Synthetic duocarmycins: structural evolution from SAR to prodrugs and ADCs - a searchable structure/function database

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ABSTRACT: Synthetic analogues of the DNA-alkylating cytotoxins of the duocarmycin class have been extensively investigated in the past 40 years, driven by their high potency, their unusual mechanism of bioactivity, and the beautiful modularity of their structure-activity relationship (SAR). This minireview analyses how the molecular designs of synthetic duocarmycins have evolved: from (1) early SAR studies, through to modern applications for directed cancer therapy as (2) prodrugs and (3) antibody-drug conjugates in late-stage clinical development. Analysing 583 primary research articles and patents from 1978-2022, we distill out a searchable A0-format "Minard map" poster of ca. 200 key structure/function-tuning steps tracing chemical developments across these three key areas. This structure-based overview showcases the ingenious approaches to tune and target bioactivity, that continue to drive development of the elegant and powerful duocarmycin platform.

1. Introduction

The natural products CC-1065 and duocarmycin SA are irreversible DNA alkylators that react after docking in the minor groove. Since their isolation from *Streptomyces* in the 1980s,[1,2] their picomolar cytotoxic potency has attracted continuous attention. Several total syntheses have been reported,[3–5] and biochemical research has shown how their site-selectivity of DNA alkylation depends on structural features and stereochemistry.[6,7] Clinical drug[8] and prodrug[9] candidates for cancer treatment quickly advanced to phase I and II clinical trials.[10–13] Even after initial trials were discontinued due to narrow therapeutic index or strong side effects, an entire "duocarmycin family" of synthetic analogues, with a broad range of aims and applications, have been pursued. Our aim in this minireview is to distill this diversity of duocarmycin development into a rapidly grasped, yet comprehensive, format.

Medicinal chemistry around duocarmycins has focused on three key areas. **(1) SAR** studies have explored the relationship of pharmacophore structure to DNA alkylation, and simplified synthetic analogues such as the cyclopropabenz[*e*]indoles (CBIs)[14] have been developed, to retain the parent functionality while offering greater chemical tractability.[15] **(2) Prodrugs** aiming to direct activity better towards target cancer cells, have explored activatable alkylation motifs and bifunctional conjugates.[16] **(3) Antibody-drug conjugates** (ADCs) using duocarmycin-based cytotoxic payloads have also been developed for improved targeting, and in this incarnation the first duocarmycin derivative was recently FDA-approved.[17]

In this Perspective we present a systematic literature review (SLR;[18] **Figure 1a,** details in **Supplementary Information**), aiming to collate most of the prolific research on duocarmycins, for rapid analysis. We create a database classified according to research focus, use it for meta-analysis, and provide it for future researchers with an interest in the field to orient their molecular designs. We do so by providing a searchable, dynamic datafile in A0 poster format (**Figure 1b**; Poster S1) which classifies and analyses structural features and design principles with a focus on duocarmycin family (1) SAR, (2) prodrugs and bifunctional conjugates, and (3) ADCs.

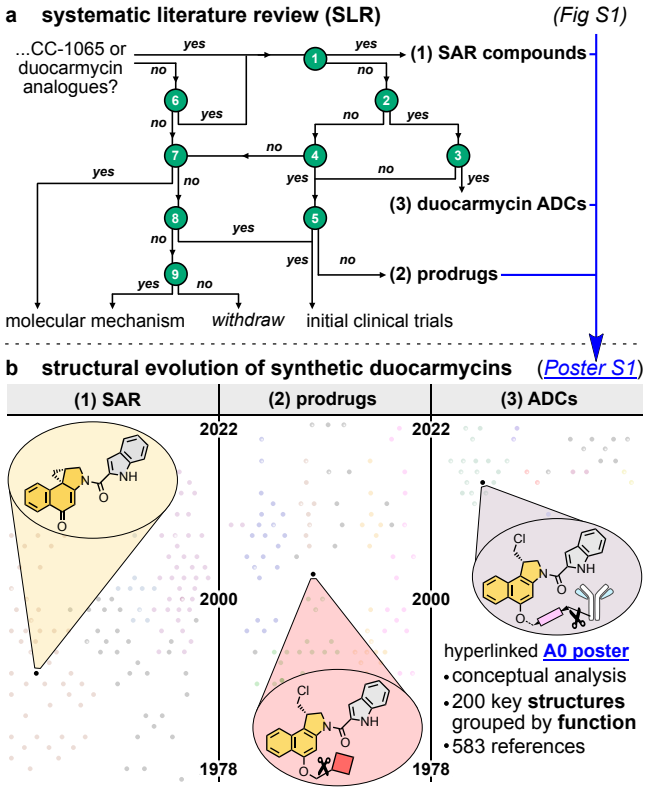

Figure 1. (**a**) Systematic literature review (SLR). Research items were classified according to a nine-point scheme, then all classed items were structurally analysed. (**b**) Cartoon of the A0-sized poster (Poster S1) that summarises the structural evolution of >200 duocarmycin-derived agents and conjugates from SAR studies to prodrugs and ADCs.

2. Systematic literature review (SLR)

SLR[18] was conducted to collate and group the vast majority of experimental literature concerning duocarmycins. Two groups with low structural diversity were split off: (a) reports of the isolation, characterisation, and mechanism of action of natural products structurally related to CC-1065; and (b) reports of preclinical and clinical trials of early cancer drug candidates. Three groups with high structural diversity are analysed here: **(1: SAR)** synthesis and cellular evaluation of derivatives in structure-activity-relationship (SAR) studies; **(2: Prodrugs)** synthesis, evaluation and/or therapeutic use of prodrugs, mainly based on bioactivation of the *seco*-duocarmycin latent alkylator functional unit, or of bifunctional small molecule conjugates bearing at least one (*seco*-)duocarmycin; **(3: ADCs)** synthesis, conjugation, and therapeutic efficacy of (multi)functional ADCs incorporating a synthetic duocarmycin or its *seco*-precursor.

Literature screening was first done by Boolean keyword search initiated with e.g. ["CC-1065" or "duocarmycin"] AND ["analog" or "prodrug" or "derivative"] then refined with more specific keywords (see **Supplementary Information**). From this, the major academic groups or pharmaceutical companies in each area of research were identified. For each group, all references reporting duocarmycin family agents were manually collected and categorized. Lastly, select recent reviews on specific topics within the field of duocarmycins[16,17,19–24] were harvested for additional references. Thus, a comprehensive duocarmycin structural library was assembled, from 583 reports - mainly of primary research.

2.1 Literature metrics

A bibliographic overview of this library is given in **Figure 2**. Of the 583 total research items, the vast majority were published in scientific journals (123 journals, 499 publications, 86%) covering all areas from basic biology, biochemistry, medicinal chemistry and molecular sciences, to physical chemistry, theoretical chemistry, and preclinical or clinical oncology. Major progress in chemical design and SAR has been published in chemistry (JACS 63, JOC 35, JMC 33, ANIE 13, Chem. Eur. J. 11) and bioorganic chemistry journals (BMC 38, BMCL 31); isolation and mechanism reports cluster in Biochemistry (21) and J. Antibiotics (13); and clinical results in oncology journals (Cancer Res. 16, Cancer Chemother. Pharmacol. 11, Mol. Cancer Ther. 9). 61 patents or patent applications filed by academic groups and pharmaceutical companies also entered this library (**Figure 2a**).

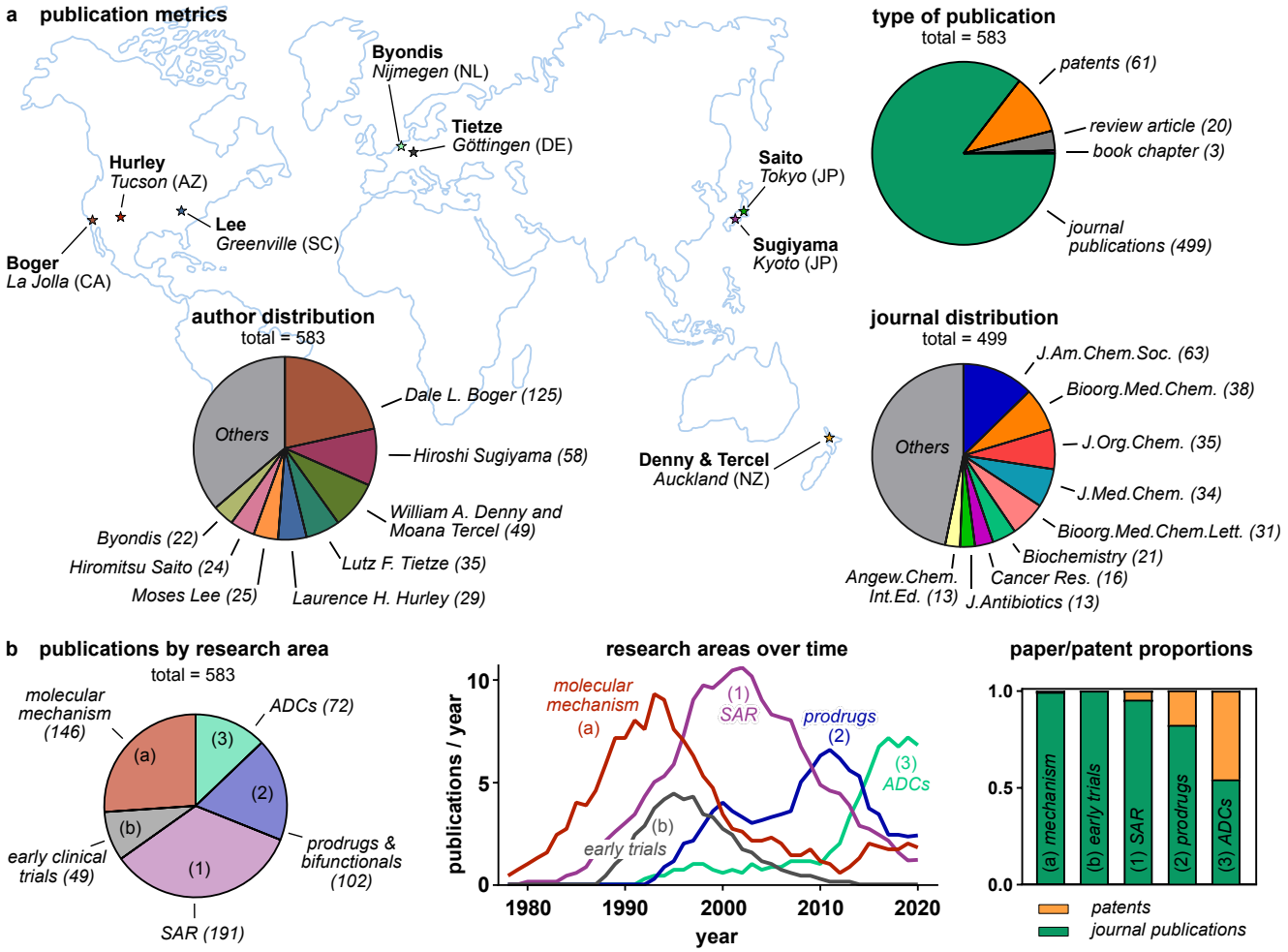

Figure 2. Literature metrics. (**a**) 583 primary research items form the literature database reviewed here; charts show the major research groups (>20 publications), their geographical locations, and major journals (>10 publications). (**b**) The literature was grouped as: (a) natural products, biochemistry, and molecular mechanism of CC-1065 and close analogues; (b) initial clinical trial compounds and reports; and then the focus groups of this Concept: **(1)** synthetic analogues and SAR studies; **(2)** duocarmycin-derived prodrug designs, and bifunctional conjugates; **(3)** duocarmycin-based ADCs and their therapeutic use. Histograms of these groups sorted by their year of publication reveal the temporal progress of duocarmycin research; and paper/patent ratios may indicate perceived commercialisation chances.

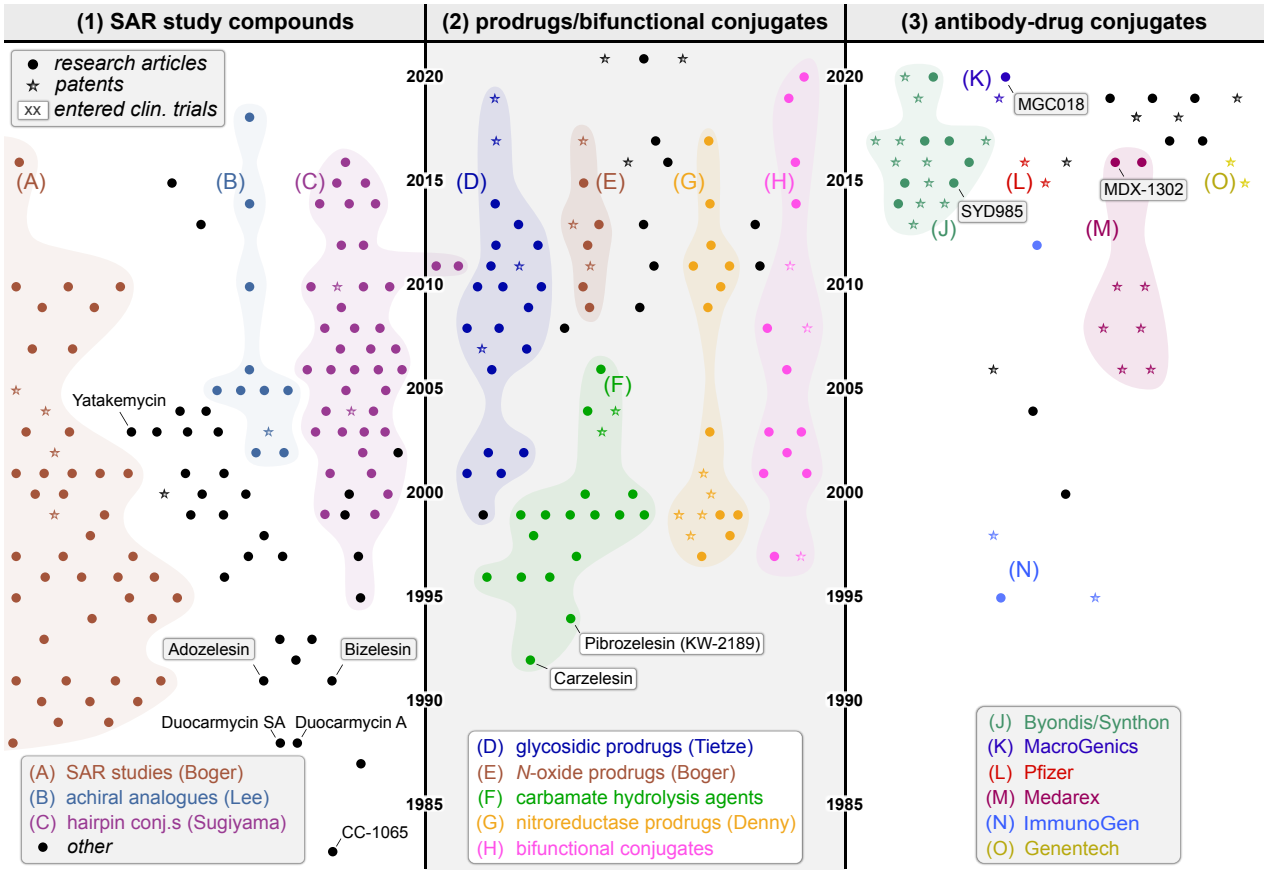

Figure 3. Timeline of the structural designs of duocarmycins (cartoon; all chemical structures in Poster S1). In Group 1 (SAR compounds), studies have resolved the molecular motifs crucial for rational tuning of bioactivity. In Group 2 (Prodrugs), non-natural activatable prodrugs (glycosides, nitroaryls, carbamates, *N*-oxides) and bifunctional conjugates have expanded the scope of duocarmycins. Industry is a main driver of research in Group 3 (duocarmycin-based ADCs).

2.2 Evolution of the focus of duocarmycin research

The sequence of duocarmycin development is easily visible after analysing the five report groups by date (**Figure 2b**). Isolation and early molecular mechanism studies (group a; 146 items) dominate the 1980s and 1990s, and have been key for further molecular designs. Rapidly following initial cytotoxicity studies, small molecule drugs (adozelesin and bizelesin) and hydrolytic prodrugs (carzelesin and pibrozelesin) were taken into initial clinical anticancer trials that were discontinued during the 2000s (group b; 49 items). Hurley (29), Krueger (17) and others were the major academic groups driving both these developments.

Exhaustive and creative structural variations during the 1990s and 2000s largely mapped the SAR in this molecular class (**Group 1, SAR:** 192 items) with major contributions by Boger (125), Sugiyama (58), and Lee (24). Innovation increasingly focused on targeting, with activatable prodrugs and bifunctional small molecule conjugates taking off during the 2000s and 2010s (**Group 2, Prodrugs:** 102 items) led by Denny and Tercel (49), Tietze (34), Saito (23) and others. Finally, since the 2010s, conjugates of duocarmycins with monoclonal antibodies (**Group 3, ADCs:** 71 items) have opened up a new future for this class of bioactives. Combining the tunable potency and molecular flexibility of the duocarmycins, with the potential for enriched delivery to cancers, has led to a new wave of duocarmycin antibody-drug conjugates in clinical trials, driven by Byondis B.V. (22), Medarex Inc. (7) and others. With their increasing therapeutic relevance, the share of patents in the last two areas of research is also significantly higher (**Figure 2b**).

3. Structural evolution of duocarmycin analogues

The structural evolution of duocarmycins across these groups can also be best understood along a time axis, that resolves both the stepwise and the disruptive innovations that have driven this field from 1978 to 2022. **Figure 3** is a cartoon representation showing a datapoint for each research item in the three focus groups (circle: journal; star: patent); the A0-size Poster S1 in the supplementary information maps these datapoints one-to-one onto representative chemical structures from each research item, colour-coded for functionality, and DOI-hyperlinked for access.

3.1. Group 1: SAR compounds

The lead natural product CC-1065 was isolated in 1978[1] and its first total synthesis was reported in 1988, laying the grounds for much synthetic development.[3] During the 1990s and 2000s, systematic variations of both the core alkylator motif ("segment A", typically an activated cyclopropane) and the DNA-docking motif ("segment B") led to our current understanding of the structural features that need to be arranged for DNA association and sequence-selective alkylation (succinctly described by Hurley[7]).

Many heterocyclic systems beyond the native cyclopropa[*c*]pyrroloindole (CPI) of duocarmycin SA[25] can serve as segment A. Much research has focused on the chemically tractable CBI, with optional substitutions;[14] cyclopropaindole[26] (CI) and others[27] also alkylate DNA with the reactivity trend (CBI~CPI>CI) (**Figure 4a**).

The activated cyclopropane electrophile must be in its native (*S*)-configuration for DNA alkylation[28,29], but high potency can be maintained with 'proagent' *seco*-variants, that use *in situ* intramolecular Winstein spirocyclisation to unfurl their activated cyclopropane, relying on the *para*-phenol.[30] Good leaving groups (-Cl, -Br, -OMs)[9,31] and several alternatives to the dihydroindole (5-, 6-, 7-membered rings)[32] are all tolerated. Alternatively, masking this phenol suppresses spirocyclisation[33]: a disruptive step that opened the door for rational tuning of prodrug candidates in later years (see below). The group of Lee also introduced achiral *seco*-variants that are similarly reactive, but structurally simpler and more accessible.[34,35]

Segment B heterocycles have mainly clustered around indole-based rings that strengthen DNA binding. Stepwise simplification of the native dimeric segment B (in CC-1065) gave variously the deoxygenated CDPI dimer,[36] 3,4,5-trimethoxyindole (TMI),[29] mono-alkoxylated (DEI)[37] or even simple mono/oligoindoles and other heterocycles[38] can also be used. These impact DNA binding, alkylation site-selectivity, and potency; but overall, the tolerance for segment B variance is high (**Figure 4a**).

Assembling the A and B segments has also received attention. A remarkable class of hairpin duocarmycin conjugates was driven by Lown and Sugiyama in the 2000s.[39] Using synthetic oligo-pyrroles/imidazoles from the minor-groove binder distamycin A as segment B binding domains, gave potent duocarmycin analogues allowing sequence-selective alkylation in specific areas of DNA.[40,41] 'Standard' duocarmycins consist of segments A and B linked by an amide bond, but the natural product Yatakemycin,[42,43] has revealed that multiple B segments may be used, and randomly shuffled around, without losing bioactivity.[44] Dimeric bisalkylators with two A segments, allowing interstrand DNA crosslinking, also give extremely high potency.[45,46]

3.2. Group 2: Activatable prodrugs and bifunctionals

Early trials already exploited duocarmycin prodrugs where *seco*-duocarmycin bioactivity was to be triggered *in situ* by unmasking a *para*-phenol, to avoid parasitic loss of a preformed cyclopropane *en route* to target tissues. These carbamate hydrolysis designs (Carzelesin,[47,48] Pibrozelesin/KW-2189[9,49]) were discontinued in clinical trials due to their side effects profile and low therapeutic index.[13,50] Follow-up work mined esters, solubilised carbamates, phosphates and others as other hydrolytic activation methods (**Figure 4b**),[51–56] although none of these promises any greater mechanistic selectivity for cancer.

Steps towards cancer-selective prodrugs were initiated by the lab of Denny in the late 1990s. They introduced nitro-*seco*-CBIs that can be irreversibly reduced to the amino-*seco*-CBI in the low-oxygen conditions found in solid tumors. These amines then undergo Winstein cyclisation becoming DNA-alkylators (**Figure 4b**).[57–59] In the early 2000s, Tietze developed glycosidic prodrugs that can be built modularly, aiming at antitumor uses relying on glycosidases.[37,60,61] Adopting novel chemistries in the 2010s, Boger introduced *O*-amino-*N*-acyl-*seco*-CBIs that are also subject to bioreductive activation.[62–64] The field of masked *seco*-CBIs has by now exploited the full arsenal of chemical biology, passing through reducible Co-complexes,[65] Fe(II)-reactive peroxides,[66] photoactivated designs,[67,68] and oxidisable naphthalenes.[69] Recently, cyclic dichalcogenides (that resist monothiol exchange, but can be reductively activated by specific oxidorectases like thioredoxin) have joined this panoply of prodrugs.[70,71] Finally, bifunctional conjugates of duocarmycins with other pharmaceuticals (glucuronide,[72] biotin,[73] antibiotics,[74] pyrrolobenzodiazepine (**Figure 4b**),[75,76] albumin,[77] peptides[78]) show the wide applicability and adaptability of this unique class of bioactives.

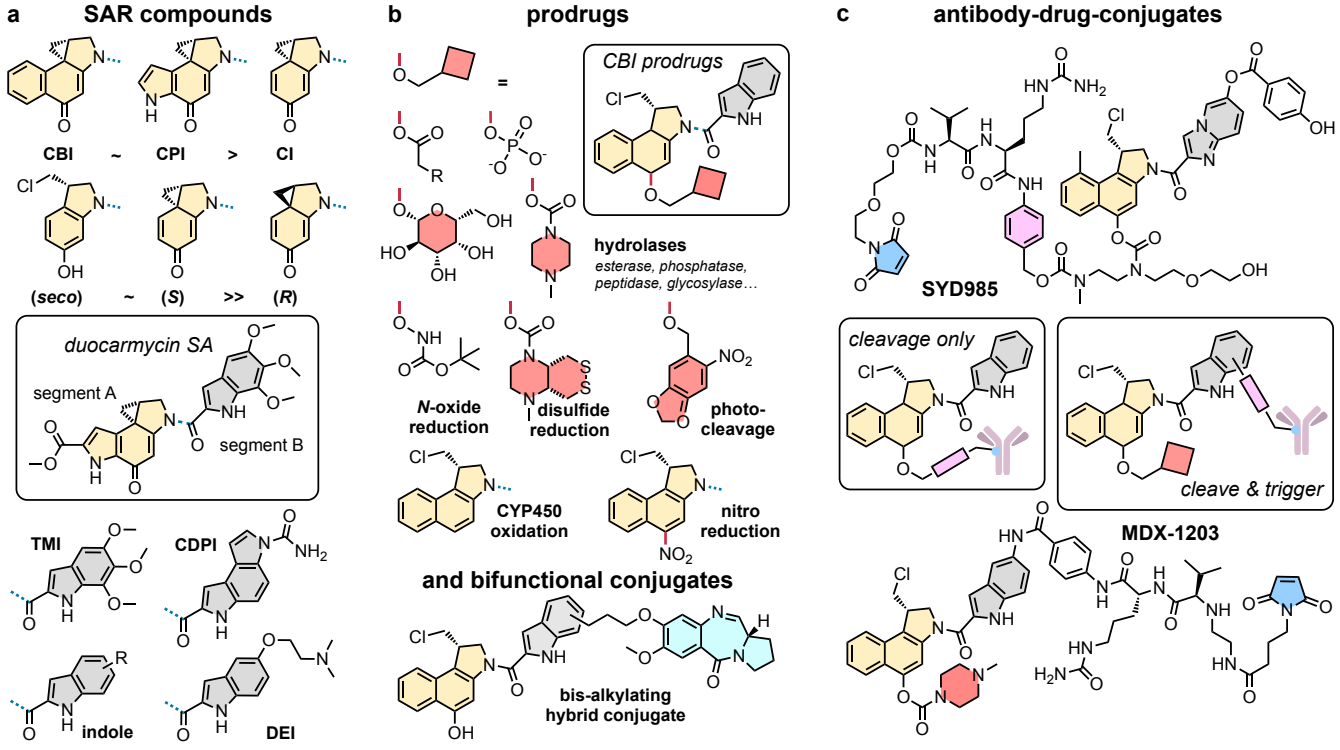

Figure 4 Structural elements of duocarmycin therapeutics. (a) SAR analysis: variations of segments A and B. (b) Activatable prodrugs: diverse strategies to trigger bioactivity. (c) Antibody-drug conjugates: CBI-ADCs including clinical candidates SYD985 and MDX-1302. See also **Poster S1**.

3.3. Group 3: Antibody-drug conjugates (ADCs)

There is general potential for monoclonal antibodies against suitably selective biomarkers of cancerous states, to deliver high-potency cytotoxic cancer drugs in a targeted and therapeutically effective manner. Given the duocarmycins' outstanding cytotoxic potency, they have long been the subject of ADC research, with two general designs emerging: either masking the *seco*-CBI phenol with a cleavable linker extending to the antibody, for spirocyclisation activation only after linker cleavage (Type A); or else attaching a phenolic prodrug of the duocarmycin to the antibody by a peripheral site, permitting an extra layer of targeting selectivity if pre-release activation can be avoided (Type B) (**Figure 4c**). Beyond biomarker and payload choice however, ADC development must balance factors from choice of the conjugation site and linker[79,80] to conjugation techniques.[81] Various self-immolative linkers have been used in Type A designs (dipeptides such as ValCit that are prone to lysosomal proteolysis, hydrolysable phosphates, reducible disulfides), often invoking cyclisation or elimination cascades to liberate the CBI phenol.[82–86] Designs for ADCs of Type B have been IP-protected by various pharmaceutical companies.[87–90]

The late-2000s rebirth of preclinical/clinical development in the duocarmycin class has been driven by these ADCs, with a variety of designs achieving *in vivo* efficacy in mouse cancer models.[56,91–95] The ADCs SYD985, MGC018 and MDX-1203 all reached clinical trials with promising results including high anticancer efficacy.[96–98] While MDX-1203 was denied approval due to insufficient improvement of therapeutic benefit compared to alternative therapeutics, SYD985 was recently given fast-track approval as a follow-up or co-treatment for patients with HER2-positive metastatic breast cancer.[99] This is the first duocarmycin therapeutic approved for use in man, and its success may spur the developments of the future.

4. Conclusions

Duocarmycins have undergone great development efforts across biochemistry, organic and medicinal chemistry, aiming to develop targeted, fine-tuned cancer therapeutics. A careful understanding of their unusual mechanism of bioactivity, leveraging spirocyclisation and docking to give high-potency and site-selective DNA alkylation, has enabled creative approaches exploiting them as a modular bioactive platform. Here, we have provided a structured literature review tracking the chemical developments of the last forty years that have led from isolation to basic understanding, early trials and setback, re-engineering, and ultimately to a first clinical anticancer agent.

We hope that our concise, structure-based analytical overview will promote the understanding, rational design, and use of duocarmycin-based bioactives. We also add to the tradition of Njarðarson's Posters[100] with the A0-size **Poster S1** accompanying this Perspective, that can be printed and hung up in hallways for graphical overview and discussions, or used digitally for easy follow-up of its 200 embedded structures and hyperlinks.

The modularity of duocarmycin bioactivity should encourage researchers to interchangeably select structural features according to their needs. A structure-based overview to guide the choice and understanding of these features, with easy direction to the corresponding references, may be very helpful for gaining a *coup d'œil* when entering new scientific territory: particularly where the frontiers of research are increasingly interdisciplinary. We can still expect much from the duocarmycins; and we hope this Concept and its **Poster** bring a graphic understanding of how to design, incorporate and exploit this powerful molecular class.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

An A0-size **Poster S1** (>200 structures with hyperlinked references) is available as a vectorial PDF, with corresponding CDX structure file.

The ~600-work literature citation database is available as a RIS file.

The SLR workflow for this article is available as a PDF.

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REFERENCES

(1) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. CC-1065 (NSC-298223), A New Antitumor Antibiotic. Production, in Vitro Biological Activity, Microbiological Assays and Taxonomy of the Producing Microorganism. *J. Antibiot.* **1978**, *31* (12), 1211–1217. https://doi.org/10.7164/antibiotics.31.1211.

(2) Takahashi, I.; Takahashi, K.-I.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. Duocarmycin A, a New Antitumor Antibiotic from Streptomyces. *J. Antibiot.* **1988**, *41* (12), 1915–1917. https://doi.org/10.7164/antibiotics.41.1915.

(3) Boger, D. L.; Coleman, R. S. Total Synthesis of (+)-CC-1065 and Ent-(-)-CC-1065. *J. Am. Chem. Soc.* **1988**, *110* (4), 1321–1323. https://doi.org/10.1021/ja00212a067.

(4) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. Total Synthesis and Preliminary Evaluation of (+)- and Ent-(-)-Duocarmycin SA. *J. Am. Chem. Soc.* **1993**, *115* (20), 9025–9036. https://doi.org/10.1021/ja00073a019.

(5) Okano, K.; Tokuyama, H.; Fukuyama, T. Total Synthesis of (+)-Yatakemycin. *J. Am. Chem. Soc.* **2006**, *128* (22), 7136–7137. https://doi.org/10.1021/ja0619455.

(6) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. Reaction of the Antitumor Antibiotic CC-1065 with DNA: Structure of a DNA Adduct with DNA Sequence Specificity. *Science* **1984**, *226* (4676), 843–844. https://doi.org/10.1126/science.6494915.

(7) Hurley, L. H.; Lee, C. S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. Molecular Basis for Sequence-Specific DNA Alkylation by CC-1065. *Biochemistry* **1988**, *27* (10), 3886–3892. https://doi.org/10.1021/bi00410a054.

(8) Bhuyan, B. K.; Smith, K. S.; Adams, E. G.; Wallace, T. L.; Von Hoff, D. D.; Li, L. H. Adozelesin, a Potent New Alkylating Agent: Cell-Killing Kinetics and Cell-Cycle Effects. *Cancer Chemother. Pharmacol.* **1992**, *30* (5), 348–354. https://doi.org/10.1007/BF00689961.

(9) Kobayashi, E.; Okamoto, A.; Asada, M.; Okabe, M.; Nagamura, S.; Asai, A.; Saito, H.; Gomi, K.; Hirata, T. Characteristics of Antitumor Activity of KW-2189, a Novel Water-Soluble Derivative of Duocarmycin, against Murine and Human Tumors. *Cancer Res.* **1994**, *54*, 2404–2410.

(10) Shamdas, G. J.; Alberts, D. S.; Modiano, M.; Wiggins, C.; Power, J.; Kasunic, D. A.; Elfring, G. L.; Earhart, R. H. Phase I Study of Adozelesin (U-73,975) in Patients with Solid Tumors. *Anti-Cancer Drugs* **1994**, *5*, 10–14. https://doi.org/10.1097/00001813-199402000-00002.

(11) Alberts, S. R.; Erlichman, C.; Reid, J. M.; Sloan, J. A.; Ames, M. M.; Richardson, R. L.; Goldberg, R. M. Phase I Study of the Duocarmycin Semisynthetic Derivative KW-2189 given Daily for Five Days Every Six Weeks. *Clin. Cancer Res.* **1998**, *4*, 2111–2117.

(12) Cristofanilli, M.; Bryan, W. J.; Miller, L. L.; Chang, A. Y.-C.; Gradishar, W. J.; Kufe, D. W.; Hortobagyi, G. N. Phase II Study of Adozelesin in Untreated Metastatic Breast Cancer. *Anti-Cancer Drugs* **1998**, *9*, 779–782. https://doi.org/10.1097/00001813-199810000-00006.

(13) Markovic, S. N.; Suman, V. J.; Vukov, A. M.; Fitch, T. R.; Hillman, D. W.; Adjei, A. A.; Alberts, S. R.; Kaur, J. S.; Braich, T. A.; Leitch, J. M.; Creagan, E. T. Phase II Trial of KW2189 in Patients With Advanced Malignant Melanoma: *Am. J. Clin. Oncol.* **2002**, *25* (3), 308–312. https://doi.org/10.1097/00000421-200206000-00022.

(14) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. A Potent, Simple Derivative of an Analog of the CC-1065 Alkylation Subunit. *Bioorg. Med. Chem. Lett.* **1991**, *1* (1), 55–58. https://doi.org/10.1016/S0960-894X(01)81090-8.

(15) MacMillan, K. S.; Boger, D. L. Fundamental Relationships between Structure, Reactivity, and Biological Activity for the Duocarmycins and CC-1065. *J. Med. Chem.* **2009**, *52* (19), 5771–5780. https://doi.org/10.1021/jm9006214.

(16) Jukes, Z.; Morais, G. R.; Loadman, P. M.; Pors, K. How Can the Potential of the Duocarmycins Be Unlocked for Cancer Therapy? *Drug Discovery Today* **2021**, *26* (2), 577–584. https://doi.org/10.1016/j.drudis.2020.11.020.

(17) Yao, H.-P.; Zhao, H.; Hudson, R.; Tong, X.-M.; Wang, M.-H. Duocarmycin-Based Antibody–Drug Conjugates as an Emerging Biotherapeutic Entity for Targeted Cancer Therapy: Pharmaceutical Strategy and Clinical Progress. *Drug Discovery Today* **2021**, *26* (8), 1857–1874. https://doi.org/10.1016/j.drudis.2021.06.012.

(18) Boell, S. K.; Cecez-Kecmanovic Dubravka. On Being "Systematic" in Literature Reviews in IS. *Journal of Information Technology* **2015**, *30* (2), 161–173. https://doi.org/10.1057/jit.2014.26.

(19) Ghosh, N.; Sheldrake, H.; Searcey, M.; Pors, K. Chemical and Biological Explorations of the Family of CC-1065 and the Duocarmycin Natural Products. *Curr. Top. Med. Chem.* **2009**, *9* (16), 1494–1524. https://doi.org/10.2174/156802609789909812.

(20) Patil, P.; Satam, V.; Lee, M. A Short Review on the Synthetic Strategies of Duocarmycin Analogs That Are Powerful DNA Alkylating Agents. *Anti-Cancer Agents in Med. Chem.* **2015**, *15* (5), 616–630. https://doi.org/10.2174/1871520615666141216144116.

(21) Searcey, M. Duocarmycins - Natures Prodrugs? *Curr. Pharm. Des* **2002**, *8* (15), 1375–1389. https://doi.org/10.2174/1381612023394539.

(22) Cacciari, B.; Romagnoli, R.; Baraldi, P. G.; Ros, T. D.; Spalluto, G. CC-1065 and the Duocarmycins: Recent Developments. *Exp. Op. Ther. Patents* **2000**, *10* (12), 1853–1871. https://doi.org/10.1517/13543776.10.12.1853.

(23) Tietze, L. F.; Krewer, B. Antibody-Directed Enzyme Prodrug Therapy: A Promising Approach for a Selective Treatment of Cancer Based on Prodrugs and Monoclonal Antibodies. *Curr. Biol. Drug Des.* **2009**, *74* (3), 205–211. https://doi.org/10.1111/j.1747-0285.2009.00856.x.

(24) F. Tietze, L.; Schmuck, K. Prodrugs for Targeted Tumor Therapies: Recent Developments in ADEPT, GDEPT and PMT. *Curr. Pharm. Des.* **2011**, *17* (32), 3527–3547. https://doi.org/10.2174/138161211798194459.

(25) Boger, D. L.; Coleman, R. S. Total Synthesis of (.+-.)-N2-(Phenylsulfonyl)-CPI, (.+-.)-CC-1065, (+)-CC-1065, Ent-(-)-CC-1065, and the Precise, Functional Agents (.+-.)-CPI-CDPI2, (+)-CPI-CDPI2, and (-)-CPI-CDPI2 [(.+-.)-(3bR*,4aS*)-, (+)-(3bR,4aS)-, and (-)-(3bS,4aR)-Deoxy-CC-1065]. *J. Am. Chem. Soc.* **1988**, *110* (14), 4796–4807. https://doi.org/10.1021/ja00222a043.

(26) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. Demonstration of a Pronounced Effect of Noncovalent Binding Selectivity on the (+)-CC-1065 DNA Alkylation and Identification of the Pharmacophore of the Alkylation Subunit. *Proc. Natl. Acad. Sci. USA* **1991**, *88* (4), 1431–1435. https://doi.org/10.1073/pnas.88.4.1431.

(27) Boger, D. L.; Garbaccio, R. M.; Jin, Q. Synthesis and Evaluation of CC-1065 and Duocarmycin Analogues Incorporating the Iso-CI and Iso-CBI Alkylation Subunits: Impact of Relocation of the C-4 Carbonyl. *J. Org. Chem.* **1997**, *62* (25), 8875–8891. https://doi.org/10.1021/jo971686p.

(28) Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. DNA Alkylation Properties of the Duocarmycins: (+)-Duocarmycin A, Epi-(+)-Duocarmycin A, Ent-(-)-Duocarmycin A and E&Ent-(-)-Duocarmycin A. *Bioorg. Med. Chem. Lett.* **1992**, *2* (7), 759–765. https://doi.org/10.1016/S0960-894X(00)80407-2.

(29) Boger, D. L.; Yun, W. CBI-TMI: Synthesis and Evaluation of a Key Analog of the Duocarmycins. Validation of a Direct Relationship between Chemical Solvolytic Stability and Cytotoxic Potency and Confirmation of the Structural Features Responsible for the Distinguishing Behavior of Enantiomeric Pairs of Agents. *J. Am. Chem. Soc.* **1994**, *116* (18), 7996–8006. https://doi.org/10.1021/ja00097a006.

(30) Baird, R.; Winstein, S. Neighboring Carbon and Hydrogen. Li. Dienones from Ar1[UNK]-3 Participation. Isolation and Behavior of Spiro(2,5)Octa-1,4-Diene-3-One. *J. Am. Chem. Soc.* **1963**, *85*, 12. https://doi.org/10.1021/ja00888a020.

(31) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. (+)-CC-1065 DNA Alkylation: Key Studies Demonstrating a Noncovalent Binding Selectivity Contribution to the Alkylation Selectivity. *J. Am. Chem. Soc.* **1991**, *113* (10), 3980–3983. https://doi.org/10.1021/ja00010a046.

(32) Boger, D. L.; Hertzog, D. L.; Bollinger, B.; Johnson, D. S.; Cai, H.; Goldberg, J.; Turnbull, P. Duocarmycin SA Shortened, Simplified, and Extended Agents: A Systematic Examination of the Role of the DNA Binding Subunit. *J. Am. Chem. Soc.* **1997**, *119* (21), 4977–4986. https://doi.org/10.1021/ja9637208.

(33) Tietze, L. F.; Lieb, M.; Herzig, T.; Haunert, F.; Schuberth, I. A Strategy for Tumor-Selective Chemotherapy by Enzymatic Liberation of Seco-Duocarmycin SA-Derivatives from Nontoxic Prodrugs. *Bioorg. Med. Chem.* **2001**, *9* (7), 1929–1939. https://doi.org/10.1016/S0968-0896(01)00098-0.

(34) Daniell, K.; Stewart, M.; Madsen, E.; Le, M.; Handl, H.; Brooks, N.; Kiakos, K.; Hartley, J. A.; Lee, M. Design, Synthesis, and Biological Evaluation of Achiral Analogs of Duocarmycin SA. *Bioorg. Med. Chem. Lett.* **2005**, *15* (1), 177–180. https://doi.org/10.1016/j.bmcl.2004.10.021.

(35) Sato, A.; McNulty, L.; Cox, K.; Kim, S.; Scott, A.; Daniell, K.; Summerville, K.; Price, C.; Hudson, S.; Kiakos, K.; Hartley, J. A.; Asao, T.; Lee, M. A Novel Class of in Vivo Active Anticancer Agents: Achiral *Seco* -Amino- and *Seco* -Hydroxycyclopropylbenz[*e*]Indolone (*Seco* -CBI) Analogues of the Duocarmycins and CC-1065. *J. Med. Chem.* **2005**, *48* (11), 3903–3918. https://doi.org/10.1021/jm050179u.

(36) Boger, D. L.; Ishizaki, T.; Wysocki, R. J.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. Total Synthesis and Evaluation of (.+-.)-N-(Tert-Butoxycarbonyl)-CBI, (.+-.)-CBI-CDPI1, and (.+-.)-CBI-CDPI2: CC-1065 Functional Agents Incorporating the Equivalent 1,2,9,9a-Tetrahydrocyclopropa[1,2-c]Benz[1,2-e]Indol-4-One (CBI) Left-Hand Subunit. *J. Am. Chem. Soc.* **1989**, *111* (16), 6461–6463. https://doi.org/10.1021/ja00198a089.

(37) Tietze, L. F.; Major, F.; Schuberth, I. Antitumor Agents: Development of Highly Potent Glycosidic Duocarmycin Analogues for Selective Cancer Therapy. *Angew. Chem. Int. Ed.* **2006**, *45* (39), 6574–6577. https://doi.org/10.1002/anie.200600936.

(38) Boger, D. L.; Yun, W.; Han, N. 1,2,9,9a-Tetrahydrocyclopropa[c]Benz[e]Indol-4-One (CBI) Analogs of CC-1065 and the Duocarmycins: Synthesis and Evaluation. *Bioorg. Med. Chem.* **1995**, *3* (11), 1429–1453. https://doi.org/10.1016/0968-0896(95)00130-9.

(39) Fregeau, N. L.; Wang, Y.; Pon, R. T.; Wylie, W. A.; Lown, J. W. Characterization of a CPI-Lexitropsin Conjugate-Oligonucleotide Covalent Complex by 1H NMR and Restrained Molecular Dynamics Simulation. *J. Am. Chem. Soc.* **1995**, *117* (35), 8917–8925. https://doi.org/10.1021/ja00140a004.

(40) Bando, T.; Narita, A.; Asada, K.; Ayame, H.; Sugiyama, H. Enantioselective DNA Alkylation by a Pyrrole–Imidazole *S* -CBI Conjugate. *J. Am. Chem. Soc.* **2004**, *126* (29), 8948–8955. https://doi.org/10.1021/ja049398f.

(41) Tao, Z.-F.; Fujiwara, T.; Saito, I.; Sugiyama, H. Rational Design of Sequence-Specific DNA Alkylating Agents Based on Duocarmycin A and

Pyrrole–Imidazole Hairpin Polyamides. *J. Am. Chem. Soc.* **1999**, *121* (21), 4961–4967. https://doi.org/10.1021/ja983398w.

(42) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. Yatakemycin, a Novel Antifungal Antibiotic Produced by Streptomyces Sp. TP-A0356. *J. Antibiot.* **2003**, *56* (2), 107–113. https://doi.org/10.7164/antibiotics.56.107.

(43) Tichenor, M. S.; Kastrinsky, D. B.; Boger, D. L. Total Synthesis, Structure Revision, and Absolute Configuration of (+)-Yatakemycin. *J. Am. Chem. Soc.* **2004**, *126* (27), 8396–8398. https://doi.org/10.1021/ja0472735.

(44) Tichenor, M. S.; MacMillan, K. S.; Trzupek, J. D.; Rayl, T. J.; Hwang, I.; Boger, D. L. Systematic Exploration of the Structural Features of Yatakemycin Impacting DNA Alkylation and Biological Activity. *J. Am. Chem. Soc.* **2007**, *129* (35), 10858–10869. https://doi.org/10.1021/ja072777z.

(45) Fukuda, Y.; Seto, S.; Furuta, H.; Ebisu, H.; Oomori, Y.; Terashima, S. The Novel Cyclopropapyrroloindole(CPI) Bisalkylators Bearing 3,3′-(1,4-Phenylene)Diacryloyl Group as a Linker. *Bioorg. Med. Chem. Lett.* **1998**, *8* (15), 2003–2004. https://doi.org/10.1016/S0960-894X(98)00346-1.

(46) Bando, T.; Iida, H.; Saito, I.; Sugiyama, H. Sequence-Specific DNA Interstrand Cross-Linking by Imidazole–Pyrrole CPI Conjugate. *J. Am. Chem. Soc.* **2001**, *123* (21), 5158–5159. https://doi.org/10.1021/ja003660c.

(47) Li, L. H.; DeKoning, T. F.; Kelly, R. C.; Krueger, W. C.; McGovren, J. P.; Padbury, G. E.; Petzold, G. L.; Wallace, T. L.; Ouding, R. J.; Prairie, M. D.; Gebhard, I. Cytotoxicity and Antitumor Activity of Carzelesin, a Prodrug Cyclopropylpyrroloindole Analogue. *Cancer Res.* **1992**, *52*, 4904–4913.

(48) van Tellingen, O.; Nooijen, W. J.; Schaaf, L. J.; Henrar, R. E. C.; Beijnen, J. H. Comparative Pharmacology of the Novel Cyclopropylpyrroloindole-Prodrug Carzelesin in Mice, Rats, and Humans. *Cancer Res.* **1998**, *58*, 2410–2416.

(49) Ogasawara, H.; Nishio, K.; Takeda, Y.; Ohmori, T.; Kubota, N.; Funayama, Y.; Ohira, T.; Kuraishi, Y.; Isogai, Y.; Saijo, N. A Novel Antitumor Antibiotic, KW-2189 Is Activated by Carboxyl Esterase and Induces DNA Strand Breaks in Human Small Cell Lung Cancer Cells. *Jpn. J. Cancer Res.* **1994**, *85*, 418–425. https://doi.org/10.1111/j.1349-7006.1994.tb02375.x.

(50) Pavlidis, N.; Aamdal, S.; Awada, A.; Calvert, H.; Fumoleau, P.; Sorio, R.; Punt, C.; Verweij, J.; van Oosterom, A.; Morant, R.; Wanders, J.; Hanauske, A.-R. Carzelesin Phase II Study in Advanced Breast, Ovarian, Colorectal, Gastric, Head and Neck Cancer, Non-Hodgkin's Lymphoma and Malignant Melanoma: A Study of the EORTC Early Clinical Studies Group (ECSG). *Cancer Chemother. Pharmacol.* **2000**, *46* (2), 167–171. https://doi.org/10.1007/s002800000134.

(51) Boger, D. L. CBI Prodrug Analogs of CC-1065 and the Duocarmycins. *Synthesis* **1999**, *1999* (S1), 1505–1509. https://doi.org/10.1055/s-1999-3658.

(52) Nagamura, S.; Kanda, Y.; Kobayashi, E.; Gomi, K.; Saito, H. Synthesis and Antitumor Activity of Duocarmycin Derivatives. *Chem. Pharm. Bull.* **1995**, *49* (9), 1530–1535.

(53) Wang, Y.; Li, L.; Tian, Z.; Jiang, W.; Larrick, J. W. Synthesis and Antitumor Activity of CBI-Bearing Ester and Carbamate Prodrugs of CC-1065 Analogue. *Bioorg. Med. Chem.* **2006**, *14* (23), 7854–7861. https://doi.org/10.1016/j.bmc.2006.07.062.

(54) Junutula, J. R.; Smith, S. W.; Borkin, D.; Degrado, S. Isoquinolidinobenzodiazepine (ICQ) - 1-(Chloromethyl)-2,3-Dihydro-1H-Benzo8e9indole (CBI) Dimers. Wo 2018/071455 A1, April 19, 2018.

(55) Zhao, R. Y.; Chari, R. V. J. Prodrugs of CC-1065 Analogs. US007655660B2, June 29, 2004.

(56) Zhao, R. Y.; Erickson, H. K.; Leece, B. A.; Reid, E. E.; Goldmacher, V. S.; Lambert, J. M.; Chari, R. V. J. Synthesis and Biological Evaluation of Antibody Conjugates of Phosphate Prodrugs of Cytotoxic DNA Alkylators for the Targeted Treatment of Cancer. *J. Med. Chem.* **2012**, *55* (2), 766–782. https://doi.org/10.1021/jm201284m.

(57) Atwell, G. J.; Wilson, W. R.; Denny, W. A. Synthesis and Cytotoxicity of Amino Analogues of the Potent DNA Alkylating Agent Seco-CBI-TMI. *Bioorg. Med. Chem. Lett.* **1997**, *7* (12), 1493–1496. https://doi.org/10.1021/jm990136b.

(58) Hay, M. P.; Sykes, B. M.; Denny, W. A.; Wilson, W. R. A 2-Nitroimiazole Carbamate Prodrug of 5-Amino-1-(Chloromethyl)-3-[(5,6,7-Trimethoxyindol-2-Yl)Carbonyl]-1,2-Dihydro-3H-Benz[e]Indole (Amino-Seco-CBI-TMI) for Use with ADEPT and GDEPT. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2237–2242. https://doi.org/10.1016/s0960-894x(99)00381-9.

(59) Tercel, M.; Atwell, G. J.; Yang, S.; Ashoorzadeh, A.; Stevenson, R. J.; Botting, K. J.; Gu, Y.; Mehta, S. Y.; Denny, W. A.; Wilson, W. R.; Pruijn, F. B. Selective Treatment of Hypoxic Tumor Cells In Vivo: Phosphate Pre-Prodrugs of Nitro Analogues of the Duocarmycins. *Angew. Chem. Int. Ed.* **2011**, *50* (11), 2606–2609. https://doi.org/10.1002/anie.201004456.

(60) Tietze, L. F.; von Hof, J. M.; Müller, M.; Krewer, B.; Schuberth, I. Glycosidic Prodrugs of Highly Potent Bifunctional Duocarmycin Derivatives for Selective Treatment of Cancer. *Angew. Chem. Int. Ed.* **2010**, *49* (40), 7336–7339. https://doi.org/10.1002/anie.201002502.

(61) Tietze, L. F.; Schuster, H. J.; Krewer, B.; Schuberth, I. Synthesis and Biological Studies of Different Duocarmycin Based Glycosidic Prodrugs for Their Use in the Antibody-Directed Enzyme Prodrug Therapy. *J. Med. Chem.* **2009**, *52* (2), 537–543. https://doi.org/10.1021/jm8009102.

(62) Lajiness, J. P.; Robertson, W. M.; Dunwiddie, I.; Broward, M. A.; Vielhauer, G. A.; Weir, S. J.; Boger, D. L. Design, Synthesis, and Evaluation of Duocarmycin *O* -Amino Phenol Prodrugs Subject to Tunable Reductive Activation. *J. Med. Chem.* **2010**, *53* (21), 7731–7738. https://doi.org/10.1021/jm1010397.

(63) Vielhauer, G. A.; Swink, M.; Parelkar, N. K.; Lajiness, J. P.; Wolfe, A. L.; Boger, D. Evaluation of a Reductively Activated Duocarmycin Prodrug against Murine and Human Solid Cancers. *Cancer Biol. & Ther.* **2013**, *14* (6), 527–536. https://doi.org/10.4161/cbt.24348.

(64) Wolfe, A. L.; Duncan, K. K.; Parelkar, N. K.; Brown, D.; Vielhauer, G. A.; Boger, D. L. Efficacious Cyclic *N* -Acyl *O* -Amino Phenol Duocarmycin Prodrugs. *J. Med. Chem.* **2013**, *56* (10), 4104–4115. https://doi.org/10.1021/jm400413r.

(65) Lu, G.-L.; Stevenson, R. J.; Chang, J. Y.-C.; Brothers, P. J.; Ware, D. C.; Wilson, W. R.; Denny, W. A.; Tercel, M. N-Alkylated Cyclen Cobalt(III) Complexes of 1-(Chloromethyl)-3-(5,6,7-Trimethoxyindol-2-Ylcarbonyl)-2,3-Dihydro-1H-Pyrrolo[3,2-f]Quinolin-5-Ol DNA Alkylating Agent as Hypoxia-Activated Prodrugs. *Bioorg. Med. Chem.* **2011**, *19* (16), 4861–4867. https://doi.org/10.1016/j.bmc.2011.06.076.

(66) Spangler, B.; Fontaine, S. D.; Shi, Y.; Sambucetti, L.; Mattis, A. N.; Hann, B.; Wells, J. A.; Renslo, A. R. A Novel Tumor-Activated Prodrug Strategy Targeting Ferrous Iron Is Effective in Multiple Preclinical Cancer Models. *J. Med. Chem.* **2016**, *59* (24), 11161–11170. https://doi.org/10.1021/acs.jmedchem.6b01470.

(67) Tietze, L. F.; Müller, M.; Duefert, S.-C.; Schmuck, K.; Schuberth, I. Photoactivatable Prodrugs of Highly Potent Duocarmycin Analogues for a Selective Cancer Therapy. *Chem. Eur. J.* **2013**, *19* (5), 1726–1731. https://doi.org/10.1002/chem.201202773.

(68) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Yamamoto, T.; Ivanic, J.; Kobayashi, H.; Schnermann, M. J. *In Vivo* Activation of Duocarmycin–Antibody Conjugates by Near-Infrared Light. *ACS Cent. Sci.* **2017**, *3* (4), 329–337. https://doi.org/10.1021/acscentsci.7b00026.

(69) Pors, K.; Loadman, P. M.; Shnyder, S. D.; Sutherland, M.; Sheldrake, H. M.; Guino, M.; Kiakos, K.; Hartley, J. A.; Searcey, M.; Patterson, L. H. Modification of the Duocarmycin Pharmacophore Enables CYP1A1 Targeting for Biological Activity. *Chem. Commun.* **2011**, *47* (44), 12062. https://doi.org/10.1039/c1cc15638a.

(70) Thorn-Seshold, O.; Felber, J.; Thorn-Seshold, J.; Zeisel, L. Disulfide-Based Prodrug Compounds. PCT/EP2022/057483, 2021.

(71) Thorn-Seshold, O.; Zeisel, L.; Felber, J. G. Dichalcogenide Prodrugs. PCT/EP2022/059280, 2021.

(72) Wang, Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. Synthesis and Preliminary Cytotoxicity Study of Glucuronide Derivatives of CC-1065 Analogues. *Bioorg. Med. Chem.* **2003**, *11* (7), 1569–1575. https://doi.org/10.1016/S0968-0896(02)00603-X.

(73) Wang, Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. Synthesis and Cytotoxicity of a Biotinylated CC-1065 Analogue. *BMC Chem. Bio.* **2002**, *2*, 1–4. https://doi.org/10.1186/1472-6769-2-1.

(74) Wang, Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. Synthesis and Preliminary Cytotoxicity Study of a Cephalosporin-CC-1065 Analogue Prodrug. *BMC Chem. Bio.* **2001**, *1*, 4–8.

(75) Zhou, Q.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. Design and Synthesis of a Novel DNA–DNA Interstrand Adenine–Guanine Cross-Linking Agent. *J. Am. Chem. Soc.* **2001**, *123* (20), 4865–4866. https://doi.org/10.1021/ja005658r.

(76) Purnell, B.; Sato, A.; O'Kelley, A.; Price, C.; Summerville, K.; Hudson, S.; O'Hare, C.; Kiakos, K.; Asao, T.; Lee, M.; Hartley, J. A. DNA Interstrand Crosslinking Agents: Synthesis, DNA Interactions, and Cytotoxicity of Dimeric Achiral Seco-Amino-CBI and Conjugates of Achiral Seco-Amino-CBI with Pyrrolobenzodiazepine (PBD). *Bioorg. Med. Chem. Lett.* **2006**, *16* (21), 5677–5681. https://doi.org/10.1016/j.bmcl.2006.08.005.

(77) Wang, Y.; Jiang, J.; Jiang, X.; Cai, S.; Han, H.; Li, L.; Tian, Z.; Jiang, W.; Zhang, Z.; Xiao, Y.; Wright, S. C.; Larrick, J. W. Synthesis and Antitumor Activity Evaluations of Albumin-Binding Prodrugs of CC-1065 Analog. *Bioorg. Med. Chem.* **2008**, *16* (13), 6552–6559. https://doi.org/10.1016/j.bmc.2008.05.025.

(78) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D. A Small-Molecule Drug Conjugate for the Treatment of Carbonic Anhydrase IX Expressing Tumors. *Angew. Chem. Int. Ed.* **2014**, *53* (16), 4231–4235. https://doi.org/10.1002/anie.201310709.

(79) McCombs, J. R.; Owen, S. C. Antibody Drug Conjugates: Design and Selection of Linker, Payload and Conjugation Chemistry. *AAPS* **2015**, *17* (2), 339–351. https://doi.org/10.1208/s12248-014-9710-8.

(80) Peters, C.; Brown, S. Antibody–Drug Conjugates as Novel Anti-Cancer Chemotherapeutics. *Bioscience Reports* **2015**, *35* (4), e00225. https://doi.org/10.1042/BSR20150089.

(81) Kasper, M.; Stengl, A.; Ochtrop, P.; Gerlach, M.; Stoschek, T.; Schumacher, D.; Helma, J.; Penkert, M.; Krause, E.; Leonhardt, H.; Hackenberger, C. P. R. Ethynylphosphonamidates for the Rapid and Cysteine-Selective Generation of Efficacious Antibody–Drug Conjugates. *Angew. Chem. Int. Ed.* **2019**, *58* (34), 11631–11636. https://doi.org/10.1002/anie.201904193.

(82) Gangwar, S.; Sufi, B. Cytotoxic Compounds and Conjugates with Cleavable Substrates. US 20060247295A1, November 2, 2006.

(83) Boyd, S. E.; Chen, L.; Gangwar, S.; Guerlavais, V.; Horgan, K.; Sufi, B.; Huang, H.; King, D. J.; Pan, C.; Cardarelli, J. M. Antibody-Drug Conjugates and Methods of Use. US20080279868A1, November 13, 2008.

(84) Maderna, A.; Doroski, M. D.; Chen, Z.; Risley, H. L.; Casavant, J. M.; O'Donnell, C. J.; Porte, A. M.; Subramanyam, C. Bifunctional Cytotoxic Agents. US20150209445A1, July 30, 2015.

(85) Maderna, A.; Subramanyam, C.; Tumey, L. N.; Chen, Z.; Casavant, J. M. Bifunctional Cytotoxic Agents Containing the CTI Pharmacophore. WO 2016/151432A1, September 29, 2016.

(86) Beusker, P. H.; Coumans, R. G. E.; Elgersma, R. C.; Menge, W. M. P. B.; Joosten, A. F.; Spijker, H. J.; de Groot, F. M. H. Novel Conjugates of CC-1065 Analogs and Bifunctional Linkers. US20130224227A1, August 29, 2013.

(87) Janda, K. D.; Wirsching, P.; Boger, D. L. Compositions and Methods for Delivery of Antitumor Agents. WO 2006/002895A2, January 12, 2006.

(88) Chen, L.; Gangwar, S.; Guerlavais, V.; Lonberg, N.; Zhang, Q. Chemical Linkers with Single Amino Acids and Conjugates Thereof. US20100113476A1, May 6, 2010.

(89) Jackson, P. M. J.; Thurston, D. E.; Rahman, K. M. Asymmetric Conjugate Compounds. US20190144443A1, May 16, 2019.

(90) Helin, J.; Saarinen, J.; Satomaa, T.; Ekholm, F. S. Saccaride Derivative of a Toxic Payload and Antibody Conjugates Thereof. US20180228906A1, August 16, 2018.

(91) Dokter, W.; Ubink, R.; van der Lee, M.; van der Vleuten, M.; van Achterberg, T.; Jacobs, D.; Loosveld, E.; van den Dobbelsteen, D.; Egging, D.; Mattaar, E.; Groothuis, P.; Beusker, P.; Coumans, R.; Elgersma, R.; Menge, W.; Joosten, J.; Spijker, H.; Huijbregts, T.; de Groot, V.; Eppink, M.; de Roo, G.; Verheijden, G.; Timmers, M. Preclinical Profile of the HER2-Targeting ADC SYD983/SYD985: Introduction of a New Duocarmycin-Based Linker-Drug Platform. *Mol. Cancer Ther.* **2014**, *13* (11), 2618–2629. https://doi.org/10.1158/1535-7163.MCT-14-0040-T.

(92) Yu, L.; Lu, Y.; Yao, Y.; Liu, Y.; Wang, Y.; Lai, Q.; Zhang, R.; Li, W.; Wang, R.; Fu, Y.; Tao, Y.; Yi, S.; Gou, L.; Chen, L.; Yang, J. Promiximab-Duocarmycin, a New CD56 Antibody-Drug Conjugates, Is Highly Efficacious in Small Cell Lung Cancer Xenograft Models. *Oncotarget* **2018**, *9* (4), 5197–5207. https://doi.org/10.18632/oncotarget.23708.

(93) Fu, Y.; Urban, D. J.; Nani, R. R.; Zhang, Y.-F.; Li, N.; Fu, H.; Shah, H.; Gorka, A. P.; Guha, R.; Chen, L.; Hall, M. D.; Schnermann, M. J.; Ho, M. Glypican-3-Specific Antibody Drug Conjugates Targeting Hepatocellular Carcinoma. *Hepatology* **2019**, *70* (2), 563–576. https://doi.org/10.1002/hep.30326.

(94) Scribner, J. A.; Brown, J. G.; Son, T.; Chiechi, M.; Li, P.; Sharma, S.; Li, H.; De Costa, A.; Li, Y.; Chen, Y.; Easton, A.; Yee-Toy, N. C.; Chen, F. Z.; Gorlatov, S.; Barat, B.; Huang, L.; Wolff, C. R.; Hooley, J.; Hotaling, T. E.; Gaynutdinov, T.; Ciccarone, V.; Tamura, J.; Koenig, S.; Moore, P. A.; Bonvini, E.; Loo, D. Preclinical Development of MGC018, a Duocarmycin-Based Antibody–Drug Conjugate Targeting B7-H3 for Solid Cancer. *Mol. Cancer Ther.* **2020**, *19* (11), 2235–2244. https://doi.org/10.1158/1535-7163.MCT-20-0116.

(95) Tong, X.-M.; Feng, L.; Suthe, S. R.; Weng, T.-H.; Hu, C.-Y.; Liu, Y.-Z.; Wu, Z.-G.; Wang, M.-H.; Yao, H.-P. Therapeutic Efficacy of a Novel Humanized Antibody-Drug Conjugate Recognizing Plexin-Semaphorin-Integrin Domain in the RON Receptor for Targeted Cancer Therapy. *J. Immunotherapy Cancer* **2019**, 7 (1), 250. https://doi.org/10.1186/s40425-019-0732-8.

(96) Banerji, U.; van Herpen, C. M. L.; Saura, C.; Thistlethwaite, F.; Lord, S.; Moreno, V.; Macpherson, I. R.; Boni, V.; Rolfo, C.; de Vries, E. G. E.; Rottey, S.; Geenen, J.; Eskens, F.; Gil-Martin, M.; Mommers, E. C.; Koper, N. P.; Aftimos, P. Trastuzumab Duocarmazine in Locally Advanced and Metastatic Solid Tumours and HER2-Expressing Breast Cancer: A Phase 1 Dose-Escalation and Dose-Expansion Study. *The Lancet Oncology* **2019**, *20* (8), 1124–1135. https://doi.org/10.1016/S1470-2045(19)30328-6.

(97) Owonikoko, T. K.; Hussain, A.; Stadler, W. M.; Smith, D. C.; Kluger, H.; Molina, A. M.; Gulati, P.; Shah, A.; Ahlers, C. M.; Cardarelli, P. M.; Cohen, L. J. First-in-Human Multicenter Phase I Study of BMS-936561 (MDX-1203), an Antibody-Drug Conjugate Targeting CD70. *Cancer Chemother. Pharmacol.* **2016**, *77* (1), 155–162. https://doi.org/10.1007/s00280-015-2909-2.

(98) Jang, S.; Powderly, J. D.; Spira, A. I.; Bakkacha, O.; Loo, D.; Bohac, G. C.; Sharma, M. Phase 1 Dose Escalation Study of MGC018, an Anti-B7-H3 Antibody-Drug Conjugate (ADC), in Patients with Advanced Solid Tumors. *J. Clin. Onc.* **2021**, *39* (15_suppl), 2631–2631. https://doi.org/10.1200/JCO.2021.39.15_suppl.2631.

(99) FDA Updates. Fast Track Designation Granted for Breast Cancer Treatment. *Oncology Times* **2018**, *40* (5), 16. https://doi.org/10.1097/01.COT.0000531194.80509.6d.

(100) McGrath, N. A.; Brichacek, M.; Njardarson, J. T. A Graphical Journey of Innovative Organic Architectures That Have Improved Our Lives. *J. Chem. Educ.* **2010**, *87* (12), 1348–1349. https://doi.org/10.1021/ed1003806.

SYNOPSIS/TOC. We present an A0-size poster of 200 colour-coded duocarmycin structures, and narrate a structure-and-chronology overview of their 600 primary research reports. We concisely analyse their mechanisms, SAR, prodrugs, antibody-drug conjugates, and clinical trials. This tracks the structural features that have led the duocarmycin family to become a platform design, which now offers unique opportunities for rationally designed prodrugs and therapeutics.