The Mobile Proton: What Benzimidazole SAR Can and Cannot Say

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***Abstract*—Benzimidazole is among the most heavily worked scaffolds in medicinal chemistry, and almost every paper on it opens with the same two sentences: the ring is a purine bioisostere, and 2-substituted derivatives show antimicrobial and antioxidant activity. This review takes the scaffold's defining feature the single acidic N–H, with pK_a 12.8 for deprotonation and 5.6 for the conjugate acid and follows its consequences through synthesis, characterisation and biological interpretation. That proton moves fast enough to make the 5- and 6-positions of an N-unsubstituted benzimidazole one position rather than two: the NMR signals of C4/C7, C5/C6 and C3a/C7a coalesce, the two tautomers are a single substance, and N-alkylation of a 5(6)-substituted ring gives an approximately 1:1 mixture of two genuinely different compounds. A published structure–activity relationship that distinguishes a 5-substituted from a 6-substituted N–H benzimidazole is therefore reporting a distinction that does not exist. On the synthetic side, the condensation of benzene-1,2-diamine with an aldehyde is an oxidation, not a dehydration, and the oxidant air, metabisulfite, iodine, peroxide, benzoquinone belongs in the green accounting that microwave and solvent-free protocols usually omit; the same reaction competes with formation of the 1,2-disubstituted product, which is a different compound with a different SAR. On the biological side, the only firmly established benzimidazole mechanism is β-tubulin binding with 25- to 400-fold selectivity for nematode over mammalian tubulin, while reported radical-scavenging activity generally belongs to an appended phenol or thioamide rather than to the ring. Tables set out the routes, the evidence that fixes a structure, and the activity data worth carrying forward.**

***Index Terms*—Benzimidazole; annular tautomerism; Phillip's condensation; microwave-assisted synthesis; β-tubulin; DPPH; structure–activity relationship**

I. INTRODUCTION

The benzimidazole ring has been in medicinal chemistry since 5,6-dimethylbenzimidazole was identified as the axial ligand of cobalt in vitamin B_{12}, and the comparison with purine that this invited has shaped how the scaffold is described ever since [3,20]. It is a fair comparison. The ring is planar, electron-rich, carries a hydrogen-bond donor and an acceptor in a 1,3-relationship, and appears in drugs as different as albendazole, omeprazole and telmisartan (Fig. 1) [21]. It is also the starting point of a great many dissertation projects, because the chemistry is cheap and forgiving: benzene-1,2-diamine plus a carboxylic acid or an aldehyde, a few hours of reflux, and a crystalline solid. The compounds are then screened for antimicrobial and antioxidant activity, a structure–activity relationship is proposed, and the series is reported.

This review follows a single thread through that sequence: the N–H proton. It is the reason the ring is amphoteric, the reason the 5- and 6-positions are not independent, the reason the aldehyde condensation needs an oxidant, and when it is replaced by an alkyl group the reason a one-compound starting material becomes a two-compound product. Several of the most common errors in the benzimidazole literature are traceable to that one proton, and all of them can be avoided without additional equipment.

The argument is not that the published work is worthless. It is that the scaffold is unusually unforgiving of two habits that are otherwise harmless: naming a compound more precisely than the evidence allows, and describing a reaction by its intention rather than by its stoichiometry. Where those two habits meet in a table of 5- and 6-substituted analogues prepared

by an undeclared oxidation the structure–activity relationship that results cannot be reproduced, because the compounds it names are not the compounds that were tested.

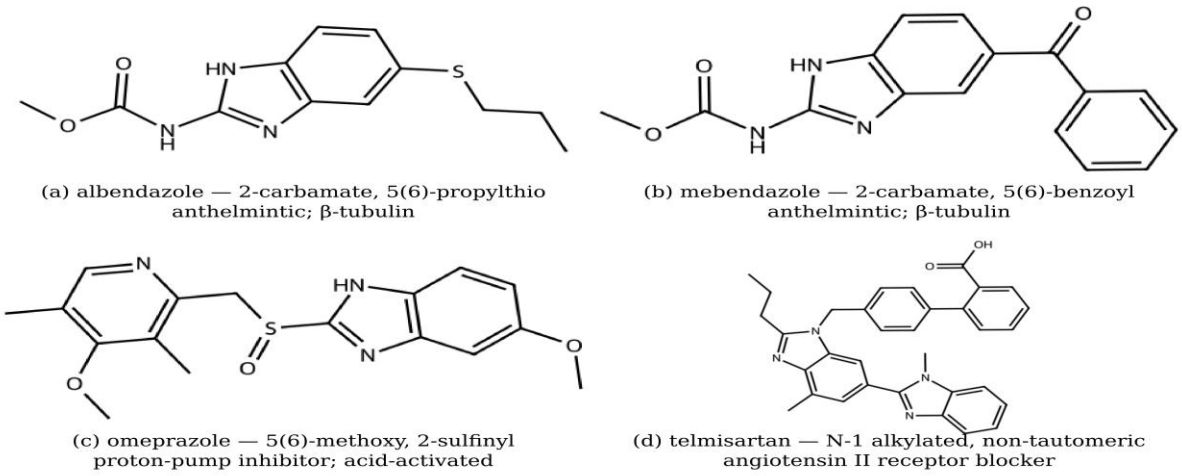


Fig. 1. Marketed benzimidazoles. The 2-substituent sets the pharmacophore and the N-1 substituent decides whether benzo-ring regiochemistry exists at all: (a) and (b) are N–H compounds, so their acyl and thioether groups are correctly described as 5(6)-; (c) is likewise 5(6)-methoxy; (d) is N-alkylated and therefore has a defined, non-tautomeric substitution pattern.

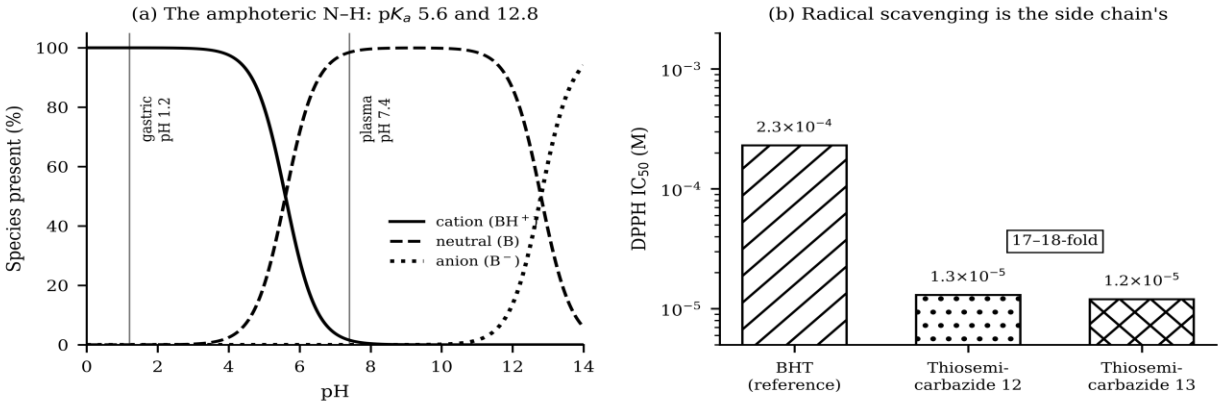


Fig. 2. (a) Speciation of the benzimidazole N–H system computed from pK_a 5.6 (conjugate acid) and 12.8 (N–H); the cation dominates at gastric pH and the neutral species at plasma pH. (b) Reported DPPH IC_{50} values for two benzimidazole-linked thiosemicarbazides against butylated hydroxytoluene; the activity was attributed to the side-chain N–H groups rather than to the ring [28].

II. THE RING AND ITS PROTON

2.1 An amphoteric N–H

Benzimidazole is amphoteric. The pK_a for loss of the N–H proton is 12.8, and the pK_a of the conjugate acid, protonated at N-3, is 5.6, making the ring appreciably less basic than imidazole [3,21]. Figure 2a plots the resulting speciation. Three features matter pharmaceutically. At gastric pH the ring is essentially fully protonated, which is what omeprazole exploits: the drug is a prodrug that rearranges to a sulfenamide only in the acidic secretory canaliculus [27]. At plasma pH the neutral species dominates, so transport and partitioning behaviour should be modelled on the free base. And the anion does not appear in appreciable amounts below about pH 11, so the acidity of the N–H, though real, is not a source of ionisation under assay conditions but it is a source of hydrogen-bond

donation, and of susceptibility to N-alkylation during work-up with alkylating agents.

Substituents shift both values. Electron-withdrawing groups on the benzo ring raise N–H acidity and lower basicity; the 2-carbamate of albendazole and mebendazole does both. Reporting a calculated log P for a new series without the corresponding pK_a, as most papers do, leaves the species that actually faces the organism unspecified.

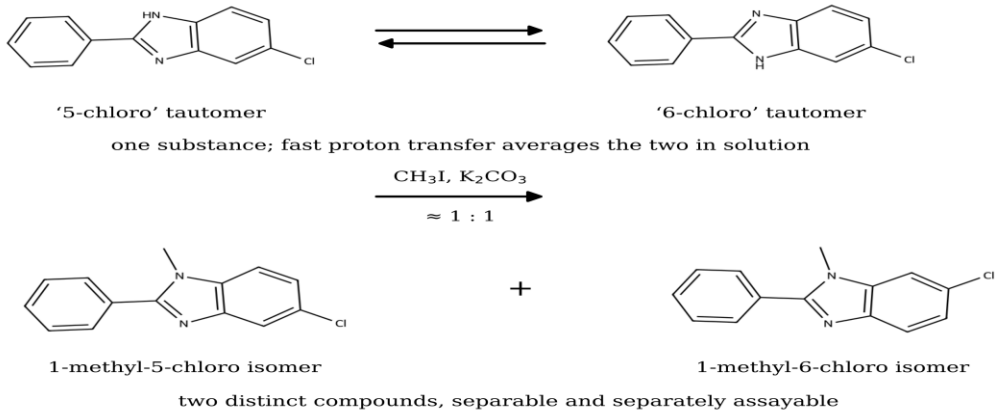
The same N–H underwrites the purine comparison. Benzimidazole presents a donor and an acceptor nitrogen in a 1,3-relationship on a planar, electron-rich bicycle, which is the hydrogen-bonding pattern that adenine offers to a kinase hinge or a nucleic acid base pair, and it is the reason benzimidazole nucleosides were among the earliest synthetic nucleoside analogues [20]. The comparison is useful as a design heuristic and misleading as an argument: sharing a hydrogen-bonding motif with purine predicts nothing about which of the thousands of purine-binding proteins a given derivative will engage, and it certainly does not predict antibacterial selectivity, since bacteria and their hosts both run on purines.

2.2 Annular tautomerism makes 5 and 6 the same position

In an N-unsubstituted benzimidazole the proton exchanges between N-1 and N-3 fast on the NMR timescale. The consequence is not subtle: the carbon signals of the benzo ring coalesce in pairs C3a with C7a, C4 with C7, C5 with C6 into averaged resonances, and the two nitrogen environments likewise average [1]. A careful NMR study of the parent system measured the barrier to proton transfer directly, obtaining $\Delta G^{\ddagger} = 58.0$ kJ mol^{-1} at 277.5 K in HMPA-d_{18}, and showed that the 2-substituent modulates the rate: 2-H and 2-CF_3 exchange quickly, 2-methyl and 2-benzyl more slowly [1]. Blocking the tautomerism, either by N-methylation or in the solid state, restores the separate signals.

The practical implication is set out in Scheme 3. A compound written as 5-chloro-2-phenylbenzimidazole and one written as 6-chloro-2-phenylbenzimidazole are the same substance. They cannot be separated, they cannot be assayed separately, and no spectrum will distinguish them, because there is nothing to distinguish. Correct nomenclature for such a compound is 5(6)-chloro, and this is why mebendazole is formally methyl (5-benzoyl-1H-benzimidazol-2-yl) carbamate while the benzoyl group is equally at the 6-position [21]. Only when N-1 carries a substituent do the two become distinct: alkylation of a 5(6)-substituted benzimidazole typically gives a roughly 1:1 mixture of the 1-alkyl-5- and 1-alkyl-6-substituted isomers, which are separable and which may well differ in activity [1,2].

A structure–activity relationship that contrasts 5-substituted with 6-substituted N–H benzimidazoles is therefore reporting an artefact. Conversely, a series of N-alkylated compounds in which the regiochemistry was never resolved is reporting the average of two compounds in unknown ratio. Both failures are common, and both are avoidable.



Scheme 3. Why 5- and 6-substitution are not separate variables. Above: the two tautomers of a 5(6)-chloro compound are one substance, averaged by fast proton transfer. Below: N-methylation converts that one substance into two distinct, separable regioisomers in roughly equal amounts.

III. MAKING THE RING

3.1 From carboxylic acids: the Phillips condensation

Heating benzene-1,2-diamine with a carboxylic acid in 4 M hydrochloric acid, or in polyphosphoric acid at higher temperature, gives the 2-substituted benzimidazole directly (Scheme 1) [3,4]. The reaction is a double condensation with loss of two molecules of water: the amide forms first, then cyclodehydration closes the ring. No oxidant is needed, because the carboxyl carbon already sits at the oxidation level of the product's C-2. Atom economy for the parent case is high and the by-product is water.

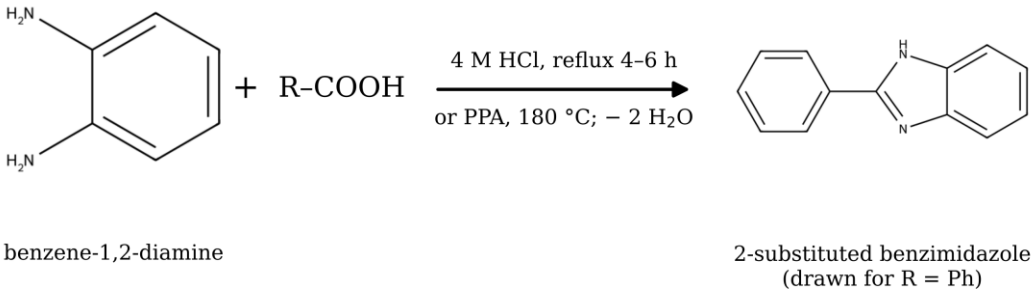
Its limitations are the forcing conditions, which are incompatible with acid-sensitive substituents, and the restricted availability of some carboxylic acids compared with the corresponding aldehydes. That second consideration is why most contemporary series are built from aldehydes instead and why they inherit a different problem.

Historically the aldehyde route came second. Weidenhagen showed in 1936 that benzene-1,2-diamine and an aldehyde give the benzimidazole in the presence of copper (II) acetate, the copper serving as the oxidant and being recovered as copper(I) [5]. Nearly every modern protocol is a variation on that observation with a different oxidant, and the historical record is a useful reminder of what the modern experimental sections leave out: Weidenhagen named his oxidant in the title.

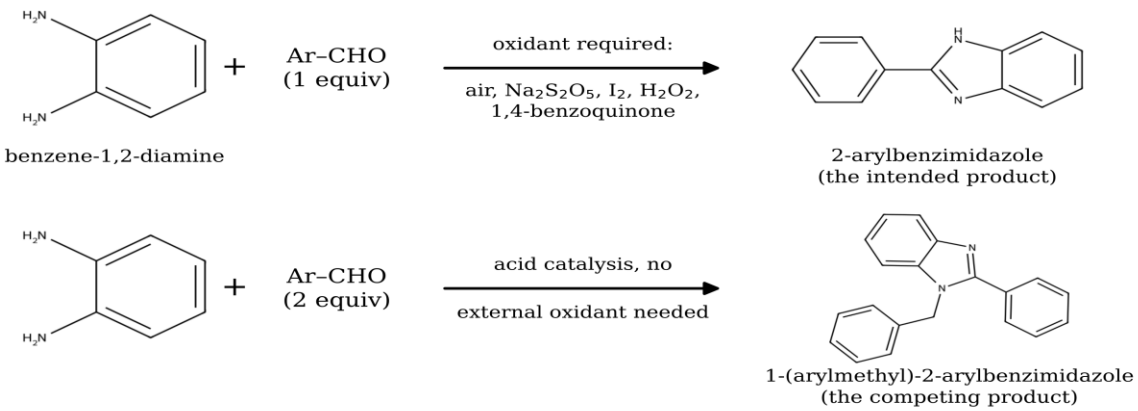
3.2 From aldehydes: an oxidation in disguise

Condensation of benzene-1,2-diamine with an aldehyde first gives an imine, which cyclises to a 2,3-dihydro-1H-benzimidazole. That intermediate is at a lower oxidation level than the aromatic product, so aromatisation requires the removal of two hydrogens. The oxidant may be atmospheric oxygen, or it may be added deliberately: sodium metabisulfite, iodine, hydrogen peroxide, 1,4-benzoquinone, copper (II) acetate and Oxone have all been used, as have catalytic systems that use air as the terminal oxidant [6–10]. Describing this step as a condensation, as many experimental sections do, misstates the chemistry and conceals a reagent.

The second difficulty is selectivity (Scheme 2). With two equivalents of aldehyde, or under strong acid catalysis, the reaction diverts to the 1-(arylmethyl)-2-arylbenzimidazole, in which a second molecule of aldehyde has been incorporated and reductively attached at N-1. This product needs no external oxidant, which is part of why it forms so readily. It is a different compound with a different molecular formula, a different mass spectrum, and crucially no tautomerism, and the two are routinely obtained together [6,11]. Control is achievable: pre-forming the bisulfite adduct of the aldehyde, or using sodium bisulfite in water, strongly favours the 2-substituted product by making the second condensation slow [8,11], while slow addition of the aldehyde to excess diamine has the same effect. A paper that reports only the intended product without saying which control was applied has left the reader unable to judge the purity claim.



Scheme 1. The Phillips condensation. The carboxyl carbon is already at the oxidation level of C-2, so the only by-product is water and no oxidant is required.



Scheme 2. The aldehyde route and its competing product. Formation of the 2-arylbenzimidazole requires removal of two hydrogens from the cyclised intermediate; the 1-(arylmethyl)-2-aryl compound consumes a second aldehyde instead and needs no oxidant, which is why it competes so effectively.

3.3 Microwaves, grinding and the word green

Microwave-assisted and solvent-free protocols dominate recent work, and the yield and time advantages are real: minutes instead of hours is routine [19]. The mechanistic claim that often accompanies them is less secure. Careful studies that controlled for bulk temperature found no evidence for non-thermal microwave effects in the great majority of reactions examined; the acceleration is thermal, arising from rapid, uniform, superheated heating of a polar medium [17,18]. That is a good enough reason to use a microwave, and not a reason to call a route green without measuring anything.

What a green claim requires is arithmetic, and Table 1 lists the terms. Atom economy, computed from the balanced equation, is a property of the route [12]. The E-factor kilograms of waste per kilogram of product is a property of the process, and for pharmaceutical chemistry it typically falls between 25 and over 100 [13]. Process mass intensity is the same quantity counted as input and equals the E-factor plus one [15]. Solvent dominates all of them, which is why solvent selection guides exist, and why a reaction run neat but worked up through three extractions and two recrystallisations may be worse than the reflux it replaced [14,16]. For the aldehyde route, the oxidant and its reduced form belong in the same accounting: stoichiometric benzoquinone generates stoichiometric hydroquinone.

Table 1. Routes to substituted benzimidazoles, the oxidant each does or does not consume, and what an honest experimental section must state [3–11,17–19].

Route	Typical conditions	Oxidant	Principal side reaction	Reporting requirement
Phillips (carboxylic acid)	4 M HCl, reflux 4–6 h; or polyphosphoric acid, 180 °C	None required	Amide intermediate surviving if cyclisation is incomplete	Water is the only by-product; report atom economy and solvent volumes
Aldehyde condensation	EtOH or water, 60–80 °C, acid or Lewis's acid catalysis	Required	1-(Arylmethyl)-2-aryl product; unoxidised 2,3-dihydro compound	Name the oxidant and include it and its reduced form in the mass balance
Bisulfite-adduct variant	$Na_2S_2O_5$ or $NaHSO_3$, water or DMF, 80–100 °C	Metabisulfite serves as oxidant and as selectivity control	Strongly suppressed	State the bisulfite stoichiometry; it is a reagent, not an additive

Route	Typical conditions	Oxidant	Principal side reaction	Reporting requirement
Microwave-assisted	Sealed vessel, 100–150 °C, 5–20 min, EtOH or solvent-free	As for the underlying route	As for the underlying route; local overheating	Report bulk temperature; acceleration is thermal, not a special effect
N-Alkylation of a 5(6)-substituted ring	Alkyl halide, K_2CO_3, DMF or acetone	None	Approximately 1:1 mixture of 1-alkyl-5- and 1-alkyl-6- isomers	Separate the isomers and assign each by NOE, HMBC or diffraction

IV. PROVING WHAT YOU MADE

Benzimidazoles are easy to make and surprisingly easy to misassign. Three questions must be answered for any new compound, and Table 2 summarises what each kind of evidence can settle.

The first is whether the product is the 2-substituted or the 1,2-disubstituted benzimidazole. Here the molecular ion is decisive, and so is the ^{1}H NMR integration: the 1,2-disubstituted compound has no N–H resonance near δ 12–13 and carries an additional benzylic CH_2 singlet near δ 5.5. Reporting an N–H signal is positive evidence for the 2-substituted product; reporting neither is a gap.

The second is whether the ring aromatised. A 2,3-dihydrobenzimidazole has an sp³ C-2 with a characteristic ^{1}H signal near δ 6.5 and a ^{13}C signal near δ 65–75, far from the δ 150–152 of an aromatic C-2. Incomplete oxidation is the commonest unreported impurity in aldehyde-route preparations.

The third is regiochemistry, and only for N-alkylated compounds, since for N–H compounds the question is void. Nuclear Overhauser enhancement between the N-alkyl group and the adjacent aromatic proton, or an HMBC correlation from the N-alkyl protons to C-7a, distinguishes the isomers; a melting point and an elemental analysis do not, because the two isomers share a formula. Where a single crystal can be grown, diffraction settles it outright.

Table 2. What each kind of evidence settles for a new benzimidazole. The recurring limitation is that prototropic exchange erases benzo-ring regiochemistry in N–H compounds [1,2].

Evidence	What it establishes	What it cannot establish
Melting point and elemental analysis	Purity and empirical formula	Nothing about regiochemistry; the 1-alkyl-5- and 1-alkyl-6- isomers share a formula
Mass spectrum (molecular ion)	Whether the product is 2-substituted or 1,2-disubstituted, since these differ in formula	Which benzo-ring position carries a substituent
^{1}H NMR: N–H near δ 12–13; absence of a benzylic CH_2 near δ 5.5	That the N–H is present and no second aldehyde was incorporated	Regiochemistry, because tautomeric exchange averages the benzo-ring environments
^{13}C NMR: C-2 near δ 150–152 rather than δ 65–75	That aromatisation is complete and no dihydro compound remains	Regiochemistry, for the same reason
NOE between N-alkyl and the adjacent aromatic H; HMBC to C-7a	Regiochemistry of an N-alkylated compound	Anything about an N–H compound, where the question does not arise
Single-crystal X-ray diffraction	The complete structure, including the solid-state tautomer	The tautomer population in solution, which may differ

V. ANTIMICROBIAL ACTIVITY: ONE PROVEN MECHANISM AND MANY UNASSIGNED ONES

5.1 What β-tubulin binding actually established

The benzimidazole anthelmintics are the scaffold's clearest success and its best-characterised mechanism. Thiabendazole entered use in 1961, and mebendazole, albendazole, flubendazole and fenbendazole followed [22,24]. They bind β-tubulin and prevent polymerisation into microtubules, and their selectivity is quantitative: the inhibition constant for nematode tubulin is 25- to 400-fold more favourable than for mammalian tubulin, with the difference attributed largely to a much slower dissociation rate from parasite tubulin [22,23]. Resistance in the field maps to the same site, most often a phenylalanine-to-tyrosine substitution at residue 200 of β-tubulin isotype 1 [25,26].

Two lessons follow for a new series. First, a mechanism counts as established when a binding site, a selectivity ratio and a resistance mutation all point to the same protein not when a docking score is favourable. Second, the pharmacophore that achieves it is specific: a 2-carbamate with a 5(6)-acyl or 5(6)-thioether substituent. A 2-aryl benzimidazole is a different molecule and should not inherit the mechanism by association.

5.2 Antibacterial and antifungal reports

Reported antibacterial potencies for synthetic 2-substituted benzimidazoles sit mostly in the tens of micrograms per millilitre. A recent survey of the field records 2-(1H-benzimidazol-2-yl) aniline and 2-benzyl-1-(phenylsulfonyl) benzimidazole at a minimum inhibitory concentration of 15.63 μg/mL, which is representative of the better results rather than exceptional [6]. Table 3 collects the data worth carrying forward and marks, for each entry, what the measurement establishes.

Three qualifications apply across the set. Zones of inhibition are not potency measurements; diffusion through agar depends on lipophilicity, charge, molecular weight and agar depth, so a ranking by zone diameter is partly a ranking by diffusion [34,35]. Broth microdilution is the reference method and is inexpensive [36,37]. And an MIC without a mammalian cytotoxicity counter-screen cannot distinguish a selective antibacterial from a general cytotoxin, which matters particularly here, because the scaffold's one proven mechanism is inhibition of tubulin a protein that mammalian cells also possess.

Antifungal results deserve the same caution for a sharper reason. The agricultural benzimidazole fungicides, carbendazim and its precursor benomyl, act on fungal β-tubulin by the same mechanism as the anthelmintics, and resistance in field isolates maps to the equivalent β-tubulin substitutions [22,23]. A new 2-substituted benzimidazole that inhibits Candida albicans is therefore not a novel observation unless it is shown to act somewhere else, and the natural control measuring activity against a mammalian cell line in parallel is the same control that establishes selectivity in the first place.

Table 3. Benzimidazole bioactivity data arranged by what the measurement supports rather than by potency. Only the first-row rests on evidence sufficient to call a mechanism established.

Compound class	Endpoint	Reported value	What is established	Ref.
2-Carbamate anthelmintics (albendazole, mebendazole)	β-Tubulin binding; nematode vs mammalian	25- to 400-fold more favourable inhibition constant for nematode tubulin	A mechanism: binding site, selectivity ratio and resistance mutation all agree	[22–25]
2-(1H-Benzimidazol-2-yl) aniline; 2-benzyl-1-(phenylsulfonyl) derivative	Antibacterial MIC	15.63 μg/mL	Potency against the strains tested; no selectivity index reported	[6]
2-(Hydroxyphenyl)benzimidazoles	Radical scavenging	Potency tracks the number and position of phenolic OH groups	That the phenol, not the ring, carries the activity	[6]
Benzimidazole thiosemicarbazides	DPPH IC_{50}	$1.2–1.3 \times 10^{-5}$ M vs 2.3×10^{-4} M for BHT	Hydrogen donation by side-chain N–H groups in one solvent system	[28]
Benzimidazole and simple 2-alkyl derivatives	Autoxidation retardation	Little direct scavenging; retards metal-catalysed autoxidation	That the unadorned ring is not itself an antioxidant	[6]

VI. ANTIOXIDANT ACTIVITY: WHOSE ELECTRON IS IT?

Almost every benzimidazole series is now also run through a DPPH assay, and strong results are frequently reported. They deserve closer reading. Benzimidazole itself and its simple 2-alkyl derivatives have little direct radical-scavenging activity; what they show is retardation of metal-catalysed autoxidation, which is a chelation effect rather than hydrogen-atom donation [6].

Where potent scavenging is observed, the responsible group is almost always appended rather than intrinsic. The reported series in which benzimidazole-linked

thiosemicarbazides were 17- to 18-fold more potent than butylated hydroxytoluene against DPPH (Fig. 2b) attributed the activity explicitly to hydrogen donation by the side-chain N–H groups [28]. Similarly, 2-(hydroxyphenyl) benzimidazoles are active because they contain a phenol, and the number and position of the hydroxyl groups control the potency [6]. Calling the resulting compound an antioxidant benzimidazole is accurate; calling the benzimidazole the antioxidant is not, and the distinction determines what should be varied next.

The assay itself constrains interpretation further. DPPH and ABTS measure reactivity toward one stable, sterically hindered radical in organic solvent, and the apparent mechanism switches between hydrogen-atom transfer and sequential proton-loss electron transfer with solvent and with added acid or base, so an IC_{50} measured in methanol need not rank compounds the same way as one measured in ethanol [29–32]. Bond dissociation enthalpy, not the heterocycle, is what predicts phenolic antioxidant activity [33]. There is also a second mechanism the radical assays do not see at all. The 1,3-diaza arrangement chelates transition metals the property that lets 5,6-dimethylbenzimidazole coordinate cobalt in vitamin B_{12} and sequestering iron or copper suppresses Fenton chemistry without any radical being scavenged [6]. A compound inactive in DPPH may therefore still protect a lipid system, and one potent in DPPH may do nothing in a metal-driven one. A series intended as antioxidants should therefore report at least two mechanistically distinct assays, state the solvent, and include a lipid-peroxidation or cell-based endpoint before the claim is generalised.

VII. THE STRUCTURE–ACTIVITY STATEMENTS THAT SURVIVE

Filtering the literature for claims that are mechanically possible and experimentally supported leaves a short list.

Position 2 governs the pharmacophore. A 2-carbamate gives the anthelmintic tubulin binders; a 2-aryl group gives planar, lipophilic compounds whose reported activity is mostly Gram-positive and modest; a 2-sulfinylmethyl pyridine gives the acid-activated proton pump inhibitors [21,27].

Position 1 is a genuine variable, and the most under-used one. N-alkylation removes the tautomerism, fixes the regiochemistry of any benzo-ring substituent, removes a hydrogen-bond donor and raises lipophilicity; telmisartan and candesartan both exploit it [21]. For a student series it has the additional merit of making a 5-substituted and a 6-substituted compound separately testable for the first time.

The benzo ring positions are real variables only in N-alkylated compounds. In N–H compounds the correct statement is 5(6)-substituted, and any activity difference claimed between 5 and 6 should be read as noise.

Finally, lipophilicity is not a free parameter. Compounds that accumulate in Gram-negative bacteria tend to be relatively polar and to carry an ionisable amine; 2-arylbenzimidazoles are neither, which is consistent with the repeated observation that their activity is better against Gram-positive organisms [40,41].

VIII. A CHECKLIST FOR THE NEXT SERIES

For the chemistry: state the oxidant for every aldehyde-route condensation and include it in the mass balance; state the measure taken to suppress the 1,2-disubstituted product; report atom economy from the balanced equation and an E-factor or process mass intensity computed from the actual solvent volumes, for both the new protocol and the one it replaces.

For characterisation: report the N–H resonance and the C-2 chemical shift; use 5(6)- nomenclature for all N–H compounds; and for every N-alkylated compound, establish the regiochemistry by NOE, HMBC or diffraction, and say which.

For biology: determine MICs by broth microdilution rather than zone diameters, add a minimum bactericidal concentration and a mammalian cell line so that a selectivity index can be quoted, report the vehicle and a vehicle control, and use at least two antioxidant assays with the solvent stated. Treat a docking result as a hypothesis and label it as one [38]; screen the series against the published assay-interference substructures before building a programme on it [39].

IX. CONCLUSION

Benzimidazole deserves its reputation. It is cheap to access, chemically robust, present in four distinct classes of marketed drug, and the carrier of one of the

best-characterised selective mechanisms in antiparasitic pharmacology. What it does not tolerate is casual reporting, because its single mobile proton silently merges two ring positions, quietly demands an oxidant, and competes for a second molecule of aldehyde. The three corrections proposed here name and account for the oxidant, use 5(6)- nomenclature and resolve regiochemistry only where it exists, and measure rather than assert both greenness and potency cost nothing beyond attention. Applied to a new series, they turn a set of compounds into a structure–activity relationship that another laboratory could reproduce.

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