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Re-Designing of Existing Pharmaceuticals for Environmental Biodegradability: A Tiered Approach with β-Blocker Propranolol as an Example

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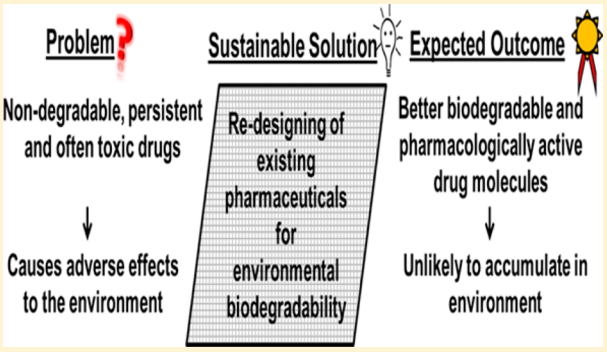
Supporting Information

ABSTRACT: Worldwide, contamination of aquatic systems with micropollutants, including pharmaceuticals, is one of the challenges for sustainable management of water resources. Although micropollutants are present at low concentrations, many of them raise considerable toxicological concerns, particularly when present as components of complex mixtures. Recent research has shown that this problem cannot be sustainably solved with advanced effluent treatment. Therefore, an alternative that might overcome these environmental problems is the design of new pharmaceutical molecules or the redesign of existing pharmaceutical molecules that present the functionality needed for their application and have improved environmental biodegradability. Such redesigning can be performed by small molecular changes in the drug molecule with intact drug moiety which could incorporate the additional attribute such as biodegradability while retaining its pharmacological potency. This proof of concept study provides an approach for the rational redesign of a given pharmaceutical (Propranolol as an example). New derivatives with small molecular changes as compared to propranolol molecule were generated by a nontargeted photolysis process. Generated derivatives with intact drug moieties (an aromatic ring and a β-ethanolamine moiety) were further screened for aerobic biodegradability and pharmacological potency. The feasibility of the approach of redesigning an existing pharmaceutical through nontargeted generation of new derivatives with intact drug moiety and through subsequent screening was demonstrated in this study. Application of such approaches in turn might contribute to the protection of water resources in a truly sustainable manner.



INTRODUCTION

Clean water is fundamental for water quality, food security, health, societal well-being, and economic growth. However, worldwide, freshwater systems, including drinking water, are contaminated by micropollutants, derived from personal care products, pesticides, pharmaceuticals, etc. and the unwanted products of their incomplete degradation, which have undetermined chemical structure and unknown toxicity.[1−4] Although present at low concentrations, many of these molecules raise considerable toxicological concerns, particularly as they are present as complex mixtures, making chemical pollution a key environmental problem of our era.[5−8] The presence of active pharmaceutical ingredients (APIs) in the aquatic environment is increasingly seen as one of the major challenges to the sustainable management of water resources worldwide because of the expensive, ineffective and/or nonsustainable nature of strategies for the prevention of entrance of pharmaceuticals into the aquatic environment.[9−11] Therefore, it is urgently necessary to encourage the design of APIs of increased environmental biodegradability, which was historically not considered necessary.[12,13]

The tenth principle of green chemistry is "Design for Degradation".[14] This principle relates to the design of molecules of improved biodegradability. Accordingly, the functionality of a pharmaceutical should not only include the properties required for successful application as a pharmaceutical per se, but also properties required for the fast and easy degradation of the unmetabolized drug excreted from human body in the environment. The concept of benign by design is the key element in this respect.[12,15] The concept of benign by design can be applied on two levels: the design of completely new molecules or the redesign of existing molecules. However, the feasibility of the tenth principle of green chemistry "Design for Degradation" has not yet been demonstrated for pharmaceuticals, nor is a proper working scheme yet available. The present study demonstrates the feasibility of this principle for pharmaceuticals.

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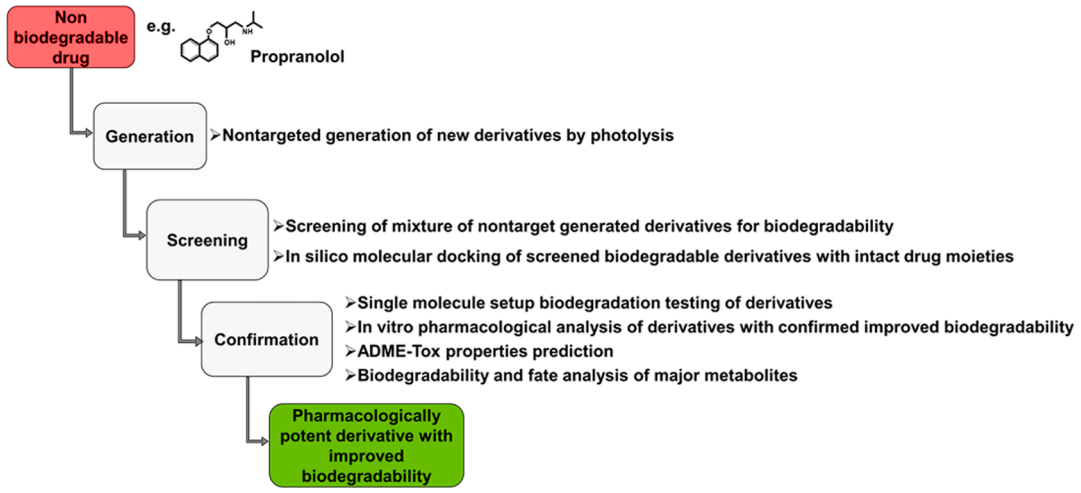

Figure 1. Illustration of the systematic tiered approach of redesigning existing pharmaceuticals for environmental biodegradability through nontargeted generation of new derivatives and subsequent screening.

The present study is a proof of concept and focuses on the redesign of the β-blocker class of APIs. Propranolol (PPL), a nonselective β-blocker[16] which binds to both β_1 and β_2 adrenergic receptors[17] as the essential step of its activity, was selected as an example for the investigations. In 2012, a total amount of 3.216 tonnes of PPL was consumed by individuals in Germany who were ensured by public health insurance.[18,19] PPL has been reported as a nonbiodegradable and highly persistent chemical[20] and was detected in sewage treatment plant effluent at concentrations ranging from 30 to 373 ng L^{-1}.[21]

The goal of this proof of concept was to incorporate additional attribute biodegradability into PPL molecule through small molecular alteration in its structure while conserving its substructures responsible for β adrenergic receptor blocker activity. An aromatic ring and a β-ethanolamine moiety are considered the essential substructures of β-blockers responsible for their receptor blocking activity.[22,23] The challenge is the generation and selection of such drug derivatives. Thus, the present study is the proof of concept for demonstrating a working scheme for the nontargeted generation and targeted selection of biodegradable and pharmacologically active derivatives of a nonbiodegradable β-blocker drug PPL as an example.

EXPERIMENTAL SECTION

The tiered systematic approach of redesigning nonbiodegradable existing pharmaceuticals into biodegradable drug-like molecules through nontarget generation of derivatives, screening and confirmation is illustrated as Figure 1. The very first step for designing biodegradable β-blocker derivatives is to generate new molecules that possess substructures (an aromatic ring and a β-ethanolamine moiety) providing their specific action as β-blockers.

A simple and nonselective direct photolysis process was selected for the nontargeted generation of new derivatives (photoderivatives, PDs); this resulted in numerous derivatives. In the next step, aerobic biodegradation tests employing different bacterial densities (closed bottle test [CBT] and manometric respiratory test [MRT]) were applied to the mixtures resulting from photolytic treatment in order to assess the derivatives' biodegradability in the environment. Thereby, the PDs that had improved biodegradability compared to the parent compound and that still possessed an aromatic ring and a β-ethanolamine moiety were selected. The ones that were found to be biodegradable with intact drug moieties were further assessed with in silico molecular docking and in vitro analysis for the investigation of their pharmacological activity.

In addition, the retention of drug-like properties (absorption, distribution, metabolism and excretion; ADME) of a derivatives which were pharmacologically active with improved biodegradability was investigated. The final step was in silico toxicity (mutagenicity/genotoxicity) assessment for the improved biodegradable and yet pharmacologically active derivatives. The biodegradability of human metabolite of these derivatives, if any, was also investigated.

Chemicals. (±)-Propranolol hydrochloride (CAS 318−96−9) and Isoprenaline hydrochloride (CAS 51−30−9) were purchased from Sigma-Aldrich (Steinheim, Germany). (±)-4-hydroxypropranolol hydrochloride (CAS 10476−53−6), 5-hydroxypropranolol hydrochloride (CAS 62117−35−5), (±)-7-hydroxypropranolol (CAS 81907−81−5) and 4-hydroxypropranolol β-D-glucuronide (CAS 94731−13−2) were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). All the solutions and standards for analytical measurement and photodegradation were prepared in ultrapure water (water resistivity at 25 °C Q_1: 16.6 mΩ-cm and Q_2: 18.2 mΩ-cm).

Nontargeted Generation of Photoderivatives of Propranolol. Nontargeted generation of new derivatives (photoderivatives, PDs) were performed through UV photolysis. Propranolol (PPL) solution was exposed to a medium pressure mercury lamp (TQ150, UV Consulting Peschl, Mainz) for 256 min in a 1 L batch photo reactor (LRS2, UV Consulting Peschl, Mainz). PPL was dissolved in ultrapure water for direct photolytic experiments with no pretreatment in order to exclude any other constituents that could interfere with the formation of derivatives and/or to avoid the initiation of scavenger effects of any absorbing or photosensitizing chemical or other species during degradation. The experimental setup, procedure and characteristics of UV lamp were described in detail in Text S1 in Supporting Information (SI).

Analysis. A Shimadzu Prominence HPLC system (Duisburg, Germany) was used to measure the primary elimination of the parent compound (PPL). The Agilent LC 1100 series

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coupled to a Bruker Daltonic Esquire 6000+ ion-trap mass spectrometer (IT-MS) with electrospray ionization interface was used for identification and structure elucidation of the formed photoderivatives (PDs). Dissolved organic carbon (DOC) was measured by Shimadzu TOC-V_{CPN} analyzer equipped with an ASI-V auto sampler. The detail description of the LC method, operating parameters of the source and ion-trap and DOC measurement were summarized in Text S2 in SI.

Biodegradation. The aerobic biodegradability of PPL and the mixture of its generated photoderivatives (PDs) were investigated according to OECD guidelines through closed bottle test [CBT] 301D[24] and manometric respiratory test [MRT] 301 F.[25] The CBT was employed as the most stringent biodegradation test working at low bacterial densities and diversities in order to avoid any false positive results. The MRT works with higher bacterial densities and diversities. The ready biodegradability of 4-, 5-, and 7-hydroxypropranolol was tested through the CBT only. The test principles, procedures and composition of the both investigated aerobic biodegradation test series are described in detail in Text S3 (SI).

In Silico Analysis. *Molecular Docking.* Nontargetedly generated derivatives (PDs) of PPL which were observed to be of improved biodegradability in aerobic biodegradation test assays and possessed the intact drug moieties (an aromatic ring and a β-ethanolamine moiety) were considered to be relevant for assessing their pharmacological potency. The prediction of the pharmacological properties of PPL and its relatively biodegradable PDs was performed by in silico molecular docking with Schrodinger Maestro Version 2012 (v.9.3) software. PPL is known to have a considerable affinity toward both β_1 and β_2 adrenergic receptor.[17] As the human β_1 adrenergic receptor crystal structure was not publically available, PPL and its derivatives were docked on the structures of the turkey β_1 and human β_2 adrenergic receptors.

The X-ray structures of both β_1 and β_2 adrenergic receptor were derived from RCSB protein database.[26] The codes for the applied X-ray structures of the turkey β_1 receptor was 2YCW[27] and 2RH1 for the human β_2 receptor,[28] respectively. The receptor grid and ligands preparation are described in detail in SI as Text S4.

The docking score (output from in silico molecular docking analysis) estimates the binding interaction of the molecule (ligands) to the protein or enzyme of interest. The lower the docking scores, the better the molecule is supposed to bind to the protein and in return provides an indication of the pharmacological potency of the investigated molecules. In order to identify the PDs which have comparable binding ability as compared to PPL, their docking scores were evaluated.

However, in order to keep the workload manageable, the derivatives with docking score ≤ -8 were selected as of main interest to shortlist the pharmacologically comparable biodegradable derivatives and to perform further analysis.

ADME Properties Prediction. The ADME (absorption, distribution, metabolism, and excretion) properties prediction prior to expensive experimental procedures of pharmacokinetic and/or clinical trials can eliminate unnecessary testing of compounds that are doomed to fail, which will significantly reduce the amount of wasted time and resources, and streamline the overall drug development process. Therefore, in the present study, ADME properties were predicted using Schrödinger's QikProp 3.8 software developed by Jorgensen et al.,[29] for the improved biodegradable and pharmacologically potent derivatives shortlisted after the experimental assessment.

Thus, incorporating ADME properties predictions as a part of the "green" drug development process will result in lead derivatives that are more likely exhibiting satisfactory ADME performances during experimental clinical trials. In the present study, the ADME properties of the lead candidates (i.e., the ones with better biodegradability, intact drug moieties and pharmacologically active) were also compared with PPL and the known selective β_1 blockers Atenolol and Metoprolol. The information about the database of the software and various ADME properties, which were predicted by QikProp, are summarized and described in detail in Text S5 in SI.

Toxicity Prediction. The toxicological end points such as carcinogenicity, mutagenicity and genotoxicity were predicted for the improved biodegradable and pharmacologically potent derivative by the various models of OASIS Catalogic (Laboratory for Mathematical Chemistry, Bulgaria), CASE Ultra v.1.4.5.1 (MultiCASE Inc., Ohio) and Leadscope software (Leadscope, Inc., Ohio).

The mutagenicity assessment as suggested by the recently implemented ICH M7 guideline by the combination of statistical and rule-based models from different software packages CASE Ultra v. 1.5.2.0[30] and Leadscope Model Applier v. 1.8.6.[31] The above-mentioned software's models and their outputs and end points are described in detail in SI in Text S6.

In Vitro Pharmacological Assay. In vitro pharmacological analysis was performed through the PathHunter eXpress β-Arrestin Human and Ortholog GPCR kits (DiscoveRx Corporation Ltd., UK). The kit contains a genetically modified CHO-K1 cell line expressing the human β_1 adrenergic receptor.[32] The kit monitors GPCR (G protein-coupled receptors) activity by detecting the interaction of β-Arrestin with the activated GPCR using β-galactosidase (β-gal) enzyme fragment complementation.[33] Isoprenaline hydrochloride, an agonist for the β_1 adrenergic receptor, was used as a positive control in the test assay. PPL and lead candidates were tested in the assay for their antagonistic activity. The principle and working procedure of the in vitro assay is detailed described in SI in Text S7.

RESULTS AND DISCUSSION

Generation of Photoderivatives. In total, 16 photoderivatives (PDs) of PPL were observed to be formed during photolysis. According to the LC-MS/MS data (Table S13, SI), several of the identified PDs were constitutional isomers. Formation of constitutional isomers of PPL derivatives was previously reported.[34−37] On the basis of observed results and literature finding a scheme as well as reaction pathways of the generation of PDs of PPL are illustrated in Figure S7 (under Text S8, SI). The attachment of hydroxyl radical ($HO^{\bullet}$) on the aromatic naphthalene ring of PPL was observed to be the major mechanism for the synthesis of derivatives of PPL. This attachment of $HO^{\bullet}$ often resulted in the opening of the naphthalene ring which ends up forming the alcohol and/or aldehyde moieties.

The other two mechanisms which were observed for the generation of derivatives were the dealkylation (elimination of isopropyl moiety from the side chain) and/or the cleavage of ethanolamine side chain from the aromatic ring. The observed mechanism for the generation of PDs were also reported earlier.[35,37,38] The detailed description of the results and structures of PDs are described in detail in Text S8 and Table S13 in SI.

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Biodegradability Screening of Photoderivatives. The mixture of PDs of PPL resulting from nontargeted generation was submitted to two aerobic biodegradation test assays and the results are summarized in Figure S8 and S9, and Table S8 in SI. The results of both aerobic biodegradation assays confirmed that PPL is not at all biodegradable. LC-MS analyses of samples from each biodegradation test confirmed these findings (Figure S9, SI), which were also supported by data in the literature, that is, that PPL was not eliminated through biodegradation, hydrolysis, sorption, or abiotic oxidation. Therefore, it can be concluded that the degradation observed to have been achieved during the biodegradation tests was due to the PDs. All the PDs with improved biodegradability (Table S8, SI) were found to be hydroxylated derivatives of PPL, with or without the opening of the naphthalene ring as identified after photo treatment. This phenomenon is consistent with the observation that attachments of electron-donating functionalities such as oxygen atoms (e.g., hydroxyl, aldehyde, carboxylic acid groups, etc.) or amines to the aromatic ring of a molecule generally increase its aerobic biodegradability.[39]

Also, the addition of hydroxyl groups onto the aromatic ring is considered the first step of the aerobic biodegradation pathway of aromatic compounds (such as aniline and the *p*-nitrophenol), leading to the opening of the aromatic ring to form alcohols and acids, which then subsequently mineralize.[40,41] Stereochemistry also plays an important role for biodegradability. However, there are no specific rules for determining which enantiomer will be preferably degraded.[42,43] Therefore, formation of enantiomers of certain derivatives of different biodegradability cannot be excluded.

Pharmacological Potency Screening of Photoderivatives (In Silico). The binding interactions between chemical species and the amino acids of the receptor grid have been reported to be stereoselective.[44,45] Therefore, all the identified PDs with improved biodegradability (Table S8, SI) with intact drug moieties (an aromatic ring and a β-ethanolamine moiety), including all their possible constitutional and enantiomeric isomers, were in silico docked on the turkey β_1 and human β_2 adrenergic receptors to estimate their pharmacological potency. As expected, PPL showed good docking with both receptor grids due to its nonselective nature toward the β_1 and β_2 subtype receptors.[16] The results of molecular docking of PPL and PDs are summarized in Table S9 in SI. The docking results indicate that the hydroxylation on the naphthalene ring of PPL (as in PD_{1-7} 276) and the opening of the ring (as in PD 266) during photolysis lead to derivatives which may have comparable or even improved pharmacological potency as compared to PPL. The binding interactions of the turkey β_1 adrenergic receptor grid generated by in silico docking software with both enantiomers (R and S) of PPL, 4-OH PPL (PD_3 276) and 7-OH PPL (PD_6 276) are detailed described in SI as Text S9.

The docking results showed that small changes are possible without losing pharmacological potency, even on the moieties responsible for the particular mode of action of the parent compound. The only condition is that such small molecular changes should not disturb or rupture the drug moieties of the molecule. Such changes in the molecule with intact drug moiety could incorporate additional attributes into the molecule (such as biodegradability) while conserving its pharmacological potency. Thus, derivatives such as PD_{1-7} 276 and PD 266 (Table S9, SI) could be considered new lead candidates that might have pharmacological potency and are, at the same time, comparably improved environmentally biodegradable. Therefore, further experimental analyses were performed to confirm their properties such as biodegradability and pharmacological potency.

Confirmation of Biodegradability. To confirm the properties of the shortlisted lead (proposed to be pharmacologically active) PDs, it was necessary to test them in a single molecule setup in the respective test assays. Three hydroxyl derivatives of PPL (i.e., 4-OH PPL (PD_3 276), 5-OH PPL (PD_4 276) and 7-OH PPL (PD_6 276)), commercially available as racemic mixtures, were tested for their biodegradability in the CBT along with PPL. The highest % of biodegradation was observed for 4-OH PPL (23%), while the other selected derivatives showed no biodegradation (Figure S11 in SI). These observations of biodegradation of the lead PDs were in accordance with Boethling et al.[46] There, the authors suggested that the number of substituent groups attached to the base structure (aromatic ring) will affect biodegradability.

LC-MS analysis of the CBT samples (Figure 2a) shows that there was no elimination of PPL itself or of 5-OH PPL or 7-OH

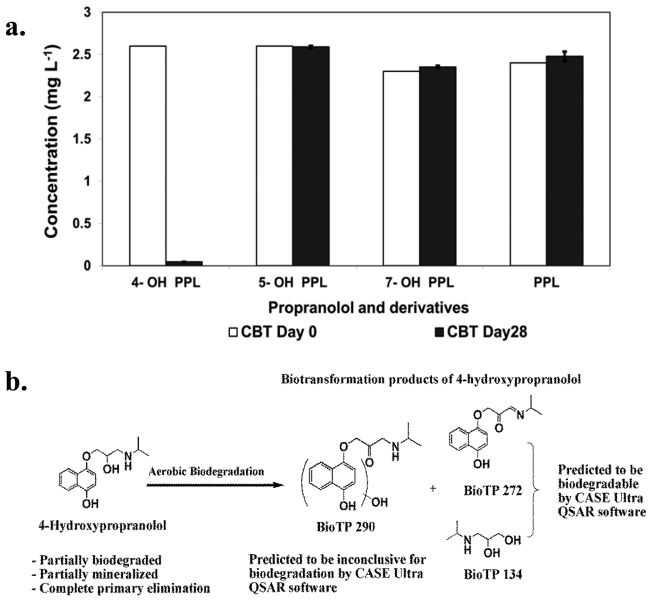

Figure 2. (a) Results of LC-MS of CBT samples of Propranolol and hydroxyl derivatives of Propranolol (n = 2; error bar indicate the difference between the measured values and the average value); (b) Proposed biodegradation pathways and biodegradation transformation products of 4-hydroxypropranolol.

PPL during the test. Dissolved organic carbon (DOC) measurements supported these results (no decrease in DOC). In contrast 4-OH PPL was completely primarily eliminated and a mineralization of 48% was observed. Hence, these analyses confirmed that 4-OH PPL has an improved biodegradability and will be much more mineralized compared to PPL and the other lead derivatives (5- and 7-OH PPL) under aerobic environmental biodegradation conditions and is unlikely to accumulate in the aquatic environment.

However, the incomplete mineralization of 4-OH PPL indicates the formation of biodegradation transformation products (bioTPs) during the test. This is confirmed by the detection of three bioTPs ($bioTP_{1-6}$ 290, bioTP 272 and bioTP 134, Figure 2b) that were formed during biodegradation testing of 4-OH PPL. Several peaks were observed with the

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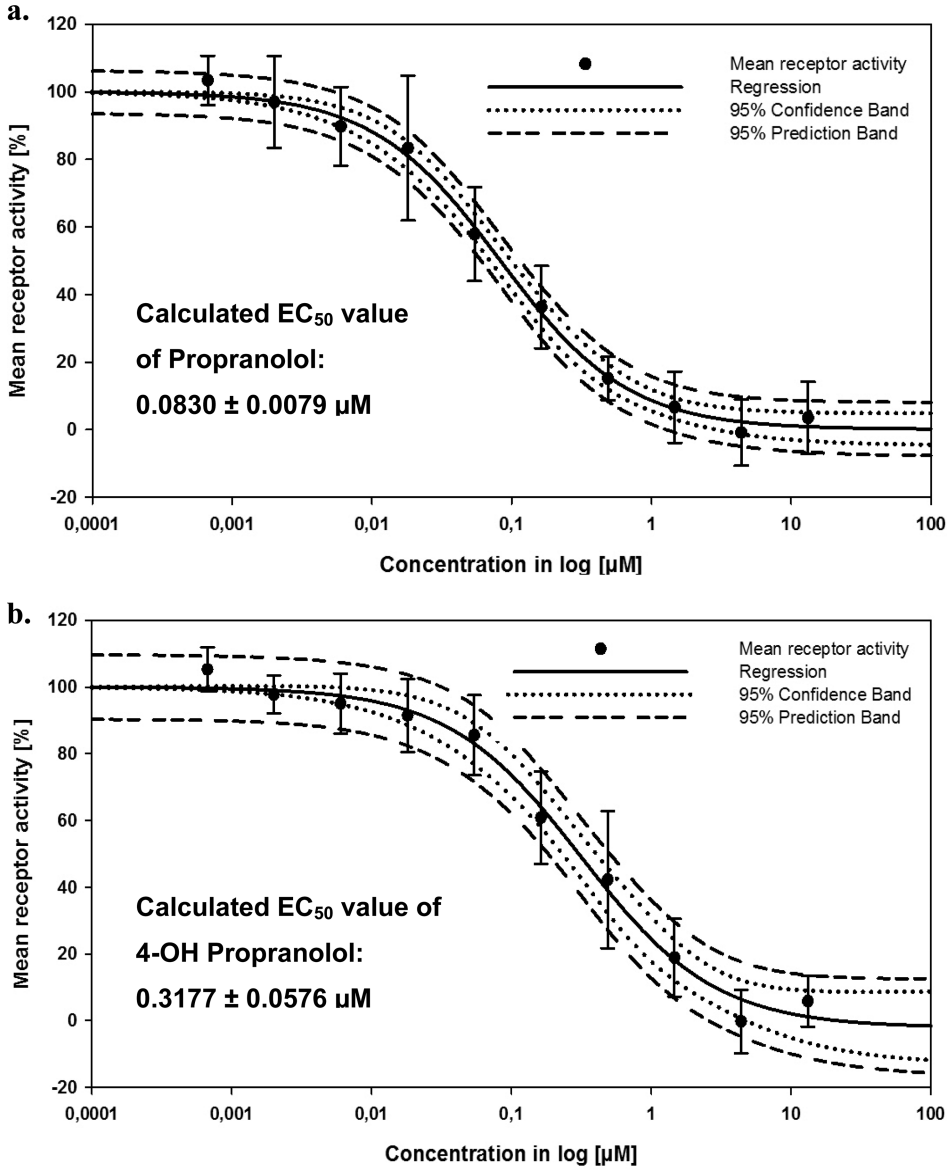

Figure 3. In vitro analysis of the pharmacological potency of tested chemicals as β adrenergic receptor antagonists: (a) Propranolol and (b) 4-hydroxypropranolol (n = 3; error bar indicate the difference between the measured values and the average value).

same MS^2 fragmentation pattern during LC-MS/MS analysis for the specific mass m/z 290 (bioTP 290), indicating the formation of constitutional isomers of bioTP 290 (see Table S13 in SI).

These proposed structures, which are not commercially available, were characterized in silico for ready biodegradability (Table S10, SI). BioTP 134 and bioTP 272 were predicted with positive alerts for ready biodegradability while constitutional isomers of $bioTP_{1-6}$ 290 were predicted either with "out of domain" or "inconclusive" predictions. This means that these isomers of $bioTP_{1-6}$ 290 were not included in the applicability domain of the model, or there were positive alerts but the calculated probability fell below the classification threshold (50.0%) of the respective model.

For greater safety, bacterial mutagenicity was therefore assessed for all bioTPs, as suggested in the only guideline, the very recently implemented ICH M7,[47] and as acknowledged for this purpose by the U.S. Food and Drug Administration (U.S. FDA) and the European Medical Agency (EMA). As in silico predictions are somewhat uncertain, therefore several software packages were applied for individual end points ("in silico testing battery") when the compounds and/or tests for reliable predictions were not available. Therefore, the proposed structures of all bioTPs of 4-OH PPL were characterized for bacterial mutagenicity as per ICH M7 guideline by the combination of statistical and rule-based models from different software packages (Table S10, SI).

The in silico mutagenicity predictions of the statistical models of CASE Ultra software package reported that all bioTPs would be nonmutagenic. The rule-based model "Expert rules for genotoxicity" from CASE Ultra provided a positive alert for genotoxicity due to the presence of naphthalene analogs (Table S10, SI). However, this model also provided the same positive alert for PPL due to the presence of the naphthalene ring, but this prediction was overruled because PPL is a known nongenotoxic compound. Thus, on the basis of in silico read-across approach, these predictions by the expert

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rule model might be overruled for the bioTPs. Therefore, these bioTPs may be nonmutagenic, as is PPL itself.

Both the statistical and expert models of Leadscope provided a negative prediction for all bioTPs except bioTP$_2$ 290 (Table S10, SI). This bioTP$_2$ 290 was predicted with an indeterminate alert. The negative prediction for all bioTPs except bioTP$_2$ 290 indicates that they might be nonmutagenic. The indeterminate alerts for bioTP$_2$ 290 for both the expert and statistically based models indicate that there was insufficient evidence or conflicting data to provide a positive or negative alert with confidence. However, the ICH M7 genotox consensus predicted that all the bioTPs, including bioTP$_2$ 290, might be nonmutagenic.

Confirmation of Pharmacological Activity (In Vitro). Based on the encouraging results for 4-OH PPL (PD$_3$ 276), the pharmacological potency of 4-OH PPL as a β_1 adrenergic receptor antagonist was confirmed and compared with PPL by in vitro tests. Figure 3a and b show the dose response curve for PPL and 4-OH PPL, respectively. The graph shows the inhibition of the β_1 adrenergic receptor triggering due to the addition of agonist isoprenaline in the test assay. Increasing doses of both PPL and 4-OH PPL reduces the agonistic activity in the test assay. Such a phenomenon was expected from PPL, as PPL is a known antagonistic drug for the β_1 adrenergic receptors. 4-OH PPL also showed a similar dose response (reduction of agonistic activity) which confirms that it is most likely also an antagonistic chemical for β_1 adrenergic receptor.

However, the EC$_{50}$ value of 4-OH PPL was higher than PPL in the in vitro assay. That indicates the respective extents of blocking of the β_1 adrenergic receptors by both 4-OH PPL and PPL are different. In a two tailed t test performed for the EC$_{50}$ values of PPL and 4-OH PPL, no statistically significant difference was found (p = 0.135).

This is in accordance with the in vivo data from Fitzgerald et al.,[48] who reported that 4-OH PPL is approximately pharmacologically equipotent to the parent drug PPL as a β-adrenoceptor blocking agent in vivo in rats, cats, dogs and guinea pigs. Therefore, it is reasonable to assume that approximately the same dose of 4-OH PPL might be required to have the same therapeutical effect in humans as PPL.

To this point it can be concluded that 4-OH PPL is a pharmacologically active β_1 adrenergic receptor blocking molecule with improved biodegradability and is unlikely to accumulate in the aquatic environment.

Assessment of Drug-Like Properties (In Silico ADME Properties). In order to augment the data already established for 4-OH PPL as a β_1 blocker, ADME properties (absorption, distribution, metabolism and excretion) of 4-OH PPL were assessed by a software package QikProp 3.8 (see Table S11, SI), and compared with nonselective β-blocker PPL and selective β_1-blocker Atenolol (ATL) and Metoprolol (MTL). The predictions for drug-like properties for 4-OH PPL were in the recommended range of the software and comparable with PPL and selected β_1-blockers (see Table S11, SI). Predictions for Lipinski's rule of five and for Jorgensen's rule of three properties showed no violation by 4-OH PPL, similar to ATL, MTL and PPL. This further supports that 4-OH PPL is a drug-like molecule and most likely to be orally available. The predicted partition coefficient (log $P_{o/w}$) values were within the recommended range and indicate that 4-OH PPL is more hydrophilic than PPL.

The inhibitory concentration (IC$_{50}$) values for the blockage of the human Ether-à-go-go-Related Gene (hERG) K+ channel were predicted by the Qikprop software. It was observed that the predicted value was below the recommended range for 4-OH PPL but however comparable to both PPL and MTL (Table S11, SI). The Qikprop software predicted apparent Caco-2 cell permeability and apparent MDCK cell permeability (P Caco and P MDCK) for 4-OH PPL less than PPL and MTL but was still in the recommended range (see Table S11, SI).

The Qikprop software predicted 4-OH PPL to have a high human oral absorption, similar to PPL. The skin permeability prediction for 4-OH PPL was in the recommended range and comparable to the other investigated β-blockers. The predicted brain/blood partition coefficient (log BB) was lower than that for PPL (Table S11, SI), which is a satisfactory indication that 4-OH PPL will tend to partition in the blood instead of the brain. This means more distribution of 4-OH PPL to heart, muscles and circulation which are the target areas of it.

Toxicity Assessment (In Silico Toxicity Prediction). In addition to their pharmacological activity, efficacy and efficiency drugs should also be nontoxic. First of all, mutagenicity, genotoxicity and carcinogenicity have to be absent. Therefore, 4-OH PPL was assessed and compared with PPL for mutagenicity by an "in silico testing battery" (with different software packages for individual end points) described in detail in Text S6 in SI. The results of *in silico* mutagenicity assessment as suggested by the ICH M7 guideline for PPL and 4-OH PPL are summarized in Table S12. The statistical mutagenicity models of CASE Ultra predicted 4-OH PPL might be nonmutagenic while the rule-based model predicted 4-OH PPL to be mutagenic. This rule-based model predicted a positive alert due the presence of a naphthalene analog in the 4-OH PPL. However, both the statistical and expert models from the Leadscope predicted PPL and 4-OH PPL might be nonmutagenic. Predictions by other models indicated that the 4-OH PPL is probably not a carcinogen, nor genotoxic or mutagenic.

Human Metabolite 4-Hydroxypropranolol Glucuronide. It has been reported that 4-OH PPL [PD$_3$ 276] generally undergoes sequential metabolism to 4-hydroxypropranolol glucuronide (4-OH PPL GLU).[49] Therefore, if 4-OH PPL is administered as a drug, it will be excreted in part as 4-OH PPL GLU. In this study, it was found that the metabolite 4-OH PPL GLU was 22% degraded in the CBT (Figure 4), according to oxygen consumption, and partially mineralized to 36%, according to DOC elimination. Furthermore, by employing LC-MS/MS it was demonstrated that 4-OH PPL GLU was completely cleaved back into the biodegradable parent compound 4-OH PPL, as shown in Figure 4b. Such a behavior is reported in the literature for glucuronides of other pharmaceuticals such as Diclofenac. Lee et al.[50] reported that Diclofenac glucuronide, a major metabolite of Diclofenac, deconjugated to equimolar Diclofenac during microbial degradation testing.

The reformed parent compound 4-OH PPL undergoes further elimination and transformations into the three already identified bioTPs (bioTP 134, bioTP 272, and bioTP$_{1-6}$ 290) (Figure 2b and Table S10). Thus, 4-OH PPL GLU will not persist in the environment after its release and strengthens the potential that 4-OH PPL can be a greener β-blocker derivative.

4-OH PPL can be considered a lead structure for an environmentally friendly β_1 adrenergic receptor blocker, although it must be taken into account that further analysis such as biodistribution, pharmacokinetic and/or clinical trials should be carried out for 4-OH PPL. Thus, after successful

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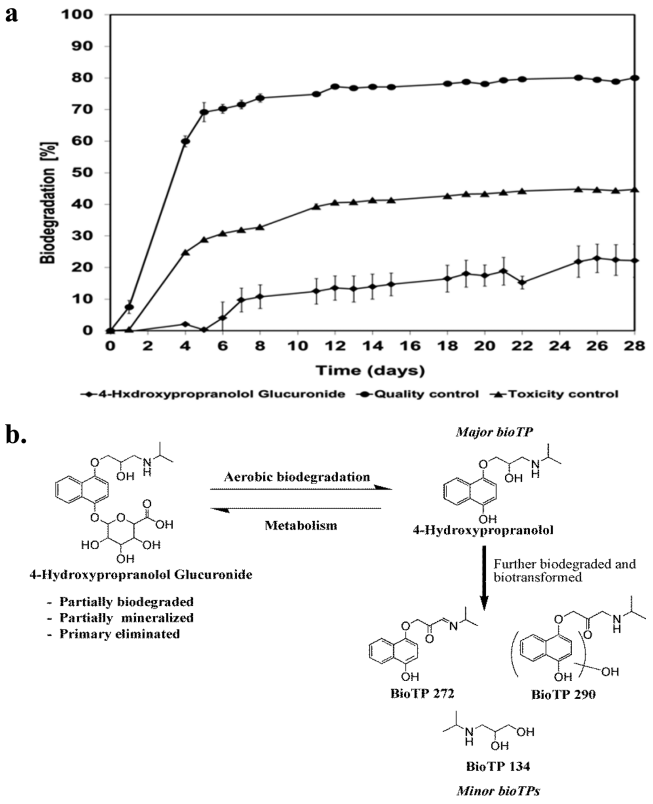

Figure 4. Results of aerobic biodegradation of 4-hydroxypropranolol glucuronide, a metabolite of 4-hydroxypropranolol: (a) Kinetics of aerobic biodegradation (n = 2, error bar indicate the difference between the measured values and the average value). The test was valid according to OECD guidelines and no inhibitory effects were observed in the toxicity control; (b) Proposed scheme for aerobic biodegradation of 4-hydroxypropranolol glucuronide and the formation of bio-TPs.

trials it can be labeled and used as a β_1 adrenergic receptor blocker drug with the additional advantage of being improved biodegradable after its release to the aquatic environment.

The study demonstrated the feasibility and provided a working scheme for rationally redesigning β-blockers. This approach promises a solution to the global challenge of micropollutants in the aquatic system even if there is no sewage treatment available. It may also lead to new drug candidates useful for patients and industries, and can therefore be called a sustainable solution in the broadest sense. The authors acknowledge that the tiered approach to redesigning pharmaceuticals presented here should also be tested for additional classes of drugs. This might lead to innovative follow-up studies and will widen the knowledge and experience in order to integrate environmental aspects into drug design and development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b03051.

Descriptive experimental methodology, supporting results, tables and figure (PDF)

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Notes

The authors declare no competing financial interest.

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