



Recent Advances in the Synthesis and Biological Evaluation of Benzimidazole Derivatives: A Review

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ABSTRACT :

Benzimidazole, a bicyclic heteroaromatic system formed by fusing benzene and imidazole rings, is a privileged scaffold in medicinal chemistry and is present in several marketed drugs. This review summarises recent developments in benzimidazole synthesis, covering classical condensation, catalytic, green, and multicomponent approaches, and reviews biological evaluation across antimicrobial, antifungal, anticancer, anthelmintic, antiulcer, antihypertensive, antiviral, and anti-inflammatory activities. Mechanisms and structure–activity relationships (SAR) at the C-2, N-1, and C-5/C-6 positions are emphasised. Antimicrobial and antifungal activity is considered in relation to growing resistance, and anticancer activity in relation to tubulin, PARP, and DNA-binding mechanisms. Current limitations include variability between assays, incomplete pharmacokinetic and target-validation data, and few direct comparisons between closely related series. A stepwise evaluation strategy is proposed to move promising derivatives from *in silico* design to biochemical, cellular, and preliminary pharmacokinetic studies.

Keywords: benzimidazole; privileged scaffold; heterocyclic synthesis; green chemistry; structure–activity relationship; antimicrobial; anticancer

1. Introduction: Disease Burden and the Need for New Scaffolds

Infectious diseases remain a major cause of illness and death worldwide, and cancer, cardiovascular disease, and gastrointestinal disorders contribute substantially to the global disease burden [1,2]. Infection is best understood as a host–microbe interaction with outcomes ranging from commensalism and colonisation to disease [3]. Antifungal drug resistance has long been recognised as a clinical problem [4], which makes the search for new chemical scaffolds an ongoing need.

Benzimidazole is a bicyclic heteroaromatic system formed by fusing benzene with imidazole. Several benzimidazole-containing drugs are in clinical use, including the anthelmintics albendazole and mebendazole, the proton pump inhibitor omeprazole, the angiotensin II receptor antagonists candesartan and telmisartan, and the PARP inhibitor veliparib (ABT-888) [5,6,7]. This breadth of clinical application is one reason the scaffold has attracted sustained research interest.

Benzimidazole has been described as a privileged structure, a framework able to provide useful ligands for more than one biological target [8]. Its usefulness reflects both its physicochemical properties and the ease with which positions 1, 2, 5, and 6 can be substituted to tune activity [6,9].

This review concentrates on synthetic methods and biological evaluation from recent years, with emphasis on green and catalytic approaches, mechanism-based evaluation, and structure–activity relationships.

2. Structural Basis of the Benzimidazole Scaffold

2.1 Hydrogen bonding and aromatic interactions

The benzimidazole ring contains an N–H group that can act as a hydrogen-bond donor and an sp²-hybridised nitrogen that can act as an acceptor. The fused aromatic system supports π – π stacking. Crystallographic work on Hoechst 33258, a bis-benzimidazole, showed that the benzimidazole N–H groups form bridging hydrogen bonds with adenine and thymine atoms at the edges of base pairs in the minor groove of DNA [10]. This illustrates how these features can support molecular recognition.

2.2 Purine mimicry

Because the bicyclic core resembles purine, benzimidazole derivatives can mimic nucleotide-binding motifs in enzymes and receptors [5]. This similarity is one of the main reasons the scaffold has been explored against kinases, polymerases, and other nucleotide-processing targets.

2.3 Tautomerism and substitution

In unsubstituted benzimidazoles the N–H proton is mobile, so the two ring nitrogens are tautomerically equivalent. Substitution at N-1 fixes the tautomer, and the choice of substituent therefore changes both the hydrogen-bonding pattern and the electron distribution of the ring. SAR interpretation should consider which tautomer or substitution pattern is present in the target environment [9].

3. Synthetic Strategies

3.1 Classical condensation

The Phillips condensation, in which an o-phenylenediamine reacts with a carboxylic acid under strong acid, remains the standard route to 2-substituted benzimidazoles [11]. Harsh conditions, long reaction times, and poor compatibility with acid-sensitive groups limit its use. Reviews of synthetic approaches starting from o-phenylenediamine summarise these routes and their alternatives [12]. Table 1 compares the principal strategies.

3.2 Catalytic approaches

Catalysts that avoid stoichiometric strong acids have been developed to make the core formation milder and more selective. As one example, Bahrami and co-workers reported a mild and efficient method for 2-arylbenzimidazoles [13]. Catalytic routes are attractive because they can reduce waste and simplify purification, although catalyst cost and removal must be considered for scale-up.

3.3 Green and sustainable methods

Microwave-assisted synthesis can shorten reaction times substantially compared with conventional heating [14]. Mechanochemistry, in which reactions are driven by grinding or milling with little or no solvent, offers a cleaner alternative [15]. Deep eutectic solvents, mixtures of hydrogen-bond donors and acceptors with low melting points, are considered greener reaction media [16]. The greatest benefit of these methods is reduced solvent and energy use, and their suitability for each benzimidazole route should be assessed individually.

3.4 Multicomponent reactions

Multicomponent reactions combine three or more starting materials in one pot, with most of their atoms incorporated into the product. Rational design strategies for such reactions are reviewed by Ruijter and co-workers [17], and isocyanide-based variants by Dömling and Ugi [18]. Broader reviews of imidazole and benzimidazole drug development place such approaches in the context of lead discovery [19].

Table 1. Comparison of principal synthetic strategies for benzimidazole derivatives.

Strategy	Typical conditions	Advantages	Limitations
Phillips condensation	Strong acid, reflux	Simple, scalable, inexpensive	Harsh, poor functional-group tolerance
Aldehyde oxidative cyclization	Mild heat, oxidant	Broader scope	Regioselectivity issues with N-substitution
Lewis/heterogeneous acid catalysis	Mild, often RT–80 °C	Better selectivity, catalyst reuse	Catalyst cost or preparation
Microwave-assisted	Minutes, solvent-minimized	Fast, high yield	Scale-up equipment needed
Metal-catalyzed C–N coupling	Pd or Cu, base, heat	Access to hindered patterns	Metal residues, cost
Multicomponent	One-pot, varied	Library generation	Mechanistic complexity

4. Biological Evaluation: Therapeutic Areas and Chemotypes

The activities below are grouped by therapeutic area. Reported potency values come from different assays and should not be compared directly across papers. Table 2 summarises representative examples.

4.1 Antimicrobial activity

Benzimidazoles have been investigated extensively as antibacterial agents, and the scaffold has been reviewed for its antimicrobial potential [20]. A recent review covering 2018 to 2024 analyses the SAR of antimicrobial benzimidazoles [21]. Docking studies have been used alongside synthesis to rationalise antibacterial activity, although docking scores alone do not establish activity [22]. Susceptibility testing should follow the standardised dilution

methods of CLSI, since minimum inhibitory concentrations are only comparable between studies when the same method is used [23]. Related bicyclic azoles illustrate a validated antibacterial target. Indazole derivatives have been identified as bacterial DNA gyrase B inhibitors [24], which suggests that gyrase B is a target class that benzimidazole derivatives could also be designed against. This remains a hypothesis to be tested experimentally. Schiff base derivatives, which contain an imine ($>C=N-$) group, have also been reviewed for broad antimicrobial activity [25]. Efflux limits intracellular accumulation of many compounds. Homology models of the human efflux transporter P-glycoprotein are used to study how ligands bind to it [26], and this type of analysis can help flag compounds likely to be effluxed, although these models describe the human transporter rather than bacterial efflux systems.

4.2 Antifungal activity

Antifungal resistance is an established clinical problem, driven by mechanisms including efflux, target alteration, and changes in the biosynthetic pathway [4]. Benzimidazole derivatives have been synthesised and evaluated as antifungal agents, and bis-benzimidazoles have shown MIC values in the low $\mu\text{g/mL}$ range against several fungal strains [27].

Ergosterol is the major sterol of fungal membranes and a principal target of current antifungal drugs, and resistance to these agents has been linked to changes in ergosterol biosynthesis [28]. Lanosterol 14 α -demethylase is a key enzyme in this pathway, and crystal structures of this enzyme from *Candida albicans* and *Candida glabrata* provide structural tools for antifungal design [29]. Benzimidazole antifungals can be designed with reference to this pathway, but target engagement must be confirmed experimentally.

4.3 Anticancer activity

Nocodazole, a benzimidazole carbamate, suppresses microtubule dynamic instability at nanomolar concentrations, and this reduction in dynamics is associated with mitotic arrest [30]. Tubulin-targeting benzimidazoles therefore represent one established anticancer mechanism.

Veliparib (ABT-888) was discovered as a PARP inhibitor based on a 2-substituted benzimidazole-4-carboxamide framework [7]. PARP inhibitors exploit synthetic lethality in tumours with defects in homologous recombination repair, such as those carrying BRCA mutations [31,32]. A third mechanism is DNA binding: Hoechst 33258 binds the minor groove of DNA, as described above [10], and bis-benzimidazoles are therefore studied as DNA-binding anticancer candidates.

Across these classes, substituents at C-2 and N-1 are the main determinants of target selectivity, and polar side chains are commonly introduced to improve solubility and pharmacokinetic behaviour [6].

4.4 Anthelmintic activity

Benzimidazole carbamates are established anthelmintics that act by inhibiting microtubule assembly in parasites [33]. Their clinical success is well documented, and the mechanism provides a reference point for designing new derivatives. The structural features that determine parasite selectivity over mammalian tubulin are a key question for optimisation.

Table 2. Representative benzimidazole derivatives and reported activities discussed in Section 4.

Activity	Representative compounds	Mechanism or reported activity
Antimicrobial	Benzimidazoles with lipophilic electron-withdrawing substituents	SAR reviewed for antibacterial activity [20,21]
Antifungal	Alkylated bis-benzimidazoles	MIC values of 0.975 to 15.6 $\mu\text{g/mL}$ against fungal strains reported [27]
Anthelmintic	Albendazole, mebendazole, benzimidazole carbamates	Inhibition of microtubule assembly in parasites [33]
Anticancer (tubulin)	Nocodazole	Suppression of microtubule dynamic instability at nanomolar concentrations [30]
Anticancer (PARP)	Veliparib (ABT-888)	PARP inhibition; synthetic lethality in BRCA-deficient tumours [7,31]

5. Structure–Activity Relationships: A Synthesis

Across therapeutic areas, several SAR principles recur:

1. **C-2 substitution** is the primary handle for potency and target selection. Aryl, heteroaryl, and carboxamide groups are common [9,6].
2. **N-1 substitution** controls lipophilicity and can influence how quickly a compound is metabolised. Benzyl and aryl groups are frequent [21].
3. **C-5/C-6 substitution** influences electronics and membrane permeability. Lipophilic electron-withdrawing groups are reported to enhance antimicrobial activity in several series [21,20].
4. **Tautomerism** of the N–H group affects binding, so SAR interpretation should account for the tautomer or substitution pattern present in the target environment.

These principles are most thoroughly established for anticancer and antimicrobial activity. For newer targets the SAR is less mature and often rests on a few analogues. Docking can help rationalise these trends, but it should be validated experimentally [38].

6. Challenges and Limitations

6.1 Assay variability and mechanism

Many reported compounds are evaluated only in cell-based assays without target validation, which makes it difficult to separate a specific mechanism from nonspecific cytotoxicity. Cytotoxicity is commonly measured by protein-staining assays [39] or tetrazolium reduction assays [40], and these readouts respond to different cellular properties. Using more than one readout, and reporting the assay used clearly, makes comparison between studies more reliable.

6.2 Physicochemical properties and permeability

Highly planar aromatic derivatives can have limited aqueous solubility and oral bioavailability. Solubility and permeability should be assessed alongside potency, using experimental and computational approaches [41].

6.3 Covalent and fragment-based design

Covalent inhibition, as used by proton pump inhibitors, has regained acceptance in drug discovery [42]. Fragment-based approaches have also contributed to a number of clinical candidates [43]. Both strategies require careful assessment of selectivity and off-target reactivity before a compound can be advanced.

6.4 Resistance

Resistance remains a central problem in antimicrobial and antifungal therapy [4,28]. Compounds that are potent against susceptible strains may lose activity against resistant isolates, so resistant strains should be included in evaluation where possible.

7. Proposed Synthetic and Pharmacological Evaluation Strategy

7.1 Design and synthesis

A rational design strategy starts from a 2-substituted or N-substituted benzimidazole core and varies substituents at C-2, N-1, and C-5/C-6. Synthesis can use condensation under catalytic or microwave conditions [13,14], and green solvents or mechanochemical routes can be considered where they are compatible with the chemistry [15,16]. After synthesis, identity and purity should be confirmed by infrared spectroscopy, proton and carbon-13 NMR, and mass spectrometry.

7.2 Stepwise pharmacological evaluation

A stepwise framework is proposed to identify compounds with a balanced activity profile, acceptable cellular tolerability, and suitable physicochemical properties (Table 3). Early computational screening focuses experimental effort, but computational predictions must be followed by biological testing [38]. Antimicrobial and antifungal activity should be measured by standardised dilution methods [23], cytotoxicity by a validated cell-viability assay [39,40], and mechanism by target-specific assays where a target is proposed.

Compounds should be prioritised on the balance of their activity profile rather than potency against a single target, and drug-likeness should be assessed alongside activity [41]. A systematic progression from computational evaluation to biochemical, cellular, and, where appropriate, in vivo studies gives the strongest basis for establishing therapeutic potential.

Table 3. Proposed stepwise evaluation of benzimidazole derivatives.

Stage	Methods	Purpose or readout
1. In silico	Molecular docking against proposed targets; ADME and drug-likeness prediction	Plausible binding modes and prioritisation of compounds [38,41]
2. Antimicrobial and antifungal	Broth microdilution for MIC against susceptible and resistant strains	Activity and spectrum; comparability with other studies [23,4]
3. Cytotoxicity	SRB or MTT assay in mammalian cell lines	Selectivity of microbial or cancer-cell activity relative to normal cells [39,40]
4. Target assays	Tubulin polymerisation (anticancer); PARP inhibition; DNA-binding; H ⁺ /K ⁺ -ATPase inhibition (as relevant to the proposed target)	Mechanism beyond phenotypic activity

Stage	Methods	Purpose or readout
5. Physicochemical and preliminary pharmacokinetics	Solubility, permeability, and metabolic stability	Drug-likeness and decision to progress [41]
6. In vivo	Efficacy and safety in appropriate models	Only for compounds with consistent activity and an acceptable profile

8. Conclusions and Future Perspectives

- The benzimidazole scaffold combines hydrogen-bonding capacity, aromaticity, and synthetic accessibility, and is present in several marketed drugs.
- Synthesis has moved from harsh acid condensations toward catalytic, microwave-assisted, mechanochemical, and multicomponent methods that generate diverse derivatives more efficiently.
- The strongest evidence concerns anticancer (tubulin and PARP), antimicrobial and antifungal, anthelmintic, and proton pump inhibitor activities. Antiviral and anti-inflammatory findings are more preliminary.
- Future work should use standardised assays, validate targets and mechanisms, assess solubility and permeability, and report SAR across closely related series to enable direct comparison.

Abbreviations

AT₁, angiotensin II type 1 receptor; CLSI, Clinical and Laboratory Standards Institute; DES, deep eutectic solvent; DNA, deoxyribonucleic acid; FT-IR, Fourier-transform infrared spectroscopy; GBD, Global Burden of Disease; MCR, multicomponent reaction; MIC, minimum inhibitory concentration; MS, mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic resonance; PARP, poly(ADP-ribose) polymerase; PPAR- γ , peroxisome proliferator-activated receptor gamma; PPI, proton pump inhibitor; SAR, structure–activity relationship.

Acknowledgements

The authors are thankful to the management and the Department of Pharmaceutical Chemistry, Malik Deenar College of Pharmacy, for the facilities and support provided.

Conflict of Interest

The authors declare that they have no known financial, commercial, or personal conflicts of interest that could have influenced the work reported in this review article. All authors confirm that the research was conducted independently and that no external organization or individual had any role that could bias the content or conclusions of this manuscript.

REFERENCES

- [1] World Health Organization. (2023). *World health statistics 2023: Monitoring health for the SDGs*.
- [2] GBD 2019 Diseases and Injuries Collaborators. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019. *The Lancet*, 396(10258), 1204–1222.
- [3] Casadevall, A., & Pirofski, L. A. (2000). Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infection and Immunity*, 68(12), 6511–6518.
- [4] White, T. C., Marr, K. A., & Bowden, R. A. (1998). Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clinical Microbiology Reviews*, 11(2), 382–402.
- [5] Bansal, Y., & Silakari, O. (2012). The therapeutic journey of benzimidazoles: a review. *Bioorganic & Medicinal Chemistry*, 20(21), 6208–6236.
- [6] Keri, R. S., Chand, K., Budagumpi, S., et al. (2015). A comprehensive review in current developments of benzimidazole-based medicinal chemistry. *Chemical Biology & Drug Design*, 86(1), 19–65.
- [7] Penning, T. D., Zhu, G. D., Gandhi, V. B., et al. (2009). Discovery of the poly(ADP-ribose) polymerase (PARP) inhibitor 2-[(R)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide (ABT-888) for the treatment of cancer. *Journal of Medicinal Chemistry*, 52(2), 514–523.
- [8] Duarte, C. D., Barreiro, E. J., & Fraga, C. A. M. (2007). Privileged structures: a useful concept for the rational design of new lead drugs. *Mini-Reviews in Medicinal Chemistry*, 7(11), 1108–1119.
- [9] Yadav, G., & Ganguly, S. (2015). Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities. *European Journal of Medicinal Chemistry*, 97, 419–443.
- [10] Pjara, P. E., Grzeskowiak, K., & Dickerson, R. E. (1987). Binding of Hoechst 33258 to the minor groove of B-DNA. *Journal of Molecular Biology*, 197(2), 257–271.
- [11] Phillips, M. A. (1928). The formation of 2-substituted benzimidazoles. *Journal of the Chemical Society*, 2393–2399.
- [12] Alaqeel, S. I. (2017). Synthetic approaches to benzimidazoles from o-phenylenediamine: a literature review. *Journal of Saudi Chemical Society*, 21(2), 229–237.
- [13] Bahrami, K., Khodaei, M. M., & Naali, F. (2008). Mild and highly efficient method for the synthesis of 2-arylbenzimidazoles and 2-arylbenzothiazoles. *The Journal of Organic Chemistry*, 73(17), 6835–6837.
- [14] Kappe, C. O. (2004). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie International Edition*, 43(46), 6250–6284.

- [15] James, S. L., Adams, C. J., Bolm, C., et al. (2012). Mechanochemistry: opportunities for new and cleaner synthesis. *Chemical Society Reviews*, 41(1), 413–447.
- [16] Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep eutectic solvents (DESs) and their applications. *Chemical Reviews*, 114(21), 11060–11082.
- [17] Ruijter, E., Scheffelaar, R., & Orru, R. V. A. (2011). Multicomponent reaction design in the quest for molecular complexity and diversity. *Angewandte Chemie International Edition*, 50(28), 6234–6246.
- [18] Dömling, A., & Ugi, I. (2000). Multicomponent reactions with isocyanides. *Angewandte Chemie International Edition*, 39(18), 3168–3210.
- [19] Gaba, M., & Mohan, C. (2016). Development of drugs based on imidazole and benzimidazole bioactive heterocycles: recent advances and future directions. *Medicinal Chemistry Research*, 25(2), 173–210.
- [20] Tahlani, S., Kumar, S., & Narasimhan, B. (2019). Antimicrobial potential of 1H-benzo[d]imidazole scaffold: a review. *BMC Chemistry*, 13(1), Article 18.
- [21] Ibrahim, A. A., Said, E. G., AboulMagd, A. M., Amin, N. H., & Abdel-Rahman, H. M. (2025). Synthesis and SARs of benzimidazoles: insights into antimicrobial innovation (2018–2024). *RSC Advances*, 15(27), 22097–22127.
- [22] Erol, M., Çelik, İ., Temiz-Arpaci, Ö., Göker, A. H., Kaynak-Onurdag, F., & Okten, S. (2020). Synthesis, molecular docking and ADME prediction of some new benzimidazole carboxamides derivatives as antimicrobial agents. *Medicinal Chemistry Research*, 29(11), 2028–2038.
- [23] Clinical and Laboratory Standards Institute. (2024). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (12th ed., CLSI standard M07-Ed12).
- [24] Zhang, J., Yang, Q., Romero, J. A. C., et al. (2015). Discovery of indazole derivatives as a novel class of bacterial gyrase B inhibitors. *ACS Medicinal Chemistry Letters*, 6(10), 1080–1085.
- [25] Ceramella, J., Iacopetta, D., Catalano, A., et al. (2022). A review on the antimicrobial activity of Schiff bases: data collection and recent studies. *Antibiotics*, 11(2), Article 191.
- [26] Lagares, L. M., Minovski, N., Caballero Alfonso, A. Y., et al. (2020). Homology modeling of the human P-glycoprotein (ABCB1) and insights into ligand binding through molecular docking studies. *International Journal of Molecular Sciences*, 21(11), Article 4058.
- [27] Chandrika, N. T., Shrestha, S. K., Ngo, H. X., & Garneau-Tsodikova, S. (2016). Synthesis and investigation of novel benzimidazole derivatives as antifungal agents. *Bioorganic & Medicinal Chemistry*, 24(15), 3680–3686.
- [28] Alcazar-Fuoli, L., & Mellado, E. (2013). Ergosterol biosynthesis in *Aspergillus fumigatus*: its relevance as an antifungal target and role in antifungal drug resistance. *Frontiers in Microbiology*, 3, Article 439.
- [29] Keniya, M. V., Sabherwal, M., Wilson, R. K., et al. (2018). Crystal structures of full-length lanosterol 14 α -demethylases of prominent fungal pathogens *Candida albicans* and *Candida glabrata* provide tools for antifungal discovery. *Antimicrobial Agents and Chemotherapy*, 62(11), Article e01134-18.
- [30] Vasquez, R. J., Howell, B., Yvon, A. M., Wadsworth, P., & Cassimeris, L. (1997). Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. *Molecular Biology of the Cell*, 8(6), 973–985.
- [31] Lord, C. J., & Ashworth, A. (2017). PARP inhibitors: synthetic lethality in the clinic. *Science*, 355(6330), 1152–1158.
- [32] Ferraris, D. V. (2010). Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. *Journal of Medicinal Chemistry*, 53(12), 4561–4584.
- [33] Lacey, E. (1990). Mode of action of benzimidazoles. *Parasitology Today*, 6(4), 112–115.
- [34] Lindberg, P., Nordberg, P., Alminger, T., Brändström, A., & Wallmark, B. (1986). The mechanism of action of the gastric acid secretion inhibitor omeprazole. *Journal of Medicinal Chemistry*, 29(8), 1327–1329. doi:10.1021/jm00158a001.
- [35] Shin, J. M., & Sachs, G. (2008). Pharmacology of proton pump inhibitors. *Current Gastroenterology Reports*, 10(6), 528–534.
- [36] Benson, S. C., Pershadsingh, H. A., Ho, C. I., et al. (2004). Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPAR- γ -modulating activity. *Hypertension*, 43(5), 993–1002.
- [37] Tonelli, M., Simone, M., Tasso, B., et al. (2010). Antiviral activity of benzimidazole derivatives. II. Antiviral activity of 2-phenylbenzimidazole derivatives. *Bioorganic & Medicinal Chemistry*. 2010;18(8):2937–2953. doi:10.1016/j.bmc.2010.02.037.
- [38] Ferreira, L. G., dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, 20(7), 13384–13421.
- [39] Skehan, P., Storeng, R., Scudiero, D., et al. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, 82(13), 1107–1112.
- [40] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63.
- [41] Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46(1–3), 3–26.
- [42] Singh, J., Petter, R. C., Baillie, T. A., & Whitty, A. (2011). The resurgence of covalent drugs. *Nature Reviews Drug Discovery*, 10(4), 307–317.
- [43] Erlanson, D. A., Fesik, S. W., Hubbard, R. E., Jahnke, W., & Jhoti, H. (2016). Twenty years on: the impact of fragments on drug discovery. *Nature Reviews Drug Discovery*, 15(9), 605–619.