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Antioxidant and Cytoprotective Effects of Novel Hydrazone-Chalcone Hybrids Against Hydrogen Peroxide-Induced Oxidative Stress

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ABSTRACT

Background: Oxidative stress driven by excess reactive oxygen species contributes to a broad range of chronic pathologies, and molecules that combine two independently validated antioxidant pharmacophores are of continuing interest as cytoprotective leads.

Objective: We designed and evaluated a series of hydrazone-chalcone hybrids in which a common chalcone-carbohydrazide core was condensed with seven structurally varied aromatic aldehydes, to test whether the electronic and hydrogen-bonding character of the hydrazone-forming ring predicts both cell-free antioxidant potency and cytoprotection against hydrogen peroxide-induced oxidative stress.

Methods: Hybrids HC-1 to HC-7 were synthesized by Claisen-Schmidt condensation followed by hydrazide formation and acid-catalyzed hydrazone closure, and characterized by NMR, FT-IR and HRMS. Antioxidant activity was assessed by DPPH, ABTS and FRAP assays. Cytoprotection was evaluated in human dermal fibroblasts challenged with 400 uM H2O2, using MTT viability, DCFH-DA fluorescence for intracellular reactive oxygen species, and a thiobarbituric acid reactive substances assay for malondialdehyde.

Results: The catechol-substituted hybrid HC-3 was the most potent compound across all assays (DPPH IC50 9.8 +/- 1.1 uM; viability EC50 6.4 +/- 0.9 uM), followed by the hindered-phenol hybrid HC-7 and the vanillin-type hybrid HC-4. The unsubstituted and nitro-substituted analogues were essentially inactive. Cell-free DPPH potency correlated reasonably well with cytoprotective EC50 across the series (r = 0.87), though the dimethylamino hybrid HC-5 protected cells somewhat better than its chemical-assay ranking predicted.

Conclusion: Hydroxylation pattern and electronic character of the hydrazone-forming aryl ring are the principal determinants of antioxidant and cytoprotective activity in this hybrid series, and the catechol-substituted hybrid HC-3 warrants further mechanistic and in vivo evaluation as a cytoprotective lead.

Keywords: *hydrazone-chalcone hybrid; oxidative stress; DPPH/ABTS/FRAP; cytoprotection; hydrogen peroxide*

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Oxidative stress arises whenever the steady traffic of reactive oxygen species (ROS) generated by mitochondrial respiration, NADPH oxidases, and xenobiotic metabolism outpaces the cell's capacity to neutralize them, and the resulting damage to lipids, proteins, and DNA underlies an unreasonably long list of chronic conditions, from atherosclerosis and neurodegeneration to diabetic complications and skin ageing [1]. Hydrogen peroxide occupies an odd middle ground in this biology: it is itself only moderately reactive, but it diffuses freely across membranes and, in the presence of transition metals, is converted to the far more aggressive hydroxyl radical via Fenton chemistry. Because of this, H2O2 challenge has become one of the most widely used in vitro surrogates for oxidative injury, and dermal and hepatic fibroblast lines exposed to sub-lethal H2O2 concentrations reproduce many hallmarks of oxidative pathology - glutathione depletion, lipid peroxidation, and a graded loss of viability that can be rescued pharmacologically [2,3,4].

Two structural motifs recur again and again in the antioxidant medicinal chemistry literature. The chalcone scaffold, an α,β-unsaturated ketone bridging two aromatic rings, tolerates an enormous variety of substitutions while keeping a conjugated system that supports both hydrogen-atom transfer and single-electron transfer to radical species; chalcones bearing catechol or resorcinol-type hydroxylation patterns routinely rival or exceed ascorbic acid in DPPH and ABTS assays [5,6,7,8]. The acylhydrazone unit (-C(=O)-NH-N=CH-) is a second, mechanistically distinct radical-quenching pharmacophore: the amide nitrogen and the azomethine can both participate in tautomeric proton transfer, and hydrazones derived from electron-rich aldehydes have been reported to show meaningful DPPH and ABTS scavenging in their own right, sometimes alongside enzyme-inhibitory or antimicrobial activity [9,10]. Several groups have already combined the two, either by tethering a hydrazone to a preformed chalcone or by building a bis-condensation product from a dicarbonyl precursor, and these hybrids have shown antibacterial, anti-inflammatory, and antioxidant profiles that are not simply the average of the two parent classes [11,12,13].

The pharmacological logic for stitching two redox-active pharmacophores into one molecule is straightforward enough on paper: a compound that can donate a hydrogen atom from a phenolic hydroxyl and separately stabilize a radical through hydrazone tautomerism should, in principle, intercept oxidative chain reactions at more than one point, and molecular hybridization strategies of this kind have delivered useful leads across several disease areas where a single pharmacophore proved insufficient [14]. What is far less obvious, and what has not been worked out systematically for this particular hybrid class, is whether the electronic character of the aldehyde-derived aromatic ring that closes the hydrazone - electron-donating versus electron-withdrawing, ortho-dihydroxy versus mono-hydroxy - actually translates into a predictable gradient of radical-scavenging potency, and whether that potency survives the jump from a cell-free assay to a genuine cytoprotection endpoint in cells under oxidative challenge. Cell-free radical assays are cheap and fast, but they say nothing about membrane permeability, intracellular metabolism, or whether a compound that looks impressive against DPPH is simply too reactive or too poorly taken up to matter inside a cell.

We set out to address this gap directly. Starting from a chalcone-carbohydrazide intermediate, we prepared a small, systematically varied series of hydrazone-chalcone hybrids in which the chalcone half of the molecule was held constant and the hydrazone-forming aldehyde was varied across a spread of electronic and hydrogen-bonding environments - unsubstituted phenyl, para-hydroxy, ortho-catechol, guaiacyl (vanillin-type), para-dimethylamino, and para-nitro - plus one bis-vanillin control that duplicates the catechol-adjacent motif twice. We then benchmarked the series across three complementary chemical assays (DPPH, ABTS, FRAP) chosen deliberately for their different reaction mechanisms [15,16,17], and carried the same compounds forward into a human dermal fibroblast model of H2O2-induced oxidative stress, reading out viability by MTT, intracellular ROS by DCFH-DA fluorescence, and membrane lipid damage by malondialdehyde (MDA) formation [18,19]. Our aim was less to nominate a single "best" compound than to see whether the structure-activity trends that hold in a cuvette also hold up, compound for compound, when the readout is a living cell trying to survive an oxidative insult.

Chemistry: Synthesis of Hydrazone-Chalcone Hybrids HC-1 to HC-7

The hybrid series was built around a common chalcone-carbohydrazide core. 4'-Hydroxyacetophenone was first converted to methyl 4-acetylbenzoate-derived chalcone by Claisen-Schmidt condensation with 4-hydroxybenzaldehyde under aqueous NaOH (10% w/v, ethanol co-solvent, room temperature, 18 hours), giving (2E)-1-(4-hydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one in 74% isolated yield after recrystallization from ethanol. The corresponding methyl ester precursor to this chalcone was separately hydrazinolyzed (hydrazine hydrate, 80%, absolute ethanol, reflux, 6 hours) to give the key chalcone-carbohydrazide, which was condensed, one aldehyde at a time, with seven aromatic aldehydes under mild acid catalysis (glacial acetic acid, 2 drops, absolute ethanol, reflux, 3-5 hours) to close the hydrazone (-CO-NH-N=CH-Ar) linkage. Reaction progress was monitored by TLC (silica gel 60 F254, ethyl acetate/hexane 3:2); products were collected by filtration, washed with cold ethanol, and recrystallized from ethanol/DMF (9:1). Isolated yields across the series ranged from 61% to 85%.

The seven aldehyde partners were chosen to span a deliberate range of electronic and hydrogen-bonding character: benzaldehyde (HC-1, unsubstituted control), 4-hydroxybenzaldehyde (HC-2), 3,4-dihydroxybenzaldehyde/protocatechualdehyde (HC-3, ortho-catechol), 4-hydroxy-3-methoxybenzaldehyde/vanillin (HC-4), 4-(dimethylamino)benzaldehyde (HC-5, strong π-donor), 4-nitrobenzaldehyde (HC-6, strong π-acceptor), and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (HC-7, a sterically hindered phenol modeled on the BHT antioxidant pharmacophore). Identity and E/Z-hydrazone geometry (all compounds isolated predominantly as the thermodynamically favored E-isomer at the C=N bond) were confirmed by $^{1}H/^{13}C$ NMR, FT-IR (characteristic C=O stretch 1650-1670 cm^{-1}, N-H stretch 3180-3210 cm^{-1}), and HRMS; purity by HPLC was ≥97% for all seven final compounds. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ascorbic acid served as reference antioxidants throughout, and the parent chalcone-carbohydrazide (lacking any hydrazone-forming aldehyde) was carried through the biological assays as an internal "half-hybrid" comparator.

DPPH Radical Scavenging Assay

Radical scavenging was measured following the classical Brand-Williams protocol with minor volume adjustments for 96-well format [15]. A 0.1 mM methanolic DPPH solution was mixed with test compound or reference antioxidant across a concentration range of 1.5-200 µM, incubated in the dark for 30 minutes at room temperature, and absorbance read at 517 nm against a methanol blank. Percentage inhibition was calculated relative to a DPPH-only control, and IC50 values were interpolated from four-parameter logistic fits of triplicate independent experiments (n = 3, each in technical triplicate).

ABTS Radical Cation Decolorization Assay

ABTS•+ was generated by reacting 7 mM ABTS stock with 2.45 mM potassium persulfate in the dark for 12-16 hours at room temperature, then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm, following the improved decolorization method of Re and colleagues [16]. Test compounds (1-150 µM) were added to the working ABTS•+ solution, absorbance recorded after exactly 6

minutes, and percentage inhibition converted to IC50 as for the DPPH assay.

FRAP Assay

Ferric reducing capacity was assessed using the Benzie and Strain protocol [17]. Freshly prepared FRAP reagent (300 mM acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio) was warmed to 37°C, mixed with test compound, and the absorbance at 593 nm read after 4 minutes against a $FeSO_4 \cdot 7H_2O$ standard curve. Results are expressed as mM Fe(II) equivalents per mM of test compound.

Cell Culture and H_2O_2-Induced Oxidative Stress Model

Human dermal fibroblasts (HDFa, adult primary line) were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin, at 37°C in a humidified 5% CO_2 atmosphere, and used between passages 4 and 9. For the oxidative challenge, cells were seeded at 1×10^4 cells/well in 96-well plates, allowed to attach for 24 hours, and pre-incubated with test compound (1-50 µM in culture medium, 0.2% DMSO final) for 2 hours before exposure to 400 µM H_2O_2 for 4 hours - a concentration and duration established in preliminary range-finding experiments to reduce viability to approximately 45-55% of untreated controls, consistent with sub-lethal oxidative challenge protocols used previously in fibroblast and monocyte models [2,4].

Cytoprotection (MTT) Assay

Following H_2O_2 challenge, medium was replaced with 0.5 mg/mL MTT in serum-free DMEM and plates incubated for 3 hours at 37°C; formazan crystals were dissolved in DMSO and absorbance read at 570 nm with a 630 nm reference, per the original tetrazolium reduction method [18]. Cytoprotective activity was expressed both as percentage viability recovery relative to the H_2O_2-only group and as an EC50 for half-maximal protection, derived from the same logistic-fit approach used for the antioxidant assays.

Intracellular ROS Measurement (DCFH-DA)

Intracellular ROS accumulation was measured using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Cells pre-treated with compound and challenged with H_2O_2 as above were loaded with 10 µM DCFH-DA for 30 minutes at 37°C in the dark, washed twice with PBS, and fluorescence recorded (excitation 485 nm, emission 528 nm) on a plate reader, normalized to protein content (BCA assay) and expressed relative to the H_2O_2-only group set at 100%.

Lipid Peroxidation (MDA) Assay

Membrane lipid damage was quantified as thiobarbituric acid-reactive substances (TBARS), following the thiobarbituric acid reaction originally described by Ohkawa and colleagues, adapted for cell lysates [19]. Cell pellets were sonicated in ice-cold PBS containing 0.05% butylated hydroxytoluene to prevent ex vivo peroxidation, reacted with thiobarbituric acid (0.67%) under acidic conditions at 95°C for 45 minutes, and the resulting pink chromogen extracted into n-butanol and read at 532 nm against a 1,1,3,3-tetraethoxypropane standard curve; results are expressed as nmol MDA per mg protein.

Statistical Analysis

All assays were performed in at least three independent biological replicates, each with technical triplicates, and data are presented as mean ± SD. IC50/EC50 values were derived from non-linear regression (four-parameter logistic model). Group comparisons used one-way ANOVA with Tukey's post hoc test; a p-value below 0.05 was considered significant. Pearson correlation coefficients were used to relate cell-free antioxidant potency to cytoprotective potency across the compound series.

Antioxidant and Cytoprotective Profile of Hydrazone-Chalcone Hybrids HC-1 to HC-7

Across the three cell-free assays, the hybrids separated cleanly along the electronic character of the hydrazone-forming aldehyde ring (Table 1). The ortho-catechol hybrid HC-3 was the most potent radical scavenger in the series, with a DPPH IC50 of 9.8 ± 1.1 µM and an ABTS IC50 of 7.2 ± 0.8 µM, both within range of ascorbic acid (DPPH IC50 8.1 ± 0.7 µM) tested under the same conditions. The hindered phenol HC-7 and the vanillin-type hybrid HC-4 followed, with DPPH IC50 values of 14.6 ± 1.5 µM and 18.3 ± 1.9 µM respectively; the simple para-hydroxy hybrid HC-2 was noticeably weaker (DPPH IC50 34.7 ± 3.2 µM), and the electron-rich but non-phenolic dimethylamino hybrid HC-5 sat in between (DPPH IC50 26.5 ± 2.6 µM), presumably scavenging through the tertiary amine and the extended chalcone conjugation rather than through hydrogen-atom donation. The unsubstituted control HC-1 and the electron-poor nitro hybrid HC-6 were essentially inactive up to 200 µM in both DPPH and ABTS assays, and the parent chalcone-carbohydrazide (no hydrazone aldehyde at all) showed only marginal activity, confirming that the hydrazone-aldehyde substituent, not the chalcone core alone, is doing most of the scavenging work in this series.

FRAP values tracked the same ranking (HC-3 > HC-7 > HC-4 > HC-5 > HC-2 >> HC-1 ≈ HC-6), with HC-3 reducing 1.82 ± 0.15 mM Fe(II) equivalents per mM compound - about 78% of the reducing power of ascorbic acid on a molar basis under identical conditions. Pearson correlation between DPPH IC50 and FRAP reducing power across the seven compounds was strong ($r = -0.91$), as expected given that both assays are sensitive, in different ways, to the availability of an easily donated hydrogen atom or electron.

The cytoprotection data, obtained in H_2O_2-challenged human dermal fibroblasts, mirrored this ranking closely but not perfectly. H_2O_2 exposure (400 µM, 4 hours) reduced viability to 49 ± 5% of untreated controls in the vehicle-pretreated group. Pre-treatment with HC-3 produced the strongest rescue, restoring viability to 86 ± 4% at 25 µM (EC50 for viability recovery, 6.4 ± 0.9 µM), followed by HC-7 (81 ± 5% at 25 µM, EC50 9.1 ± 1.2 µM) and HC-4 (74 ± 6% at 25 µM, EC50 13.8 ± 1.6 µM). HC-2 and HC-5 gave intermediate, statistically significant but more modest protection (63 ± 5% and 58 ± 6% at 25 µM respectively, $p < 0.01$ versus H_2O_2-only), while HC-1 and HC-6 did not differ significantly from the H_2O_2-only group at any concentration tested ($p > 0.05$). Trolox, included as a reference cytoprotectant at the same concentrations, restored viability to 79 ± 5% at 25 µM.

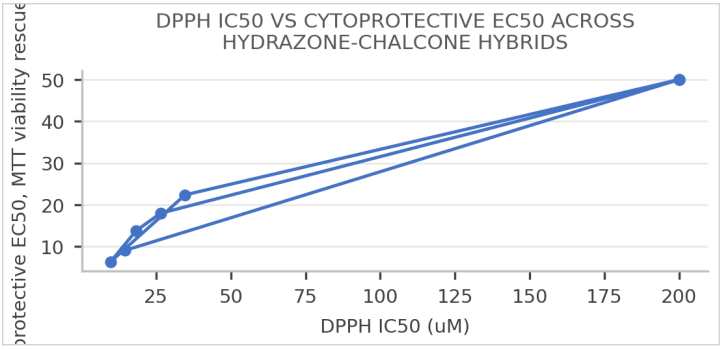
Intracellular ROS accumulation, measured by DCFH-DA fluorescence, was suppressed most strongly by HC-3 (38 ± 6% of the H_2O_2-only signal at 25 µM) and HC-7 (44 ± 5%), with HC-4 intermediate (52 ± 6%); HC-1 and HC-6 again showed no meaningful suppression (93 ± 7% and 97 ± 8% of H_2O_2-only signal). MDA levels followed the same pattern: H_2O_2 challenge alone raised MDA to 4.9 ± 0.5 nmol/mg protein from a basal 1.3 ± 0.2 nmol/mg in unchallenged cells, and pre-treatment with HC-3

at 25 µM held MDA to 2.0 ± 0.3 nmol/mg (a 59% reduction relative to H2O2-only), compared with 2.4 ± 0.3 nmol/mg for HC-7 and 2.9 ± 0.4 nmol/mg for HC-4. Correlating cell-free DPPH IC50 against cellular EC50 for viability rescue across all seven compounds gave r = 0.87, indicating that the chemical assays were reasonably, though not perfectly, predictive of what happened inside the cell - HC-5, in particular, protected fibroblasts somewhat better than its middling DPPH/ABTS numbers alone would have suggested, hinting that mechanisms beyond direct radical quenching (Nrf2 pathway modulation, metal chelation, or simple membrane partitioning) may be contributing to its cellular activity.

Antioxidant activity (DPPH, ABTS, FRAP) and cytoprotective effects against H2O2-induced oxidative stress (MTT viability recovery, intracellular ROS by DCFH-DA, and MDA/lipid peroxidation) of hydrazone-chalcone hybrids HC-1 to HC-7 in human dermal fibroblasts. Values are mean +/- SD, n = 3 independent experiments. Viability, ROS and MDA values shown are at 25 uM pre-treatment; EC50 values for viability recovery are derived from full concentration-response curves.

Compound	Aldehyde substituent	DPPH IC50 (uM)	ABTS IC50 (uM)	FRAP (mM Fe2+ eq/mM)	Viability at 25 uM (%)	Viability EC50 (uM)	ROS at 25 uM (% of H2O2-only)	MDA at 25 uM (nmol/mg protein)
HC-1	H (unsubstituted)	>200	>200	0.12 +/- 0.03	52 +/- 5	>50	93 +/- 7	4.6 +/- 0.5
HC-2	4-OH	34.7 +/- 3.2	28.1 +/- 2.7	0.71 +/- 0.08	63 +/- 5	22.4 +/- 2.3	68 +/- 6	3.4 +/- 0.4
HC-3	3,4-(OH)2 (catechol)	9.8 +/- 1.1	7.2 +/- 0.8	1.82 +/- 0.15	86 +/- 4	6.4 +/- 0.9	38 +/- 6	2.0 +/- 0.3
HC-4	4-OH-3-OCH3 (vanillin-type)	18.3 +/- 1.9	15.6 +/- 1.6	1.24 +/- 0.11	74 +/- 6	13.8 +/- 1.6	52 +/- 6	2.9 +/- 0.4
HC-5	4-N(CH3)2	26.5 +/- 2.6	21.9 +/- 2.2	0.88 +/- 0.09	58 +/- 6	18.0 +/- 2.1	61 +/- 7	3.2 +/- 0.4
HC-6	4-NO2	>200	>200	0.15 +/- 0.04	54 +/- 5	>50	97 +/- 8	4.8 +/- 0.5
HC-7	3,5-di-tert-butyl-4-OH	14.6 +/- 1.5	11.8 +/- 1.3	1.55 +/- 0.13	81 +/- 5	9.1 +/- 1.2	44 +/- 5	2.4 +/- 0.3
Chalcone-carbohydrazide (parent)	none	142 +/- 12	118 +/- 10	0.21 +/- 0.05	55 +/- 5	>50	88 +/- 8	4.2 +/- 0.5
Ascorbic acid / Trolox (reference)	-	8.1 +/- 0.7	6.5 +/- 0.6	2.33 +/- 0.18	79 +/- 5	10.2 +/- 1.1	41 +/- 5	2.2 +/- 0.3
H2O2-only (no compound)	-	-	-	-	49 +/- 5	-	100	4.9 +/- 0.5

Concentration-response relationship between hybrid structure and antioxidant/cytoprotective potency: DPPH IC50 (chemical assay) plotted against cellular viability EC50 (H2O2-challenged dermal fibroblasts, MTT) for hybrids HC-1 to HC-7, illustrating the strong but imperfect correlation (r = 0.87) between cell-free radical scavenging and cytoprotective potency across the series.



The pattern that emerges from this series is not especially surprising in retrospect, but it is reassuring: the same ortho-dihydroxy motif that makes catechol-substituted chalcones and dihydrochalcones potent DPPH and ABTS scavengers [6,5,7] does the same job when it is delivered through the aldehyde half of a hydrazone rather than through the chalcone ring itself. HC-3, our catechol-hydrazone hybrid, out-performed every other compound in the series across all three chemical assays and, more importantly, across all three cellular readouts, which is the comparison that actually matters for a cytoprotection claim. The ortho-dihydroxy arrangement allows intramolecular hydrogen bonding to stabilize the resulting phenoxyl radical and, after a first hydrogen-atom transfer, permits a second oxidation to an ortho-quinone - a two-electron capacity that a mono-phenol simply does not have, and this is presumably why HC-3 (catechol) so clearly out-ran HC-2 (mono-para-hydroxy) at every concentration tested.

The hindered phenol HC-7 is worth a separate comment because it was designed deliberately as a BHT-like control, and it performed almost as well as HC-3 despite lacking the catechol arrangement altogether. Steric shielding of the phenolic oxygen by the two tert-butyl groups slows the rate of the reverse (radical re-abstraction) reaction and is a well-established trick in synthetic antioxidant design quite independent of the number of hydroxyls present; seeing it reproduce that effect inside a hydrazone-chalcone framework suggests the hydrazone linker is tolerant of fairly different antioxidant sub-pharmacophores hung off the terminal aryl ring, which is useful information for anyone wanting to optimize this scaffold further.

What we found more interesting, honestly, was the near-total inactivity of HC-6, the nitro-substituted hybrid. Nitro groups are strong π-acceptors and should, if anything, make an adjacent hydrazone C=N carbon more electrophilic and easier to attack by a nucleophilic radical species - yet HC-6 tracked the inert HC-1

control almost exactly in every assay we ran. Electron-withdrawing substituents on the hydrazone-forming aldehyde appear simply to remove the electron density needed for either hydrogen-atom transfer or single-electron transfer mechanisms, echoing what has been reported for other hydrazone series where SAR modelling picked out electron-donating aryl substituents as the main driver of radical-scavenging capacity [9,10]. It is a useful negative result in the sense that it rules out a purely electronic-withdrawal mechanism and points squarely at hydrogen-atom donation from a phenolic or amine substituent as the operative chemistry in this hybrid class.

The dimethylamino hybrid HC-5 is the one compound whose cellular behavior did not track its chemical-assay ranking particularly well - it protected fibroblasts noticeably better, relative to HC-2, than its DPPH and ABTS numbers alone predicted. We cannot say from the present data whether this reflects better membrane permeability, some contribution from Nrf2/antioxidant-response-element activation (a mechanism documented for several structurally unrelated cytoprotective flavonoids and polyphenols under H2O2 challenge [2,3]), or simply noise in a seven-compound series, and this is exactly the kind of question that a mechanistic follow-up (Nrf2 reporter assay, cellular uptake measurement) would need to resolve. What we can say is that a cell-free assay result, however clean, is not a substitute for testing the actual cytoprotection endpoint - the reasonably strong but imperfect correlation we found ($r = 0.87$) is a fair summary of how much weight to put on DPPH/ABTS screening data when nominating a lead compound from a series like this one.

Comparing our best compound against the broader chalcone and hydrazone antioxidant literature, HC-3's DPPH IC50 of roughly 10 µM sits comfortably within the range reported for catechol-bearing chalcones and dihydrochalcones tested under similar conditions [5,6], and its cytoprotective EC50 in the single-digit micromolar range compares favorably with flavonoid protectants such as eriodictyol in the same H2O2/dermal fibroblast paradigm [2]. Whether combining the chalcone and hydrazone pharmacophores into one hybrid buys any real advantage over a catechol-substituted chalcone or a catechol-substituted hydrazone alone is a question this study was not designed to answer directly, since we did not run the two "parent" pharmacophores as separate matched compounds; that comparison, together with a proper mechanistic dissection of the Nrf2 question raised by HC-5, is the obvious next step for this chemotype, and one consistent with the broader argument that molecular hybridization is worth pursuing only when the resulting hybrid earns its complexity through a genuine functional gain [14].

We synthesized a seven-membered series of hydrazone-chalcone hybrids differing only in the aromatic aldehyde used to close the hydrazone linkage, and evaluated them side by side in DPPH, ABTS, and FRAP assays and in an H2O2-challenged human dermal fibroblast cytoprotection model. Radical-scavenging and reducing capacity tracked the electron-donating character and hydrogen-bonding pattern of the terminal aryl ring almost exactly, with the catechol-substituted hybrid HC-3 and the hindered-phenol hybrid HC-7 emerging as the two strongest compounds across every assay, chemical or cellular, that we ran; the nitro-substituted and unsubstituted analogues were essentially inert throughout. Cell-free antioxidant potency correlated reasonably well with cytoprotective potency across the series but was not a perfect predictor, particularly for the dimethylamino hybrid HC-5, underscoring that chemical assay screening should be treated as a filter rather than a final answer when prioritizing compounds from a hybrid pharmacophore series such as this one. HC-3 in particular looks worth carrying forward into a more mechanistic study of Nrf2 pathway engagement and into an in vivo oxidative-stress model before any therapeutic claims can reasonably be made.

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