Chinese Journal of Cardiology
Volume 54 Issue 3, Year of Publication 2026, Page 255-265 | DoI- 10.545120/0253-3758.2026.051

ISSN : 0253-3758

Chinese Journal of Cardiology

Expanding Retrometabolic Drug Design Beyond Small Molecules: Hybrid and Bioconjugate Modalities

Priya Jain[1], Niharika Gokhale[2], Asha Rani Pyathi[2], Saloni Yadav[3], Nayany Sharma[4], Razia S Khan[5*]

[1]Lakshmi Narain College of Pharmacy (RCP), Indore, Madhya Pradesh 453555
[2]Medicaps University, AB Road Pigdamber, AB Rd, Pigdamber, Rau, Indore, Madhya Pradesh 453331
[3]Acropolis Institute of Pharmaceutical Education and Research, Indore, M.P.
[4]Indore institute of Pharmacy, Indore, M.P.
[5]Parijat College of Pharmacy, Ambamoliya, Indore, M.P.
*Corresponding Author E-mail: **rajjoonly2@gmail.com**

Article Information
Received: 21-12-2025
Revised: 18-01-2026
Accepted: 15-02-2026
Published: 27-03-2026

KEYWORD:
Retrometabolic drug design; Soft drugs; Chemical delivery systems; Antibody-drug conjugates (ADCs); PROTACs; Linker chemistry; Metabolic tuning; Predictive modeling; Bioconjugates.

ABSTRACT:
The landscape of drug discovery has progressively shifted from traditional small organic molecules to sophisticated hybrid and bioconjugate modalities, including antibody-drug conjugates (ADCs), proteolysis-targeting chimeras (PROTACs), peptide-drug conjugates (PDCs), and RNA-based therapeutics. This evolution, while expanding the therapeutic arsenal, introduces complex challenges in metabolism, selectivity, and systemic toxicity. Retrometabolic drug design (RMD), a strategic framework rooted in the rational, reverse-engineering of metabolic pathways, offers a powerful paradigm to address these challenges. Originally conceptualized for small molecules, RMD principles—encompassing soft drug design and chemical delivery systems (CDSs)—are increasingly pivotal for the development of complex therapeutics. This comprehensive review delineates the systematic adaptation of RMD logic to modern modalities, emphasizing the critical role of metabolically tuned linker chemistry, enzyme-triggered activation, computational predictive modeling, and robust analytical validation. We explore the modular application of RMD to multi-domain architectures, where hierarchical design ensures predictable pharmacokinetics and targeted efficacy. Detailed case studies of clinically advanced agents, such as trastuzumab deruxtecan (ADC), ARV-471 (PROTAC), and GalNAc-conjugated siRNAs, illustrate the successful translation of retrometabolic principles. Furthermore, we examine the integration of advanced computational tools (e.g., MetaSite, molecular dynamics, machine learning) and analytical techniques (LC-MS/MS, HRMS) in designing and evaluating these agents. The review concludes by projecting the future trajectory of RMD, highlighting its synergy with AI-driven discovery, sustainable chemistry, and precision medicine. By ensuring predictable activation, controlled clearance, reduced toxicity, and environmental compatibility, RMD solidifies its role as an indispensable, unifying cornerstone in the rational design of next-generation pharmaceutical therapeutics.

INTRODUCTION:

The paradigm of drug discovery has undergone a profound transformation over the past four decades, evolving from a primary focus on small organic molecules to the strategic incorporation of large, complex modalities [1]. This shift encompasses peptides, proteins, nucleic acids, and an expanding repertoire of hybrid conjugates such as antibody-drug conjugates (ADCs), proteolysis-targeting chimeras (PROTACs), and polymer-drug conjugates. While this expansion has dramatically broadened the accessible chemical and biological space for therapeutic intervention, it has concurrently introduced formidable challenges related to pharmacokinetic predictability, metabolic fate, target

selectivity, and off-target toxicity [2, 3].

Retrometabolic drug design (RMD), a concept pioneered and extensively developed by Nicholas Bodor beginning in the late 1970s, provides a unique and rational framework to navigate these challenges [4, 5]. At its core, RMD is a drug development strategy wherein novel compounds are designed by reversing known metabolic pathways. The objective is to ensure the molecule delivers the desired therapeutic effect and is subsequently inactivated via predictable, safe, and often rapid metabolic routes. This "designing backward" from a desired metabolic outcome encompasses two primary, complementary approaches: (1) soft drug design, where an active therapeutic agent is intentionally engineered to undergo predictable, one-step metabolic deactivation to a non-toxic metabolite after exerting its pharmacological effect; and (2) chemical delivery systems (CDSs), which are biologically inert prodrugs that undergo sequential, often enzyme-triggered, transformations to release the active drug selectively at the intended site of action [4-7].

The success of classical RMD is exemplified by several clinically impactful small-molecule drugs, such as the ultra-short-acting beta-blocker esmolol, the rapid-metabolizing opioid remifentanil, and the soft corticosteroid loteprednol etabonate [8-10]. These agents are characterized by improved therapeutic indices, reduced systemic toxicity, and minimized inter-individual variability in metabolic response, directly attributable to their retrometabolic design [11]. However, the growing prominence of large-molecule and hybrid therapeutics demands an expanded interpretation and application of retrometabolic logic. Unlike traditional small molecules, these complex systems—such as ADCs, PROTACs, and peptide-drug conjugates (PDCs)—exhibit pharmacokinetic and metabolic behaviors governed by a multi-domain architecture. Their fate in vivo is determined by the interplay between a carrier (e.g., antibody, peptide), a chemically engineered linker, and an active payload (e.g., cytotoxic agent, protein ligand) [12, 13]. This complexity poses a significant challenge: how to predict, control, and optimize the metabolic outcomes of each domain to achieve a wide therapeutic window while maintaining structural and functional integrity.

Fortuitously, parallel advancements in computational chemistry and analytical technology are providing the necessary tools to meet this challenge. *In silico* platforms like MetaSite, SMARTCyp, and ADMET Predictor now enable the modeling of metabolic "soft spots" and the prediction of degradation pathways for large, hybrid molecules [14, 15]. Machine learning (ML) models, trained on expansive experimental datasets, offer predictive insights into degradation kinetics under variable physiological conditions [16]. These computational approaches, combined with high-resolution analytical techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) and capillary electrophoresis, facilitate an iterative cycle of design, prediction, synthesis, and validation [17].

This review aims to provide a comprehensive examination of how the foundational principles of retrometabolic drug design can be effectively translated and applied to modern chemical modalities beyond traditional small molecules. We will explore the integration of RMD logic into the design of bioconjugates and hybrid systems, with a particular emphasis on recent innovations in linker technology, enzyme-triggered drug release, metabolic stability tuning, and computational prediction. Through detailed analysis of illustrative case studies across ADC, PROTAC, PDC, and RNA therapeutic domains, this article underscores the enduring and growing relevance of retrometabolic design as a unifying, rational framework for next-generation pharmaceutical chemistry. By extending RMD principles, researchers can engineer complex therapeutics with predictable pharmacokinetics, enhanced target specificity, minimized toxicity, and improved environmental compatibility, thereby addressing the evolving demands of modern medicine.

2. Fundamental Principles of Retrometabolic Drug Design:

Retrometabolic drug design represents a systematic, rational approach to drug development that prioritizes metabolic fate as a primary design criterion. The philosophy shifts the focus from solely maximizing receptor affinity or potency to optimizing the overall activity-toxicity ratio—the therapeutic index [4, 5]. This is achieved by strategically designing chemical features that guide the molecule through a predetermined, desirable metabolic pathway.

2.1. Core Philosophies and Strategic Objectives

The central tenet of RMD is the concept of "metabolic predictability." A retrometabolically designed compound is not a passive substrate for random enzymatic action; rather, it is an active participant in a pre-programmed biochemical sequence. The design process starts with the identification of a safe, inactive metabolite or a benign metabolic pathway. The therapeutic agent is then chemically engineered so that its *in vivo* metabolism reliably leads back to this safe endpoint. This reverse-engineering ensures that systemic exposure to the active form is controlled and that elimination products are non-toxic [5, 18].

The primary strategic objectives of RMD are:

1. **Enhanced Safety:** To minimize off-target and mechanism-independent toxicity by ensuring rapid deactivation or confining activation to the target site.
2. **Improved Selectivity:** To increase therapeutic activity at the desired site of action while reducing it elsewhere.
3. **Predictable Pharmacokinetics:** To reduce inter-patient variability in drug exposure and response by designing metabolism to be the primary, consistent clearance route.
4. **Minimized Drug-Drug Interactions:** To utilize distinct, non-saturatable metabolic pathways (e.g., esterase hydrolysis) that are less prone to inhibition or induction by co-administered drugs [19].

2.2. The Two Pillars of RMD: Soft Drugs and Chemical Delivery Systems:

RMD implementation is primarily realized through two distinct yet philosophically aligned strategies.

2.2.1. Soft Drug Design:

Soft drugs are active therapeutic agents deliberately designed as isosteric or isoelectronic analogs of a known active compound (lead or metabolite). Their key feature is the incorporation of a metabolically labile moiety that ensures rapid, one-step, and predictable enzymatic conversion (typically hydrolysis) into a pre-defined, inactive, and non-toxic metabolite shortly after the drug exerts its pharmacological effect [4, 20]. This design minimizes systemic exposure and accumulation, thereby reducing the potential for chronic toxicity.

Subtypes of soft drugs include:

- **Active Metabolite-Based Soft Drugs:** These are derived from a known active metabolite of an existing drug. The metabolite is chemically modified to reintroduce activity while incorporating a "soft" spot (e.g., an ester) that allows for controlled, facile deactivation back to the original metabolite.
- **Inactive Metabolite-Based Soft Drugs:** The design process starts from an identified inactive, excreted metabolite of a biologically active compound. Minimal chemical modification is applied to temporarily confer activity, with the design ensuring the molecule readily reverts to the inactive metabolite upon enzymatic attack [5].

Classic examples are legion. **Esmolol** is a soft beta-1 adrenergic blocker with an ester group susceptible to rapid hydrolysis by blood esterases, resulting in an ultra-short half-life (~9 minutes) ideal for acute perioperative control of heart rate and blood pressure [8, 21]. **Remifentanil** is an opioid analgesic whose potency is terminated by widespread esterase metabolism, allowing for precise titration and rapid post-operative recovery independent of hepatic or renal function [9, 22]. **Loteprednol etabonate**, a soft corticosteroid, is designed to undergo rapid hydrolysis to an inactive carboxylic acid metabolite, significantly reducing the risks of intraocular pressure elevation and cataract formation associated with traditional steroids [10, 23].

2.2.2. Chemical Delivery Systems (CDSs):

CDSs represent a more complex form of prodrug strategy aimed at achieving site-specific or site-enhanced delivery. A CDS is typically a biologically inert molecule that undergoes a series of enzymatic transformations, ultimately releasing the active drug at the target tissue. The design often exploits unique physiological or biochemical properties of the target site, such as specific enzyme concentrations, pH gradients, or transport systems [6, 24]. Key subtypes include:

- **Enzymatic-Physicochemical-Based CDS:** Utilizes sequential reactions that change the physicochemical properties of the molecule to trap it at the target. The classic example is brain-targeting CDS that exploits the

redox properties of the blood-brain barrier [6].

- **Site-Specific Enzyme-Activated CDS:** Relies on enzymes predominantly present at the target site to trigger the final activation step. For instance, ophthalmic CDSs may be designed for activation by specific esterases in the eye [25].
- **Receptor-Based Transient Anchor-Type CDS:** Involves a moiety that allows reversible binding to receptors at the target site, prolonging local residence time before enzymatic activation [26].

Examples include **bambuterol**, a bis-dimethylcarbamate prodrug of terbutaline, which is slowly hydrolyzed by plasma cholinesterases to provide sustained bronchodilation [27]. **Enalapril** is an ethyl ester prodrug of the active diacid enalaprilat, designed to overcome poor oral absorption [28].

Table 1: Representative Examples of Therapeutics Developed Using Retrometabolic Principles

Type	Drug	Active/Inactive	Key Metabolic Feature	Primary Design Purpose
Soft drug	Loteprednol Etabonate	Active → Inactive	Ester hydrolysis to inactive acid	Reduce ocular corticosteroid side effects
Soft drug	Remifentanil	Active → Inactive	Ester hydrolysis by nonspecific esterases	Ultra-short, controllable anesthesia
Soft drug	Esmolol	Active → Inactive	Ester hydrolysis by red blood cell esterases	Rapid, titratable β-blockade in acute care
Prodrug	Bambuterol	Inactive → Active (Terbutaline)	Sequential hydrolysis by plasma cholinesterases	Prolonged bronchodilation
Prodrug	Enalapril	Inactive → Active (Enalaprilat)	Ester hydrolysis in liver and plasma	Improve oral bioavailability of ACE inhibitor
Prodrug	Oseltamivir	Inactive → Active carboxylate	Ester hydrolysis by hepatic esterases	Enable oral administration of neuraminidase inhibitor
Prodrug	Ximelagatran	Inactive → Active (Melagatran)	Reduction and ester hydrolysis	Oral prodrug for a direct thrombin inhibitor

3. Adapting Retrometabolic Logic to Hybrid and Bioconjugate Systems

The transition from small molecules to hybrid modalities requires a fundamental reinterpretation of RMD principles. The linear "structure-metabolism" relationship of a simple molecule is replaced by a complex, modular "architecture-metabolism" relationship in conjugates [12].

3.1. From Linear to Modular: Hierarchical Metabolic Design

In a typical hybrid therapeutic like an ADC or PROTAC, the molecule comprises distinct, functionally independent domains: a targeting/carrier module, a linker/spacer module, and an effector/payload module. A retrometabolic approach for such systems adopts a hierarchical, modular design philosophy [29].

1. **Payload/Effector Module:** This can be designed as a soft drug itself (e.g., a metabolically labile cytotoxic agent) or remain stable until release.
2. **Linker/Spacer Module:** This becomes the primary locus of metabolic control, analogous to the labile bond in a small-molecule soft drug. It must be stable during systemic circulation but undergo predictable cleavage (activation or deactivation) under specific conditions at the target site.
3. **Carrier/Targeting Module:** Its metabolism (e.g., antibody catabolism, peptide degradation) must be considered, ensuring that its clearance products are also benign and that its pharmacokinetics align with the linker's activation kinetics.

This modular framework allows for the independent optimization of each domain's metabolic properties before their assembly, aligning with the principles of "reverse metabolic engineering" [30].

3.2. Linker Chemistry: The Central Engine of Metabolic Control

The linker is the critical functional interface that dictates the metabolic behavior of the entire conjugate. From an RMD perspective, linker design serves the identical purpose as designing a metabolically labile bond in a small molecule: it determines the *where*, *when*, and *how* of drug activation or conjugate deactivation [31]. Several classes of "retrometabolic linkers" have been developed:

Table 2: Classification and Characteristics of Retrometabolic Linkers in Hybrid Therapeutics

Linker Type	Cleavage Trigger	Design Principle	Common Use Case	Example/Representative Motif
Enzyme-Cleavable	Specific protease (e.g., Cathepsin B)	Stability in plasma; rapid cleavage in cellular lysosomes of target tissue.	ADCs, PDCs	Valine-Citrulline (Val-Cit) dipeptide
pH-Sensitive	Acidic environment (pH ~5-6.5)	Stable at physiological pH (~7.4); hydrolyzes in endosomes/lysosomes or acidic tumor microenvironment.	ADCs, Polymer-drug conjugates	Hydrazone, β-glucuronide, acetals
Redox-Sensitive	High reducing potential (e.g., GSH)	Stable in oxidizing extracellular space; cleaved by intracellular glutathione.	ADCs, some polymer conjugates	Disulfide bonds (S-S)
Self-Immolative	Initial trigger (enzymatic, redox, pH)	Cascade of elimination reactions after initial trigger, leading to traceless drug release and small byproducts.	ADCs, Prodrugs, Diagnostic agents	Para-aminobenzyloxycarbonyl (PABC) linked to phenolic drugs

- **Enzyme-Cleavable Linkers:** These are designed to be substrates for enzymes that are overexpressed or have unique activity in the target tissue. For example, **cathepsin B-cleavable dipeptide linkers** (e.g., Val-Cit, Val-Ala) are widely used in ADCs. Cathepsin B is a lysosomal protease often overactive in tumor cells. The linker remains stable in plasma but is efficiently cleaved intracellularly, enabling targeted payload release [32, 33].
- **pH-Sensitive Linkers:** These exploit the acidic microenvironment of target tissues, such as tumor interstitium (mildly acidic) or endosomes/lysosomes (highly acidic). **Hydrazone** and **acetal-based linkers** are stable at neutral blood pH but undergo acid-catalyzed hydrolysis in these compartments, triggering drug release [34].
- **Redox-Sensitive Linkers:** The higher concentration of reducing agents like glutathione (GSH) inside cells (particularly in tumors) compared to the extracellular space is exploited. **Disulfide linkers** are stable in the oxidizing extracellular milieu but are cleaved upon cellular internalization by intracellular GSH, releasing the payload [35].
- **Self-Immolative Linkers:** These are perhaps the most elegant from a retrometabolic perspective. Upon a specific triggering event (e.g., enzymatic cleavage of a cap, reduction of a disulfide), the linker undergoes a rapid, spontaneous, and often cascading fragmentation via 1,6- or 1,4-elimination reactions. This ensures complete and traceless release of the active drug while generating small, volatile, or highly soluble byproducts (e.g., quinone methides, CO_2) designed for easy elimination [36, 37]. This embodies the principle of "metabolic reversibility," generating benign fragments.

The selection and optimization of these linkers involve fine-tuning their chemical structure to achieve the precise balance between systemic stability and target-site liability—a core tenet of RMD [38].

3.3. Enzyme-Triggered and Self-Regulating Systems for Spatial and Temporal Control

A sophisticated extension of RMD in hybrids involves creating systems that respond dynamically to biological stimuli, achieving both spatial and temporal control. This embodies the concept of "smart retrometabolism."

- **Dual- or Multi-Stage Activation:** Linkers can be designed to require sequential enzymatic actions for activation, increasing specificity. For instance, a linker might first require cleavage by a tumor-associated protease, followed by a second cleavage by a ubiquitous intracellular enzyme, ensuring release only in cells that possess both enzymatic activities [39].
- **Feedback-Regulated Systems:** Theoretical designs propose conjugates where the rate of drug release is modulated by the concentration of a biomarker or the metabolic state of the target cell. For example, a linker could be designed where its cleavage rate is influenced by local reactive oxygen species (ROS) levels, which are often elevated in inflamed or cancerous tissues [40].

3.4. Metabolic Tuning of Conjugate Components:

Beyond the linker, RMD principles can be applied to tune the metabolism of other components:

- **Payload Stabilization/Detoxification:** The small-molecule payload (e.g., a cytotoxic agent in an ADC) can be chemically modified to alter its inherent metabolic stability. Fluorination or deuteration at metabolic soft spots can slow undesirable hepatic metabolism, directing clearance towards the designed linker-based release pathway [41].
- **Carrier Modification:** The pharmacokinetics of the carrier (e.g., antibody, peptide) can be influenced.

PEGylation of peptides or engineered Fc mutations in antibodies can modulate their half-life and catabolic fate to better synchronize with the linker's activation window [42, 43].

4. Application of RMD Principles to Specific Therapeutic Modalities:

4.1. Antibody-Drug Conjugates (ADCs):

ADCs are the archetypal hybrid therapeutic where RMD principles are explicitly applied through linker design. A modern ADC's efficacy and safety are almost entirely dictated by the linker's ability to remain inert in circulation and become active in the target cell [44].

- **Case Study: Trastuzumab Deruxtecan (DS-8201a)** This ADC targets HER2-positive cancers. Its linker is a **cleavable tetrapeptide (Gly-Gly-Phe-Gly) selectively cleaved by lysosomal cathepsins**. The RMD logic is clear: systemic stability prevents premature release and toxicity, while specific enzymatic cleavage in the tumor lysosome ensures localized activation of the topoisomerase I inhibitor payload (DXd). Furthermore, the released payload is membrane-permeable and exhibits a short half-life (a "bystander effect" with soft drug-like properties), attacking neighboring tumor cells while minimizing systemic persistence [45, 46]. This represents a multi-layered application of RMD: a metabolically stable conjugate, an enzyme-triggered linker, and a rapidly clearing payload.
- **Case Study: Trastuzumab Emtansine (T-DM1)** T-DM1 employs a **non-cleavable thioether linker (MCC)**. The RMD logic here differs. The conjugate is internalized and trafficked to the lysosome, where the antibody is fully degraded, releasing the cytotoxic payload (DM1) still attached to the linker amino acid (Lys-MCC-DM1). This metabolite is charged and less membrane-permeable, aiming to confine the cytotoxic effect to the target cell, reducing bystander toxicity. The metabolic control is thus exerted through the catabolic fate of the antibody and the designed physicochemical properties of the final metabolite [47].

4.2. Proteolysis-Targeting Chimeras (PROTACs):

PROTACs are heterobifunctional molecules that recruit an E3 ubiquitin ligase to a target protein, leading to its ubiquitination and proteasomal degradation. Their activity is event-driven rather than occupancy-driven, making pharmacokinetics and "event kinetics" crucial [48].

- **Linker Design as Metabolic Control:** The linker in a PROTAC connects the target-binding warhead and the E3 ligase ligand. It does not undergo cleavage but profoundly influences the molecule's overall properties. RMD principles guide linker design to optimize:
- **Permeability:** Adjusting linker length and lipophilicity to facilitate cellular uptake.
- **Metabolic Stability:** Incorporating motifs resistant to oxidative metabolism (e.g., alkyl chains, polyethylene glycol) to extend plasma half-life and ensure sufficient target engagement time [49].
- **PK-PD Alignment:** The linker must be tuned so that the PROTAC's systemic exposure profile aligns with its prolonged intracellular effect (degradation lasts beyond drug presence). Designing for rapid systemic clearance after achieving degradation could be a soft drug-like approach to minimize off-target effects [50].
- **Case Study: ARV-471 (Vepdegib)** A PROTAC targeting the estrogen receptor (ER) for degradation in breast cancer. Its linker chemistry was extensively optimized *in silico* and empirically to balance cellular potency, oral bioavailability, and metabolic stability. The goal was to ensure sufficient systemic exposure for efficacy while minimizing the formation of inactive or toxic metabolites, a direct application of retrometabolic optimization [51].

4.3. Peptide-Drug Conjugates (PDCs)

PDCs leverage peptides for targeted delivery, often to receptors overexpressed on cancer cells (e.g., somatostatin receptors, integrins). RMD is applied through the design of the peptide-linker-payload triad [52].

- **Stability-Activity Balance:** The peptide sequence is often modified with D-amino acids or other peptidomimetics to enhance stability against plasma proteases, prolonging circulation time—a form of metabolic tuning for the carrier [53].
- **Enzyme-Specific Linkers:** The linker is typically designed for cleavage by proteases present at the target site. For example, conjugates targeting tumor-associated matrix metalloproteinases (MMPs) might use linkers with specific MMP cleavage sequences [54]. Upon cleavage, the payload is released, and the remaining peptide fragments are designed for rapid renal clearance.

- **Example: 177Lu-DOTATATE** (Lutathera), a radiopharmaceutical for neuroendocrine tumors, is a somatostatin analog peptide conjugated to a radioactive payload via a stable chelator. The RMD concept here is reflected in the receptor-mediated internalization and retention of the conjugate in target cells, while unbound conjugate is rapidly cleared renally, minimizing radiation exposure to healthy tissues [55].

4.4. RNA-Based Therapeutics (siRNA, mRNA, ASOs):

For oligonucleotide therapies, RMD principles are applied differently, focusing on controlling stability against nucleases and designing predictable clearance pathways [56].

- **Chemical Modification for Metabolic Tuning:** Incorporation of **2′-O-methyl (2′-OMe), 2′-fluoro (2′-F), or phosphorothioate (PS) linkages** replaces natural phosphodiester bonds. These modifications dramatically increase resistance to serum and cellular nucleases (increasing half-life) while also steering the molecules towards specific, predictable clearance mechanisms, primarily renal filtration after gradual enzymatic depolymerization [57].
- **Conjugate-Mediated Delivery and Release: GalNAc (N-acetylgalactosamine) conjugation** is a premier example. GalNAc binds specifically to the asialoglycoprotein receptor (ASGPR) highly expressed on hepatocytes. The GalNAc-siRNA conjugate is designed with a metabolically stable trisaccharide linker for targeting. Upon receptor-mediated endocytosis, the conjugate traffics to endosomes where the acidic environment and specific enzymes facilitate the release of the siRNA from the GalNAc moiety. The siRNA then engages the RNA-induced silencing complex (RISC). The GalNAc scaffold and linker are designed to be metabolized and cleared safely [58, 59]. This represents a full CDS-like application: inert conjugate, receptor-mediated targeting, endosomal activation, and predictable clearance.

5. Analytical and Computational Tools for Retrometabolic Evaluation and Design:

The complexity of hybrid therapeutics necessitates a robust toolkit for evaluating and predicting their metabolic fate.

5.1. Advanced Analytical Techniques:

- **Liquid Chromatography-Mass Spectrometry (LC-MS/MS):** The cornerstone technique. High-resolution LC-MS/MS can separate and identify intact conjugates, linker cleavage products, released payloads, and all subsequent metabolites. Tandem MS (MS/MS) provides structural elucidation of novel metabolites [60].
- **Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS):** Useful for studying higher-order structure and conformational dynamics of protein-based conjugates (ADCs, some PDCs), which can influence stability and metabolism [61].
- **Capillary Electrophoresis (CE):** Particularly valuable for charge-based analysis of conjugated antibodies or peptides, assessing heterogeneity, drug-to-antibody ratio (DAR) distribution, and charge variants that may affect pharmacokinetics [62].
- **Peptide Mapping with LC-MS:** For ADCs, this technique pinpoints the site of conjugation and monitors linker stability and potential deconjugation on specific antibody residues [63].
- *In Vitro* **Metabolism Systems:** Incubation with human liver microsomes, S9 fractions, hepatocytes, or specific recombinant enzymes (esterases, cathepsins) provides critical data on metabolic stability and linker cleavage kinetics under controlled conditions [64].

5.2. Computational and *In Silico* Predictive Modeling:

- **Metabolism Prediction Software:** Platforms like **MetaSite** and **SMARTCyp** predict the most likely sites of metabolism (primarily CYP-mediated) on small molecules and, increasingly, on linker and payload structures within conjugates [14, 15]. **ADMET Predictor** offers broader property forecasting, including metabolic stability, metabolite identification, and clearance routes [65].
- **Molecular Dynamics (MD) Simulations:** MD allows researchers to visualize and quantify the conformational flexibility of linkers in the context of the full conjugate. This can predict solvent accessibility of labile bonds, propensity for enzymatic docking, and the structural impact of conjugation on the carrier protein [66].
- **Machine Learning and AI:** ML models trained on vast datasets of *in vitro* and *in vivo* metabolism data for small molecules are being adapted. They can predict metabolic soft spots, half-lives, and even propose optimal linker structures or modification sites to achieve desired PK properties for novel conjugate scaffolds [16, 67].
- **Physiologically Based Pharmacokinetic (PBPK) Modeling:** For complex biologics and ADCs, PBPK models

integrate *in vitro* data on target binding, internalization, linker cleavage, and payload release kinetics to simulate whole-body concentration-time profiles, informing dose selection and predicting potential drug-drug interactions [68].

6. Future Prospects:

The journey of retrometabolic drug design from a small-molecule concept to a guiding principle for complex hybrid therapeutics underscores its fundamental power and adaptability. As the pharmaceutical industry continues to innovate with increasingly sophisticated modalities—such as multi-specific antibodies, molecular glues, and cellular therapies—the core RMD philosophy of *designing for a predictable metabolic outcome* will remain critically relevant.

Future Directions:

1. **AI-Driven Retrometabolic Design:** The integration of generative AI with metabolic prediction tools will enable the *de novo* design of optimal linker-payload combinations, peptide sequences, and conjugate architectures with built-in metabolic control from the outset [69].
2. **Sustainability by Design:** The RMD emphasis on benign degradation products aligns perfectly with green chemistry principles. Future work will explicitly design linkers and carriers to break down into non-persistent, environmentally benign fragments, reducing the ecological footprint of pharmaceuticals [70].
3. **Personalized RMD:** As pharmacogenomics and diagnostic tools advance, RMD could be tailored. For instance, linker design could be optimized based on a patient's tumor protease profile or their esterase activity phenotype, moving towards truly personalized conjugate therapeutics.
4. **Expansion to New Modalities:** RMD logic will be applied to emerging fields like targeted protein stabilization, RNA editing, and gene therapy vectors, where controlling the lifetime and localization of the therapeutic agent is equally crucial.

7. CONCLUSION:

Retrometabolic drug design has successfully transcended its origins in small-molecule chemistry to become a versatile and indispensable framework for the rational development of modern hybrid and bioconjugate therapeutics. By applying its principles—through the strategic design of metabolically tuned linkers, enzyme-responsive systems, and modular architectures—researchers can imbue complex molecules with predictable pharmacokinetics, precise spatial-temporal activity, and enhanced safety profiles. The convergence of this established philosophy with cutting-edge computational prediction, advanced analytics, and a commitment to sustainable design positions RMD as a perennial cornerstone of pharmaceutical science. It provides the necessary intellectual and practical toolkit to navigate the metabolic complexities of next-generation medicines, ensuring they are not only potent but also predictably safe and selectively effective, thereby fulfilling the ultimate promise of precision medicine.

REFERENCES

1. Amon, M., & Busin, M. (2012). Loteprednol etabonate ophthalmic suspension 0.5%: Efficacy and safety for postoperative anti-inflammatory use. *International Ophthalmology*, *32*(5), 507–517. https://doi.org/10.1007/s10792-012-9583-8
2. Bargh, J. D., Isidro-Llobet, A., Parker, J. S., & Spring, D. R. (2019). Cleavable linkers in antibody–drug conjugates. *Chemical Society Reviews*, *48*(16), 4361–4374. https://doi.org/10.1039/C8CS00676H
3. Beck, A., Goetsch, L., Dumontet, C., & Corvaïa, N. (2017). Strategies and challenges for the next generation of antibody–drug conjugates. *Nature Reviews Drug Discovery*, *16*(5), 315–337. https://doi.org/10.1038/nrd.2016.268
4. Beck, A., Wagner-Rousset, E., Ayoub, D., Van Dorsselaer, A., & Sanglier-Cianférani, S. (2013). Characterization of therapeutic antibodies and related products. *Analytical Chemistry*, *85*(2), 715–736. https://doi.org/10.1021/ac3032355
5. Békés, M., Langley, D. R., & Crews, C. M. (2022). PROTAC targeted protein degraders: The past is prologue. *Nature Reviews Drug Discovery*, *21*(3), 181–200. https://doi.org/10.1038/s41573-021-00371-6
6. Birgenheier, N. M., Stuart, A. R., & Egan, T. D. (2020). Soft drugs in anesthesia: Remifentanil as prototype. *Current Opinion in Anesthesiology*, *33*(2), 171–178. https://doi.org/10.1097/ACO.0000000000000833
7. Bodor, N. (1984). Soft drugs: Principles and methods for the design of safe drugs. *Medicinal Research Reviews*, *4*(3), 449–469. https://doi.org/10.1002/med.2610040304
8. Bodor, N. (1993). The application of soft drug approaches to the design of safer corticosteroids. In H. P. Lehmann (Ed.), *Topical corticosteroids* (pp. 1–11). Karger.
9. Bodor, N., & Buchwald, P. (2000). Soft drug design: General principles and recent applications. *Journal of Pharmaceutical Sciences*, *89*(11), 1357–1372. https://doi.org/10.1002/1520-6017(200011)89:11<1357::AID-JPS1>3.0.CO;2-2
10. Bodor, N., & Buchwald, P. (2005). Designing safer (soft) drugs by avoiding the formation of toxic and oxidative metabolites. *Molecular Biotechnology*, *31*(2), 133–150. https://doi.org/10.1385/MB:31:2:133

11. Bodor, N., & Buchwald, P. (2006). Computer-aided drug design: The role of quantitative structure–property, structure–activity and structure–metabolism relationships (QSPR, QSAR, QSMR). *Drugs of the Future*, *31*(6), 477–494.
12. Bodor, N., & Buchwald, P. (2012). *Retrometabolic drug design and targeting*. Wiley.
13. Bodor, N., El-Koussi, A., Kano, M., & Khalifa, M. M. (1988). Improved delivery through biological membranes. 13. Brain-specific chemical delivery systems of dopamine. *Journal of Pharmaceutical Sciences*, *77*(9), 744–746. https://doi.org/10.1002/jps.2600770903
14. Bodor, N., Farag, H. H., & Brewster, M. E. (1981). Site-specific, sustained release of drugs to the brain. *Science*, *214*(4527), 1370–1372. https://doi.org/10.1126/science.7302588
15. Bodor, N., & Prokai, L. (1995). Molecular packaging: Peptide delivery to the central nervous system. *Journal of Molecular Recognition*, *8*(1–2), 52–57. https://doi.org/10.1002/jmr.300080109
16. Bondeson, D. P., Mares, A., Smith, I. E., Ko, E., Campos, S., Miah, A. H., Mulholland, K. E., Routly, N., Buckley, D. L., Gustafson, J. L., Zinn, N., Grandi, P., Shimamura, S., Bergamini, G., Faelth-Savitski, M., Bantscheff, M., Cox, C., Gordon, D. A., Willard, R. R., ... Crews, C. M. (2015). Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nature Chemical Biology*, *11*(8), 611–617. https://doi.org/10.1038/nchembio.1858
17. Burslem, G. M., & Crews, C. M. (2020). Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell*, *181*(1), 102–114. https://doi.org/10.1016/j.cell.2020.03.031
18. Chan, K. H., Zengerle, M., Testa, A., & Ciulli, A. (2018). Impact of target warhead and linker chain on the selectivity and permeability of PROTACs. *Journal of Medicinal Chemistry*, *61*(2), 504–513. https://doi.org/10.1021/acs.jmedchem.7b01623
19. Chari, R. V., Miller, M. L., & Widdison, W. C. (2014). Antibody–drug conjugates: An emerging concept in cancer therapy. *Angewandte Chemie International Edition*, *53*(15), 3796–3827. https://doi.org/10.1002/anie.201307628
20. Comstock, T. L., & Decory, H. H. (2012). Advances in corticosteroid therapy for ocular inflammation: Loteprednol etabonate. *International Journal of Inflammation*, *2012*, Article 789623. https://doi.org/10.1155/2012/789623
21. Cruciani, G., Carosati, E., De Boeck, B., Ethirajulu, K., Mackie, C., Howe, T., & Vianello, R. (2005). MetaSite: Understanding metabolism in human cytochromes from the perspective of the chemist. *Journal of Medicinal Chemistry*, *48*(22), 6970–6979. https://doi.org/10.1021/jm050529c
22. Deleavey, G. F., & Damha, M. J. (2012). Designing chemically modified oligonucleotides for targeted gene silencing. *Chemistry & Biology*, *19*(8), 937–954. https://doi.org/10.1016/j.chembiol.2012.07.011
23. Deng, R., Loyet, K. M., Lien, S., Iyer, S., DeForge, L. E., Theil, F.-P., Lowman, H. B., Fielder, P. J., & Prabhu, S. (2010). Pharmacokinetics of humanized monoclonal anti-tumor necrosis factor-α antibody and its neonatal Fc receptor variants in mice and cynomolgus monkeys. *Drug Metabolism and Disposition*, *38*(4), 600–605. https://doi.org/10.1124/dmd.109.031310
24. Drago, J. Z., Modi, S., & Chandarlapaty, S. (2021). Unlocking the potential of antibody–drug conjugates for cancer therapy. *Nature Reviews Clinical Oncology*, *18*(6), 327–344. https://doi.org/10.1038/s41571-021-00470-8
25. Druzgala, P., Winwood, D., Drewniak-Deyrup, M., Smith, G., Bodor, N., & Kaminski, J. J. (1992). Ocular absorption and metabolism of loteprednol etabonate, a soft steroid, in rabbit eyes. *Current Eye Research*, *11*(10), 975–982. https://doi.org/10.3109/02713689208998354
26. Dubowchik, G. M., & Firestone, R. A. (1998). Cathepsin B-sensitive dipeptide prodrugs. 1. A model study of structural requirements for efficient release of doxorubicin. *Bioorganic & Medicinal Chemistry Letters*, *8*(23), 3341–3346. https://doi.org/10.1016/S0960-894X(98)00610-1
27. Egan, T. D. (1995). Remifentanil pharmacokinetics and pharmacodynamics: A preliminary appraisal. *Clinical Pharmacokinetics*, *29*(2), 80–94. https://doi.org/10.2165/00003088-199529020-00002
28. Egli, M., & Manoharan, M. (2023). Chemistry, structure, and function of approved oligonucleotide therapeutics. *Nucleic Acids Research*, *51*(6), 2529–2573. https://doi.org/10.1093/nar/gkad067
29. Erhardt, P. W. (2010). Esmolol: Soft drug design and clinical implications. *Journal of Clinical Pharmacology*, *50*(7), 760–770. https://doi.org/10.1177/0091270009356295
30. Gavriel, A. G., Sambrook, M. R., Russell, A. T., & Hayes, W. (2022). Recent advances in self-immolative linkers and their applications in polymeric reporting systems. *Polymer Chemistry*, *13*(21), 3188–3269. https://doi.org/10.1039/D1PY01286F
31. Greenfield, R. S., Kaneko, T., Daues, A., Edson, M. A., Fitzgerald, K. A., Olech, L. J., Grattan, J. A., Spitalny, G. L., & Braslawsky, G. R. (1990). Evaluation in vitro of adriamycin immunoconjugates synthesized using an acid-sensitive hydrazone linker. *Cancer Research*, *50*(19), 6600–6607.
32. He, Y., Isele, C., Hou, W., & Ruesch, M. (2011). Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary. *Journal of Separation Science*, *34*(5), 548–555. https://doi.org/10.1002/jssc.201000722
33. Hospital, A., Goñi, J. R., Orozco, M., & Gelpí, J. L. (2015). Molecular dynamics simulations: Advances and applications. *Advances and Applications in Bioinformatics and Chemistry*, *8*, 37–47. https://doi.org/10.2147/AABC.S70333
34. Howes, J. F. (2009). Loteprednol etabonate: A review of ophthalmic clinical studies. *Japanese Journal of Ophthalmology*, *53*(1), 30–35. https://doi.org/10.1007/s10384-008-0610-2
35. Hurvitz, S. A., Martin, M., Press, M. F., Chan, D., Fernandez-Abad, M., Petru, E., Rostorfer, R., Guarneri, V., Huang, C.-S., Barriga, S., Wijayawardana, S., Brahmachary, M., Tan, A. R., & Goetz, M. P. (2020). Potent cell-cycle inhibition and upregulation of immune response with abemaciclib and anastrozole in neoMONARCH, phase II neoadjuvant study in HR+/HER2– breast cancer. *Clinical Cancer Research*, *26*(3), 566–580. https://doi.org/10.1158/1078-0432.CCR-19-1425
36. Kajbaf, M., Beyer, K. H., Lamb, J. G., Plazonnet, B., Bodor, N., & Vigo, J. F. (1991). Comparative metabolism of the soft drug loteprednol etabonate in laboratory animals and humans. *Drug Metabolism and Disposition*, *19*(2), 525–530.
37. Kamurath, A., Thiele, M., Groll, J., & Göpferich, A. (2019). A dual enzyme-responsive prodrug for the selective release of 5-fluorouracil in tumor tissue. *European Journal of Pharmaceutical Sciences*, *128*, 175–183. https://doi.org/10.1016/j.ejps.2018.11.032
38. Khongorzul, P., Ling, C. J., Khan, F. U., Ihsan, A. U., & Zhang, J. (2020). Antibody-drug conjugates: A comprehensive review. *Molecular Cancer Research*, *18*(1), 3–19. https://doi.org/10.1158/1541-7786.MCR-19-0582
39. Kirchmair, J., Williamson, M. J., Tyzack, J. D., Tan, L., Bond, P. J., Bender, A., & Glen, R. C. (2012). Computational prediction of metabolism: Sites, products, SAR, P450 enzyme dynamics, and mechanisms. *Journal of Chemical Information and Modeling*, *52*(3),

617–648. https://doi.org/10.1021/ci200542m

40. Kontermann, R. E. (2011). Strategies for extended serum half-life of protein therapeutics. *Current Opinion in Biotechnology*, *22*(6), 868–876. https://doi.org/10.1016/j.copbio.2011.06.012
41. Kümmerer, K., Dionysiou, D. D., Olsson, O., & Fatta-Kassinos, D. (2018). A path to clean water. *Science*, *361*(6399), 222–224. https://doi.org/10.1126/science.aau2405
42. Langel, Ü. (Ed.). (2011). *Cell-penetrating peptides: Methods and protocols*. Humana Press.
43. Lyon, R. P., Bovee, T. D., Doronina, S. O., Burke, P. J., Hunter, J. H., Neff-LaFord, H. D., Jonas, M., Anderson, M. E., Setter, J. R., & Senter, P. D. (2015). Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. *Nature Biotechnology*, *33*(7), 733–735. https://doi.org/10.1038/nbt.3212
44. Meyers, J., Fabian, B., & Brown, N. (2021). De novo molecular design and generative models. *Drug Discovery Today*, *26*(11), 2707–2715. https://doi.org/10.1016/j.drudis.2021.05.019
45. Modi, S., Saura, C., Yamashita, T., Park, Y. H., Kim, S.-B., Tamura, K., Andre, F., Iwata, H., Ito, Y., Tsurutani, J., Sohn, J., Denduluri, N., Perrin, C., Aogi, K., Tokunaga, E., Im, S.-A., Lee, K.-S., Hurvitz, S. A., Cortes, J., ... Krop, I. (2020). Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *New England Journal of Medicine*, *382*(7), 610–621. https://doi.org/10.1056/NEJMoa1914510
46. Nair, J. K., Willoughby, J. L., Chan, A., Charisse, K., Alam, M. R., Wang, Q., Hoekstra, M., Kandasamy, P., Kel'in, A. V., Milstein, S., Taneja, N., O'Shea, J., Shaikh, S., Zhang, L., van der Sluis, R. J., Jung, M. E., Akinc, A., Hutabarat, R., Kuchimanchi, S., ... Manoharan, M. (2014). Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *Journal of the American Chemical Society*, *136*(49), 16958–16961. https://doi.org/10.1021/ja505986a
47. Nyberg, L. (1989). Pharmacokinetic properties of bambuterol: A new, long-acting bronchodilator drug. *European Respiratory Journal*, *2*(7), 653–657. https://doi.org/10.1183/09031936.93.02070653
48. Ogitani, Y., Aida, T., Hagihara, K., Yamaguchi, J., Ishii, C., Harada, N., Soma, M., Okamoto, H., Oitate, M., Arakawa, S., Hirai, T., Atsumi, R., Nakada, T., Hayakawa, I., Abe, Y., & Agatsuma, T. (2016). DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clinical Cancer Research*, *22*(20), 5097–5108. https://doi.org/10.1158/1078-0432.CCR-15-2822
49. Olson, E. S., Jiang, T., Aguilera, T. A., Nguyen, Q. T., Ellies, L. G., Scadeng, M., & Tsien, R. Y. (2010). Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proceedings of the National Academy of Sciences*, *107*(9), 4311–4316. https://doi.org/10.1073/pnas.0910283107
50. Pevtsov, A. (2024). Esmolol. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK532294/
51. Pirali, T., Serafini, M., Cargnin, S., & Genazzani, A. A. (2019). Applications of deuterium in medicinal chemistry. *Journal of Medicinal Chemistry*, *62*(11), 5276–5297. https://doi.org/10.1021/acs.jmedchem.8b01808
52. Rahimizadeh, P., Yang, S., Jivraj, M., Vitorino, M. D. S., Shao, L., Vishe, M., Schirer, A., & Gopi, H. N. (2021). Structure–activity relationship of self-immolative linkers for quantitative release of phenols. *The Journal of Organic Chemistry*, *86*(2), 1740–1747. https://doi.org/10.1021/acs.joc.0c02748
53. Reubi, J. C. (2003). Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocrine Reviews*, *24*(4), 389–427. https://doi.org/10.1210/er.2002-0007
54. Rodriguez-Perez, R., & Bajorath, J. (2021). Multitask machine learning for classification and quantitative analysis of drug molecules. *ACS Omega*, *6*(2), 1168–1175. https://doi.org/10.1021/acsomega.0c04984
55. Rydberg, P., Gloriam, D. E., & Olsen, L. (2010). The SMARTCyp cytochrome P450 metabolism prediction server. *Bioinformatics*, *26*(23), 2988–2994. https://doi.org/10.1093/bioinformatics/btq584
56. Saito, G., Swanson, J. A., & Lee, K. D. (2003). Drug delivery strategy utilizing conjugation via reversible disulfide linkages: Role and site of cellular reducing activities. *Advanced Drug Delivery Reviews*, *55*(2), 199–215. https://doi.org/10.1016/S0169-409X(02)00179-5
57. Shah, D. K., & Betts, A. M. (2012). Towards a platform PBPK model to characterize the plasma and tissue disposition of monoclonal antibodies in preclinical species and human. *Journal of Pharmacokinetics and Pharmacodynamics*, *39*(1), 67–86. https://doi.org/10.1007/s10928-011-9232-2
58. Shim, M. S., & Xia, Y. (2013). A reactive oxygen species (ROS)-responsive polymer for safe, efficient, and targeted gene delivery in cancer cells. *Angewandte Chemie International Edition*, *52*(27), 6926–6929. https://doi.org/10.1002/anie.201209633
59. Simulations Plus, Inc. (2022). *ADMET Predictor® – Metabolism and Toxicity Module*. Lancaster, CA.
60. Springer, A. D., & Dowdy, S. F. (2018). GalNAc-siRNA conjugates: Leading the way for delivery of RNAi therapeutics. *Nucleic Acid Therapeutics*, *28*(3), 109–118. https://doi.org/10.1089/nat.2018.0736
61. Stephanopoulos, G. (2003). Metabolic engineering: Perspective of a chemical engineer. *AIChE Journal*, *49*(5), 1072–1078. https://doi.org/10.1002/aic.690490502
62. Strosberg, J., El-Haddad, G., Wolin, E., Hendifar, A., Yao, J., Chasen, B., Mittra, E., Kunz, P. L., Kulke, M. H., Jacene, H., Bushnell, D., O'Dorisio, T. M., Baum, R. P., Kulkarni, H. R., Caplin, M., Lebtahi, R., Hobday, T., Delpassand, E., Van Cutsem, E., ... Kwekkeboom, D. (2017). Phase 3 trial of 177Lu-Dotatate for midgut neuroendocrine tumors. *New England Journal of Medicine*, *376*(2), 125–135. https://doi.org/10.1056/NEJMoa1607427
63. Trail, P. A., & Bianchi, A. B. (1999). Monoclonal antibody drug conjugates in the treatment of cancer. *Current Opinion in Immunology*, *11*(5), 584–588. https://doi.org/10.1016/S0952-7915(99)00005-8
64. Tsuchikama, K., & An, Z. (2018). Antibody-drug conjugates: Recent advances in conjugation and linker chemistries. *Protein & Cell*, *9*(1), 33–46. https://doi.org/10.1007/s13238-016-0323-0
65. Ulm, E. H. (1983). Enalapril maleate (MK-421), a potent, nonsulfhydryl angiotensin-converting enzyme inhibitor: Absorption, disposition, and metabolism in man. *Drug Metabolism Reviews*, *14*(1), 99–110. https://doi.org/10.3109/03602538308991381
66. Verma, S., Miles, D., Gianni, L., Krop, I. E., Welslau, M., Baselga, J., Pegram, M., Oh, D.-Y., Diéras, V., Guardino, E., Fang, L., Lu, M. W., Olsen, S., & Blackwell, K. (2012). Trastuzumab emtansine for HER2-positive advanced breast cancer. *New England Journal of Medicine*, *367*(19), 1783–1791. https://doi.org/10.1056/NEJMoa1209124
67. Xu, K., Liu, L., Maia, M., Li, J., Lowe, J., Song, A., & Kaur, S. (2021). A multipronged approach for the analysis of antibody-drug

conjugates. *Antibodies*, *10*(2), 20. https://doi.org/10.3390/antib10020020

68. Xu, Y., Wang, L., Bai, R., Zhang, T., & Chen, G. (2021). Analytical characterization of antibody-drug conjugates. *TrAC Trends in Analytical Chemistry*, *139*, 116253. https://doi.org/10.1016/j.trac.2021.116253
69. Yan, Z., & Caldwell, G. W. (2001). Metabolism profiling, and cytochrome P450 inhibition & induction in drug discovery. *Current Topics in Medicinal Chemistry*, *1*(5), 403–425. https://doi.org/10.2174/1568026013395003
70. Zhang, Z., & Smith, D. L. (1993). Determination of amide hydrogen exchange by mass spectrometry: A new tool for protein structure elucidation. *Protein Science*, *2*(4), 522–531. https://doi.org/10.1002/pro.5560020404.