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"Towards the Design of Active Pharmaceutical Ingredients Mineralizing Readily in the Environment"

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Highlights:

- **Examples for environmentally (bio)degradable APIs from industry and academia demonstrate the feasibility of Benign by Design.**
- **The optimization phase as most appropriate step to implement environmental considerations**
- **Pharmacological parameters are not necessarily in conflict with biodegradability in the environment.**
- **Optimization of pharmacological parameters of APIs impacts their environmental biodegradability.**

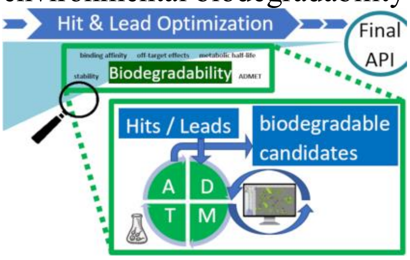
Abstract

Active pharmaceutical ingredients (APIs), their metabolites, and transformation products (TPs) occur globally in the environment. They pose a risk to both environmental and human health. These alarming circumstances highlight the strong need for efficient measures, which is also reflected in EU strategies on sustainable chemicals and on pharmaceuticals in the environment. The design of APIs mineralizing in the environment according to the concept Benign by Design (BbD) is a promising approach to tackle this challenge. However, its implementation into the industrial API discovery process has not been discussed yet. To stimulate such a discussion and to better understand the applicability and limitations of this approach, the generic API discovery

process is reviewed, including procedure, principles, and paradigms based on publicly available information. In addition to the concept of BbD itself, workflow scenarios such as *de novo* design and re-design are presented to provide a better understanding of its feasibility.
Bringing these aspects together, we conclude that the optimization phase within drug discovery seems to be the most appropriate place to implement environmental considerations. At this early stage, costs are low and the potential impact of design and structural variation on the outcome is high. We found that pharmacological parameters required for application are sometimes even in line with biodegradability in the environment, since the conditions in the human body and the environment differ. However, the effects of optimizing pharmacological parameters such as toxicity and stability on environmental biodegradability of APIs must be considered together with design rules for biodegradability.
Understanding the feasibility of BbD can mitigate the concerns pointed out by stakeholders and encourage them to invest in research and development, as well as support pharmaceutical companies to be prepared for upcoming regulations, since the aforementioned EU-strategies announce further political regulations. We found also that successful implementation of BbD depends on the availability of suitable tools and methods as well as on incentives for research and development within constructive collaboration of industry, academia, and authorities.

Graphical Abstract

The optimization phase within API discovery is suitable for environmental considerations through Benign by Design. Pharmacological parameters are not necessarily in conflict with environmental biodegradability as an additional parameter.



Keywords: Benign by Design, Green Pharmacy, Sustainable Pharmacy, Active Pharmaceutical Ingredient (API), Drug Discovery, Environmental Biodegradation

Introduction

Active pharmaceutical ingredients (APIs) and their metabolites occur ubiquitously in the environment. They enter the environment mainly via wastewater as a result of patient use since, on average, an estimated 60-70% of APIs and their metabolites are excreted via urine, with varying excretion rates for the individual substances.[1] Most APIs do not degrade completely in wastewater treatment plants (WWTPs), despite the fact that much research and effort already having been made in the field of wastewater treatment.[2] Furthermore, 80% of wastewater worldwide is not treated at all because of a lack of treatment facilities.[3] Another entry path of APIs into the environment is via contaminated manure spread on fields. In the environment, some APIs are fully mineralized, i.e. degraded to water, carbon dioxide, and inorganic salts, for example acetylsalicylic acid.[4] Some are persistent, e.g. carbamazepine[5], whilst others are only partly mineralized, like fluoroquinolones.[6] Incomplete mineralization results in a highly diverse cocktail of transformation products (TPs) since several or many TPs are often formed based on one parent API.[7] Additionally, several thousand APIs are marketed worldwide and the diversity and volume will only increase due to increasing living standards, population, and age. Thus, there is a ubiquitous occurrence of APIs, their metabolites, and TPs in the environment. Although their environmental fate and effects are often unknown, some effects of APIs in environmental concentrations on environmental organisms, but also at a population level, have been demonstrated, e.g. cytological effects on fish.[8,9] Nevertheless, the chemical formulas, fate, and effects of TPs are mostly unknown. In some cases, it has been found that TPs are even more toxic than the parent API, as is the case for methadone and its TP *N*-Nitrosodimethylamine.[10,11] Adverse effects of compound mixtures, including metabolites and TPs, are difficult to predict.[12]

These alarming circumstances highlight the strong need for efficient measures against APIs, their metabolites, and TPs, which is also reflected in the EU Chemicals Strategy for Sustainability Towards a Toxic-Free Environment[13] based on the Zero Pollution Action Plan[14], as well as in the Pharmaceutical Strategy for Europe.[15] In addition to measures like adapted emission management or discouraging unnecessary use, the design of greener APIs according to the concept of Benign by Design (BbD) is a promising method of input prevention: It is an effective measure at the source ("beginning of the pipe")[16] and a sustainable long-term solution alongside the across-the-board approach and the precautionary principle.[17] Thus, the implementation of BbD is urgently needed. However, according to the results of the consultation of targeted stakeholders, short-term solutions are favored by the industry, likely due to concerns about the high costs and technical challenges associated with long-term solutions such as BbD. On the other hand, the pharmaceutical industry has not yet taken significant steps to implement the concept BbD into the generic API discovery process,[16] albeit it would offer economic advantages as new compounds will result in new business opportunities.[2] The reasons for this reluctance have to be better understood.

Even through an environmental risk assessment (ERA) has been required within the marketing authorisation application in the EU since 2006,[18] authorization cannot be denied because of the result of the ERA. Accordingly, it is typically conducted late during drug development.[19] Thus, environmental considerations are not integrated effectively, i.e. early within the API discovery process, as would be desirable for a greener design to gain the full functionality needed within the product life cycle (*eco*-pharmacovigilance).[20] The implementation of BbD into regular API discovery could be stimulated by providing an appropriate strategy. Workflows showing how environmental biodegradability can be considered beside the indispensable pharmaceutical activity[21] and demonstrating the feasibility of BbD[22,23] are available in existing literature. On a laboratory scale, the combination of *in silico* prediction and *in vitro* confirmation has been used as a very powerful strategy to achieve BbD.[24,25] This combination is already implemented during the industrial process for endpoints such as toxicity: *In silico* prediction is well integrated due to cost and time savings. However, tools predicting ready biodegradability are scarce, even though rules of thumb for structure-biodegradability relationships are well-described.[26–30]

To the best of our knowledge, there is no article discussing the implementation of the concept BbD within the industrial API discovery process. Therefore, the overarching goal of this study is to foster a better understanding of the pros and cons and motivate academia and industry to investigate the feasibility and opportunities of the concept of BbD for the discovery of APIs mineralizing readily in the environment. Herein, we provide an overview on the feasibility of the concept of BbD from a scientific point of view to mitigate the concerns pointed out by stakeholders and encourage them to invest in research and development. Furthermore, this overview is intended to support pharmaceutical companies to be prepared for upcoming regulations since the aforementioned EU-strategies announce further political regulations.

To achieve these objectives, the generic API discovery process, including the generic procedure, principles, and paradigms, was studied based on publicly available information. Next, the concept of BbD is presented including suggested test methods and tools. Thereby, the role of abiotic degradation, e.g., by direct and indirect photodegradation, is not discussed in this article although such processes may play a role under environmental conditions (e.g., access to light in contrast to the human body) and could in some cases initiate or at least support and accelerate biodegradation. However, photolytic and hydrolytic degradation are not expected to lead to complete mineralization. For example, photolysis is mediated by radicals (directly formed or by hydroxy radicals), which are of low selectivity due to their reactivity. The absorption spectrum has changed after the reaction. Often, no further absorption of light with sufficiently high energy takes place and/or no hydrogen atoms can be abstracted anymore resulting in stable TPs. In general, more polar TPs can be formed via abiotic degradation that are not necessarily better at mineralizing environmentally, or being of lower ecotoxicity, but which are more mobile in the aquatic environment and also under discussion as persistent, mobile, and toxic (PMT) substances with growing concerns.[31] Biotic degradation, on the other hand, is mediated by enzymes, leading to a lower TP diversity as the reaction pathways are specific. Therefore, we focus in this review on biodegradability of APIs due to the potential for complete mineralization.

Furthermore, we present different BbD workflow scenarios for varying starting points, such as *de novo* design ("from scratch") and re-design ("re-engineering", i.e. improvement of already existing molecules by structure variation) to present a variety of perspectives. In the final discussion, we highlight where the BbD concept can be included the generic, well-established API discovery process.

The Generic Workflow for the Discovery of APIs: Procedures, Principles, and Paradigms

The generic discovery of APIs is well established[32–35] and therefore only briefly described, including its principles and paradigms, before we discuss the needed improvements.

Traditional target-based drug discovery in the pharmaceutical industry (Figure 1) is subdivided into i) target identification, ii) hit selection, iii) from hit to lead optimization, iv) from lead to candidate optimization, and v) preclinical trials for candidates as a stepping stone to further drug development.[36]

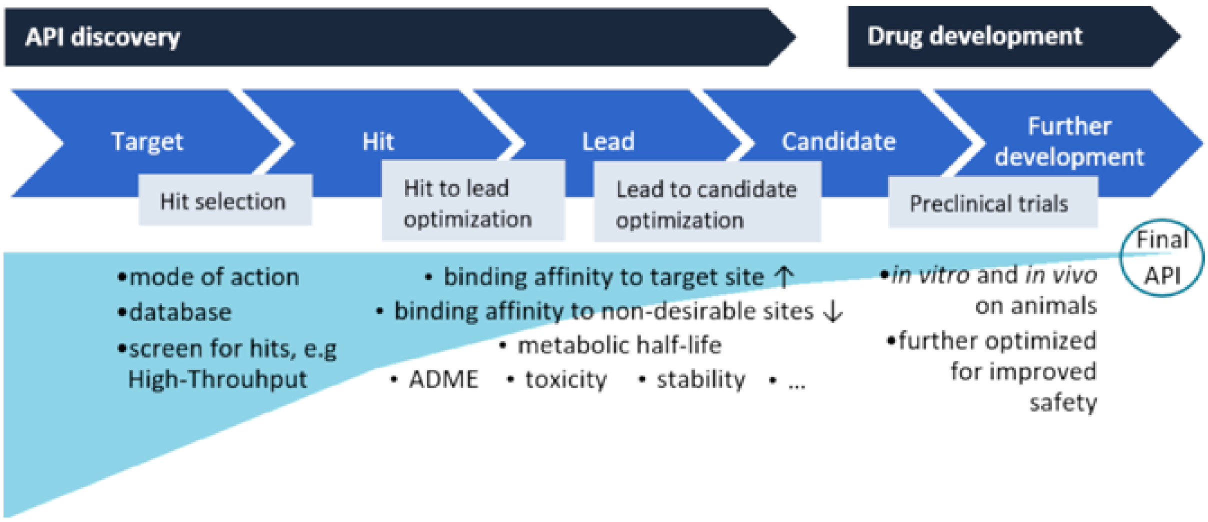


Figure 1: Scheme of a traditional research process in drug discovery, inspired by Messinger et al.[36]

Target identification and hit selection

First, the target for the disease needs to be identified, which is typically assumed to be a protein. For this selected target, large molecule libraries are used to find compounds with potential activity on the selected target, resulting in up to tens of thousands of compounds. By an appropriate screening method, e.g. high throughput screening, structures with confirmed activity on the chosen target are selected. These so-called hits can then be further optimized.[33]

From hit to lead to candidate optimization

The optimization steps 'from hit to lead' and 'from lead to candidate' follow. Leads are the most promising optimized hits. They are safe, patentable, have desired *in vitro* pharmacokinetic properties (absorption, distribution, metabolism, and excretion (ADME)) and show activity in preliminary *in vivo* models. Next, selected candidates are suitable for preclinical development.

This optimization strategy is challenging for medicinal chemists due to the many parameters that need to be investigated.[32] At the same time, it is most crucial to include all the required properties in this multiparameter space, such as activity, selectivity, ADME, toxicology, target specificity, drugability and patentability.[36] The synthetic route of the "optimal" candidate compound needs to be highly flexible to allow for the implementation of bioactive groups. Therefore, the optimization process is conceived as a repetitive cycle, the so-called design-make-test-analyse (DMTA) cycle,[37,38] that is executed many times. Since drug discovery is a very competitive field, the factor *time* affects the process significantly. To reduce the cycle time for optimization as much as possible and to enable many runs during the optimization phase (1 - 2 years), automated, combinatorial, or parallel concepts are employed.[36] Selected assays of the *in vitro* test phase that run in parallel can provide 80% of all data within just ten working days. In order to cope with the challenge of multi-parameter design and to improve the quality and the effectiveness of the DMTA cycle, effective collaboration within a dedicated multidisciplinary design team consisting of medicinal, synthetic, and computational chemists as well as drug metabolism and pharmacokinetics experts is essential.

It must be emphasized that the quality of a candidate compound which dictates the success of development is decided already at the point of design where cost of idea finding is low and the chances of success are variable.[37] Therefore, most companies perform *in vitro* pharmacology profiling already at this early stage, even though only one *in vitro* pharmacology assay is required by regulatory authorities: the assay for effects on the human ether-à-go-go-related gene (hERG) cardiac potassium channel (ICH Guideline S7B).[39] Importantly, an early screening of compounds against a broad range of targets (receptors mainly of the G protein coupled receptor superfamily, ion channels, enzymes, transporters) that are different from the intended therapeutic target(s) can reduce safety-related attrition (i.e. due to adverse drug reactions) in the later stages of drug discovery and development. In order to perform an effective *in vitro* pharmacological profiling early during API design, Browes et al.[40] recommend 44 targets as a minimal panel and the use of data for the development of *in silico* tools.

Preclinical trials for candidates

After optimization, candidates that fulfil all criteria are transferred to preclinical development to ensure safe application of the drug using *in vitro* tests *and in vivo* tests on animals. At this point, the required quantities of compounds increase from grams to kilograms and up to tons, for which process chemists are developing the optimal synthetic route. The final clinical development of the promising candidate(s) follows via tests on humans which, if successful, will ultimately lead to an effective and safe drug.[32,33,35,36,41]

Overarching principles and paradigms

In all of the steps in the drug discovery process, quality assurance is an overarching principle. For this purpose, organic and genotoxic impurities are analysed and physical properties and stability, e.g. regarding temperature and humidity (stress testing), are controlled according to defined standards (ICH Guideline Q1A(R2)).[42] However, these experimental tests take time and are usually only conducted once the optimal synthetic route is decided. Therefore, the Quality by Design approach, recommended by the European Medicines Agency (EMA), would be more effective, as it applies statistical, analytical, and risk-management methodology in each stage of the process from discovery to manufacturing.[43] For example, a control strategy within the development of a robust aniline-containing API could limit the amount of potential genotoxic aryl nitroso and hydroxylamine impurities that arise from the reduction of an aromatic nitro-compound.[44]

Another overarching principle is to further improve the efficacy of the process under competitive conditions. For this purpose, computational tools are indispensable today throughout the entire process from target selection to drug development. *In silico* tools are diverse and have various pros and cons.[45,46] In the broad field of computational screenings for hits, structure-based screenings are predominant compared to ligand-based screenings, which in turn tend to provide more potent hits.[46] Starting out with fragments, hot spot analysis can be applied. Continuing with further optimization, druggability prediction, (quantitative) structure-property/activity relationship ((Q)SAR) methods, molecular docking, machine learning, and deep learning models play a role for endpoints such as bioactivity, solubility, synthetic feasibility, ADME properties, and toxicity (ADMET).[45]

Computational calculations routinely predict characteristics defined by Lipinski's rule of five, e.g., Log P <5 using (Q)SAR, for prospective structures within API discovery.[32] Through molecular docking, on-target as well as off-target effects can be calculated. The limitations of the accurate simulation of relative binding free energy[46] can be overcome using the guidelines provided by Cournia et al. based on examples encouraging free energy methods.[47] A prominent example of successful application is the prediction of effects on the hERG cardiac potassium channel early during the design phase of the DMTA cycle, which has led to reduced safety-related attrition rates. Based on the predicted effects on the hERG channel, a prioritization for subsequent synthesis and *in vitro* testing is possible.[48] Driven by business needs, i.e. minimizing overall attrition and costs, machine learning is also recently being implemented at all stages of the drug discovery and development process. For example, machine learning was successfully applied to lead optimization by designing compounds with desirable properties, but this required an enormous amount of training data.[49] In general, API discovery could benefit more from artificial intelligence (AI), as it is currently only partially implemented; AI has the potential to become a more integral part of API discovery.[50]

These principles, i.e. quality assurance and process efficacy, are both well-established in contrast to the concept of Benign by Design, here aimed specifically at the principle *Design for Environmental Degradation*[51]. This is further explained in the following section.

The Concept Benign by Design

Benign by Design (BbD) is the targeted design of a compound with optimized characteristics for its application and fast and complete mineralization after it has entered the environment. It means that ready degradability after use or application is considered even before an API's synthesis.[52] BbD is based on the tenth principle of green chemistry described by Anastas and Warner: "Design for Degradation".[51] The design of degradable APIs will contribute to United Nations Sustainable Development Goals 3 (health), 6 (clean water), 9 (sustainable industries), 11 (sustainable cities), and 12 (responsible production and consumption).[53] Beyond ready environmental degradability as part of full functionality in terms of BbD, an ideal and modern API is expected to be effective and efficient, receptor specific, with reduced or without unwanted effects, and metabolized to harmless metabolites.[27]

BbD takes advantage of the fact that changes in the chemical structure of a molecule alter its chemical and physical properties including its biodegradability. If changes are made at parts of the molecule that are not mandatory for its activity, environmental biodegradability can be improved without compromising its activity.[25] However, changes at the core structure delivering activity are possible as well, and may go hand in hand with improved properties needed for application and at the same time increased environmental biodegradability.[23,54,55] Thus, this BbD concept can be used to design APIs to be fully biodegradable without decreased pharmacological activity or increased human toxicity during use.[21]

Ideally, the entire life cycle of a product should be made circular, meaning that there are no resource inputs and outputs and only limited energy inputs during its entire life cycle.[56,57] Achieving resource circularity is particularly difficult in the case of APIs, as they are emitted at non-point sources into sewage systems, soil, and surface water. After emission, collection is impossible as it is too energetically, financially, and environmentally taxing to derive them for recycling purposes.[52] However, if cleverly designed APIs mineralize at the end of their life cycles through complete environmental degradation into carbon dioxide, water, and inorganic salts there will be no end of life issues.[58] These resulting inorganic degradation products can then re-enter the biological cycles by incorporation into plant and microbial biomass, which can then in theory be used as a renewable resource for pharmaceutical production, thereby closing the cycle to the best currently feasible extent.[52,58] Furthermore, such an approach protects water resources without upgrading sewage treatments. This saves financial resources, chemicals, and the need for advanced treatment as well as the resulting associated issues related to products of incomplete mineralization.[57,59] In fact, this concept can be applied everywhere, even if there is no wastewater treatment available, which is the case for 80% of effluent on the planet.[3] Furthermore, because of climate change there will be an increased need for water reuse, e.g. in agriculture which requires non-polluted water.[53,59,60]

Tools and test methods needed for BbD

In silico

In silico models based on (Q)SAR are very efficient in predicting biodegradability because of lower costs and time savings when compared to *in vitro* tests. Therefore, *in silico* methods are an appropriate tool when dealing with a huge number of compounds, which is often the case during API discovery. However, their application is not trivial and requires expertise, as only (Q)SAR models with defined and validated applicability domains should be used. Additionally, the application of more than one model makes the predictions more reliable. Ideally, statistical models should be used in combination with expert rule-based models as recommended by the ICH M7 guidelines for toxicity.[61] The user needs to review and evaluate the predicted results, and be aware of which experimental tests provide the data for the *in silico* model. Rücker and Kümmerer[62] have provided practical information e.g. on the availability and limitations of reviewed prediction models like the Biowin1-7 of the US EPA, and Multicase, which could be of interest for an API designer. Two essential challenges for the prediction of biodegradation are the poor reproducibility of data caused by the variability of bacterial populations and the lack of qualitative experimental data.[62] However, high quality experimental data is a prerequisite for good predictions and needs to be generated by further research. Available QSAR software packages (e.g. CASE Ultra from Multicase and ModelApplier from Leadscope) allow the user to integrate new data into models. Thus, within our experimental research, results will be used to amend existing databases and establish models for prediction of biodegradability in order to improve the quality of predictions.

To increase the reliability of results, predictions via (Q)SAR based models can be expanded, i.e. as *in silico* test battery, using the Read-Across approach which is accepted for the assessment of chemicals according to the REACH regulation, particularly Annex XI, 1.5. Read-Across is a technique used for the prediction of a property for one substance (target), by using data on the same property for (an)other reference substance(s). Structural similarity, e.g. regarding common functional groups, is a prerequisite for the grouping of substances and subsequent application of the Read-Across approach.[63,64] The ECHA developed the Read-

Across Assessment Framework as an internal tool for examining predictions about environmental fate based on Read-Across and according to the REACH regulation. It is emphasized that expert judgement is required to use this approach.[63] To use Read-Across to determine fate during biodegradation, environmental conditions must be defined using proper judgement during the testing of the reference substances. However, recently Fenner et al.[65] have demonstrated broader application of Read-Across: Half-lives from biodegradation tests with mixtures of chemicals in activated sludge can be used to predict half-lives in soils. Thus, extensive work and resource-intense regulatory simulation studies might no longer be needed. In theory, bacterial populations in soil and activated sludge could be functionally similar regarding the enzymatic degradation of diverse molecules of environmentally relevant concentrations.[65] A practical quantitative Read-Across procedure is proposed for the prediction of Ames mutagenicity by Benigni.[66] Although environmental biodegradability has not been addressed, this specific approach can help in using Read-Across in a simplified, standardized, and reliable manner, as is the case for (Q)SAR. This approach can be especially useful for parameters for which (Q)SAR has limitations.[66]

In vitro

A plethora of *in vitro* biodegradation tests are available.[67–69] However, EU standards, e.g. ISO 7827, ISO 9408, ISO 14593,[70] and OECD tests reflecting the latest state of science and techniques are accepted internationally as standard methods. OECD tests (series 301 for ready biodegradability, test 308 for aquatic sediments) are recommended by the EMA for the environmental risk assessment of pharmaceuticals.[71]

Since the biodegradation tests vary in conditions (type of inoculum, concentration of substance(s), light and oxygen availability, and detection and evaluation method), different types of biodegradability can be determined (Table 1).

Table 1: Definitions Regarding Biodegradability.[72]

Ultimate biodegradation	Complete metabolism and thus mineralization
Primary biodegradation	Any chemical change caused by microorganism involvement
Readily biodegradable	Generally used to indicate a chemical having passed OECD tests for biodegradability, typically agreed upon to indicate that a chemical will biodegrade completely in aquatic environments with aerobic conditions
Inherently biodegradable	Undeniable evidence of biodegradation in a test has been demonstrated
Half-life ($t_{0.5}$)	Time it takes for 50% of a substance's chemical composition to change in a biodegradability test when first-order kinetics can be used to describe this change
Disappearance time 50 (DT50)	The time it takes for the initial concentration of a compound to halve during a biodegradability test

According to the concept of BbD, ready and ultimate biodegradation is the goal, i.e. complete degradation resulting in full mineralization that rapidly initiates once a pharmaceutical has exited the body, in order to enable complete degradation even in surface water.[72–74] This is needed since 80% of wastewater worldwide is not treated due to of a lack of treatment facilities.[3] Thus, the tests for ready biodegradability (OECD test series 301)[75] are highly recommended during drug discovery. The test choice within series 301 is primarily dependent on solubility, volatility, and absorbance of the compound (Table 2). For example, the Closed Bottle Test (CBT) and the Manometric Respirometry Test (MRT) are suitable for a broad range of properties, i.e. solubility, volatility, and absorbance.[75]

Table 2: Biodegradability Test Choice.[75]

Test	**Analytical method**	**Suitability for compounds which are:**		
		poorly soluble	**volatile**	**adsorbing**
DOC Die-Away (301 A)	Dissolved organic carbon	-	-	+/-
CO2 Evolution (301 B)	Respirometry: CO2 evolution	+	-	+
MITI (I) (301 C)	Respirometry: oxygen consumption	+	+/-	+
Closed Bottle (301 D)	Respirometry: dissolved oxygen	+/-	+	+
Modified OECD Screening (301 E)	Dissolved organic carbon	-	-	+/-
Manometric Respirometry (301F)	Oxygen consumption	+	+/-	+

When putting BbD into practice, the CBT and MRT were found to function as suitable elements of BbD.[67,68,76,77–79] During the CBT (OECD 301 D), the compounds to be tested are treated with an inoculum of low concentration (10^4 – 10^6 cells L^{-1}) received from secondary effluent from a domestic sewage treatment plant or from surface water. The inoculum for the MRT (OECD 301 F), on the other hand, is received, e.g., from activated sludge or sludge effluent and has a higher concentration (10^7 – 10^8 cells L^{-1}). Thus, ready biodegradability during the CBT is the gold standard for BbD, because a compound that biodegrades readily during the CBT will degrade readily in the environment. Furthermore, during the MRT, solubility problems may occur for poorly soluble compounds, since 10 times as much test substance as in the CBT (50 - 100 mg ThOD L^{-1} vs. 5 - 10 mg ThOD L^{-1}) is used.[75]

However, the 301 OECD tests take 28 days and may be time intensive from an industry perspective. As mentioned previously, the test phase of the DMTA cycle is attempted to be shortened by the industry.[37] Approaches to OECD test simplification already exist. During the OECD test 301 D, the monitoring of oxygen consumption using the optode method instead of the laborious electrode method saves time and effort as well as having analytical advantages.[80] Furthermore, efforts have been made to simplify the elaborate water-sediment test 308 of the OECD. Thereby, the use of an artificial and standardized medium and the measurement of pressure difference in closed vessels (OxiTop®) turned out to be an easier screening method.[81] These examples indicate that there are opportunities to simplify the standard tests.

Furthermore, high throughput screenings are demanded for competitive drug design where time is a crucial factor. AstraZeneca developed a high throughput biodegradation screening test (HT-BST) based on a colorimetric assay for aromatic compounds. It was thereby evaluated for use as a high volume test method for the screening and prioritization of compounds based on their biodegradability equal to OECD tests for ready biodegradability. 20,000 tests could run in parallel and for the same cost as an analogue OECD standard test.[82] This HT-BST reveals to be a good indicator of relative biodegradability and a tool to prioritize APIs and to investigate the structural rules of biodegradation toward establishing reliable *in silico* models.[62,83] Structure-property relationships have been derived from this HT-BST. One example of this is the influence of the electrophilicity of the substituent group at the *meta* position of phenols on their biodegradability, as indicated by the statistically significant correlation with the substituent's Hammett constant on this *meta* position. A general trend was found for increased biodegradability through decreased electrophilicity of the substituent at the benzene ring. These findings could serve as future design rules. However, the HT-BST has limitations as mineralization is unexplored and TPs that may interact with the color reagent are not considered, which could lead to a false-negative result. Thus, the combination with high throughput screenings developed for ultimate degradation[84] is recommended, so as to take advantage of both methods.[82]

Workflow scenarios of BbD and case studies of greener APIs

We can differentiate between two procedures of BbD: *de novo* design and re-design. The *de novo* approach is defined as design from scratch, whereas re-design aims to improve already existing molecules by structure variation.[2] In the case of re-design, the complex optimization process discussed above might be less challenging, as already existing knowledge about the starting molecule is available.

Furthermore, we can differentiate between two types of molecule generation as the crucial starting point for the workflow of BbD: targeted and non-targeted synthesis.[21] In targeted synthesis, structural features known to improve properties are intentionally inserted into the molecule, e.g. computer aided. In doing this, expert knowledge on design rules regarding desired properties can help lead to successful development of compounds satisfying the concept BbD. However, the optimization process via stepwise structural variation can be time-intensive. Every step includes an *in silico* screening ((Q)SAR, Docking) for pharmaceutically important properties (e.g. ADMET) and further *in vitro* testing of promising compounds (selected ADMET endpoints, stability, and environmental biodegradability). Finally, the most promising candidate can be investigated during subsequent preclinical development. Non-targeted synthesis, on the other hand, is based on an undirected, arbitrary generation of a large pool of compounds, for example via photolysis, which can be screened simultaneously. Therefore, less expertise is necessary for this quick and dirty approach, which is also cheaper than targeted synthesis.[21] Even an arbitrarily generated hit without any grounded influence can be found, as a matter of luck, and there are examples of success presented as a case study for non-targeted re-design at the end of this section.

The combination of procedures and molecule generation leads to four approaches to designing new compounds, namely non-targeted *de novo* design, targeted *de novo* design, non-targeted re-design, and targeted re-design (Table 3).

Table 3: Four approaches according to the concept of BbD by the combination of procedures and molecule generation.

Approach	**Description**
Non-targeted *De-novo* Design	Computational screening of large number of *in silico*-compounds for required properties
Targeted *De-novo* Design	Combination of small structural fragments to new more complex molecules by expert knowledge
Non-targeted Redesign	Non-targeted structural variation of existing molecules and testing to improve properties
Targeted Redesign	Targeted modification of existing molecules by expert knowledge to improve properties

Non-targeted de novo design

Typical API discovery presents an appropriate example for non-targeted *de novo* design. Starting with thousands of molecules from large databases, this process can be modified for the purpose of BbD if environmental considerations are implemented in the screening and optimization process. The typical API discovery process and potential environmental considerations are explained in detail in the next section.

Targeted de novo design

Fragment-based methods for API discovery are a good example of targeted *de novo* design. Small structural fragments are combined to make new more complex molecules through the use of expert knowledge and information on the structure of fragments and their activity.[85] Considering the above-listed design rules for biodegradability, API discovery using this method can be modified according to BbD. For example, 3-ß-D-galactopyranosyloxymethyl-4-sulfatomethylfuran was selected as the starting compound for a new mode of action. By systematic structural variation, a pool of compounds was designed and tested regarding efficacy. At the same time, biodegradability was considered by environmental chemists involved in the design and selection of compounds with improved properties.[86]

Non-targeted re-design

Studies by Rastogi et al.[24,25,79] present examples of non-targeted re-design for environmental biodegradability, using non-biodegradable β-blockers such as propranolol (Figure 2). In this approach, mixtures of derivatives of the starting compounds were generated arbitrarily, e.g. via photolysis (alternative to the strategy of Rastogi et al.: *in silico* prediction of TPs). As a rule, oxidation such as hydroxylation took place, so that degradation-favored oxygen could be introduced into the molecule. Subsequently, the mixtures were screened using an *in vitro* assay for environmental biodegradability, e.g. the CBT (OECD 301D[75]). After structure elucidation of potential biodegradable derivatives with an intact drug moiety, these were investigated regarding druglikeness, absence of mutagenicity and genotoxicity, as well as other ADMET parameters using *in silico* tools (QSAR, Docking). Then, promising candidates were synthesized and experimentally tested for environmental biodegradability, pharmaceutical activity, selected ADMET endpoints, and behavior of main metabolites.[24,25,79] In the case of propranolol, the derivative 4-hydroxypropranolol was identified to be an appropriate candidate, having an experimentally determined EC_{50} in a receptor assay for the desired activity in the same range as propranolol.[25] Most promising candidates could be investigated during subsequent preclinical development.[21]

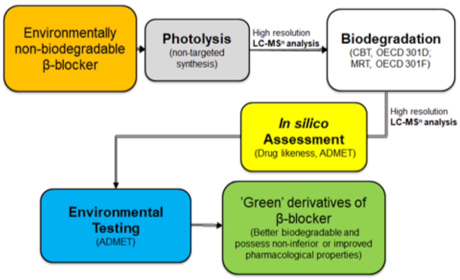


Figure 2: The re-design of β-blockers for environmental biodegradability through non-targeted synthesis of derivatives and subsequent screening, taken from Kümmerer[2] and marginally modified.

Targeted re-design

The targeted re-design approach was applied to improve the poorly biodegradable antibiotic ciprofloxacin, since it is poorly biodegradable, mainly because of the fluorine atom on the aromatic ring.[54,55] As a rule of thumb, electron-withdrawing substituents make a ring less favorable for an attack by oxygenase enzymes using electrophilic oxygen.[26] Occurrence in the environment, given the highly antibiotic effect of this fluoroquinolone, is quite concerning with regards to potential increased microbial resistance. Ciprofloxacin was re-designed via systematic variation of functional groups at the R^1 and R^2 positions on the core structure (Figure 3) using multiple types of side chains.

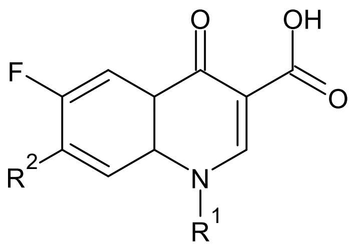


Figure 3: Core structure of antibiotic ciprofloxacin. R^1 and R^2: positions for systematic substitution.

In silico prediction of the properties of these designed derivatives led to promising candidates. The structures Cipro-Prolin and Cipro-P2C were patented as biodegradable quinolone antibiotics.[22] Another patent applies for environmentally degradable quinolone antibiotics with hemiaminal structural feature, i.e. the Hemi-linker of Cip-Hemi and CG008-Hemi as well as further substituents at C7 of fluoroquinolone.[23] Cipro-Prolin and Cip-Hemi are the most successful candidates (Figure 4) with increased degradability in the environment and only slightly decreased antibiotic activity compared to ciprofloxacin, which is one of the most active fluoroquinolones. Their degradation via hydrolytic cleavage of the amide or the hemiaminal, respectively, leads to a core structure (still including R^1 or R^2, respectively) with reduced antibacterial activity and consequently to a possibly reduced selection pressure in the environment. The hemiaminal is unstable at low pH values, but showed sufficient stability in humans (liver, blood serum).[54,55] The success of this project is demonstrated by the acquisition of patents for these greener active compounds. However, further effort is needed to design an environmentally degradable core structure to achieve the gold standard, complete mineralization. Research is currently being conducted on this.

a.) Cipro-Prolin b.) Cip-Hemi

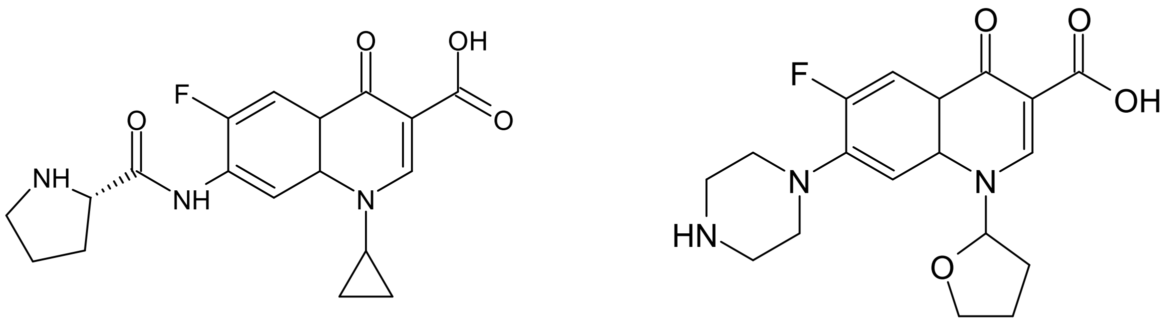


Figure 4: Structures of the most successful antibiotic candidates. Cipro-Prolin[22] (left) and Cip-Hemi[23] (right).

Further examples for (unintendedly) biodegradable APIs

There are examples of biodegradable APIs from the anti-cancer drug group that typically are quite stable and have concerning effects on the environment. The chemical structures of anti-cancer drugs, such as ifosfamide and 5-fluorourcil, were changed by glycosylation to improve drug efficacy, incidentally leading to increased environmental biodegradability.[77,78,87] For example, the anti-cancer drug 5-fluorouracil, acting as a uracil anti-metabolite, was modified to gemcitabine and cytarabine using doubly fluorinated arabinose and arabinose, respectively. Gemcitabine was biodegraded to 42% and 50% in the CBT and Zahn-Wellens Test (ZWT), respectively. Cytarabine showed between 40% to almost 80% biodegradation in the CBT, depending on the treatment period, and more than 95% in the ZWT.[77] The molecular structures and results of these tests are illustrated in Figure 5.[73] Even if these results are mainly below the 60% threshold required to deem a substance readily biodegradable according to OECD guidelines, they are better biodegradable than 5-fluorouracil, which is due to the added arabinose and doubly fluorinated arabinose moieties. Cytarabine is better biodegradable than gemcitabine, because the sugar moiety without fluorine atoms allows for the biodegradation of the cytosine moiety.[77] There are exceptions to this. Gemcitabine and cytarabine were found to have the same mode of action and similar or improved pharmacological properties.[73] Thus, cytarabine and gemcitabine present greener alternatives to 5-fluorouracil. However, since they are less stable than their parent compound and degrade under acidic conditions in the human stomach, they must be administered intravenously instead of orally.[88] Improving the delivery during drug development, however, can help researchers to overcome this challenge.[89]

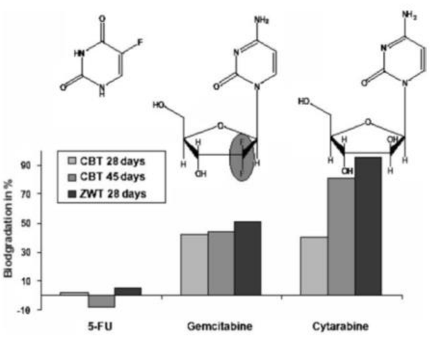


Figure 5: Results of the CBT and ZWT reported by Kümmerer[73], which illustrate the increased biodegradability after the re-design of 5-fluorouracile via addition of the arabinose or doubly fluorinated arabinose moiety to the structure of cytosine.

Another example of the fact that glucosidation can increase biodegradability is that of glufosfamide (β-D-glucosylisophosphoramidmustard), which resulted from improving structurally related ifosfamide (not biodegradable) for increased resorption and reduced side effects. Although the core structure of ifosfamide is not present anymore, glufosfamide (readily biodegradable) also belongs to the class of nitrogen mustard-derived alkylating agents[78] and is already in late clinical development.[87]

Furthermore, there are existing marketed high-volume APIs that are biodegradable in the environment.[27] These active compounds (Table 4) demonstrate that pharmacologically relevant properties are not generally speaking in contrast to environmental biodegradability.

Table 4: Biodegradability of selected pharmaceuticals from various classes in different OECD tests, taken from Kümmerer and Hempel.[27] Criterion for ready and inherent biodegradability (301 and 302 tests, respectively) is >60%.

Active compound	**OECD 301 D**	**OECD 301 F**	**OECD 302 B**
Isosobiddinitrate	>90		
Mesalazine	>90	>90	
Acetylsalicylic acid		81	
Penicillin V	27		>90
Glufosfamide	53		72
Piracetam		>90	>90
Hydroxamic acid	50	90	
Valproic acid	72	78	
Cytarabine	40		>90

Some pharmaceutical companies published the results of environmental risk assessments, including environmental degradation tests, in order to show their engagement in tackling the issue of pharmaceuticals in the environment.[90,91] Among the listed pharmaceuticals, there are examples of APIs showing inherent biodegradation, e.g. Amoxicillin and Rosiglitazone,[90,91] APIs even showing ready biodegradation, e.g. Interferon alfa-2a and Enfuvirtide, as well as the complete group of monoclonal antibodies, such as Emicizumab and Ocrelizumab.[91] This again shows that pharmacological properties are not necessarily in conflict with biodegradability in the environment.

Discussion: Implementation of BbD into the generic design process

The main goal of the common drug discovery process is to find new APIs that result in a new medicine that is safe, efficacious, and of assured quality according to legal requirements. The BbD approach simply adds environmental aspects to be included into the understanding of safe, efficacious, and of assured quality. Accordingly, the understanding of pharmacovigilance is extended to *eco*-pharmacovigilance, thereby including unwanted side effects of APIs in the environment. To reduce or even better to avoid unwanted effects by the presence of APIs in the environment, targeted design for environmental degradation or complete mineralization, respectively, is indispensable. The shown examples of marketed APIs and APIs designed by academia that are environmentally biodegradable, whether intended or not, indicate that it is feasible to design compounds that are both more environmentally biodegradable and (still) active.

Tailored to the industrial process, environmental considerations according to BbD should be included early during the drug discovery process, i.e. where they are most effective and the chances of introducing biodegradability are the greatest. It is proposed to add a filter for environmental biodegradability to the multi parameter space of the optimization process, since at this stage many compounds are designed and tested in cycles of many sets with high flexibility to find candidates that combine all required properties into one structure. It has been shown through many examples, such as the hypothesis-driven drug design by Plowright et al., that the optimization phase is appropriate for lowering overall attrition and costs.[37]

Within this early phase, the use of *in silico* tools can help to prioritize compounds for synthesis within the DMTA cycles. The presented workflows by Leder et al.[21] and Rastogi et al.[25] show this combinational approach for BbD, even if not tailored to the industrial API discovery process. The implemented hERG test strategy, for example, demonstrates on an industrial level that the combination of *in silico* and *in vitro* methods is a powerful strategy and very strongly demanded. In the same effective way, environmental biodegradability should be tested as one of many filters during the optimization cycles.

Another fundamental reason for early implementation is that environmental biodegradability of an API is already influenced (unintentionally) and can even be increased within the optimization phase which is discussed in the following section.

Hit and Lead Optimization – Impact on environmental biodegradability of APIs

For the improvement of ADME properties, it is suitable to apply Lipinski's rule of five as a rule of thumb for druglikeness, since most drugs are administered orally. For example, the rules on the presence of no more than 5 H-bond donors and 10 H-bond acceptors balance hydrophilicity for solubility against absorption as well as lipophilicity for membrane permeability. This balancing is also reflected in the rule stating Log P should be below 5. These rules are generally important for API's pharmacokinetic (ADME) properties and beneficial for cellular uptake, be it for human cells or for microbial cells, including those in the environment. The same holds true for the rule on molecular weights smaller than 500 Dalton,[92] which also has a positive effect on biodegradability.

Reducing human toxicity can affect biodegradability in the environment in various ways, depending on how the potential candidate is modified to alleviate a given mechanism of toxicity. For example, hypersensitivity and immune response, caused by greater compound reactivity, as well as off-target effects, caused by low drug specificity, can be alleviated through increasing size or branching with the aim of reduced reactivity and/or improved API specificity.[93] Both approaches (reduced reactivity, improved specificity) may decrease biodegradability due to a more complex structure. However, there are many exceptions since this depends on the added functional groups and the entire molecule. For example, monomethyl, in contrast to trimethyl, branching does not hinder biodegradation.[26] Further strategies, also later within drug development, like improving on-target specificity via controlled release formulations, can also reduce toxicity due to improved delivery, targeting, or specificity without the decrease in environmental biodegradability.

Increased stability is aimed at ensuring the function and quality of the API from shelf to target site. Stability is influenced by temperature, humidity, and light, among other factors, and can be tested according to ICH Q1A guidelines. The latter defines the exemplary stability data package for a new drug substance or drug product but leaves sufficient flexibility to consider varying practical situations due to specific scientific reasons.[94] High stability can strongly reduce biodegradability in the environment. We want to emphasise that extreme stability is superfluous due to the following reasons.[73]

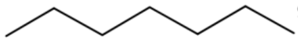
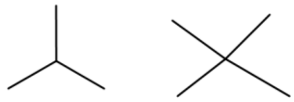
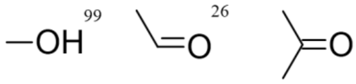
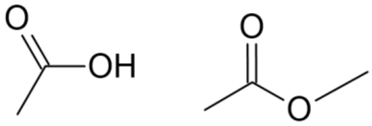
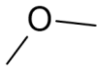
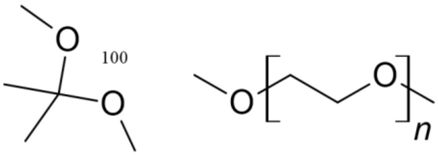
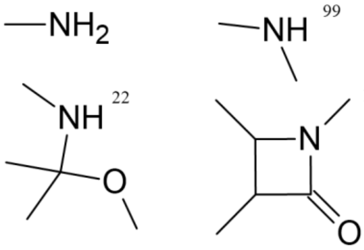
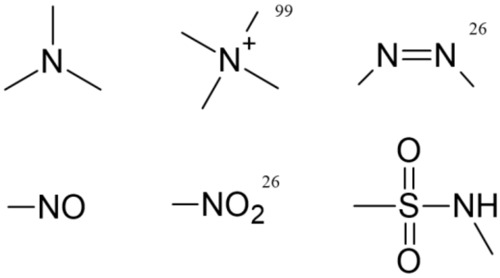
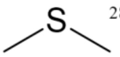
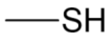
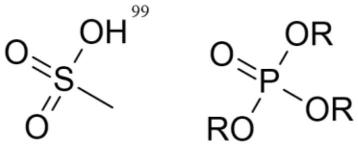
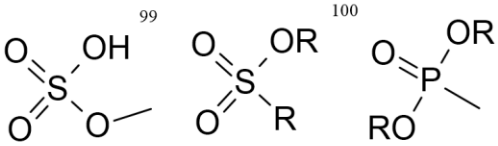
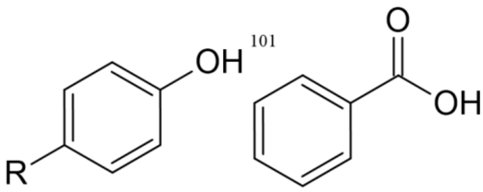
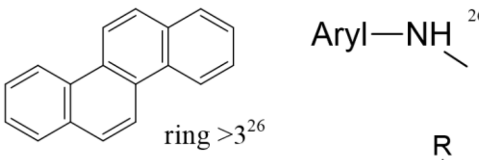
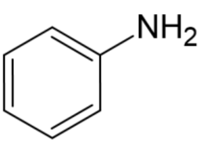
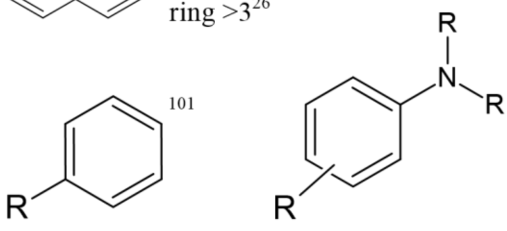
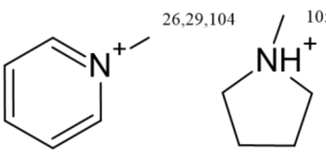
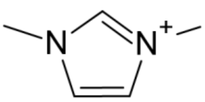
Stability, like reactivity, is not an intrinsic property; in addition to the stereochemistry and electronic properties of the molecule, it also depends strongly on the environmental conditions present. Thus, at varying stages along the life-cycle of the substance (from shelf, human body, wastewater, to the environment, e.g. surface water) with varying conditions, the potential for degradation varies as well. For example, microbes present in the human gut (mostly anaerobic) and in sewage treatment plants or surface water (mostly aerobic) as well as their enzymatic portfolio and activity are different due to differences in pH, the availability of nutrients (type and concentration), and access to light. In case compounds are sensitive to light, packing in brown glass or brown blisters protects the compound against light. This protection is not present after the API has been excreted or is present in surface water, allowing for its photolytic degradation.[73] Therefore, biodegradability in the environment is not synonymous with biodegradation in the human body, i.e. metabolization. Design for environmental degradation and mineralization of APIs is not prohibitive for stability during application, efficacy, and other properties necessary for an API to be applied as a pharmaceutical, as demonstrated with the example of the patented antibiotic Cip-Hemi.[54,55] Thus, at this point rethinking must take place, as stability from shelf to target site in the body does not necessarily contradict ready biodegradability in the environment. Small changes in the chemical structure of a pharmaceutical can drastically alter its environmental biodegradability.[73] This fact needs to be considered during the variation of lead structures.

Consequently, we have to be aware of the impact of optimization (i.e. reducing toxicity, increasing stability) on environmental biodegradability. Drug designers could balance the multi parameters for new derivatives in such a way that introduced structural features also benefit, or at the very least do not hinder, environmental biodegradability. The application of design rules could support both stability during application and environmental degradability to ensure full functionality, meaning good performance along the entire life cycle of the drug in the understanding of complete pharmacovigilance, i.e. *eco*-pharmacovigilance.[20,95]

Design rules for biodegradability

Biodegradation depends on microbial bioavailability, which is influenced by molecule size, because larger molecules (> 1000 Da) are less readily taken up by bacterial cells, and by hydrophobicity; the substance should not be too hydrophilic or hydrophobic. These criteria fit nicely with the requirements for the oral administration of drugs.[92] After uptake of the substance, its metabolism inside of the bacterial cells is affected by structural features of the molecule and impacts biodegradability. Thereby, the availability of enzymes plays a crucial role. Widespread esterases show broad specificity and are therefore ubiquitously involved in biodegradation processes. Oxygenases catalyze the insertion of oxygen when molecular oxygen is present. This initiating degradation step is beneficial for small molecules that already contain oxygen.[26] There are many well-known rules of thumb (Table 5) for structural features that favor or hinder biodegradability. For example, unbranched alkenes are better biodegradable than branched alkanes.[96] The main pathway of alkane degradation is terminal oxidation, which is widespread among bacteria. The respective alcohol is dehydrogenated by dehydrogenases via the corresponding aldehyde to form the fatty acid, which is then degraded gradually via β-oxidation to C2 units as acetyl-CoA.[97] In addition to the shown structural features, natural structural elements such as amino acids[22], peptides[98], or sugar moieties[77] can promote biodegradation as well. Thereby, the stereochemistry must be considered. Naturally occurring L-amino acids and D-saccharides are favored for BbD since they fit well with enzymes in the environment.

Table 5: Rules of thumb for structural features regarding biodegradability

Favoring biodegradability	Hindering biodegradability
 [99] Chain length: =C4[26] - C8[29]	 [99]
 [99] [26] [30]	 [99]
 [99]	 [99]
 [100] [26]	
 [99] [22] [101]	 [99] [26] [26] [102]
 [28]	 [28]
 [99] [26,100]	 [99] [100] [26]
 [101] [103]	 ring >3[26] [26]
 [99] R: strongly electron-donating[82]	 [101] [99] R: strongly electron-withdrawing, e.g. Cl[26]
 [26,29,104] [105]	In general, heterocyclic aromatics[26], e.g.:  [26,29]
oxygenated, simpler compounds	**highly substituted, more complex compounds**

A comprehensive view of the presented examples for biodegradable APIs (Table 6) reflects the rules of thumb, e.g., that oxygenated molecules of medium polarity favor biodegradation since hydroxy groups are often a good starting point for further biodegradation. In many cases, hydrolysis of bonds like glycosidic linkages, amides, and esters is expected to initiate biodegradation processes. However, in contrast to the β-lactam antibiotic Amoxicillin, related Piperacillin does not represent a fully biodegradable API in spite of the hydrolysis of β-lactam, because the benzene ring prevents further degradation.[101] As for Amoxicillin, but also 4-hydroxypropranolol, phenol rings can be oxidized at the ortho position by monooxygenases.[97]

Table 6: Comprehensive consideration of discussed biodegradable APIs: Assignment of the APIs to the expected starting reaction of the degradation process based on structural features leading to biodegradable TPs.

Expected starting reaction	**Active compound**	**Structural feature**
hydrolysis of glycosidic linkage	3-ß-D-galactopyranosyloxymethyl-4-sulfatomethylfuran	β-D-galactopyranose, furan, sulfate
	Cytarabine	arabinose, amino-pyrimidin-2-on
	Gemcitabine	fluorinated arabinose, amino-pyrimidin-2-on
	Glufosfamide	D-glucose, phosphoramide
hydrolysis of amide/peptide, lactam, hemiaminal, and/or ester	Amoxicillin	β-lactam, amide, amine, phenol, carboxylic acid
	Penicillin V	β-lactam, amide, phenol, thioether, carboxylic acid
	Piracetam	γ-lactam, amide
	Rosiglitazone	amide, thioester, phenol, 2-pyridinylamin
	Cipro-Prolin	L-proline
	Enfuvirtide	peptide
	monoclonal antibodies, e.g., Emicizumab and Ocrelizumab	peptide
	Cip-Hemi	hemiaminal ether
possible further oxidation due to electron donating substituent; and ring cleavage	4-hydroxypropranolol	1.4-dihydroxynaphthaline
	Acetylsalicylic acid	2-acetoxybenzoic acid
	Mesalazine	*o*- hydroxy-benzoic acid, amine
β-oxidation (cf. fatty acid degradation) via Acetyl-CoA pathway	Valproic acid	carboxylic acid

The design rules can be considered guidelines for the design of environmentally biodegradable APIs.[26–28,30,99] However, the entire molecule and its biodegradation mechanisms must be considered. For example, degradability depends also on the position(s) of the substituents (e.g., halogens) and on the degree of substitution (e.g. by methyl groups).[26] Furthermore, there are always exceptions. For example, the quaternary ammonium group of choline is expected to hinder biodegradability, as demonstrated for cations of ionic liquids (ILs) with cholinium covalently bound to amino acids.[29,106] However, ILs with choline (cation) and several amino acids (anion) showed ready biodegradability.[26,107] Thus, after first indications of biodegradability have been gathered using rules of thumb, the entire molecule must be evaluated considering the reaction conditions to avoid generalization. And even with the application of expert knowledge in biochemistry, testing must be carried out to determine actual biodegradability because of the complex occurring interplay outside and inside of the bacterial cell (i.e., between structural properties needed for uptake and metabolization with selective enzymes and changing reaction conditions). For example, Zumstein and Fenner[98] recently investigated the deviating stability of peptide-based antibiotics in blood plasma and in solutions with extracellular wastewater peptidases as a potential BbD approach. However, rules of thumb as well as *in silico* tools are important to give guidance and to speed up the total process of API discovery.

Enhanced process flow for the design of APIs mineralizing readily in the environment

Based on the reviewing process and the discussion regarding the implementation of the concept of BbD into generic API discovery, an enhanced process flow for the design of environmentally biodegradable APIs has been developed (Figure 6). The modified DMTA cycle as key element of the enhanced optimization phase is zoomed out and explained in the following.

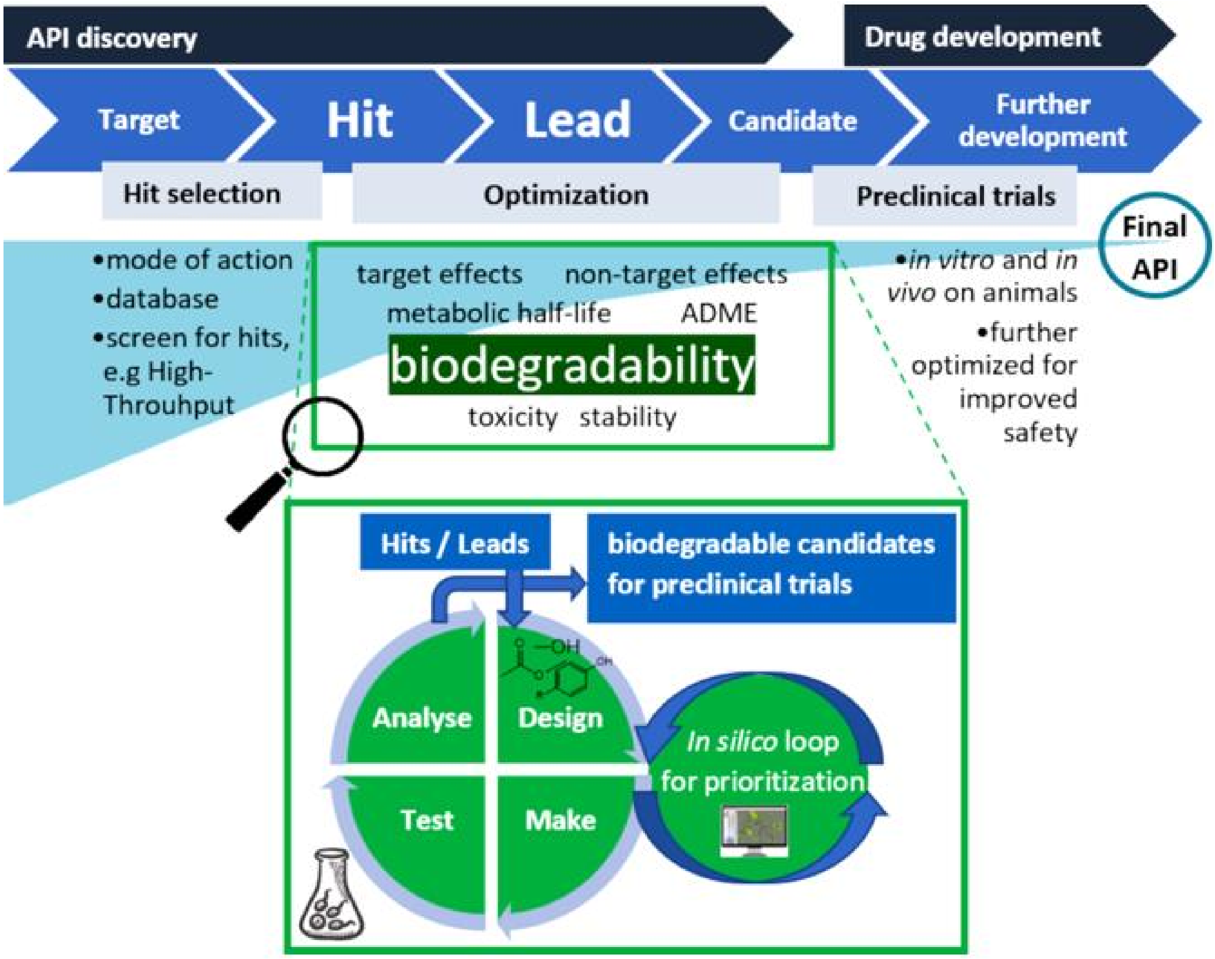


Figure 6: Common API discovery enhanced by the concept of BbD during the optimization process.

*Design of new compounds (**D**esign)*

At the start of the DMTA cycle, the lead structures are designed by considering the structural features, for which effective collaboration between experts is required. The designed leads are screened by an available and appropriate *in silico* model in order to prioritize leads for synthesis. If there is no suitable model available, e.g., because the structures do not belong to the applicability domain of the model, the proposed Read-Across approach can remedy this. Note that Read-Across should also be used to evaluate results of (Q)SAR models. In the long term, machine learning and other pattern-recognition strategies can be used in conjunction with expert-based Read-Across. Data generated according to standard methods can be of great use for later purposes, mainly as a basis for new models. Therefore, it is important that the data is made available to the community through an open database.

*Synthesis of designed compounds (**M**ake)*

The results of the computational analyses will be confirmed through *in vitro* tests. Therefore, promising candidates will be synthesized, i.e. compounds predicted to be biodegradable besides optimized regarding all pharmaco-relevant properties like activity.

*Testing in various assays (**T**est)*

For a first screening of many compounds, high throughput tests such as the outlined HT-BST by Martin et al.[82] might be an appropriate *in vitro* method to indicate biodegradability and prioritize APIs for intensive testing. For this testing, the presented standardized *in vitro* methods that are provided by OECD are suitable options, especially the CBT due to reasons discussed above.

*Analysis of test results (**A**nalyze)*

All generated data is analyzed and considered for the design of further compounds to run the next cycle. Candidates that fulfil all criteria, also environmental biodegradability, are transferred to preclinical development. All data, independent of results, should be made available to the community, preferably in open databases, and quality criteria need to be highlighted with the long-term objective of developing *in silico* models.

If minor structural changes cannot lead to success, there are other options. Namely, the improvement of drug delivery systems, e.g. by nanoencapsulation using appropriate excipients, can lead to increased bioavailability and improved delivery and efficacy.[108] This leads to smaller doses and finally to reduced input into the environment. Another possible solution is the design of biodegradable non-covalent derivatives (NCD) as cocrystals to improve physicochemical properties.[105] For example, the stability of gemcitabine (presented above) was improved reasonably via co-crystallization using *p*-toluenesulfonic acid to develop an orally available anti-cancer prodrug.[109]

Challenges and perspectives

We go along the DMTA cycle to discuss challenges and perspectives of enhancement through the concept of BbD. Starting with the design phase, the dedicated design team must consider many parameters, always giving the highest priority to safety, followed by efficacy and quality. It could be discouraging to consider yet another parameter. However, examples such as the patented CIP-Hemi[23] demonstrate business opportunities for new benign APIs.[2] Additionally, there are some industrial pharmaceuticals without targeted design which indicate the feasibility of BbD, such as valproic acid[110], acetylsalicylic acid[4], and ß-lactams.[101] When compared to chemicals, the targeted design of environmentally mineralizing pharmaceuticals poses greater challenges because of further requirements such as pharmaceutical activity. Yet, if the application of BbD is possible for this demanding substance class, it should also be possible for many other chemicals.[13] Indeed, many chemicals, such as linear alkylbenzenesulfonates, EDDS, and organic phosphorous esters, have been designed already for mineralization in the environment.[73]

Furthermore, it is essential to understand to which extent environmental biodegradability is affected by improvement of other parameters, e.g. toxicity and stability. Thereby, stability does not contradict environmental biodegradability because of their strong dependence on system conditions. In fact, sometimes these factors change parallel to one another (see above e.g. Lipinski's rule of five), assuming the absence of extreme stability. Thus, an important paradigm shift, especially among conservative stakeholders, is needed to avoid superfluous extreme stability and make BbD feasible.

To push forward this paradigm shift, it is recommended that legal requirements for API stability are adjusted to more realistically fit needs during storage and use without preventing degradation after use. Thus, a more nuanced approach to designing drugs for stability is advocated, similar to biologics (e.g. antibodies, proteins, nucleic acids or even viruses and cells). As these biopharmaceuticals are sensitive to environmental factors (e.g., temperature changes and light), specific requirements regarding stability have been developed (ICH guideline Q5C)[111] to overcome limitations regarding storage, for example. The application of biologics demonstrates that requirements for stability can be adjusted to a reasonable amount.

Within the design phase, *in silico* tools are an integral part of the conventional DMTA cycle. They can facilitate and accelerate the consideration of ready environmental biodegradability, too. However, sufficient qualitative experimental data is missing for the development of very high quality (Q)SAR models for biodegradability prediction, because not enough testing is done and because of weak reproducibility of biodegradation due to test-specific parameters. Data gaps need to be filled by intensive experimental testing of ready environmental biodegradability according to OECD methods. In terms of efficiency, pharmaceutical companies, academia, and authorities should cooperate and share knowledge in order to combine the various strengths and prevent the waste of resources by duplication. This is in line with the philosophy of the EU strategy for sustainability in the broader field of chemicals.[13] Furthermore, machine learning, which should also be used to collect and publish qualitative data, as well as the Read-Across approach are highly desirable to help overcome limitations of (Q)SAR. Therefore, open-mindedness is essential[50] which can be promoted by highlighting existing incentives.

Existing incentives include current political developments, clearly recognizable in EU strategies for a toxic-free environment.[13–15] These can be seen as upcoming unwanted limitations, but even better as new business opportunities for biodegradable APIs given increased competitiveness of sustainable EU industries. Furthermore, the effective contribution to sustainable pharmacy through the design of environmentally degradable APIs (compare Sustainable Development Goals 3, 6, 9, 11 and 12)[53] can be seen as a social mandate. Additionally, to be one of the frontrunners of greener pharmacy can be adapted as part of a marketing strategy. To add another incentive, the high costs and time effort required for an extensive environmental risk assessment can be saved since this is not necessary for environmentally biodegradable APIs. Longer patent term, fast track approval, and public reputation can all be considered as new incentives. Thus, existing and coming incentives should encourage stakeholders to try out the design phase enhanced by BbD and to fill identified data gaps by further research.

After the design phase, *in silico* predictions need to be confirmed for prioritized and synthesized compounds by an appropriate OECD method. However, the time-intensive biodegradation tests of 28 days according to OECD could be unattractive for industrial application, as currently attempts are made to shorten the DMTA cycle, e.g. to 10 days for 80% of experimental data within this phase.[37] The development of the aforementioned HT-BST offers promising solutions. These also take 28 days, which is absolutely needed to investigate complete environmental fate up to mineralization, but allow for the study of numerous compounds. Further research in this direction is needed as this approach is practice-oriented, and therefore of great interest for the proposed advanced DMTA cycle. Ideally, HT-BST should investigate ready biodegradation, including environmental mineralization of a broad range of designed lead structures.

When companies recognize that the discovery of environmentally biodegradable APIs is a business opportunity[2], the willingness to make changes grows and the identified challenges can be tackled. Examples for APIs satisfying the concept of BbD push forward the discussion on its feasibility and demonstrate business opportunities. Among them are ß-blockers with retained activity[24,25,79] and recently approved patents for new APIs[22,23], both designed for better biodegradability.

Conclusion

The design of APIs readily mineralizing in the environment (Benign by Design, BbD) is an effective measure toward avoiding the accumulation of pharmaceuticals in the environment and is therefore urgently needed. Reviewing literature regarding the common API discovery process as well as case studies of BbD, including test methods and tools for biodegradation, has enabled us to demonstrate the feasibility of BbD and to propose its implementation into commonplace API discovery procedures. These results should encourage pharmaceutical companies to invest in research and development, as well as support them to be prepared for upcoming legal changes, since the above-mentioned EU strategies announce further political regulations.

In conclusion, the optimization phase seems to be the most appropriate step to implement environmental considerations. Thereby, pharmacological parameters are not necessarily in conflict with biodegradability in the environment. However, the success of BbD within the DMTA cycle depends on the availability of suitable *in silico* tools and test methods. Since high quality data needed for the development of *in silico* test models is rare, further research and data publishing are urgently needed. Furthermore, new *in vitro* test strategies, such as HT-BST, need to be developed and integrated into everyday API discovery. Constructive collaboration of industry, academia, and authorities could accelerate the data gathering and processing. Existing incentives need to be highlighted and new ones created, so that the willingness to make changes to commonplace API discovery processes grows and the identified challenges are tackled.

Conflicts of interest

There are no conflicts of interest to declare.

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