Current Trends in Biotechnology and Pharmacy
Vol. 17 (4A) 8 - 14, October 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.4s.84

Review: Exploring the potential of Designed Multiple Ligands (DML) strategy with quinolones as anticancer

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Abstract

Multi-target-directed ligands (MTDL) or Designed Multiple Ligands (DML) use a single chemical substance to affect several ligands or targets associated with a disease to boost efficacy or safety. In recent studies, many novel quinolones have adapted this strategy by targeting many cancer ligands, including topoisomerase, tyrosine kinase, tubulin polymerisation, and formation of G-quadruplex. Moreover, the effectiveness of anticancer quinolones has been improved by the conjugation of compounds with metal complexes, such as ruthenium (III), boron, and copper (II). In the case of dual inhibitors, most of the substances target topoisomerases along with additional targets such as histone deacetylases, telomerase, microtubules, kinases, heat shock protein 90 (Hsp90), aldehyde dehydrogenase 1 (ALDH1) and proteasomes. Some of these hybrids, such as CX-5461, Q84441, and A-74441, have been shown to be effective against solid tumors with improved safety profiles. In this review, the current quinolone hybrids and DML strategy against a range of targets will be examined with the hope that the insights will aid in the development of novel quinolone derivatives for cancer treatment.

Keywords: quinolones, anticancer, topoisomerase inhibitor, tyrosine kinases, DML, hybrids

Introduction

Every year, millions of people lose their lives to cancer. With more than 277 different types of cancer, this disease remains a leading cause of morbidity and mortality worldwide, despite advances in early detection and treatment (1). The prevalence of cancer continues to rise owing to multiple factors contributing to this disease, such as obesity, air pollution, tobacco use, increased life expectancy, and cancer-causing infections (2).

According to demographic trends, there will be 15 million annual deaths by 2030 with 28.4 million new cases diagnosed by 2040 (3). As a result, the cancer crisis jeopardizes public health and undermines healthcare systems globally (4). The cancer survival statistics for patients with advanced cancer continues to be alarming despite improvements in high-resolution diagnostic equipment, tumor ablation techniques, medication cocktail compositions, and targeted therapies. The likelihood of metastasis plays a substantial role in the rise of cancer mortality (2).

There are two types of cancer treatment modalities: traditional (conventional) and advanced (modern or novel). Conventional treatment consists of surgery followed by radiotherapy and/or chemotherapy. Modern approaches involve stem cell therapies, angiogenesis inhibitors, immunotherapy, dendritic cell-based immunotherapy, and hormone therapy (5).

Both modalities involve a combination of multiple therapies to deliver effective treatments and reduce resistance to cancer cells. For instance, using multiple drugs in advanced cancer causes a high degree of cancer cell death when there is a low correlation or cross-resistance between the drugs (6). This means that the mechanism of action of each drug must be independent of each other and may target different sites.

Resistance issues and combination therapy

Drug resistance in cancer occurs when there is increased tolerance to drugs in cancer patients. The development of resistance to anticancer medications is influenced by various mechanisms, including cellular and molecular mechanisms, changes in epigenetic, genetic mutations, increase of drug efflux, and other factors. It can be classified as acquired or intrinsic resistance depending on the manifestation time. Intrinsic resistance exists prior to drug therapy, whereas acquired resistance is induced after exposure to therapy. The latter usually reduces the efficacy of drug treatments (6).

Several factors may contribute to intrinsic resistance, such as 1) activation of intrinsic pathways to counteract environmental toxins, 2) pre-existing genetic mutations that reduce cancer cell response to chemotherapy or target drugs and 3) tumour heterogeneity, which can trigger relapse in the later phases of therapeutic therapy (6). There is limited success with cancer chemotherapy with monotherapy or single-targeted agent drug due to non-selectivity and resistance problems. Combination therapies have been developed to overcome these limitations (7).

Genetic and/or epigenetic changes in cancer result in dysregulation of several biomolecular pathways Therefore, three strategies have been developed for combination therapy: (a) cocktail drugs, which combine two or more drugs which are being used synergistically, (b) promiscuous compound or multiple target drugs, and (c) hybrid compounds that can inhibit multiple cancer targets (3). Although the first approach enhances cancer treatment, it still has some drawbacks, such as tumour heterogeneity, unpredictable pharmacokinetic (PK) safety profiles, adverse effects of drug-drug interactions, and poor patient adherence (7).

On the other hand, the second strategy exhibits a wide range of biological activities with potential adverse reactions (8). Due to the limited efficacy, resistance, or toxicity associated with single-target or combination therapies, the development of multi-targeting medications has become more popular in recent years. (3). Thus, the third strategy is currently being adopted in many types of research as it possesses several advantages, such as improved bioavailability, superior pharmacokinetic and pharmacodynamic parameters, and more predictable metabolism (9). Therefore, hybrid or designed multiple ligands (DML) have emerged as appealing alternatives.

Designed multiple ligands (DML) as anticancer

DML is a single chemical that modulates several different biological receptors or targets a few biological pathways in a selective manner (10). DML is also known in numerous terms, including multiple-target directed ligands (MTDL), triple blockers, pan-agonists, heterodimers, hybrids, promiscuous medications (11), conjugates, dual ligands, codrugs, and mutual prodrugs (12). However, most current anticancer research uses hybrid or dual ligands that target two different sites.

Classification of DML drugs

There are two approaches to the concept of DML drugs in terms of how they are linked. The two approaches are either by pharmacophore combination or combining two or more entire drugs (1). In pharmacophore combinations, this approach can be classified into three types: cleavable conjugates, fused/conjugated pharmacophores, and merged/overlapping pharmacophores (11).

Cleavable conjugates comprise an ester linkage cleaved by plasma esterase (Figure: 1). It then releases two separate medicines that function independently.

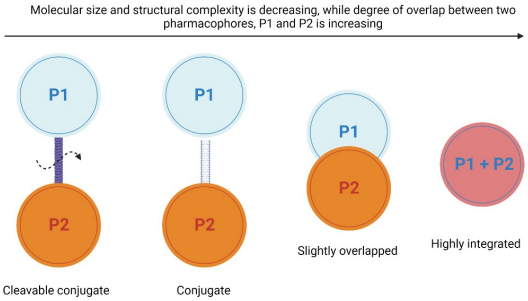

Figure: 1. Pharmacophore combination approaches in the DML. Some pharmacophores are highly integrated, whereas others are combined via cleavable or non-cleavable linkers. Created with BioRender.com

Conjugated pharmacophores combine two inhibitors via a linker or without a linker (11).

The second approach, which involves a combination of all drugs, can be performed by directly linking two drugs (fusion), merging the drugs, or connecting two drugs using a rigid or flexible spacer (12). Molecular hybridisation is also used to describe the idea of combining two pharmacophoric moieties to create a hybrid agent (13).

In anticancer therapy, most of the hybrids that modulate chemotherapy drugs use cleavable spacers or linkers, such as taxol or paclitaxel (PTX) with either epipodophyllotoxin (EP) or camptothecin (CPT) (Figure: 2), and 5-fluorouracil (5-FU) linked with cytarabine (12).

This is supported by another study that showed five novel conjugates of PTX-CPT with imine linkages were having good inhibitory activities against HCT-8 and MCF-7 cancer cells. Interestingly, these hybrids have also shown significant *in vitro* toxicity in several prostate cancer cell lines (PC3, LNCaP) sparing the non-cancerous cell lines (14).

Compared to other taxol (TXL) conjugates, compound 3 (50% effective dose, ED_{50} = 0.73 nM) and compound 4 (ED_{50} = 0.98 nM) possess significant anti-angiogenesis activity (14). Such study was

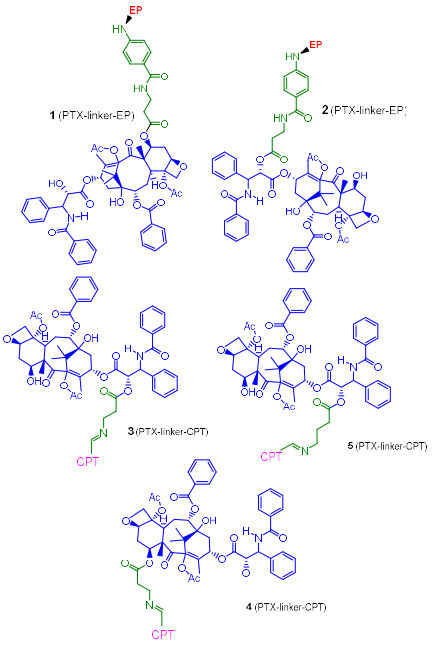

Figure: 2. Hybrid agents with linked spacers (cleavable) such as paclitaxel (PTX) with either epipodophyllotoxin (EP) or camptothecin (CPT). All the linkers are highlighted in green.

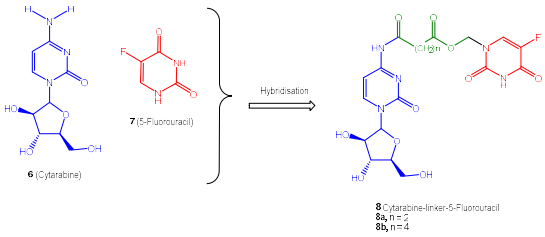

Figure: 3. DML of cytarabine with 5-fluorouracil.

the initial research which leds to hybridisation of various novel quinolones for anticancer especially in the modification of fluoroquinolone (FQ) derivatives.

Aside from these, there have been attempts to conjugate cytarabine (ara-C) with 5-FU using an amide group as a linker (Figure: 3). However, the hydrolysis of this linker was too slow. Thus, necessitating the use of a cellular enzyme to liberate release of ara-C. It was also discovered that the 5-FU attachment site of

Compound 8a was labile. In contrast, Compound 8b was found to be chemically stable at both 5-FU and ara-C attachment sites (15).

A new generation of prodrugs is trying to improve the biological activity of 5-FU while improving its delivery to cancerous tissue at the same time. Seven types of 5-FU hybrid with improved biological activities were conjugation with histone deacetylases (HDAC), deoxy- podophyllotoxin, ubenimex, oxaliplatin, parthenolide, pentacyclic triterpenes and Heme Oxygenase-1 (HO-1) inhibitor. These hybrids enhance proliferative activities, reduce side effects, and try to overcome the 5-FU resistance (16).

Apart of these, three hybrids were designed to target different sites of cancer tissues such as bone, mitochondria, and integrin (16). All the attempts addressed a useful strategy for overcoming the lack of site-specificity. However, some limitation faced by these 5-FU hybrids were chemical stability, bioavailability, and administration route (15,16).

Multiple Ligands in Cancer

Most of the new innovative anticancer therapy has dual targets, also known as hybrid or conjugate drugs. Recent studies showed that multiple molecules used in tandem targeting has been demonstrated to be effective in cancers such as using HDAC inhibitor in combination with other pharmacophores such as tyrosine kinase (TK) inhibitors, topoisomerase (TOP) inhibitors, Poly (ADP-ribose) polymerase (PARP), Janus kinase (JAK), Bromodomain and Extra-Terminal motif (BET) proteins inhibitors and B-cell lymphoma 2 (Bcl-2) (17). Interestingly, other than HDAC, combination of inhibitors is also discussed with TOP inhibitors with other target such as microtubule, protein kinases, telomerase, heat shock protein 90 (Hsp90), and proteasome (18).

Interestingly, quinolone derivatives were shown to be the most modulated scaffold as anticancer hybrids. A review done by Panda and Chakroborty found that the moiety of quinoline scaffold offers a good strategy for development of anticancer agents. The hybridisation of various pharmacophores showed good inhibition of different cancer cell lines. Among the compounds discussed, four of them showed good anticancer properties with nanomolar level of 50% inhibitory concentration (IC_{50}) (19).

Compound 9 (IC_{50} = 31.8nM) was showing a good inhibition against colorectal adenocarcinoma cell line (DLD-1) and may act as epidermal growth factor receptor (EGFR) inhibitor, while Compound 10 (IC_{50} = 7nM) was showing a significant aldehyde dehydrogenase (ALDH) inhibitory effect (20).

There has been a correlation between cancer relapse and the expression of these ALDH enzymes in many human cancers (21). Compound 11 shows good inhibition against non-small cancer cells (IC_{50} = A549: IC_{50} = 3.91 µM, and leukaemia cell lines K562: IC_{50}=1.91 µM) (22).

This quinoline-chalcone hybrid (Figure: 4) has the potential to act as PI3K inhibitor (22).

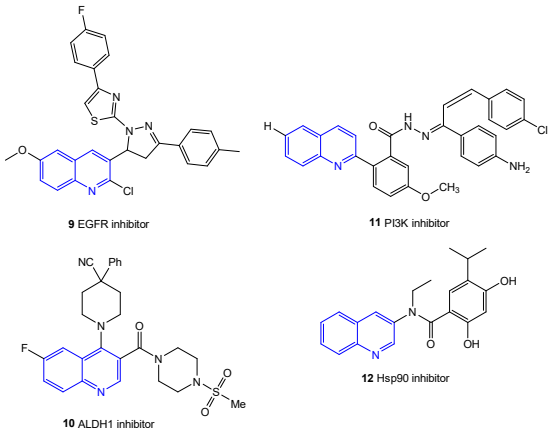

Figure: 4. Quinolines derivatives by hybridisation of different pharmacophores with multiple targets in cancer such as epidermal growth factor receptor (EGFR), phosphatidylinositol 3-kinase (PI3K), aldehyde dehydrogenase 1 (ALDH1) and heat shock protein 90 (Hsp90).

Compound 12 was evaluated against several cancer cell lines, including PC-3, Hep3B, and HCT116 (IC50 = 0.14 to 0.33 M). This compound was also discovered to inhibit Hsp90 with an IC50 of 149 nM (23). It appears that the quinoline's anticancer action is enhanced by the presence of CH_3, OCH_3, and Cl at either C-3 or C-4 or C-6 position (19).

Hybrid quinolones as anticancer agents

There have been multiple reports of antibacterial fluoroquinolones (FQs) being converted to anticancer analogues through structural changes and hybridization. Most of these approaches involved modifying the carboxylate group at position 3, the piperazine moiety at position 7, or both sites (24). Some quinolone hybrids, such as quinolone-pyrazine (CX-5461), quinolone-benzoxazine (A-84441), quinolone-pyrrole (15) and quinolone-azoles (symadex, C-1305, 18, 19a and 19b) (25).

Several *in vivo* studies have demonstrated excellent safety profiles for these hybrids. For example, a significant delay in tumour growth (TGI of 84%) was achieved when CX-5461 was administered at a dose of 25 mg/kg. It is interesting to note that A-84441 is active against 7 of 9 solid tumours, including human tumour xenografts. The anti-leukemic effect of A-84441 in murine models is over 10-fold greater than that of normal murine bone marrow cells (25).

In addition, a dose-dependent inhibition of tumour growth was observed after intraperitoneal (IP) administration of CX-5461. Daily IP administration with a dose of 50 mg/kg was also tested using Quinolone-pyrole (15) where a TGI of 65% was observed. A similar dose was used on Kun-ming mice for hybrids 19a and 19b, with no significant toxicity occured. In CX-5461, 19a, and 19b, animal body weights did not change significantly, and no other adverse effects were reported (25).

Therefore, these hybrids have shown promising *in vitro* and *in vivo* efficacy as

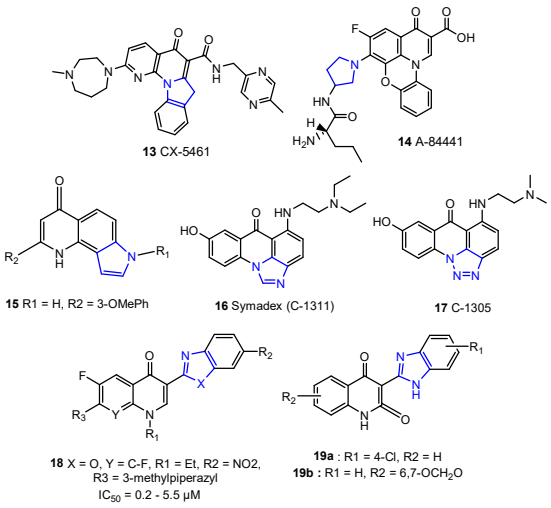

Figure: 5. Quinolones hybrids that are currently in preclinical studies as anti-cancer agents.

anticancer, and safety characteristics. Interestingly, all these hybrids possess azole groups (Figure: 5). As a result, hybrids with azole may provide ideal starting points for future study.

Most of the recent modifications involved the hybridisation of quinolone core with other pharmacophores or dimerisation of quinolone or quinoline. As a result, a few novel quinolones have been developed to target different ligands in cancer, such as topoisomerase, tyrosine kinase, tubulin polymerisation, G-quadruplex (18). It was also found that the the potency of anticancer quinolones can be enhanced by conjugation with metal complexes such as ruthenium (III), boron, and copper (II). More metal complexes have also shown potential to be used as topoisomerase inhibitors (26).

Conclusion

Using numerous medications for cancer creates a high level of cancer cell death. Thus, DML is an effective method of administering these drugs. However, multiple limitations, including as toxicity, bioavailability, and mode of administration, create

impediments. Quinolone hybrids with distinct pharmacophores are currently exhibiting strong anticancer potential. Furthermore, the azole composition is effective against a variety of cancer cell types. As a result, future research could look into improving the structures highlighted in this review to enhance their activities for cancer treatment.

Conflict of Interest

The authors declare no conflict of interest.

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