Greener by Eutectic: Deep Eutectic Solvent Routes to Anticancer 2-Arylbenzothiazoles

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***Abstract*—The pharmaceutical industry generates more waste per kilogram of product than any other chemical sector — E factors of 25 to 100 against 0.1 for oil refining — and the largest single contributor is solvent. Any synthetic route that eliminates volatile organic solvent therefore improves the environmental profile of a target molecule more than almost any other change available to the medicinal chemist. The 2-arylbenzothiazoles are a particularly worthwhile target for that treatment. Since the identification of 2-(4-amino-3-methylphenyl)benzothiazole (DF 203) and its fluorinated analogue 5F 203, the scaffold has produced a clinical candidate, the lysylamide prodrug Phortress, and a mechanism unlike that of any conventional cytotoxic: selective uptake by sensitive tumour cells, induction of cytochrome P450 1A1, bioactivation to reactive electrophiles, and formation of DNA adducts and single-strand breaks in sensitive but not resistant lines. The Phase 1 trial of Phortress did not establish a recommended dose and the programme stopped, but the pharmacophore remains actively worked, now principally through hybridisation and structure-based design against kinase and tubulin targets. The classical route to the ring — cyclocondensation of 2-aminothiophenol with an aromatic aldehyde — is simple, high-yielding and almost ideally suited to a solvent that can also act as catalyst and be recovered. Deep eutectic solvents, of which the 1:2 choline chloride–urea eutectic "reline" is the archetype, are exactly such media: cheap, biodegradable, negligibly volatile, formed by simply mixing two solids, and recoverable by aqueous precipitation of the product. This review assembles the pharmacology and structure–activity relationships of the 2-arylbenzothiazole series, sets out the green-chemistry case quantitatively, compares the reported green routes to the ring, examines deep eutectic solvents as reaction media and catalysts, and sets out what a credible DES-based synthesis with anticancer and docking evaluation would have to demonstrate.**

***Index Terms*—2-Arylbenzothiazole; deep eutectic solvent; reline; green chemistry; E factor; anticancer; molecular docking; structure–activity relationship**

I. INTRODUCTION

Green chemistry began as an accounting exercise. When Sheldon tabulated waste per kilogram of product across the chemical industry, the figures were stark: approximately 0.1 for oil refining, under 1 to 5 for bulk chemicals, 5 to 50 for fine chemicals, and 25 to 100 for pharmaceuticals [2]. The pharmaceutical sector produces the smallest tonnage and the worst ratio. The table was a provocation, and it worked; the principles codified by Anastas and Warner gave the response a framework, and atom economy and the E factor became the first and still the most widely used metrics of synthetic greenness [1,3,4].

The dominant contributor to that waste is solvent. Solvent losses are the major source of waste in pharmaceutical and fine-chemical manufacture, and solvent reduction or replacement is consequently the lever with the greatest leverage. Neoteric solvents — ionic liquids and, more recently, deep eutectic solvents — have been reviewed precisely in that context [2,41,42].

The molecules worth applying that treatment to are those with genuine therapeutic interest, and the 2-arylbenzothiazoles qualify. The benzothiazole nucleus is a privileged heterocycle whose derivatives span anticancer, antimicrobial, anti-Alzheimer and

cardiovascular activity [5,7]. Within it, the 2-aryl subclass has produced one of the more distinctive stories in modern antitumour chemistry: a simple, flat, low-molecular-weight molecule with a mechanism dependent on its own metabolic activation inside sensitive tumour cells, taken from bench to Phase 1 as the prodrug Phortress [10,13].

The synthesis is correspondingly simple. Cyclocondensation of 2-aminothiophenol with an aromatic aldehyde — a transformation whose lineage runs back to Hantzsch [30] — forms the ring in a single step with water as the only stoichiometric by-product. A reaction of that description is an unusually good candidate for solvent replacement, because there is little to optimise except the medium.

Deep eutectic solvents are the natural choice. Formed by mixing a hydrogen-bond acceptor with a hydrogen-bond donor, they melt far below either component: the archetype, a 1:2 mixture of choline chloride and urea, freezes at 12 °C although its constituents melt at 302 and 133 °C [25]. They are inexpensive, biodegradable, essentially non-volatile and can be prepared by simply heating two solids together, and in many reactions they act as catalyst as well as medium [26,27,43,44].

This review brings the two halves together. It sets out the structure–activity story of the antitumour 2-arylbenzothiazoles, quantifies the green-chemistry case, compares the reported green routes to the scaffold, examines deep eutectic solvents as media for this specific transformation, and describes what a DES-based synthesis coupled to anticancer screening and docking-based structure–activity analysis would need to establish.

II. THE 2-ARYLBENZOTHIAZOLE PHARMACOPHORE

2.1 From DF 203 to Phortress

The series began with the observation that 2-(4-aminophenyl)benzothiazoles possess potent and unusually selective activity against breast cancer cell lines in vitro and in vivo [9]. Systematic substitution then produced the lead. Bradshaw, Stevens and Westwell showed that 3′-methyl, 3′-bromo, 3′-iodo or 3′-chloro substitution on the aniline ring markedly potentiated and broadened the antitumour spectrum across ovarian, lung, renal and colon carcinoma lines, identifying 2-(4-amino-3-methylphenyl)benzothiazole (DF 203) as the potent and selective lead [10].

Two problems then had to be solved. The first was metabolic: oxidation of the series was characterised, and the C- and N-oxidation products of the antitumour 2-(4-aminophenyl)benzothiazoles were synthesised and evaluated to understand how activity was lost [39]. Fluorination at the 5-position addressed this, blocking a site of inactivating metabolism and giving 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203), which showed enhanced efficacy in vitro and superior potency in vivo against human breast and ovarian xenografts [37].

The second problem was pharmaceutical. The free amines are very poorly water-soluble, and amino-acid prodrugs were synthesised to address this [11]. The lysylamide dihydrochloride salt of 5F 203 — Phortress — proved water-soluble, chemically stable and subject to rapid, quantitative bioreversion to the parent, and was taken forward as the clinical candidate [13].

The clinical outcome deserves to be reported accurately rather than elided. A Cancer Research UK Phase 1 trial recruited 50 patients with advanced cancer. The team was unable to establish a recommended dose; Phortress and 5F 203 were measurable in blood for only a few hours after dosing and 5F 203 levels were lower than expected; DNA damage was detectable but varied considerably between cells. Of 42 evaluable patients, disease remained stable in 14 and progressed in 28. The trial was stopped early during a portfolio review and no dose could be recommended for further study [17].

That is not a reason to abandon the pharmacophore, but it is a reason to be clear about what the field now needs. The potency and selectivity in vitro were never in doubt; what failed was the translation of that potency into sustained exposure and reproducible target engagement in patients. New analogues are therefore worth making, and the most useful ones will be those that differ from 5F 203 in the properties that limited it rather than merely in decoration.

2.2 A mechanism of its own

What makes the series scientifically interesting is that its activity depends on being metabolised by the cell it is meant to kill.

Sensitive cell lines such as MCF-7 and IGROV-1 sequester the compounds efficiently and deplete them from the medium; resistant lines do not. In sensitive

cells the benzothiazole induces cytochrome P450 1A1 mRNA and protein, and the enzyme it induces then oxidises it to reactive electrophilic species. The role of CYP1A1 in modulating the antitumour properties of DF 203 was established directly in human breast cancer cells [38].

The downstream consequences have been mapped. Antitumour 2-(4-aminophenyl)benzothiazoles generate covalent DNA adducts selectively in sensitive tumour cells both in vitro and in vivo, with adducts detectable above 100 nM for 5F 203 and above 100 nM for Phortress in sensitive MCF-7 and IGROV-1 cells [12]. Alkaline elution showed that 5F 203 induces single-strand breaks and DNA–protein cross-links in sensitive MCF-7 cells, with no double-strand breaks or protein-associated breaks of the kind associated with topoisomerase inhibition, and no trapping of topoisomerase cleavage complexes; cells deficient in the nucleotide excision repair gene XPD were sensitive while XPD-complemented cells were resistant [14].

The mechanism is therefore a self-amplifying one, and selectivity is built into it: a cell that does not take up the compound and does not induce CYP1A1 does not activate it and is not killed. This is a genuinely attractive property, and it is the reason the scaffold continues to attract synthetic attention.

2.3 Beyond the aminophenyl series

Not all active 2-arylbenzothiazoles carry an aniline. A systematic exploration of alternatives identified 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648) — a simple fluorinated 2-arylbenzothiazole without the amino group — as possessing potent and selective inhibitory activity against lung, colon and breast cancer cell lines, establishing dimethoxy substitution as an alternative pharmacophore within the series [15]. Benzothiazole, benzoxazole and chromen-4-one analogues of GW 610 were then prepared, mapping the structure–activity relationship around the dimethoxyphenyl-substituted benzazole and confirming that potency is sensitive to bioisosteric replacement of the ring sulfur [16].

Contemporary work has moved toward hybridisation and structure-based design. Benzothiazole–1,2,3-triazole hybrid hydrazone and thiosemicarbazone conjugates prepared by copper-catalysed azide–alkyne cycloaddition yielded potent EGFR inhibitors with cytotoxicity against breast cancer cells, demonstrating that hydrogen-bond-donating linkers appended to the core can buy kinase affinity [18]. Benzothiazolecarbohydrazide–sulfonate conjugates, designed with docking against the tubulin colchicine-binding site and tested in vitro, produced an analogue that elevated reactive oxygen species and caused DNA fragmentation in MCF-7 cells, validating tubulin as a tractable docking target for the scaffold [19]. Reviews of the class confirm the C2-aryl position as the principal locus for optimisation of potency and selectivity [6,8].

The practical message for a synthetic programme is that the C2-aryl ring is where the chemistry should be varied, and that a method capable of installing a wide range of aryl groups cleanly and in one step is worth more than a method that is elegant for a single substrate.

III. THE GREEN-CHEMISTRY CASE, QUANTIFIED

Table 1 compares reported green routes to the 2-arylbenzothiazole ring, and Figure 1 plots the two datasets that frame the problem.

The baseline is the waste figure. Sheldon's E factor — mass of waste per mass of product — is 25 to 100 for pharmaceuticals, by far the worst of the four sectors tabulated, and the gap between that and the 0.1 of oil refining is almost entirely a matter of scale and solvent [2,3]. Process mass intensity, now widely used alongside it, is simply the E factor plus one. Atom economy, introduced by Trost as a design heuristic rather than a retrospective measure, addresses a different question: what fraction of the mass of the reactants ends up in the product [4].

The cyclocondensation of 2-aminothiophenol with an aromatic aldehyde scores well on atom economy by construction. The only stoichiometric by-products are water and the two hydrogen atoms removed on aromatisation, so for 2-phenylbenzothiazole the theoretical atom economy is high before any solvent is considered. The waste, in other words, is not in the reaction; it is in the medium, the catalyst and the work-up. That is precisely the situation in which solvent replacement delivers the largest proportional gain.

The reported green routes bear this out and are instructive about what is achievable. Urea nitrate at 15 mol% under solvent-free grinding gave 2-

arylbenzothiazoles in 98% yield in 30 seconds at room temperature [21]. Calcined eggshell, a catalyst obtained from a renewable waste stream, gave 92% in 16 minutes by the same grinding approach [22]. Dowex 50W in water provided a water-promoted protocol with a reusable catalyst, high yields and no side products [23]. Comprehensive reviews of green benzothiazole synthesis catalogue solvent-free, ionic-liquid, ultrasound-promoted, biocatalytic, photocatalytic, nanoparticle-catalysed and mechanochemical variants of the same transformation [7,20].

Two observations follow. First, this reaction is easy: many conditions give high yields, so yield alone no longer distinguishes methods and the comparison has to be made on other grounds. Second, most of these routes are catalyst-centred rather than solvent-centred. Solvent-free grinding is excellent at laboratory scale but is not always straightforward to scale, and a heterogeneous catalyst still requires a work-up solvent. A recyclable liquid medium that is itself the catalyst occupies a distinct and useful position in that landscape.

Table 1. Reported green approaches to the 2-arylbenzothiazole ring by cyclocondensation of 2-aminothiophenol with aromatic aldehydes, with conditions and outcomes as published.

Medium / catalyst	Conditions	Reported outcome	Green features	Ref.
Urea nitrate (15 mol%)	Grinding, solvent-free, room temperature	98% yield in 30 s	No solvent; no reported waste stream; very short reaction time	[21]
Calcined eggshell	Grinding, solvent-free, room temperature	92% yield in 16 min	Catalyst derived from a renewable waste stream; solvent-free	[22]
Dowex 50W	Water, water-promoted protocol	Very high yields with no side products and simple work-up	Aqueous medium; reusable solid catalyst; no anhydrous conditions required	[23]
[CholineCl][Imidazole]$_2$ deep eutectic solvent	Solvent-free, one-pot multicomponent	Up to 78% yield for 2-phenylbenzothiazole	DES acts as catalyst and medium; demonstrated reusable for this reaction class	[24]
Choline chloride/urea (reline)	Green medium for three- and four-component heterocyclic synthesis	Applied to pyrazoles and pyrano[2,3-c]pyrazoles with antimicrobial evaluation	Two cheap solids; non-volatile; biodegradable components	[29]
Various (solvent-free, ionic liquid, ultrasound, biocatalytic, photocatalytic, nanoparticle)	Reviewed conditions across the class	Generally high yields and short times	Surveyed in comprehensive reviews of green benzothiazole synthesis	[7,20]

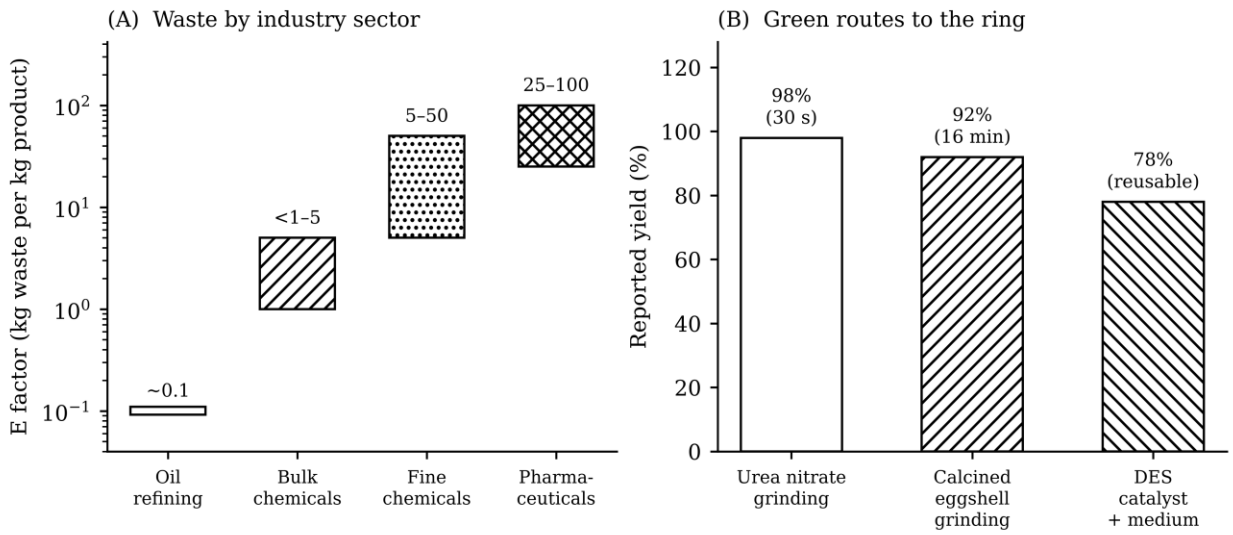


Fig. 1. (A) E factors — kilograms of waste per kilogram of product — across the four sectors of the chemical industry tabulated by Sheldon, shown on a logarithmic axis; bars span the reported ranges and the pharmaceutical sector is the worst by one to three orders of magnitude [2,3]. (B) Reported yields and reaction times for green cyclocondensation routes to 2-arylbenzothiazoles: urea nitrate under grinding [21], calcined eggshell under grinding [22], and a choline chloride-derived deep eutectic solvent acting as catalyst and medium [24]. Yields are high across all three, which is precisely why the comparison cannot be settled on yield alone.

IV. DEEP EUTECTIC SOLVENTS

4.1 What they are

The field dates precisely from the observation by Abbott and colleagues that eutectic mixtures of urea with quaternary ammonium salts are liquid at ambient temperature and have unusual solvent properties. For choline chloride and urea the eutectic falls at a urea-to-choline-chloride mole ratio of 2, and the freezing point of that mixture is 12 °C — against melting points of 302 °C for choline chloride and 133 °C for urea, a depression of 178 degrees. Nuclear magnetic resonance and mass spectrometric evidence in the same paper located the cause in hydrogen bonding between the chloride ion and the urea amide protons, and the freezing-point depression tracked hydrogen-bond strength across a series of anions [25].

The mixture, now universally called reline, is prepared by heating the two solids together at about 80 °C with stirring until a homogeneous colourless liquid forms. Its reported physical properties — density approximately 1.25 g cm^{-3}, viscosity of the order of several hundred centipoise at room temperature, and modest ionic conductivity — place it closer to an ionic liquid than to a molecular solvent. Table 2 summarises the common choline-chloride eutectics.

The green-chemistry credentials are real but should be stated carefully. Deep eutectic solvents are cheap, made from bulk commodity materials, essentially non-volatile, and in the case of choline chloride–urea composed of two substances of low toxicity and good biodegradability [26,27,45]. Natural deep eutectic solvents, built from primary metabolites such as sugars, organic acids and amino acids, extend the compositional scope further [28,45]. What they are not is automatically benign: toxicity and biodegradability depend on the specific components, and the literature contains systems whose constituents are considerably less innocuous than choline chloride and urea. The claim that should be made for reline is that it replaces a volatile organic solvent with a non-volatile, recoverable one made from two cheap, well-characterised solids — which is a substantial and defensible claim on its own.

A practical caution: reline is hygroscopic, and absorbed water changes viscosity, conductivity and melting behaviour. Water content should be measured and reported, not assumed.

4.2 Deep eutectic solvents and the benzothiazole ring

The key precedent is direct. Truong and colleagues established a choline chloride-derived deep eutectic solvent, [CholineCl][Imidazole]$_2$, as an efficient and reusable catalyst for the one-pot multicomponent synthesis of 2-substituted benzothiazoles under solvent-free conditions, reaching up to 78% yield for 2-phenylbenzothiazole and demonstrating recyclability for this exact reaction class [24]. That paper is the proof of concept on which a wider programme can be built.

Its yield is also instructive, because at 78% it is below the 92–98% achievable with grinding catalysts [21,22]. A DES route that claimed superiority on yield alone would be hard to defend. The argument has to be made on the full picture: elimination of volatile organic solvent from both reaction and work-up, recovery and reuse of the medium across multiple cycles, and a cumulative E factor across those cycles that falls as the medium is reused. A single-pass comparison of yields misses the point of a recyclable solvent entirely.

The applicability of choline chloride–urea specifically to heterocyclic construction is also established: it has been used as a green medium for three- and four-component syntheses of pyrazoles and pyrano[2,3-c]pyrazoles with antimicrobial evaluation of the products [29]. Broader reviews position deep eutectic solvents as a serious organic reaction medium rather than a curiosity [43,44].

The recovery route for this particular reaction is unusually convenient. Reline is miscible with water and 2-arylbenzothiazoles are not, so addition of water at the end of the reaction precipitates the product, which is collected by filtration; the aqueous filtrate is then concentrated to recover the eutectic for reuse. No extraction solvent is required at any stage. The honest counterpart is that the water removal step carries an energy cost that a mass-based metric does not capture, and that the recovered eutectic must be characterised rather than assumed intact — water content, composition and spectroscopic identity should be checked each cycle.

Table 2. Common choline chloride-based deep eutectic solvents and their reported properties. Melting points are those of the eutectic composition; choline chloride melts at 302 °C.

Eutectic (common name)	Hydrogen-bond donor	Molar ratio	Melting point (°C)	Notes
Choline chloride–urea (reline)	Urea (m.p. 133 °C)	1:2	12	The archetype; freezing-point depression of 178 °C attributed to Cl^-–amide hydrogen bonding; density ≈ 1.25 g cm^{-3}; viscous at room temperature; hygroscopic
Choline chloride–ethylene glycol (ethaline)	Ethylene glycol	1:2	−66	Markedly less viscous and more conductive than reline; liquid over a very wide range
Choline chloride–glycerol (glyceline)	Glycerol	1:2	−40	Intermediate viscosity; glycerol is a renewable biodiesel co-product
Choline chloride–carboxylic acids	Oxalic, citric, lactic or levulinic acid	typically 1:1 or 1:2	varies	Acidic eutectics; the Brønsted acidity can itself catalyse condensations
[CholineCl][Imidazole]$_2$	Imidazole	1:2	—	Reported as catalyst and medium for one-pot multicomponent synthesis of 2-substituted benzothiazoles, with demonstrated reusability
Natural deep eutectic solvents (NADES)	Sugars, sugar alcohols, organic acids, amino acids	various	varies	Built from primary metabolites; extends DES chemistry beyond choline chloride systems

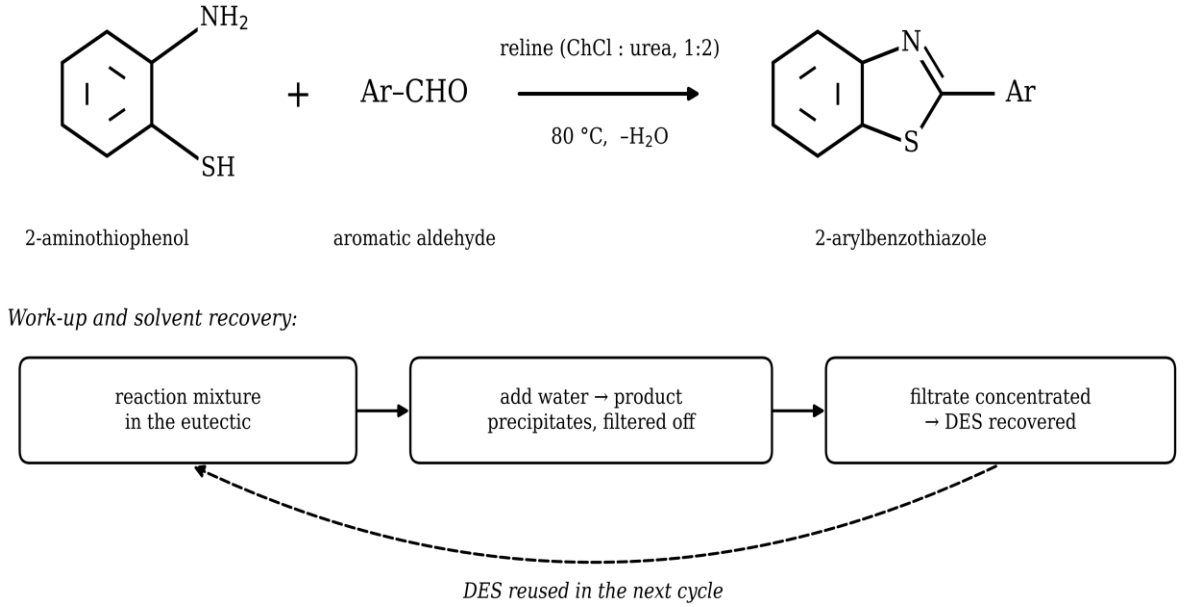


Fig. 2. The cyclocondensation of 2-aminothiophenol with an aromatic aldehyde to give a 2-arylbenzothiazole, conducted in a deep eutectic solvent that serves as both catalyst and reaction medium. Water is the only stoichiometric by-product of ring formation. Because the eutectic is water-miscible and the product is not, addition of water precipitates the benzothiazole for filtration, and concentration of the aqueous filtrate recovers the eutectic for reuse — the recycle loop shown at the foot of the scheme. No extraction solvent is required at any stage, although the evaporation step carries an energy cost that mass-based green metrics do not capture.

V. DESIGNING A DEFENSIBLE DES ROUTE

Table 3 sets out the variables and the measurements that would make such a study convincing rather than merely favourable, and Figure 2 shows the reaction and its recycle loop.

Four experimental variables dominate: the identity and molar ratio of the eutectic components, which determine viscosity, hydrogen-bond-donating ability and therefore catalytic competence; temperature, which trades reaction rate against energy input and against the viscosity that governs mass transfer; reaction time; and the electronic character of the aldehyde, since electron-poor aromatic aldehydes typically condense faster than electron-rich ones and the scope of a method is tested at its extremes rather than at its centre.

The responses that matter extend beyond isolated yield. Purity by a chromatographic method, with structures confirmed by infrared, proton and carbon nuclear magnetic resonance and mass spectrometry, is the minimum. Beyond that, a study claiming greenness should report atom economy, the E factor computed with and without water, and process mass intensity, since these are the metrics by which the claim will be judged and they are straightforward to calculate [2,3,4].

Recyclability is the central claim and should be tested as such. Yield and product purity should be reported for each successive cycle rather than only for the first and last, the recovered eutectic characterised spectroscopically and for water content at each cycle, and the cumulative E factor plotted against cycle number. A method that holds yield for five cycles has demonstrated something; one that reports a single reuse has not.

Substrate scope should be reported honestly, including substrates that fail. A table of twelve electron-rich benzaldehydes in 90% yield says less about a method than a table of eight diverse aldehydes including two that give poor conversion, because the latter tells a reader where the limits are.

Table 3. Variables, responses and reporting requirements for a defensible DES-mediated synthesis of 2-arylbenzothiazoles.

Element	What to vary or measure	Why it matters
DES composition and molar ratio	Hydrogen-bond donor identity; HBA:HBD ratio	Determines hydrogen-bond-donating ability, viscosity and catalytic competence; the ratio sets the eutectic point
Water content of the medium	Karl Fischer titration before and after each cycle	Reline is hygroscopic and absorbed water alters viscosity, conductivity and melting behaviour; uncontrolled water makes results irreproducible
Temperature and time	Reaction profile at several temperatures	Higher temperature lowers viscosity and accelerates the condensation but adds energy cost not captured by mass-based metrics
Aldehyde electronics	Electron-rich, neutral and electron-poor aromatic aldehydes; heteroaromatic substrates	Scope is tested at its extremes; reporting only favourable substrates conceals the method's limits
Yield and purity	Isolated yield; chromatographic purity; IR, ^{1}H and ^{13}C NMR, MS	Yield alone no longer discriminates between methods for this easy reaction
Green metrics	Atom economy; E factor with and without water; process mass intensity	These are the quantities that substantiate a greenness claim; they are calculable from data already in hand
Recyclability	Per-cycle yield and purity over at least five cycles; spectroscopic check of recovered DES; cumulative E factor versus cycle number	Recyclability is the central claim of a DES route and a single reuse does not demonstrate it
Energy accounting	Heating duty for the reaction and for water removal during DES recovery	Aqueous precipitation avoids extraction solvent but shifts the burden to evaporation; an honest assessment states this

VI. ANTICANCER EVALUATION: WHAT THE ASSAYS CAN AND CANNOT SHOW

Cytotoxicity screening of new 2-arylbenzothiazoles is conventionally performed by the sulforhodamine B assay, which measures total cellular protein as a surrogate for cell number [34], or by the MTT assay, which measures mitochondrial dehydrogenase activity as a surrogate for viability [35]. Both are reliable, inexpensive and well validated, and the National Cancer Institute screen built on the former provides the comparative framework within which this series was originally characterised [36].

Three cautions apply with particular force to this scaffold.

The first concerns cell line selection. The defining property of the antitumour benzothiazoles is selectivity: sensitive lines such as MCF-7 and IGROV-1 take up and activate the compounds while resistant lines such as MDA-MB-435 do not [12,14,38]. A screen conducted in a single sensitive line cannot distinguish a compound with the characteristic selective mechanism from a generically cytotoxic one. A panel containing at least one sensitive and one resistant line, and ideally a non-malignant line, is the minimum needed to make the claim that matters.

The second concerns the assays themselves. MTT reports mitochondrial reductase activity, which a compound can perturb without killing the cell; sulforhodamine B reports protein mass, which responds to cytostasis as well as cytotoxicity. Agreement between the two, or confirmation by a mechanistically independent readout, is worth more than a precise IC_{50} from one.

The third concerns mechanism. Because activity in this series depends on CYP1A1 induction and subsequent bioactivation, a compound can be inactive

simply because it is not taken up or not activated, rather than because its binding to any downstream target is poor. Potency differences within a congeneric series may therefore reflect differences in metabolism rather than in intrinsic affinity — a point that has direct implications for how docking results should be interpreted.

VII. DOCKING AND STRUCTURE–ACTIVITY ANALYSIS

Molecular docking is now routine in work of this kind, and both AutoDock Vina, which improved the speed and accuracy of the method with a new scoring function [31], and the AutoDock4 and AutoDockTools4 suite, which enables selective receptor flexibility [32], are appropriate and well validated platforms.

Target selection should be justified rather than assumed. For the benzothiazole series two choices are defensible on precedent: the epidermal growth factor receptor kinase domain, supported by the activity of benzothiazole–triazole hybrids as EGFR inhibitors [18], and the colchicine-binding site of tubulin, validated for benzothiazolecarbohydrazide–sulfonate conjugates by combined docking and in vitro work [19]. A third possibility specific to this scaffold is CYP1A1 itself, since binding to and induction of that enzyme is the activating step of the classical mechanism — a target rarely docked against but arguably the most mechanistically relevant one for the aminophenyl series.

Protocol quality determines whether the results mean anything. Redocking of the co-crystallised ligand with a reported root-mean-square deviation below 2 Å is the standard validation and should be performed and reported for every target. Protonation states and tautomers should be assigned explicitly. And docking scores should be presented as a qualitative ranking accompanied by an interaction analysis, not as predicted affinities: scoring functions are not accurate enough to support a quantitative correlation with IC_{50} across a narrow congeneric series, and reporting a high correlation coefficient between docking score and potency for eight close analogues usually indicates a coincidence rather than a finding.

Drug-likeness assessment by the Lipinski criteria is appropriate and inexpensive [33]. Here it is worth remembering the history: the parent aminophenylbenzothiazoles satisfied those criteria comfortably and still required a lysyl prodrug to be formulated at all [11,13], because aqueous solubility of a flat, planar, crystalline molecule is governed by crystal lattice energy, which the rule of five does not describe. For this scaffold specifically, a calculated solubility estimate and a measured melting point are more informative than a rule-of-five pass.

The structure–activity analysis that would be most valuable is the one that relates substitution pattern at the C2-aryl ring to both measured potency and the computed interaction profile, while remaining explicit that observed potency in this series may be modulated by uptake and bioactivation as much as by target binding.

VIII. GAPS AND OUTLOOK

Several gaps are worth naming directly.

The metric gap. Most published green syntheses of 2-arylbenzothiazoles report yield and reaction time and stop there. Very few report atom economy, E factor or process mass intensity, which are the quantities that substantiate a greenness claim and which can be calculated from data the authors already possess.

The recyclability gap. Reusability is frequently asserted and rarely characterised. Per-cycle yield, per-cycle purity, spectroscopic confirmation of the recovered medium and cumulative waste across cycles are the evidence that would make the claim meaningful.

The selectivity gap. Screening new benzothiazoles against a single sensitive cell line cannot detect the property that made this scaffold interesting. The sensitive-versus-resistant comparison is cheap and should be standard.

The mechanism gap. Docking against a kinase or tubulin is now routine for this scaffold, but the classical mechanism runs through CYP1A1 induction and DNA adduct formation [12,14,38]. A study that docks against a kinase and observes cytotoxicity in MCF-7 cells has not established which mechanism it is observing, and a simple CYP1A1 induction measurement would distinguish them.

The translational gap. Phortress failed to yield a recommended Phase 1 dose, with lower than expected plasma 5F 203 and variable DNA damage [17]. New analogues in this series are most valuable when designed against those specific liabilities — exposure

and reproducible activation — rather than against potency, which was never the limiting problem.

IX. CONCLUSION

Two arguments meet in this topic and each is stronger for the other. The pharmaceutical sector generates 25 to 100 kilograms of waste per kilogram of product, most of it solvent, and the cyclocondensation that builds the 2-arylbenzothiazole ring is a reaction whose waste lies almost entirely in the medium rather than in the chemistry. Deep eutectic solvents, and particularly the choline chloride–urea eutectic that melts at 12 °C despite being made from two high-melting solids, replace that medium with one that is cheap, non-volatile, biodegradable and recoverable by the addition of water alone — and there is a direct literature precedent for their use in exactly this transformation.

The scaffold is worth the effort. From DF 203 through 5F 203 to Phortress, the series produced a clinical candidate with a mechanism unlike any conventional cytotoxic: selective uptake, self-induced CYP1A1 bioactivation, and DNA adduct formation in sensitive cells only. The Phase 1 trial did not establish a dose and the programme stopped, but the pharmacophore remains active in current medicinal chemistry, and the C2-aryl position remains the productive locus for variation.

A study combining the two would be worth doing if it is done rigorously: a recyclable deep eutectic medium characterised across multiple cycles with mass-based green metrics reported rather than asserted; a substrate scope that includes its own failures; cytotoxicity screening in a panel that can distinguish selective from generic activity; and docking treated as a hypothesis-generating tool with validated protocols and honestly qualitative conclusions. Each of those components is standard practice somewhere in the literature. What is uncommon is finding all of them in one place.

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