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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF ISONIAZID-HYDRAZONE CONJUGATES

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Abstract

Isoniazid has anchored tuberculosis treatment since the early 1950s, yet resistance and liver toxicity continue to limit its use. Condensing its hydrazide group with aldehydes and ketones gives hydrazone conjugates that are easy to make and can be tuned for lipophilicity and cell wall penetration. This review summarises published work on the design, synthesis, characterisation and antitubercular testing of such conjugates, including the assays used, the structure-activity patterns reported, and concerns about hydrolytic stability and cytotoxicity. Many compounds show promising inhibition in vitro, but in vivo data are scarce and the mechanism is seldom proved. The review reports no new experimental data.

1. INTRODUCTION

Tuberculosis remains one of the leading infectious causes of death worldwide, and the burden of drug-resistant disease keeps the pressure on for new treatment options [1]. Standard therapy for drug-susceptible disease still rests on a four-drug regimen in which isoniazid, a small pyridine hydrazide first used in the early 1950s, plays a central part [2]. It is cheap, orally active and strongly bactericidal against dividing bacilli. Few other antitubercular agents can match that combination of properties, and no replacement for it has been found in the seventy years since its introduction. Chemists have therefore kept returning to the isoniazid scaffold, hoping to keep what works and remove what does not.

What does not work is fairly clear. Resistance to isoniazid is the commonest form of drug

resistance in tuberculosis, and it is also the first step on the path to multidrug resistance [3, 4]. Treatment can also cause hepatitis, which in some patients is severe enough to force a change of regimen [5]. Both problems are linked to the way the drug is handled, by the bacterium on one side and by the human liver on the other. A molecule that is modified at the hydrazide end may, in principle, escape some of that handling. That reasoning explains why isoniazid hydrazones, made by simple condensation with carbonyl compounds, have been among the most widely reported derivatives [6, 7].

This article reviews the published literature on isoniazid-hydrazone conjugates. It does not describe new syntheses or new biological tests. After a short account of how isoniazid works and fails, the review covers design logic, the usual synthetic and characterisation routes, the screening methods used to judge activity, the structure-activity trends that recur, and concerns about stability and safety. The main reported findings are collected in table 1. The aim is to give a pharmaceutical scientist a clear picture of what the field has shown, how much of it is solid, and what remains unproven when a new conjugate is described as promising.

2. ISONIAZID, ITS MODE OF ACTION AND ITS LIMITS

Isoniazid is a prodrug. It enters the mycobacterial cell by passive diffusion and is oxidised by the catalase-peroxidase KatG, which generates reactive species including an isonicotinoyl radical [8, 9]. That radical couples with NAD^+ to form an isonicotinoyl-NAD adduct, and the adduct binds tightly to InhA, the enoyl-acyl carrier protein reductase of the fatty acid synthase II system [10]. Blocking InhA stops the elongation of mycolic acids, the long lipids that give the cell wall its waxy, impermeable character. Genetic work confirmed InhA as a primary target, since a single point mutation in the inhA gene was enough to confer resistance [11]. The sequence matters for design, because any modification that disturbs activation by KatG may reduce activity.

Most clinical resistance comes from the same enzymes. Mutations in katG, especially at codon 315, remove the activation step and give high-level resistance, while mutations in the promoter region of inhA raise InhA expression and give lower-level resistance that also reduces the effect of ethionamide [4, 12]. Population studies show that these two routes account for a large majority of resistant isolates [4, 12]. Since the activation step is the weak point, a conjugate that still needs KatG will fail against the commonest resistant strains. A conjugate that can inhibit InhA directly, or act by another route, may keep activity. Few papers test this properly, but it is the question that matters most [9, 13].

Table 1: Published findings on isoniazid-hydrazone conjugates: synthesis, assays and activity patterns

Topic	Reported finding	Ref.
Condensation aryl aldehydes	Reflux in alcohol with catalytic acid gives crystalline hydrazones, usually in moderate to high yield.	[14, 15]
Characterisation spectral	Azomethine CH and amide NH signals and a C=N stretch identify the product; E/Z isomers can double signals.	[14, 16]
Hydrolytic stability	Rate depends on structure and pH; aromatic carbonyl partners generally give more stable products.	[17, 18]
Alamar blue assay	Dye reduction gives a minimum inhibitory concentration within about a week.	[19, 20]
Cytotoxicity MTT	Selectivity index of ten or more is a common threshold; weak surrogate for liver injury.	[21, 22]
Low-oxygen assay	Allows activity against non-replicating bacilli to be judged.	[23]
Schiff bases of isoniazid	Several retained potent activity against the standard strain in vitro and in an animal model.	[14]
Isonicotinoyl hydrazones	Lipophilic substituents often gave lower inhibitory concentrations in vitro and in vivo.	[15]
Substituted aryl rings	Ring substitution strongly altered activity across mycobacterial species.	[16]

Topic	Reported finding	Ref.
Resistant strains	Retention of activity in katG mutants is reported in some studies and denied in others.	[12, 14, 16]

Host handling is the other limit. Isoniazid is cleared mainly by N-acetylation, and the rate varies between individuals, producing slow, intermediate and fast acetylator groups [24]. Slow acetylators have higher exposure and may be more prone to neuropathy, while hepatotoxicity has been associated with reactive metabolites formed from the hydrazine moiety, including hydrazine itself [5]. Official guidance on the monitoring of liver enzymes during therapy reflects how common the problem is [2, 5]. These facts give medicinal chemists a second reason to alter the hydrazide: hiding the free NH_2 group might slow acetylation and reduce hydrazine release, provided the compound is stable enough to reach its target intact.

History explains why the scaffold has been mined so heavily. Isoniazid was first synthesised decades before its antitubercular activity was recognised, and its introduction in the early 1950s, followed by rapid clinical adoption, set off a wave of derivatisation in the hope of improving on it [25]. Early workers made alkyl and acyl derivatives and found, in general, that substitution at the terminal nitrogen reduced activity unless the group could be removed in the body. Later mechanistic studies, summarised by Vilchèze and Jacobs, recast that observation in terms of activation: a free hydrazide is needed for the radical chemistry that leads to the NAD adduct, so a blocked nitrogen must be released before the compound can act [26].

3. DESIGN RATIONALE FOR HYDRAZONE CONJUGATES

The hydrazone linkage, an azomethine group attached to an NH, is a favourite among medicinal chemists because it is easy to form and carries a wide range of biological activity. Reviews of the class list antimicrobial, anticonvulsant, anti-inflammatory and antitumour effects, which indicates that the group is tolerated by many targets but also that it can be promiscuous [6, 7]. For isoniazid the reaction is a condensation of the terminal amine with an aldehyde or ketone, giving an N-acylhydrazone. The carbonyl partner can be varied almost without limit, so large libraries are cheap to produce. This ease is both the strength and a weakness of the field, since quantity of analogues often exceeds the depth of testing [14, 15].

Lipophilicity is the most frequent argument. The mycobacterial cell wall is a thick, lipid-rich barrier, and isoniazid is very polar. Adding an aryl group through the hydrazone raises the partition coefficient and may improve uptake by passive diffusion [27, 28]. Designers often use simple property limits, such as the lipophilicity limits discussed by Waring, as a rough guide to keep the new molecules within a drug-like range, although antitubercular agents do not always follow it [27, 29]. Dartois and Barry pointed out that lipophilicity helps penetration of the wall but can also increase binding to plasma proteins and metabolic clearance, so potency measured in a broth assay may not carry over to infected tissue [30].

A second argument is the prodrug concept. If the conjugate is stable in the gut and bloodstream but hydrolyses inside the bacterium or the macrophage, it could deliver isoniazid to the site of infection while limiting systemic exposure to the free hydrazide. Hydrolysis of acylhydrazones is acid-catalysed and depends on the carbonyl partner; aromatic aldehyde-derived hydrazones tend to be more stable than aliphatic ones [17, 18]. That chemistry cuts both ways. Release in the stomach would add nothing, and release in the cell would give an isoniazid-like profile, including loss of activity against katG mutants. The prodrug and the 'new entity' views of these compounds lead to different expectations and different experiments [14, 18].

The third argument is hybridisation, where the carbonyl partner is itself a pharmacophore with antimycobacterial or efflux-pump-inhibiting potential, for example substituted benzaldehydes, heterocyclic aldehydes, isatins or quinolines. The reasoning is that two modes of action in one molecule might delay resistance. Reviews of tuberculosis drug discovery note that such hybrids have been explored widely, but few

have reached animal studies [28, 31]. A hybrid also doubles the number of ways a compound can fail. If either half is unstable or toxic, the combination suffers, and the extra mass can push the molecule outside favourable property space [27, 29].

4. SYNTHESIS AND CHARACTERISATION

The synthetic procedure reported in most papers is brief. Isoniazid and a slight excess of the aldehyde or ketone are heated under reflux in ethanol or methanol, often with a few drops of glacial acetic acid as a catalyst, until thin-layer chromatography shows the starting material has gone. The product usually crystallises on cooling and is filtered, washed and recrystallised [14, 15]. Reaction times range from under an hour to several hours, and yields are commonly moderate to high. Mechanistically the reaction is a carbinolamine formation followed by acid-catalysed dehydration, and the rate-limiting step changes with pH, which is why a mildly acidic medium works best [17]. Microwave and solvent-free variants shorten the time further.

Alternative procedures have been described as well. Microwave irradiation and solvent-free grinding are reported to shorten reaction times and cut solvent use, and the products appear to be the same hydrazones obtained by refluxing in alcohol [14, 17]. Whether such methods matter depends on scale and on equipment, since a library of a few dozen compounds is made easily either way. Purification is usually by recrystallisation, which works well for these planar, hydrogen-bonding solids. Column chromatography is less popular because acid-sensitive hydrazones can break down on silica, a detail that rarely appears in experimental sections but affects the yield and purity of the final sample [17, 18].

Characterisation follows a standard pattern. Infrared spectra show loss of the primary amine bands and appearance of a C=N stretch near the region expected for azomethine groups, along with the amide carbonyl. In proton NMR the diagnostic signals are the azomethine CH singlet, usually well downfield, and the amide NH, which exchanges with D_2O [14, 16]. Mass spectrometry and elemental analysis confirm composition. Because hydrazones can exist as E and Z isomers and as amide rotamers, spectra of some compounds show doubled signals that are easily misread as impurity [17, 18]. Good papers comment on this, and the best provide purity data from HPLC, since a minor contaminant of free isoniazid could generate activity on its own.

That last point deserves emphasis. Because the reaction is reversible and the compounds can hydrolyse in solution, a sample that has stood in DMSO stock may contain a mixture. Kalia and Raines measured the hydrolytic stability of hydrazones and oximes and showed that the rate depends strongly on structure and pH [18]. If a hydrazone partially converts to isoniazid in the assay medium, the measured minimum inhibitory concentration reflects some blend of conjugate and parent. Papers rarely report the stability of the stock or the medium. A simple control, such as HPLC analysis of the compound after incubation in assay medium for the length of the test, would resolve the doubt [14, 18].

A short word about purity and reporting. Regulatory expectations for the identification of impurities in a drug substance are far stricter than those applied in academic synthesis papers, but borrowing the basic principle is sensible for compounds that are going to be tested in cells and animals. Residual aldehyde is a particular concern, since many aromatic aldehydes are themselves cytotoxic or antimicrobial and could distort both the activity and the toxicity results [17, 22]. Reporting melting point, a single HPLC trace and a clean NMR spectrum should be regarded as the minimum for any compound whose biological data are going to be published.

5. ANTITUBERCULAR EVIDENCE

Screening for antitubercular activity relies on a handful of methods. The microplate Alamar blue assay measures reduction of the dye resazurin by living cells and gives a minimum inhibitory concentration within about a week, which is much faster than counting colonies [19]. The resazurin microtitre assay is a closely related approach adapted for detecting

drug resistance and used widely in laboratories without radiometric equipment [20]. Both are done against the avirulent or virulent H37Rv strain in most hydrazone studies. Clinical laboratories follow standard documents for susceptibility testing of mycobacteria, which define media, inocula and incubation, and academic papers sometimes cite them without following all the conditions [32]. Differences in medium alone can shift a minimum inhibitory concentration noticeably.

Within those assays, the reported pattern for isoniazid hydrazones is mixed but interesting. Hearn and colleagues prepared a series of Schiff bases of isoniazid and evaluated them in vitro and in an animal model, finding that several retained potent activity against the standard strain [14]. Sriram and co-workers reported isonicotinoyl hydrazones with in vitro and in vivo activity, with lipophilic substituents often giving lower inhibitory concentrations [15]. Maccari and colleagues screened isoniazid-related hydrazones and hydrazides against a panel of mycobacteria and noted that substitution patterns on the aromatic ring strongly altered activity [16]. The findings are summarised in table 1, which also lists the behaviour of hydrazone analogues of other hydrazide scaffolds [22].

Activity against isoniazid-resistant strains is where results become hard to interpret. Some reports describe retention of activity in strains carrying katG mutations, which would imply an action independent of KatG activation [14, 16]. Others find that potency collapses in these strains, as expected for a prodrug of isoniazid [9, 12]. The difference may reflect compound stability, the specific mutation tested, or the assay conditions. Given the importance of the question, an informative paper would test at least one katG mutant and one inhA promoter mutant alongside H37Rv. Without that, a claim that a conjugate overcomes resistance rests on thin evidence and should be read with care [4, 12].

A rough picture of the evidence base emerges when the entries in table 1 are counted by topic (fig. 1). Activity patterns form the largest group, while stability and safety contribute only two entries, which mirrors the bias of the primary literature: potency is reported in almost every paper, whereas stability, metabolism and animal exposure are reported in a minority. The counts are not a formal measure of study numbers, only a visual summary of the synthesis table prepared for this review. Even so, the imbalance matches the conclusions of broader reviews of tuberculosis drug discovery, which describe a pipeline heavy on in vitro hits and light on the data needed to choose among them [28, 30].

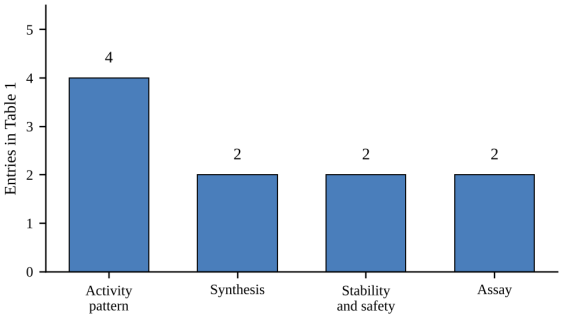


Fig. 1: Number of entries in table 1 by topic group

Selectivity is the other half of the story. Cytotoxicity is commonly assessed by the MTT assay, a colorimetric method that reports the cellular reductive activity of viable cells, usually in a Vero or macrophage-like line [21]. A selectivity index, the ratio of the cytotoxic concentration to the inhibitory concentration, of ten or more is often used as a screening threshold, as in the work by Pavan and colleagues on hydrazide and related compounds [22]. Many of the reported conjugates meet this cut-off in vitro. Cell lines are a weak surrogate for hepatic toxicity, however, and none of these assays reveals the idiosyncratic liver injury that limits isoniazid itself [5, 21].

6. STRUCTURE-ACTIVITY TRENDS

A few trends recur across series. Electron-withdrawing groups such as halogens and nitro groups on the aryl ring are frequently associated with lower inhibitory concentrations, perhaps through raised lipophilicity or altered hydrolytic stability [15, 16]. Hydroxyl and methoxy substituents give variable results. Heteroaromatic aldehydes, including pyridine, furan and thiophene derivatives, often behave similarly to simple benzaldehydes, while bulky polycyclic groups can reduce activity,

possibly because of poor solubility [16, 27]. These are tendencies rather than rules, and the opposite is seen in some series. Quantitative models built on small, congeneric sets have rarely predicted new compounds well [27, 28].

Keeping the free pyridine ring and the carbonyl-hydrazone core appears important in most series, in line with the requirement for KatG activation and for binding of the isonicotinoyl-NAD adduct [10, 13]. Where the hydrazone is replaced by a more stable linker or where the pyridine nitrogen is moved, activity usually falls sharply [27, 28]. This is consistent with the idea that many active hydrazones act at least partly by releasing isoniazid or by being activated like it. At the same time, a minority of compounds appear to retain activity when the pyridine is altered, which would point to different targets and deserves follow-up through target-engagement experiments [28, 31].

Computational work has tried to add structure to these trends. Quantitative structure-activity models for isonicotinoyl hydrazones usually find that lipophilicity, expressed as a calculated partition coefficient, and electronic descriptors of the aryl substituent explain part of the variation in activity, although the models are built on small sets and seldom validated on external compounds [27, 28]. Docking into InhA gives plausible poses but cannot tell whether a hydrazone is acting on the enzyme or releasing isoniazid. Computed properties are better treated as a way to prioritise synthesis than as proof of mechanism, and such property filters serve the same purpose [29, 30].

Safety trends are harder to read. Several papers report low cytotoxicity in mammalian cell lines for the most active compounds, but they rarely test metabolic activation, acetylation or hydrazine release [5, 21]. Janin summarised ten years of antituberculosis drug research and noted how often promising early compounds failed on exposure, toxicity or both [27]. A fair summary is that hydrazone conjugation has delivered a large number of in vitro actives but has not yet shown that it solves either of the two original problems, resistance and hepatotoxicity. The case rests on mechanism and on a handful of animal experiments that need independent confirmation [14, 28].

7. LIMITATIONS AND FUTURE DIRECTIONS

Limitations in the existing literature are fairly consistent. Studies often use a single reference strain and report one endpoint; mechanism is inferred from structure and not tested; stability of the conjugate under assay conditions is rarely checked; and animal data are sparse. Comparisons between papers are hampered by differences in media, inocula and reading methods [19, 20, 32]. Many reports also lack a positive control tested in the same plate. Publication bias is probable, since inactive compounds seldom appear in print. For these reasons, minimum inhibitory concentrations from different laboratories should not be ranked against each other without caution [22, 32].

Future work could be sharper in several ways. Series should include katG and inhA mutants and, where possible, clinical isolates with defined resistance profiles. Stability in assay medium, simulated gastric fluid and plasma should be reported, with identification of what is released [18]. Activity should be examined against non-replicating bacteria using low-oxygen models [23]. Docking against InhA may help, but only when backed by enzyme inhibition data. Early pharmacokinetic and hepatic metabolism work would prevent weeks being spent on compounds that never reach tissue [30]. If these steps become routine, the field could move from a long list of potent hydrazones towards a short list worth taking into animals.

8. CONCLUSION

Isoniazid-hydrazone conjugates are easy to prepare, easy to characterise and often show low inhibitory concentrations against the laboratory strain. Their design rests on sensible arguments, namely higher lipophilicity, masking of the hydrazine and hybridisation with a second pharmacophore. The literature does not yet show convincingly that they overcome resistance or lower hepatotoxicity, mainly because few studies test resistant mutants, stability or exposure [4, 9, 14, 18]. The balance of evidence is thick on potency and thin on stability and safety [17,

22].

Progress will depend on better controls rather than on more analogues. Stability checks, mutant strains, dormancy models and early pharmacokinetic data should accompany any claim of improvement over isoniazid [23, 30]. Standard reporting of strain, medium and controls would let results from different groups be compared [32]. Until such evidence accumulates, hydrazone conjugates are best regarded as a useful chemical platform and a source of leads, not as a proven answer to isoniazid resistance [2, 28, 31]. Early drug-metabolism work in particular would show whether masking the hydrazine truly lowers the liver risk reported with isoniazid [5, 25].

9. ABBREVIATIONS

INH-Isoniazid, InhA-Enoyl-acyl carrier protein reductase, KatG-Catalase-peroxidase, NAD-Nicotinamide adenine dinucleotide, MIC-Minimum inhibitory concentration, MTT-3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NMR-Nuclear magnetic resonance, HPLC-High-performance liquid chromatography, DMSO-Dimethyl sulfoxide, MABA-Microplate Alamar blue assay

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11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

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