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Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2024.v16.i01.pp91-98

DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF CHALCONE-TRIAZOLE HYBRIDS AGAINST MCF-7 CELLS

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Article info
Received date:26-11-2023
Accepted date:13-02-2024
Published date:04-06-2024
ISSN;0975-8216

Keywords:
Chalcone, 1,2,3-Triazole, MCF-7, Click chemistry, Anticancer hybrids

Abstract

Chalcones and 1,2,3-triazoles are two scaffolds that medicinal chemists have repeatedly joined in the search for new anticancer agents. This review summarises published work on how chalcone-triazole hybrids are designed and made, usually by Claisen-Schmidt condensation followed by copper-catalysed azide-alkyne cycloaddition, and how their activity is tested against the oestrogen receptor-positive breast cancer line MCF-7. It covers the cell model, assay methods, reported mechanisms and structure-activity patterns, and the liabilities of the enone group. Many hybrids inhibit MCF-7 growth in vitro, but data on selectivity, mechanism and exposure are sparse. The review reports no new experimental data.

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women worldwide and a leading cause of cancer death, and the tumours that express the oestrogen receptor make up the largest subtype [1, 2]. Endocrine therapy, chemotherapy and targeted agents have improved survival considerably, yet resistance and recurrence still occur and treatments carry real toxicity [2, 3]. For this reason, laboratories keep screening new chemical series against breast cancer cell lines in the hope of finding scaffolds with better potency, selectivity or resistance profiles. Early-stage screening of this kind is cheap and fast, and it is where the majority of synthetic compounds are first assessed for anticancer potential.

Two scaffolds appear again and again in that effort. Chalcones, the open-chain precursors of flavonoids, are simple to make and show antiproliferative, antimitotic and

chemopreventive properties in many studies [4, 5, 6]. The 1,2,3-triazole ring is rarely found in nature but is easily assembled by click chemistry and is stable under biological conditions, which has made it a popular linker and pharmacophore [7, 8, 9]. Putting the two together in one molecule follows the logic of molecular hybridisation, in which fragments with known activity are merged to give a compound that may act on more than one target or simply behave better than either parent [10, 11].

This article reviews the published literature on the design, synthesis and in vitro anticancer evaluation of chalcone-triazole hybrids, with particular attention to MCF-7 breast cancer cells. It does not report new compounds or new assays. The sections that follow describe the cell model, the chemistry of the two scaffolds and of the coupling reaction, the assays used to judge cytotoxicity, and the structure-activity and mechanistic trends that have emerged. Typical methods, findings and liabilities are collected in table 1. Throughout, the emphasis is on what can be stated with confidence from published work and what remains only suggested.

2. MCF-7 CELLS AS A SCREENING MODEL

The MCF-7 line was established in the early 1970s from a pleural effusion of a woman with metastatic breast carcinoma, and it has since become the most widely used breast cancer cell line in the world [12, 13]. It expresses the oestrogen and progesterone receptors, lacks amplification of HER2, and responds to oestradiol by proliferating, which makes it a model for luminal, hormone-dependent disease [13, 14]. Lee and colleagues described how work on MCF-7 shaped the understanding of endocrine therapy, including the action of tamoxifen. For a laboratory studying new cytotoxic scaffolds, MCF-7 is convenient: it grows reliably, forms colonies, and gives reproducible curves in short-term viability assays [13].

Convenience should not be mistaken for representativeness. Holliday and Speirs pointed out that cell lines drift in culture, that different stocks of MCF-7 behave differently, and that no single line captures the diversity of breast cancer [14]. MCF-7 is oestrogen-dependent, relatively slow growing and expresses wild-type p53, so a compound that works well in it may fail in triple-negative lines that carry mutant p53, or may act partly through oestrogen receptor pathways that other lines lack [13, 14]. The best papers therefore pair MCF-7 with at least one receptor-negative line, such as MDA-MB-231, and with a non-malignant breast epithelial line to estimate selectivity. Many do not include the latter, which weakens claims of tumour specificity [14].

Table 1: Chemistry, assays and findings reported for chalcone-triazole hybrids and MCF-7 cells

Topic	Reported feature or finding	Ref.
Claisen-Schmidt condensation	Base-catalysed reaction of acetophenone and benzaldehyde in alcohol gives E-chalcones in good yield.	[15, 16]
Copper-catalysed cycloaddition	Room-temperature coupling of azides and terminal alkynes gives only the 1,4-triazole.	[7, 17, 18]
Triazole as amide mimic	Stable to hydrolysis and metabolism; mimics trans amide geometry.	[9, 19]
Hybrid design	Combines pharmacophores for dual action; larger size may worsen properties.	[10, 11]
MTT assay	Measures metabolic activity; formazan location and mitochondrial effects complicate reading.	[20, 21]
SRB assay	Stains cellular protein; used in the NCI-60 screen and less metabolism-dependent.	[22, 23, 24]
Annexin V staining	Flow cytometry separates early apoptosis from necrosis.	[25]
Antimitotic chalcones	Trimethoxy and alpha-methyl chalcones inhibit tubulin polymerisation at low concentration.	[4, 16, 26]
MCF-7 model	Hormone-dependent luminal line; not representative of all breast cancers.	[12, 13, 14]

Topic	Reported feature or finding	Ref.
Enone reactivity	Michael acceptors can react with thiols and interfere with assays.	[27, 28]
IC50 estimation	Values depend on curve fitting, exposure time and replicates.	[29]

Culture conditions also affect results. The phenol red in standard medium has weak oestrogenic activity, serum contains hormones that can stimulate growth, and cell density and passage number change drug sensitivity [13, 14]. Seeding densities and treatment durations vary from forty-eight to seventy-two hours between laboratories. Because the inhibitory concentration is calculated from a fitted curve, differences in these steps change the number reported [29]. Comparing a half-maximal value from one paper with that of another therefore tells less than comparing compounds within a paper that includes the same reference drug, such as doxorubicin or tamoxifen, in the same plate and on the same day [20, 22].

Authentication of the cell line is a problem that surfaces in most reviews of cell-based work. Misidentified or cross-contaminated lines have been reported, and the original MCF-7 has itself diverged into sublines with different growth and receptor characteristics [13, 14]. Short tandem repeat profiling is now commonly expected by journals but is infrequently mentioned in papers on synthetic compounds. It is a simple control that costs little. Mycoplasma testing is another omission that can alter drug response without any visible sign. When the biological section of a chemistry paper says nothing on either point, readers have no basis for judging reliability [14, 30].

3. CHALCONES AS ANTICANCER SCAFFOLDS

Chalcones are 1,3-diaryl-2-propen-1-ones: two aromatic rings joined by a three-carbon unit that carries a ketone and a conjugated double bond. They occur in many edible plants and are biosynthetic precursors of flavonoids [5, 15]. Their synthetic simplicity is a strong attraction, since both rings can be altered independently and in large numbers. Dimmock and co-workers catalogued the bioactivities of chalcones, from antimicrobial to anti-inflammatory and cytotoxic, and tied many of them to the reactive enone, which can accept a thiol in a Michael addition [28]. Reviews of the scaffold describe it as privileged, meaning that it yields hits against many unrelated targets [6, 15].

In cancer the best-characterised mechanism is interference with microtubules. Ducki and colleagues showed that appropriately substituted chalcones, in particular those with a trimethoxyphenyl ring and an alpha-methyl group, inhibited tubulin polymerisation and arrested cells in mitosis at low concentrations [4, 16]. Microtubules are a validated target, as the taxanes and vinca alkaloids demonstrate, and drugs that bind the colchicine site are of continuing interest because some escape common efflux-based resistance [26]. Other reported actions of chalcones include inhibition of NF-kappaB signalling, induction of apoptosis, generation of reactive oxygen species and effects on angiogenesis, but the evidence for these is less uniform [5, 6].

Limitations of the plain chalcone scaffold explain why chemists modify it. Many natural chalcones have modest potency, with cell-based inhibitory concentrations in the micromolar range, and their solubility is often poor [5, 15]. The enone is a double-edged feature. It contributes to activity, but it also reacts with glutathione and other thiols, giving poor selectivity and a risk of off-target toxicity [27, 28]. Medicinal chemists have tried to tune this reactivity by changing ring substituents or by attaching heterocycles, and coupling to a triazole is one of the commonest of these strategies because the chemistry is dependable [7, 8].

4. TRIAZOLE LINKERS AND HYBRID DESIGN

The 1,2,3-triazole ring is a five-membered aromatic heterocycle with three adjacent nitrogen atoms. It is resistant to hydrolysis, oxidation, reduction and enzymatic cleavage, has a sizeable dipole moment, and can accept hydrogen bonds through its nitrogens while the C-H bond can act as a weak donor [8, 9]. Bonandi and colleagues reviewed its use as a bioisostere, noting that the 1,4-disubstituted ring mimics the geometry and electronics of a

trans amide bond but does not share the amide's vulnerability to proteases [19]. These features explain why the ring is used both as a stable connector and as a contributor to binding in its own right.

Hybridisation is the design principle that unites the two scaffolds. Viegas-Junior and colleagues defined molecular hybridisation as the combination of pharmacophoric subunits from different bioactive molecules into one structure, with the aim of improving affinity or efficacy, or of acting on several targets [11]. Morphy and Rankovic's account of designed multiple ligands set out the promise and the pitfalls: a hybrid can overcome resistance if both halves matter, but the larger molecule may have worse properties than either part [10]. For chalcone-triazoles the usual idea is that the chalcone supplies antimitotic or electrophilic activity, and the triazole-linked second fragment adds binding contacts or physicochemical benefit.

Design in practice follows a few patterns. In the first, a hydroxychalcone is converted into a propargyl ether, which is then coupled with a series of aromatic or aliphatic azides. In the second, the chalcone carries the azide and the partner holds the alkyne. In the third, a triazole-bearing aldehyde or ketone is built first and then condensed to form the chalcone itself [7, 8, 31]. The length and flexibility of the linker between the chalcone and the triazole vary between papers, often from one to several methylene units, and are sometimes treated as variables in the structure-activity analysis. Drug-likeness is commonly checked with polar surface area calculations or web calculators before synthesis, though these are guides and not tests [32, 33].

5. SYNTHESIS AND CHARACTERISATION

The chalcone core is generally made by Claisen-Schmidt condensation, the base-catalysed reaction of an acetophenone with an aromatic aldehyde in ethanol or methanol. Aqueous sodium or potassium hydroxide is the usual base, and the reaction is run at room temperature for several hours or overnight [15, 16]. The product often precipitates on acidification or on pouring into ice water and is purified by recrystallisation. Yields are frequently good, though electron-rich aldehydes react more slowly. Side reactions such as Michael addition of the enolate to the product, self-condensation of the ketone and Cannizzaro reaction of the aldehyde can lower yield, and they are managed by controlling stoichiometry and temperature [15].

Attachment of the triazole relies on the copper-catalysed azide-alkyne cycloaddition. The thermal Huisgen reaction gives a mixture of 1,4- and 1,5-regioisomers and needs heat, whereas the copper-catalysed version, described independently by Rostovtsev and co-workers and by Tornøe and co-workers in 2002, proceeds at room temperature and gives the 1,4-disubstituted triazole exclusively [17, 18]. The reaction fits the click chemistry concept introduced by Kolb, Finn and Sharpless: modular, high yielding, tolerant of water and oxygen, and generating little by-product [7]. A common recipe uses copper sulfate with sodium ascorbate in a tert-butanol and water mixture, though copper iodide or preformed copper(I) complexes are also used [17, 31].

Azides are prepared from alkyl or benzyl halides with sodium azide, or from anilines by diazotisation, and need careful handling because low-molecular-weight organic azides can be explosive. Reviews of click chemistry in medicinal chemistry describe a wide scope for the reaction [8, 31]. After coupling, the product is usually isolated by filtration or extraction and purified by chromatography or recrystallisation. Characterisation relies on NMR, where the triazole proton gives a singlet at around eight parts per million, along with mass spectrometry and infrared data. A point of concern is residual copper, which is cytotoxic and can create false positives in cell assays unless removed by chelation or washing [17, 30].

Chalcone geometry deserves attention. Claisen-Schmidt products are overwhelmingly the E isomer, recognised by a coupling constant of about fifteen hertz between the vinyl protons, and that geometry matters for biological activity [15, 16]. Photoisomerisation to the Z form can happen in solution under light, which would alter the

pharmacology of a stock stored on a bench. The structures in many papers are confirmed only by routine spectra, with crystal structures reported for a minority. Purity data from HPLC are a better guard against artefacts than elemental analysis alone, since small amounts of starting chalcone, which is itself cytotoxic, would easily mimic the effect of the hybrid [27, 28].

6. IN VITRO EVALUATION AGAINST MCF-7

Cytotoxicity is almost always judged first by a colorimetric viability assay. The MTT assay measures reduction of a yellow tetrazolium salt to a purple formazan by metabolically active cells and with the coloured product accumulating largely in lipid droplets and not only in mitochondria [20]. It is quick and suited to multi-well plates, but it measures metabolic activity, not cell number, and compounds that alter mitochondrial function can distort it. Twentyman and Luscombe showed that seeding density, incubation time and medium conditions all shift the signal, adding a further layer to interpretation [21]. The sulforhodamine B assay, which stains cellular protein and is used in the NCI-60 screen, is less affected by metabolic changes, which is why many consider it more robust [22, 23, 24].

Typical protocols expose cells to a range of concentrations of the test compound, commonly in half-log steps, for forty-eight to seventy-two hours, and then calculate the concentration giving fifty percent growth inhibition. The estimate depends on the number of concentrations, on replicates, and on the curve-fitting model, and Sebaugh set out guidelines that call for a defined top and bottom of the curve and enough points to pin the middle [29]. Many papers report single values without confidence intervals and base conclusions on differences that may lie within experimental error. Solvent control is another detail: the vehicle, usually DMSO, must be kept at a low and constant final concentration [29, 30].

Once a hybrid shows activity, follow-up assays are meant to indicate how the cells die. Annexin V staining with a DNA dye, analysed by flow cytometry, distinguishes early apoptotic from late apoptotic and necrotic cells, following the approach of Vermes and colleagues [25]. Cell cycle analysis by propidium iodide staining identifies arrest in G2/M, the expected result for antimitotic chalcones [4, 26]. Some papers add caspase activity, mitochondrial membrane potential, reactive oxygen species and Western blots for apoptotic proteins. Evasion of apoptosis is one of the recognised hallmarks of cancer, so induction of programmed cell death is a reasonable goal for any new agent [34]. Still, assays that show apoptosis do not show which target caused it.

Two-dimensional viability tests are increasingly supplemented by assays that probe longer-term or more tissue-like behaviour. Colony formation tests whether cells that survive a short exposure can still found new colonies, scratch or wound assays follow migration, and three-dimensional spheroids reproduce some of the diffusion barriers of a tumour mass [14, 23]. MCF-7 forms compact spheroids readily, and drugs that are potent in monolayers often lose strength in them, so spheroid data give a more cautious estimate of potential. Such assays are used in a minority of chalcone-triazole papers. Where they do appear they tend to confirm the direction of the monolayer result while showing a smaller effect [14, 34].

7. STRUCTURE-ACTIVITY AND MECHANISTIC TRENDS

Across series, a handful of structural trends are reported. Methoxy groups on the chalcone A ring, especially the 3,4,5-trimethoxy pattern, are associated with potent antimitotic activity, in line with the earlier chalcone work [4, 16]. Halogen and nitro substituents on the B ring or the azide-derived aryl group often lower inhibitory concentrations, perhaps through increased lipophilicity or altered electronics at the enone [6, 9]. Linker length changes results unpredictably. In some series a short linker is best and in others a longer, more flexible one. These tendencies come from small, congeneric sets, and exceptions are common, so they should be treated as rules of thumb [6, 19].

Reported potency against MCF-7 spans a wide range, from low micromolar or below

for the most active hybrids to tens of micromolar for many others. How to judge such numbers is not obvious, because the reference drugs used for comparison differ. Doxorubicin acts by DNA intercalation and topoisomerase II inhibition and is far more potent than most synthetic hits, whereas tamoxifen acts through the oestrogen receptor, so a hybrid that appears 'comparable to tamoxifen' may be compared on a very different footing [2, 3, 13]. The entries in table 1, when counted by group (fig. 1), are dominated by chemistry and assay topics, which reflects where secure knowledge lies: how these compounds are made and measured, rather than what they do in animals [20, 22].

Chemical and metabolic stability are seldom measured, though they decide whether a hit is worth following. The triazole is robust, as noted above, but the enone is not, and a chalcone-triazole may be conjugated to glutathione in cells or plasma and cleared quickly [9, 19, 28]. Reports of stability in buffer, plasma or liver microsomes, and of the formation of thiol adducts, would show how long the active species survives. Some groups argue that moderate thiol reactivity is acceptable, even useful, in oncology, as covalent drugs have shown. The counter-argument is that reactivity should be deliberate and selective, whereas that of a simple enone is neither [27, 28].

8. LIMITATIONS AND FUTURE DIRECTIONS

The literature has recognisable weaknesses. Most studies stop at two-dimensional cultures of one or two cell lines and report inhibitory concentrations after a single time point. Selectivity data, stability in medium and plasma, and solubility are often missing. Reactivity of the enone towards thiols is rarely measured, even though it governs both activity and toxicity [27, 28]. Positive controls vary, and independent replication of the activity of any named compound is uncommon. Publication practice favours positive findings, so the proportion of hybrids that fail is unknown. These shortcomings do not make the chemistry unattractive, but they limit what can be concluded about therapeutic prospects [14, 29].

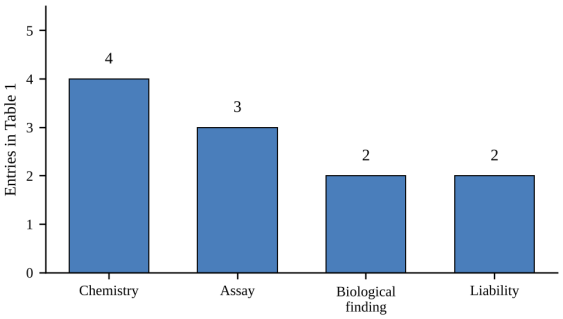


Fig. 1: Number of entries in table 1 by topic group

Reporting practice could also be tightened. Papers would be easier to compare if they stated the cell source and passage range, the seeding density, the exposure time, the vehicle concentration and the number of independent experiments, and if they gave full curves in supplementary files. Statements such as 'highly potent' should be tied to a defined threshold. The synthetic sections would gain from reporting copper content in final samples and purity by HPLC, given the cytotoxicity of both copper and the starting chalcone [17, 27, 30]. None of this requires new science. It asks only that authors give readers what they need to judge whether a result will hold up in another laboratory [20, 29].

Progress would come from a more disciplined pipeline. Compounds should be tested in several breast lines with different receptor status and in non-malignant cells; reactivity with glutathione and stability in plasma should be measured; and at least one target-directed assay should support any mechanistic statement. In silico ADME prediction with tools such as the BOILED-Egg model can screen out poorly drug-like candidates early [32, 33]. Three-dimensional spheroid models and, for the best compounds, animal pharmacokinetics and xenograft studies would show whether in vitro potency translates into exposure and effect. Until such data exist, chalcone-triazole hybrids remain promising leads and not candidates [26].

9. CONCLUSION

Chalcone-triazole hybrids combine two scaffolds with a long record in anticancer chemistry, and the synthetic route, a Claisen-Schmidt condensation followed by

copper-catalysed cycloaddition, is simple, modular and well documented. Published papers consistently report inhibition of MCF-7 growth, often with cell cycle arrest and apoptosis, and the best-supported mechanism is interference with tubulin polymerisation, as in earlier work on antimitotic chalcones [4, 7, 16, 17, 26]. Cell line choice, assay type and curve fitting each shape the numbers that are quoted [14, 20, 29].

The weaker part of the evidence is biological. Selectivity, thiol reactivity, stability, target engagement and exposure are poorly covered, and MCF-7 alone cannot represent breast cancer [13, 14, 27]. Future reports should use several cell lines, include non-malignant controls, authenticate cultures, apply robust curve-fitting and confirm mechanism with direct assays [20, 29, 33]. With that discipline the series could yield credible leads; without it the literature will keep adding potent-looking compounds of uncertain value [31].

10. ABBREVIATIONS

CuAAC-Copper-catalysed azide-alkyne cycloaddition, MTT-3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide, SRB-Sulforhodamine B, IC50-Half-maximal inhibitory concentration, ER-Oestrogen receptor, NMR-Nuclear magnetic resonance, HPLC-High-performance liquid chromatography, DMSO-Dimethyl sulfoxide, ADME-Absorption, distribution, metabolism and excretion, NCI-National Cancer Institute

11. FUNDING

Nil

12. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

13. CONFLICT OF INTERESTS

Declared none

14. REFERENCES

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