatom. These N-alkylated compounds are readily degradable if activating fragments 1 or 2 are present in the same molecule,

namely an oxo group adjacent to the alkylated N-heteroatom (molecules 1 and 10 in Figure 4). Obviously, N-alkylation does not inactivate target sites for amidohydrolases, except if the C-atom with the oxo group is framed by two methylated N-atoms as in inactivating fragment 9 (molecule 85 in Figure 4).

Seven of these N-alkylated molecules with inactivating fragment 4 do not have an oxo group in the ring, and hydroxylation of the neighboring C-atom would be the expected initiating degradation step (molecule 96 in Figure 4). Five of these N-akylated molecules are poorly degradable implicating that N-alkylation might interfere with the attack of hydroxylating enzymes, e.g., cytochrome P450 monooxygenases. A mechanistic explanation could be that the formation of the postulated aminium radical (Figure 3) is not possible with alkylated N-atoms. However, this mechanism would not explain how N-alkylation could block biodegradation of piperazine derivatives in which only one N-heteroatom is alkylated (molecule 90 in Figure 4). Apparently, there appears to be a strong influence by N-alkylation on the biodegradability of nonaromatic N-heterocycles but we could not find any publications dealing with this aspect. It was reported only recently that the ethylpiperazine-moiety of the fluoroquinolone enrofloxacin can be degraded by pure cultures of basidiomycetes (*28*). Apparently, N-alkylated N-heterocycles are degradable but the respective enzymatic mechanisms are not known.

Activating fragment 3 is found in a heterogeneous group of mainly readily degradable nonaromatic N-heterocycles that have either target sites for amidohydrolases or for cytochrome P450 monooxygenases.

Aromatic Heterocycles. 93% of the molecules carrying activating fragment 4 and 100% of those carrying activating fragment 5 are aromatic N-heterocycles, mainly pyridines and quinolines. Literature analysis suggests that the initiating step in degradation of such compounds is a hydroxylation of the *C*2-atom catalyzed by molybdenum hydroxylases (see above). Most of the readily degradable compounds (66% for fragment 4 and 75% for fragment 5) carry a secondary C-atom adjacent to the N-heteroatom which is required for a nucleophilic attack by a molybdenum-dependent hydroxylase. If the *C*2-position is blocked by carboxyl groups like in molecules 51 (pyridine-2,6-dicarboxylic acid) and 55 (quinoline-2-carboxylic acid), hydroxylation by molybdenum-dependent enzymes or oxygenases can occur at other C-atoms as well (*11*, *29*). Activating fragment 8 is found in aromatic and nonaromatic N-heterocycles and could not be related to a specific reaction initiating their biodegradation. Molecules with activating fragment 9 overlap largely with aromatic compounds carrying fragment 4 (68%) or with nonaromatic molecules carrying fragments 1 or 2. Activating fragments 10, 11, and 12 were found in 55% degradable aromatic N-heterocycles with a secondary C-atom at the *C*2 position. We could not find a mechanistic explanation why 3-hydroxy-6-methylpyridine (compound 116 in Table 1) was poorly degradable since it carries activating fragments 5 and 12 and has a target site for hydroxylation. As this molecule was the only one predicted falsely in the QSAR 1 autovalidation, we assume that the experimental classification of its biodegradability was incorrect.

Activating fragment 6 is exclusively found in imidazole derivatives. Four out of 5 molecules carrying inactivating fragment 6 are imidazoles as well. The main structural difference between the imidazole moieties linked to these two fragments is that in inactivating fragment 6, one N-atom is alkylated (molecule 89 in Figure 5) while in activating fragment 6, they all carry an H-atom (molecule 16 in Figure 5). The only detailed mechanistic knowledge about degradation of imidazoles stems from the metabolism of histidine.

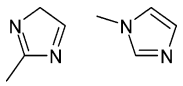

FIGURE 5. Examples for readily and poorly degradable imidazoles. Numbers below the structural formulas indicate their position in Table 1 (16). 2-Methyl-imidazole (readily degradable): Activating fragment 6; (89) 1-methyl-imidazole (poorly degradable): inactivating fragment 6. Imidazoles were found to be poorly degradable if they contain alkylated N-atoms.

There, a double bond of the heterocyclic residue is hydrated adjacent to one of the N-atoms, but not between the N-atoms. A possible explanation for the poor biodegradability of N-alkylated imidazoles might thus be that a mesomeric structure necessary for this hydration is unlikely to occur, probably due to the unfavorable formation of a quaternary N-atom. This biochemical interpretation of the QSAR models supports structure−activity relationships for imidazoles proposed in an earlier study (*30*). Degradation studies with imidazole and its derivatives strongly suggested that their degradation proceeds via a 4(5)-imidazolone intermediates (*10*, *31*). Thus, there is some evidence that the actual degradation pathway for imidazoles in nature involves indeed an urocanase-like hydratation.

Inactivating fragments 1 (including the expanded fragment) and 2 are very general inactivating fragments because organic molecules carrying sulfonate groups (*32*), nitro groups (*33*), or chloride (*34*−*36*) are generally resistant to biodegradation, and not specifically when attached to N-heterocycles.

Inactivating fragment 3 is found in molecules with triazole residues. These aromatic N-heterocycles have toxic effects on micro-organisms (*37*) and no information could be found toward their biodegradation.

Out of nine molecules carrying inactivating fragment 5, seven are poorly degradable aminopyridines. As all isomers of aminopyridine were poorly biodegradable, the amino-group may inhibit a nucleophilic attack by its electron-donating effect on the aromatic N-heterocycle independent of its position. Another possible explanation could be that aminopyridines are toxic for micro-organisms. The only degradable compound with inactivating fragment 5 was the naturally occurring vitamin B1 (compound 58 in Table 1), which was also incorrectly predicted in the autovalidation. In contrast to all other molecules with inactivating fragment 5, the aminopyrimidine ring of vitamin B1 is linked to a side chain structure that might allow attacking the ring, thereby facilitating its biodegegradation.

Biodegradation Rules Based on the Complementary Approach. Most of the relevant (i.e., occurring in at least three molecules) molecular fragments activating biodegradation of N-heterocycles could be plausibly interpreted by one specific biochemical reaction initiating degradation of the corresponding compounds. This indicates that the results of the QSAR model and the pathway-based approach are largely consistent. In most cases, the activating fragments themselves contained the actual site of enzymatic attack. However, the actual target site for the respective enzyme was not always completely displayed. With nonaromatic N-heterocycles this became evident for activating fragment 1. While almost all molecules carrying this fragment have a target site for amidohydrolases, this target site is not part of the fragment identified by MULTICASE. With aromatic N-heterocycles, fragments 4, 9, 11, and 12 do not display a target site for molybdenum hydroxylases although it is present in most of these molecules. Thus, by statistical analysis and by biochemical analysis, different substructures of the same molecule were identified as decisive for biodegradation. This indicates that for finding biochemical mechanisms involved

in degradation, the entire structure of a molecule has to be considered. In this respect, it was instrumental that the fragments identified by the statistical analysis model were largely characteristic for a specific class of N-heterocycles because it helped to focus the search for biochemical explanations. This instance clearly demonstrates that the QSAR model and the pathway-based approach are, in fact, complementary.

Some of the molecular fragments inactivating biodegradation could be explained by specific substituents that inhibit biodegradation (inactivating fragments 1 and 2) or by the probable toxicity of the class of compounds (inactivating fragments 3 and 5). These structural elements can overrule the presence of activating fragments. If no such general inactivating fragments are present, the biochemical reason for poor biodegradability was searched for in structural features that may block the initiating reaction. This procedure was used for explaining the inhibiting character of inactivating fragments 4, 6, and 9. For all three inactivating fragments, we propose an alkylation of the N-atom in the ring as inhibiting the first enzymatic step in biodegradation. These mechanistic hypotheses were the mere outcome of analyzing our dataset by the complementary approach, and we could not find any literature reference addressing them. Thus, there is also synergistic potential in the complementary approach to generate hypotheses for biochemical reactions that should be tested experimentally.

Based on the consistency and the complementarity of the biochemical interpretation of QSAR models, we propose the following structure−activity-relationships as rules for aerobic biodegradation of N-heterocycles: (1) In nonaromatic N-heterocycles, target sites for amidohydrolases enhance biodegradation. If such a target site is not present, a secondary *C*2-atom adjacent to the N-atom in the ring enhances biodegradation as a target site for hydroxylation by, e.g., cytochrome P450 monooxygenases. These enzymes create target sites for amidohydrolases or lead to ring cleavage directly. Alkylation of N-atoms in the ring inhibits biodegradation of nonaromatic N-heterocycles that have to be activated by such a hydroxylation. (2) In aromatic N-heterocycles, a secondary *C*2-atom activates biodegradation by serving as a target site for nucleophilic attack by molybdenum hydroxylases. (3) In imidazole residues, alkylated N-atoms inactivate biodegradation. A mechanistic explanation could be that an initiating hydratation step, analogous to the mechanism of urocanase, is prevented in N-alkylated imidazoles.

Our study reveals that the complementary approach to predict biodegradability is appropriate to set up a framework for the development of concrete degradation rules. Such rules could be directly implemented into designing new chemicals and synthesis strategies by considering the introduction of target sites for biodegradative enzymes. Of course, these rules should be developed further by both statistical and experimental procedures before they are used for predicting biodegradability for legislative purposes, e.g., in the context of REACH. In this study we restricted the biochemical interpretation of QSAR models on the initial enzyme reaction. Although this restriction to the first metabolic step yielded plausible explanations further improvement of degradation rules will certainly be achieved by integrating downstream metabolic pathways and further metabolic processes such as transport and gene regulation. A fascinating future perspective is to extend the complementary approach by combining QSAR models with the knowledge generated by system biology.

Acknowledgments

We acknowledge Emiel Rorije for helpful discussions and the German Ministry for Education and Research (BMBF, project no. 01RC0090) for funding. B.P. and M.H. contributed equally to this work.

Literature Cited

(1) Leisinger, T. Microorganisms and xenobiotic compounds. *Experientia* **1983**, *39*, 1183−1191.
(2) Rogers, M. D. The European Commission's White Paper "strategy for a future chemicals policy": a review. *Risk. Anal.* **2003**, *23*, 381−388.
(3) Boxall, A. B.; Sinclair, C. J.; Fenner, K.; Kolpin, D.; Maund, S. J. When synthetic chemicals degrade in the environment. *Environ. Sci. Technol.* **2004**, *38*, 368A−375A.
(4) Boethling, R. S.; Lynch, D. G.; Thom, G. C. Predicting ready biodegradability of premanufacture notice chemicals. *Environ. Toxicol. Chem.* **2003**, *22*, 837−844.
(5) Klopman, G.; Balthasar, D. M.; Rosenkranz, H. S. Application of the computer-automated structure evaluation (CASE) program to the study of structure-biodegradation relationships of miscellaneous compounds. *Environ. Toxicol. Chem.* **1993**, *12*, 231−240.
(6) Wackett, L. P.; Ellis, L. B. M. Predicting biodegradation. *Environ. Microbiol.* **1999**, *1*, 119−124.
(7) Hou, B. K.; Wackett, L. P.; Ellis, L. B. M. Microbial pathway prediction: a functional group approach. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 1051−1057.
(8) Klopman, G.; Tu, M. Structure-biodegradability study and computer-automated prediction of aerobic biodegradation of chemicals. *Environ. Toxicol. Chem.* **1997**, *16*, 1829−1835.
(9) Jaworska, J. S.; Dimitrov, S.; Nikolova, N.; Mekenyan, O. Probabilistic assessment of biodegradability based on metabolic pathways: catabol system. *SAR QSAR Environ. Res.* **2002**, *13*, 307−323.
(10) Callely, A. G. The microbial degradation of heterocyclic compounds. *Prog. Ind. Microbiol.* **1978**, *14*, 205−281.
(11) Kaiser, J. P.; Feng, Y.; Bollag, J. M. Microbial metabolism of pyridine, quinoline, acridine, and their derivatives under aerobic and anaerobic conditions. *Microbiol. Rev.* **1996**, *60*, 483−498.
(12) Schrader, T.; Schuffenhauer, G.; Sielaff, B.; Andreesen, J. R. High morpholine degradation rates and formation of cytochrome P450 during growth on different cyclic amines by newly isolated *Mycobacterium* sp. strain HE5. *Microbiology* **2000**, *146*, 1091−1098.
(13) Fetzner, S. Enzymes involved in the aerobic bacterial degradation of N-heteroaromatic compounds: molybdenum hydroxylases and ring-opening 2,4-dioxygenases. *Naturwissenschaften* **2000**, *87*, 59−69.
(14) Andreesen, J. R.; Fetzner, S. The molybdenum-containing hydroxylases of nicotinate, isonicotinate, and nicotine. *Met. Ions Biol. Syst.* **2005**, *39*, 405−430.
(15) Klopman, G. Quant. MULTICASE: A hierarchical computer-aided structure evaluation program. *Struct.-Act. Relat.* **1992**, *11*, 176−184.
(16) Organisation for Economic Co-operation and Development. *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemcal Substances and Mixtures*, OECD Series on Testing and Assessment. no. 33; OECD: Paris, 2001.
(17) Poupin, P.; Truffaut, N.; Combourieu, B.; Besse, P.; Sancelme, M.; Veschambre, H.; Delort, A. M. Degradation of morpholine by an environmental *Mycobacterium* strain involves a cytochrome P-450. *Appl. Environ. Microbiol.* **1998**, *64*, 159−165.
(18) Poupin, P.; Godon, J. J.; Zumstein, E.; Truffaut, N. Degradation of morpholine, piperidine, and pyrrolidine by mycobacteria: evidences for the involvement of a cytochrome P450. *Can. J. Microbiol.* **1999**, *45*, 209−216.
(19) Trigui, M.; Pulvin, S.; Poupin, P.; Thomas, D. Biodegradation of cyclic amines by a *Pseudomonas* strain involves an amine monooxygenase. *Can. J. Microbiol.* **2003**, *49*, 181−188.
(20) Guengerich, F. P.; Macdonald, T. L. Mechanisms of cytochrome P-450 catalysis. *FASEB* **1990**, *4*, 2453−2459.
(21) Kim, G. J.; Kim, H. S. Identification of the structural similarity in the functionally related amidohydrolases acting on the cyclic amide ring. *Biochem. J.* **1998**, *330*, 295−302.
(22) Fetzner, S. Bioconversion of pyrimidine by resting cells of quinoline-degrading bacteria. *FEMS Microbiol. Lett.* **1999**, *176*, 291−299.
(23) Vogels, G. D.; Van der Drift, C. Degradation of purines and pyrimidines by microorganisms. *Bacteriol. Rev.* **1976**, *40*, 403−468.
(24) Klepp, J.; Fallert-Müller, A.; Grimm, K.; Hull, W. E.; Retey, J. Mechanism of action of urocanase. Specific ^{13}C-labelling of the

prosthetic NAD^+ and revision of the structure of its adduct with imidazolylpropionate. *Eur. J. Biochem.* **1990**, *192*, 669−676.
(25) Lenz, M.; Retey, J. Cloning, expression and mutational analysis of the urocanase gene (hutU) from *Pseudomonas putida* Eur. *J. Biochem.* **1993**, *217*, 429−434.
(26) Xu, G.; West, T. P. Reductive catabolism of pyrimidine bases by Pseudomonas stutzeri. *J. Gen. Microbiol.* **1992**, *138*, 2459−2463.
(27) Cook, A. M. Biodegradation of s-triazine xenobiotics. *FEMS Microbiol. Rev.* **1987**, *46*, 93−116.
(28) Wetzstein, H. G.; Schneider, J.; Karl, W. Patterns of metabolites produced from the fluoroquinolone enrofloxacin by basidiomycetes indigenous to agricultural sites. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 90−100.
(29) Bubeck, B.; Tshisuaka, B.; Fetzner, S.; Lingens, F. Hydroxylation of quinaldic acid: quinaldic acid 4-monooxygenase from *Alcaligenes* sp. F-2 versus quinaldic acid 4-oxidoreductases. *Biochim. Biophys. Acta* **1996**, *1293*, 39−44.
(30) Rorije, E.; Germa, F.; Philipp, B.; Schink, B.; Beimborn, D. B. Prediction of biodegradability from structure: imidazoles. *SAR QSAR Environ. Res.* **2002**, *13*, 199−204.
(31) Oien, H. G.; Wright, L. D. Metabolism of imidazole by a pseudomonad. *J. Bacteriol.* **1971**, *105*, 1229−1231.
(32) Kertesz, M. A.; Cook, A. M.; Leisinger, T. Microbial metabolism of sulfur- and phosphorus-containing xenobiotics. *FEMS Microbiol. Rev.* **1994**, *15*, 195−215.
(33) Spain, J. C. Biodegradation of nitroaromatic compounds. *Annu. Rev. Microbiol.* **1995**, *49*, 523−555.
(34) van der Meer, J. R. Evolution of novel metabolic pathways for the degradation of chloroaromatic compounds. *Antonie Van Leeuwenhoek* **1997**, *71*, 159−178.
(35) Leisinger, T. Biodegradation of chlorinated aliphatic compounds. *Curr. Opin. Biotechnol.* **1996**, *7*, 295−300.
(36) Haggblom, M. M. Microbial breakdown of halogenated aromatic pesticides and related compounds. *FEMS Microbiol. Rev.* **1992**, *9*, 29−71.
(37) Kredich, N. M.; Foote, L. J.; Hulanicka, M. D. Studies on the mechanism of inhibition of *Salmonella typhimurium* by 1,2,4-triazole. *J. Biol. Chem.* **1975**, *250*, 7324−7331.

Received for review June 26, 2006. Revised manuscript received November 28, 2006. Accepted December 12, 2006.

ES061505D