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Review Article

TARGETING HUMAN FATTY ACID SYNTHASE IN CANCER THERAPY: STRUCTURAL INSIGHTS AND SAR OF DOMAIN SPECIFIC INHIBITOR

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ABSTRACT

Fatty acid synthase is a key enzyme in humans that drives the biosynthesis of lipids essential for energy storage and cell membrane formation. Upregulation of FAS is significantly seen in cancer cells due to increased demands of lipids and cell proliferation. Cancer cells rely heavily on fatty acid synthesis to sustain their growth and maintain their malignant characteristics. Over the past two decades, FAS has gained considerable attention as a potential target for cancer therapy, as inhibiting this enzyme could disrupt lipid biosynthesis and impair cancer cell viability. Among the various inhibitors developed so far, TVB-2640 is the only one that has advanced into clinical trials. Here we discuss in detail about the structure and function of various domains of human fatty acid synthase enzyme with its inhibitors. In this review detailed of inhibitors for each domain are discussed. Review highlights how specific modifications to the inhibitor structure can enhance their binding affinity and selectivity towards each domain of FAS. By focusing on the Structural Activity Relationship, we aim to offer insights into the rational design of novel inhibitors that can effectively target FAS in cancer cells, thereby providing new avenues for cancer therapy.

Keywords: Human fatty acid synthase, Malonyl acyl transacylase, Ketoacyl synthase, Dehydratase, Ketoacyl reductase, Thioesterase, Enoyl reductase

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INTRODUCTION

Denovo lipogenesis (DNL) mainly accounts for the contribution of lipids to cancer cells. Targeting key enzymes involved in denovo lipogenesis, such as citrate/isocitrate carrier (CIC), ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS), presents an appealing therapeutic target for anti-cancer treatment [1]. Other key players in lipid metabolism include stearoyl-CoA desaturase-1 (SCD1), which converts saturated fatty acids to unsaturated forms, CD36,which facilitates the uptake of external fatty acids into cells, carnitine palmitoyl transferase (CPT), which is responsiblefor fatty acid oxidation, acyl-CoA synthetases (ACSs) such as ACSL1 and ACSL4 which are involved in fatty acid activation, fatty acid binding proteins (FABP), and sterol regulatory element binding protein-1 (SREBP-1), which regulates genes involved in lipid synthesis. Targeting these pathways holds great promise for developing effective anti-cancer therapies [2-5]. This review will delve deeper into the various domains of fatty acid synthase enzymes and explore both natural and synthetic inhibitors.

The synthesis of fatty acids involves several key steps including production of acetyl CoA, formation of malonyl CoA or conversion of acetyl CoA to malonyl CoA, and reactions of enzyme fatty acid synthase complex. Acetyl CoA can be generated through various mechanisms including glycolysis-derived pyruvate metabolism and fatty acid oxidation. The generated acetyl CoA can be converted to malonyl CoA through carboxylation with the help of the enzyme acetyl CoA carboxylase. Elongation of fatty acid chains occurs in the third step of biosynthesis, where fatty acid synthase is an enzyme catalyzing all the reactions [6]. Human fatty acid synthase is a multifunctional enzyme complex that catalyzes de novo lipid synthesis. It's a dimer composed of two identical subunits each having a molecular weight of 240 kDa. Each monomer consists of seven distinct enzymatic domains having different activity and acyl carrier protein (ACP) binds to 4'-phosphopantetheine. The two monomer units are in an antiparallel direction and the FAS complex is divided into functional and subunit divisions. FAS is catalytically active only in its dimeric form (fig. 1). Fatty acid synthase enzyme is a polypeptide containing malonyl/acetyltransferase (MAT) which condenses malonyl CoA and acetyl CoA, Chain elongation of fatty acids occurs through the sequential addition of two-carbon units derived from acetyl-CoA and involves reduction and dehydration reactions catalysed by β-ketoacyl synthase (KS), β-ketoacyl reductase (KR), dehydratase (DH), and enoyl reductase (ER). The thioesterase (TE) domain terminates fatty acid synthesis by releasing long-chain fatty acids, such as palmitate, from the acyl carrier protein domain (fig. 2) [6-8].

The existing literature survey indicates that FAS is overexpressed in several tumour types, including prostate, breast, ovary, lung, brain cancers, due to the increased demand for lipids for cell proliferation, membrane, and protein synthesis. The increased concentration of FAS is observed in the earliest stages of cancer and becomes prominent as the tumor progresses. Inhibition of FAS induces tumour cell death by various mechanisms causing decreases in the lipid content leading to the starvation of tumor cells, accumulation of toxic malonyl CoA which is the precursor for the fatty acid biosynthesis, disrupting the multiple signal transductions like mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)–Akt and extracellular signal-regulated kinases (ERK1 and ERK2), decreases phospholipids (end product of fatty acid biosynthesis) content leads to the disturbances in the membrane function and inhibition of DNA replication leading to apoptosis by the cell cycle arrest in the G1/S phase [9, 10].

Over the decades the development of FAS inhibitors has progressed from early non-specific and reactive compounds to more selective, domain-specific, and cofactor-competitive inhibitors. Early pharmacological efforts focused on natural or reactive inhibitors such as cerulenin and its synthetic analogue C75, which established the antitumor potential of FAS inhibition but revealed significant limitations related to chemical instability, lack of selectivity, and adverse metabolic effects, including severe weight loss. These shortcomings underscored the need for more selective and drug-like FAS inhibitors. Subsequent generations of inhibitors increasingly emphasized domain specificity and improved pharmacokinetic properties, ultimately shifting attention toward cofactor-competitive inhibition of the KR domain. This evolution reflects a maturation of the field from proof-of-concept compounds to clinically viable agents, exemplified by the KR inhibitor TVB-2640.

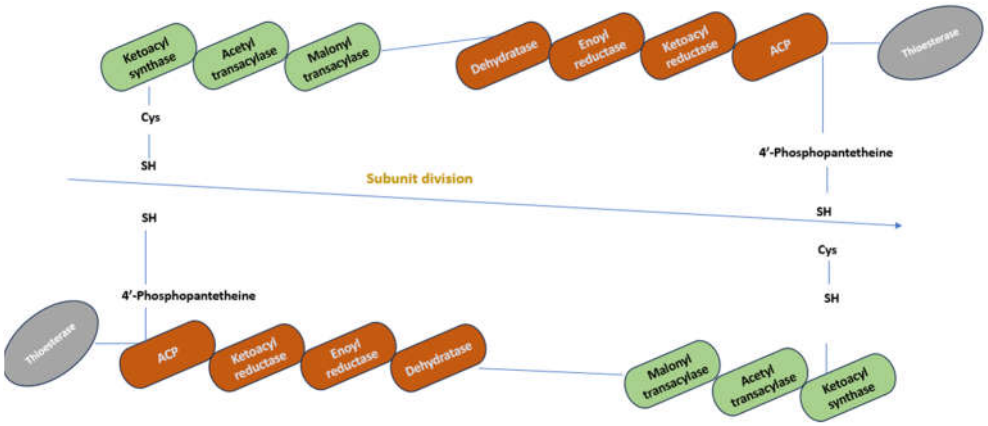

Fig. 1: The architecture of the human FAS dimer, showing head-to-tail organization of the two monomers with seven catalytic domains and the ACP, highlighting the phosphopantetheine arm that shuttles the growing acyl chain (created by author)

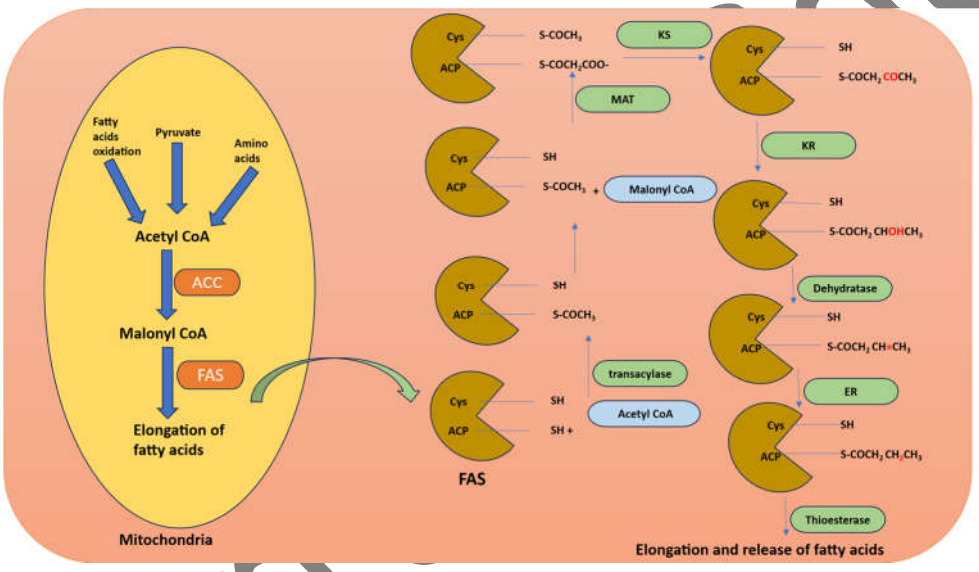

Fig. 2: Overview of de novo fatty acid synthesis mediated by human fatty acid synthase (hFASN). Acetyl-CoA derived from pyruvate, amino acids, and fatty acid oxidation is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). The multifunctional FASN complex catalyses sequential chain elongation through coordinated actions of the malonyl/acetyl transferase (MAT), ketoacyl synthase (KS), ketoacyl reductase (KR), dehydratase (DH), enoyl reductase (ER), and thioesterase (TE) domains, with the acyl carrier protein (ACP) shuttling intermediates between active sites. Repeated cycles of condensation, reduction, dehydration, and reduction result in fatty acid elongation and final release of long-chain fatty acids by the thioesterase domain. Acetyl CoA Carboxylase, followed by the synthesis of long chain fatty acids by enzyme fatty acid synthase (created by author)

Methodology

A comprehensive literature search was conducted using PubMed, Scopus, and ScienceDirect databases to identify relevant studies published between year 1950 to 2025. The search strategy employed combinations of the following keywords and Boolean operators: *"human fatty acid synthase" OR "FASN"* AND *"ketoacyl synthase (KS)" OR "malonyl/acetyl transferase (MAT)" OR "dehydratase (DH)" OR "ketoacyl reductase (KR)" OR "thioesterase (TE)" OR "enoyl reductase (ER)"*, along with *"inhibitor," "structure–activity relationship," "molecular docking," "crystal structure,"* and *"anticancer"*.

Inclusion criteria

Studies were included if they (i) reported experimental or computational data on small-molecule inhibitors of human FASN, (ii) provided domain-specific structural, biochemical, or cellular activity data, and (iii) were peer-reviewed articles published in English.

Exclusion criteria

Comprised studies focusing on non-human FASN, unrelated lipid metabolic enzymes, nutritional or nonspecific phytochemicals lacking defined molecular targets, conference abstracts, patents, editorials, and non–peer-reviewed literature. Reference lists of selected articles were manually screened to identify additional relevant studies.

Table 1: Representative fatty acid synthase inhibitors with its structures, domain potency, and key features

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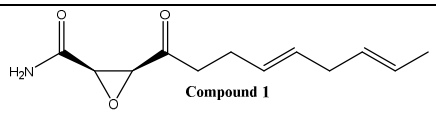
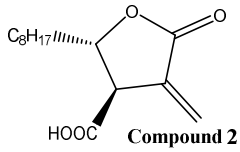
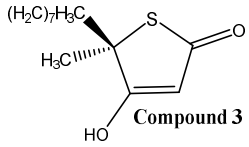
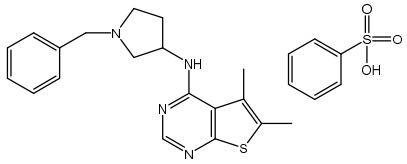
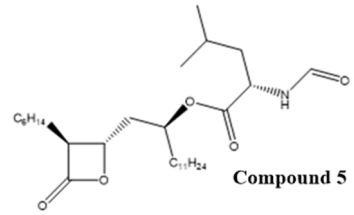
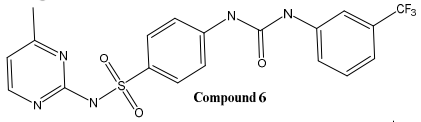
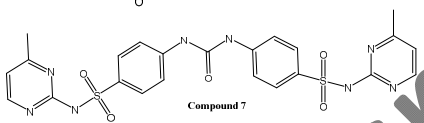
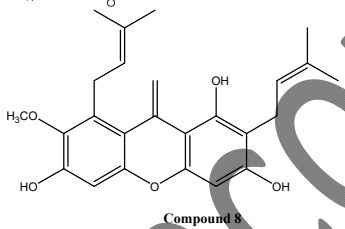
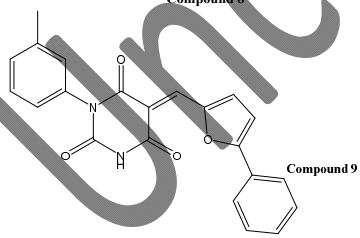
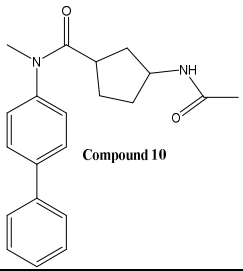
S. No.	Compound	Target FASN domain	IC_{50} (μM)	Assay type	Notes (key features/limitations)	Ref
1	 Compound 1	KS	10–20 μM	Enzyme/cell-based	Irreversible inhibitor; non-specific reactivity	[13]
2	 Compound 2	KS	32.43 μM	Cell-based	Induces weight loss via CPT-1 activation	[14, 21]
3	 Compound 3	KS	~20–80 μM	Cell-based	Designed to reduce anorexia compared to C75	[16]
4	 Compound 4	KS	~20–60 μM	Cell/*in vivo*	Suppresses Akt signaling; no weight loss observed	[17–19]
5	 Compound 5	TE	~25–30 μM	Cell-based	Poor oral bioavailability; irreversible TE inhibition	[20]
6	 Compound 6	KR	~1 μM	Enzyme-based (HTS)	Initial activity attributed to sample impurity	[22]
7	 Compound 7	KR	~0.1 μM	Enzyme-based	Selective KR-domain inhibitor	[22]
8	 Compound 8	FASN-I (unspecified)	2.6–3.5 μM	Enzyme-based	No *in vivo* efficacy reported	[24]
	 Compound 9					
9	 Compound 10	FASN-I	0.48 μM (enzyme); 0.28 μM (cell)	Enzyme/cell-based	Improved PK, good Caco-2 permeability	[25]

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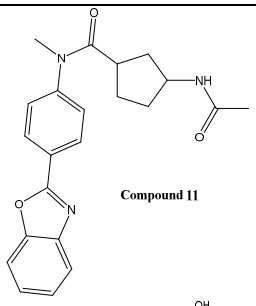
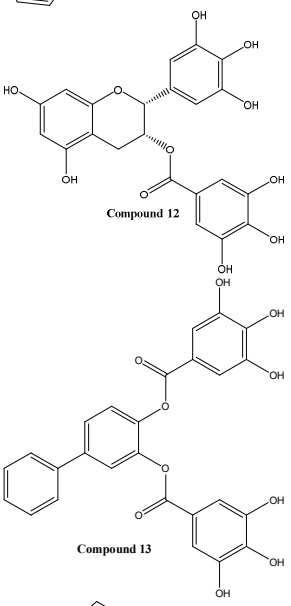
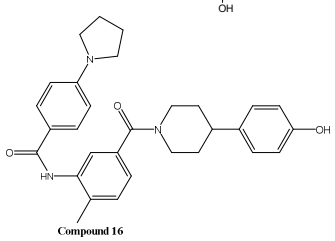
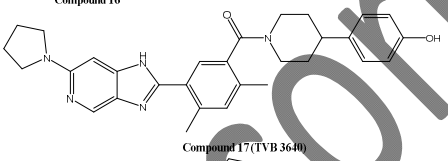
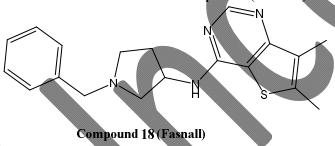
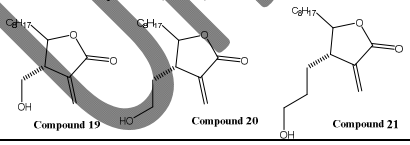
	 Compound 11					
10	 Compound 12 Compound 13	FASN-I	149±20 → 4.1±1 μM	Cell-based	SAR-driven optimization improved potency	[26]
11	 Compound 16	KR	0.050 μM	Enzyme/cell-based	Reversible KR inhibitor; blocks NADPH binding	[27]
12	 Compound 17 (TVB 3640)	KR	0.017 μM	Cell-based	Highly potent; preclinical development	[28]
13	 Compound 18 (Fasnall)	KR	0.2–3.7 μM	Cell/clinical	First-in-human FASN inhibitor; Phase II trials	[29]
14	 Compound 19 Compound 20 Compound 21	KS	1–17.9 μM	Cell/*in vivo*	Reduced anorexic side effects	[31]

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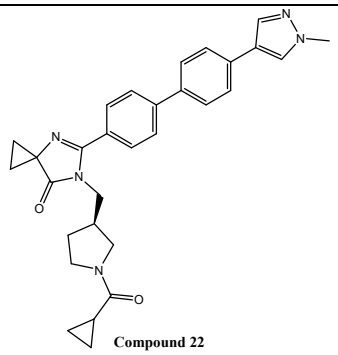
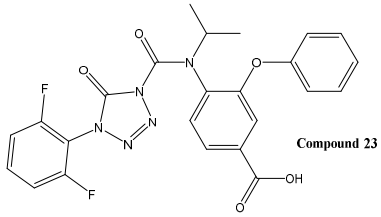
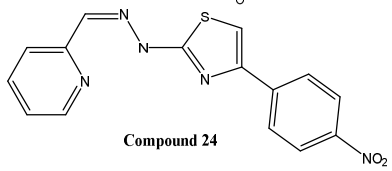
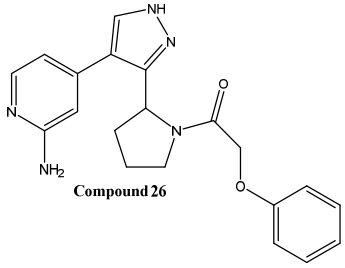
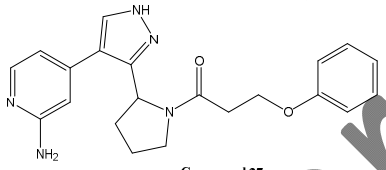
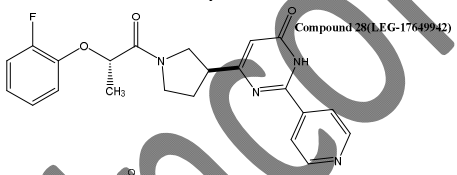
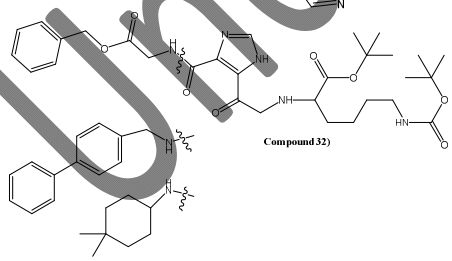
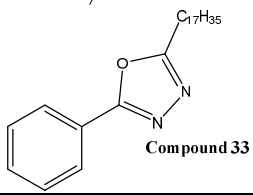
15	 Compound 22	KS	Not reported	Cell-based	SAR comparator compounds	[31]
16	 Compound 23	KR	0.11–0.5 μM	Enzyme-based	Structure-based optimization	[32]
17	 Compound 24	KS	1.5–12.7 μM	Enzyme-based	Structure-guided KS pocket inhibitors	[37]
18	 Compound 26	FASN-I	25 μM	Cell-based	Identified via virtual screening	[39]
	 Compound 27					
19	 Compound 28(LEG-17649942)	FASN-I	50 μM	Cell-based	Moderate inhibitory potency	[40]
20	 Compound 32)	KR	<0.16 μM	Enzyme-based	NADPH-competitive; high KR selectivity	[42]
21	 Compound 33	TE	1.75–2.56 μM	Enzyme/cell-based	β-lactone–based TE inhibitors	[48]

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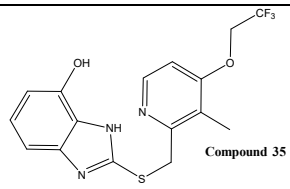
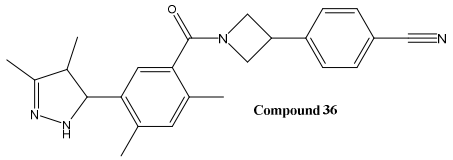
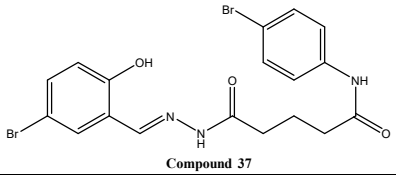
22	 Compound 35	ER	10 μM	Cell-based	Enoyl-ACP reductase inhibition	[52]
23	 Compound 36	KR (β-ketoacyl reductase)	0.02–0.04 μM	Enzyme-based KR assay; cell-based lipogenesis assays	Potent and selective KR inhibitor; orally bioavailable; strong tumor growth inhibition without inducing weight loss; preclinical tool compound leading to TVB-2640	[53]
24	 Compound 37	KS	1–5 μM	Enzyme-based KS assay; cell-based lipogenesis assays	moderate potency; limited selectivity and pharmacokinetic optimization	[53]

History of FAS inhibitors

In 1950, researchers first established a link between endogenous fatty acid synthesis and malignant tumour growth, demonstrating that rapidly proliferating neoplastic tissues utilize host-derived acetate and glucose carbon sources for lipid biosynthesis [11]. Subsequent multi-compartment metabolic analyses using radiolabelled free fatty acids and triglycerides revealed approximately 93–97% of tumour lipid fatty acids are generated via de novo lipogenesis, with only 3–7% derived from host uptake [12]. These findings firmly established fatty acid synthase as a metabolic hallmark of cancer, with its overexpression correlating strongly with increased tumour growth and metabolic demand. Consequently, extensive efforts were undertaken to develop both natural and synthetic FASN inhibitors; representative structures are summarized in table 1. The natural mycotoxin cerulenin (compound 1), isolated from *Cephalosporium caerulens*, and its synthetic analogue C75 (compound 2) were among the earliest agents evaluated in breast and ovarian cancer cell lines. FASN gene silencing studies demonstrated that both compounds downregulated p185HER2 expression and reduced tyrosine phosphorylation [13]. However, cerulenin's epoxide moiety exhibited high nonspecific reactivity, leading to off-target inhibition of proteolysis, protein palmitoylation, and cholesterol synthesis, as well as nonselective inhibition of both FASN-I and FASN-II, thereby limiting its therapeutic utility [14, 21]. Although C75 delayed mammary tumour development, its effective FASN inhibition occurred only at micromolar concentrations and was accompanied by reversible but significant weight loss in animal models, attributed to CPT-1 activation and enhanced fatty acid oxidation [15]. To address these limitations, McFadden *et al.* developed C247 (compound 3), a structurally distinct thiophenone analogue that retained anticancer activity in mammary and ovarian cancer cells while partially mitigating anorectic effects [16]. Further optimization led to the development of C93 (compound 4), which suppressed phosphorylated Akt signalling and inhibited orthotopic xenograft tumour growth in Colo680N and A549 models without inducing anorexia or weight loss, marking a clear improvement in therapeutic index over C75 [17–19]. Parallel efforts targeting individual FASN domains identified β-lactone–containing natural products as selective thioesterase inhibitors. Orlistat (compound 5), an FDA-approved anti-obesity drug, demonstrated irreversible TE inhibition via covalent binding to the catalytic serine residue, resulting in complete growth inhibition of PC-3 prostate cancer cells, while exhibiting cell line dependent potency in LnCAP and DU-145 cells [20]. However, its poor oral bioavailability restricted further oncological development. High-throughput screening of ~550,000 compounds using an NADPH consumption assay initially identified SKF-100601 (compound 6) as a putative FASN inhibitor; however, structural activity relationship (SAR) driven resynthesis failed to reproduce activity. Subsequent purification revealed GSK837149A (compound 7) as the true active component, highlighting the importance of compound purity in SAR interpretation [22]. Additional FASN-I inhibitors, including 3-aryl-4-hydroxyquinolinones (compound 8) and furanyl methylene-pyrimidinetriones (compound 9), exhibited nanomolar-to-micromolar IC_{50} values, but lacked *in vivo* efficacy data, limiting translational relevance [23, 24]. Butlin *et al.* later developed piperidine-based inhibitors with IC_{50} values of approximately 350 nM in rat models. SAR optimization of cyclopentane carboxanilide scaffolds (compound 10) through phenyl-to-heterobicyclic replacement yielded BI 99179 (compound 11), achieving a>10-fold improvement in potency, along with favourable PK–PD parameters, including high Caco-2 permeability and metabolic stability [25]. Natural polyphenols such as (−)-epigallocatechin-3-gallate (EGCG, compound 12) inhibited human FASN and induced apoptosis in breast and prostate cancer cells but failed *in vivo* due to poor stability and oral bioavailability. Subsequent polyphenolic analogues revealed that separating two galloyl units with rigid aromatic linkers reduced IC_{50} values to ~4μM, representing a 5–10-fold improvement over EGCG (fig. 3) [13]. Among these, compound 13 inhibited FASN-mediated lipogenesis without activating CPT-1, thereby avoiding anorectic effects [26]. Further SAR refinement of compound 14 identified the cyclopropylamide carbonyl and biphenyl core as essential pharmacophores. Replacement of the benzimidazole moiety with a triazolone led to GSK2194069 (compound 15), a reversible KR-domain inhibitor with EC_{50} of 15.05nM, representing a>20-fold potency enhancement relative to earlier analogues (fig. 4) [27]. Despite numerous reported inhibitors, many suffered from limited cell permeability, irreversible binding, or sub optimal PK profiles. Guided by SAR, modification of compound 16 produced an imidazopyridine scaffold in which incorporation of pyrrolidine or piperidine moieties enhanced potency by 10-fold compared to piperazine analogues (fig. 5). These efforts culminated in TVB-2640 (compound 17), the most potent and selective FASN inhibitor to date, which has progressed to Phase II clinical trials [28]. Although monotherapy trials showed no complete or partial responses, pretreated patient cohorts demonstrated early signs of clinical benefit [29]. Recognizing the role of cofactors in FASN catalysis, attention shifted toward NADPH-dependent domains (KR, ER, MAT). Screening of purine-mimetic compounds identified Fasnall (compound 18), which inhibited HepG2 cells with IC_{50} = 213nM and BT474 cells with IC_{50} = 3.71μM, inducing malonyl-CoA accumulation and CPT-1 inhibition mediated apoptosis [30]. Synthetic C75 analogues, including (±)-UB006 (compound 19), (±)-UB339 (compound 20), and (±)-UB340 (compound 21), demonstrated that conversion of the carboxylic acid to a secondary alcohol improved cytotoxicity while eliminating weight loss, with (−)-UB006 exhibiting IC_{50} values ranging from 1–17.9 μM across multiple cancer cell lines [31]. Iterative optimization of imidazolinone scaffolds yielded JNJ-54302833 (compound 22), where SAR revealed that ortho-fluoro or methyl substitution on the central phenyl ring enhanced potency by up to 100-fold, whereas heteroaryl substitutions increased IC_{50} values by 100–1000-fold. Crystallographic studies confirmed that the imidazolinone acts as a central scaffold anchoring NADPH interactions, while cyclopropylamide substituents form hydrogen bonds with Ser2021 and Tyr2034, rationalizing observed SAR trends (fig. 6) [32]. Finally,

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inhibition of de novo lipogenesis was also shown to suppress androgen receptor signalling in castration-resistant prostate cancer, where a tetrazole derivative (compound 23) irreversibly inhibited the thioesterase domain and induced cell-cycle arrest.

Early FASN inhibitors such as cerulenin and its synthetic analogue C75 were instrumental in validating FASN as a drug gable anticancer target. However, despite their potent enzymatic inhibition, the lack of domain selectivity, poor metabolic stability, and pronounced systemic toxicity most notably severe weight loss and anorexia severely limited their clinical translation. These limitations revealed a critical disconnect between *in vitro* potency and *in vivo* tolerability, emphasizing the necessity for improved pharmacokinetic properties and more refined targeting strategies. Importantly, the clinical failures of cerulenin and C75 demonstrated that complete blockade of FASN activity is not therapeutically viable, thereby directly motivating the shift toward selective inhibition of individual catalytic domains to retain efficacy while minimizing metabolic liabilities. In response, subsequent medicinal chemistry efforts prioritized domain-specific FASN inhibition, with particular emphasis on the ketoacyl reductase domain as a means to mitigate the adverse effects associated with pan-FASN inhibitors. Structure activity relationship analyses across multiple inhibitor classes indicated that selective KR engagement enables partial suppression of lipogenesis while avoiding the profound energy imbalance caused by full FASN shutdown. This body of work established that moderate enzymatic inhibition, when coupled with enhanced selectivity and favourable pharmacokinetic profiles, can preserve anticancer activity. Collectively, these findings marked a conceptual transition from maximizing enzymatic potency to optimizing therapeutic windows, firmly linking molecular selectivity to clinical feasibility.

Building on these insights, a second generation of FASN inhibitors including GSK2194069, JNJ-54302833, (−)-UB006, IPI-9119, GSK837149A, and TVB-2640 was developed (fig. 7). Among these candidates, only the KR-selective inhibitor TVB-2640 has advanced to clinical evaluation and is currently in Phase II trials, underscoring the translational advantage of domain-specific inhibition. Other synthetic inhibitors from this class are discussed in the subsequent sections of this review.

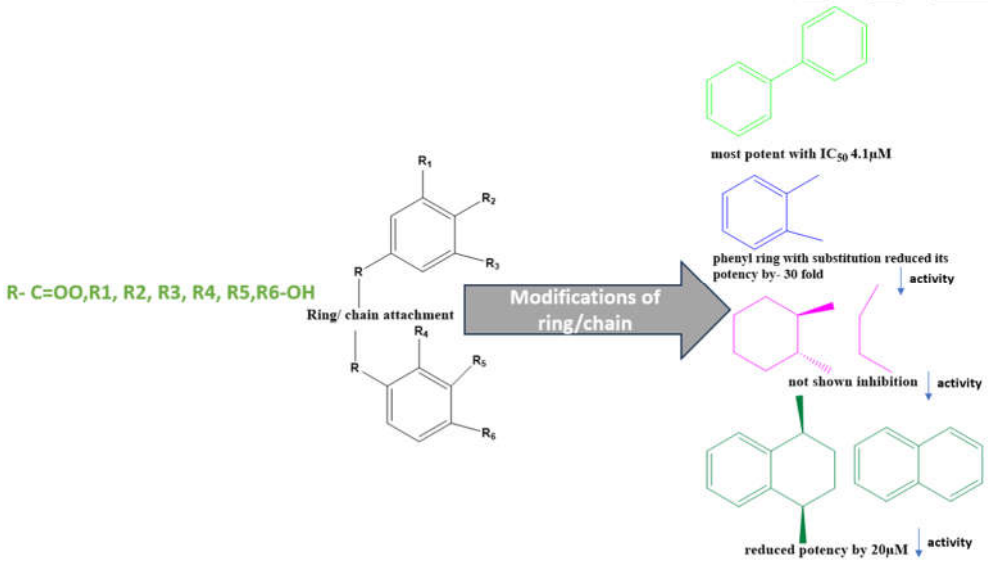


Fig. 3: Structure–activity relationship illustrating the impact of ring and chain modifications on inhibitory potency for the development of polyphenolic compounds as fatty acid synthase inhibitors(↓ indicates reduced activity) (created by author)

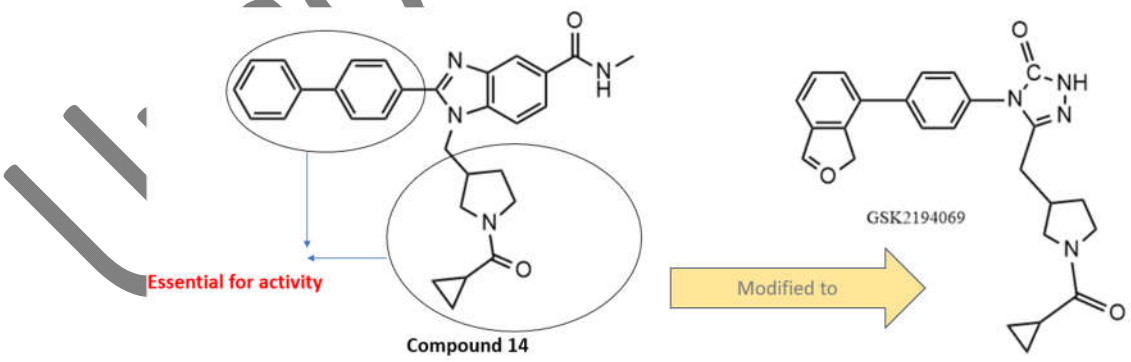


Fig. 4: Structural optimization leading to the KR domain inhibitor GSK2194069 (created by author)

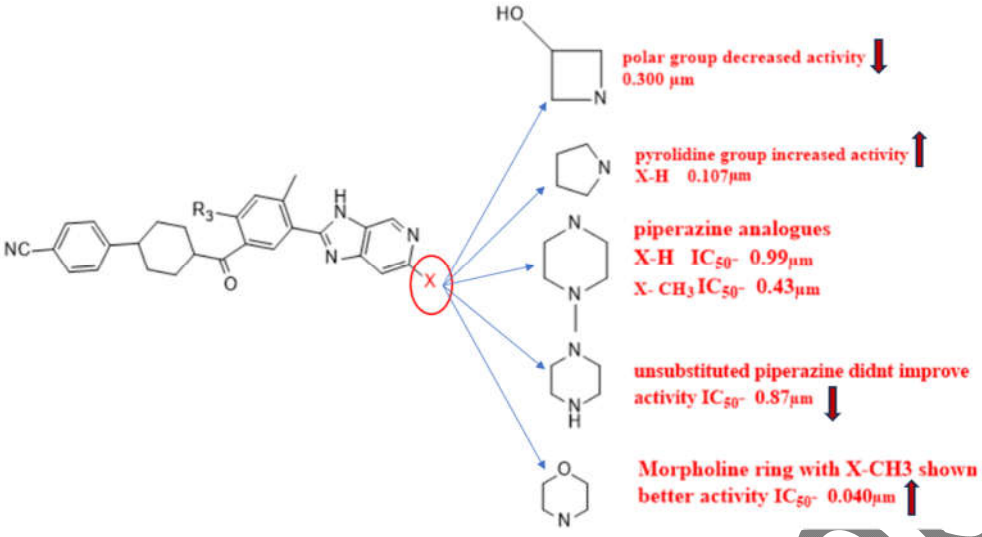


Fig. 5: Structure–activity relationship analysis of heterocyclic substitutions at the X position of the centre scaffold imidazopyridine. (↑ indicates activity increasing, and ↓ indicates reduced activity) (created by author)

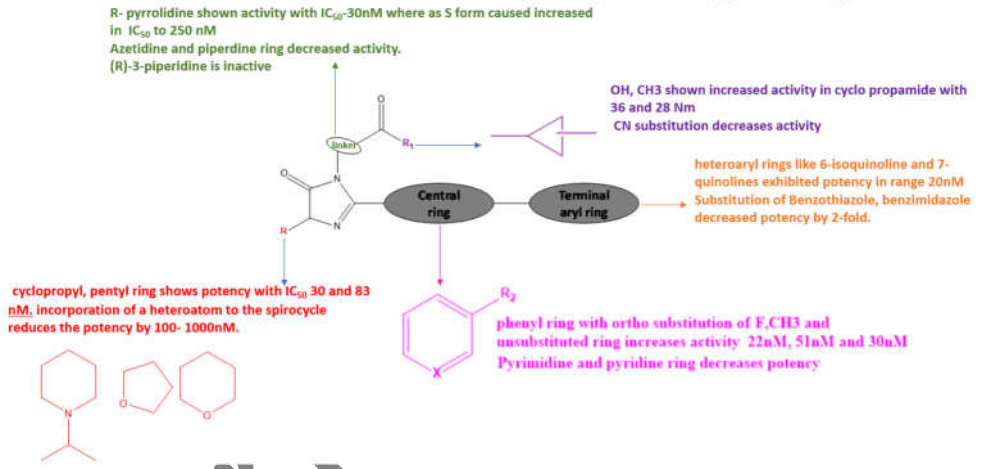


Fig. 6: Structure–activity relationship summary highlighting the influence of linker, central ring, and terminal aryl substitutions on inhibitory potency (created by author)

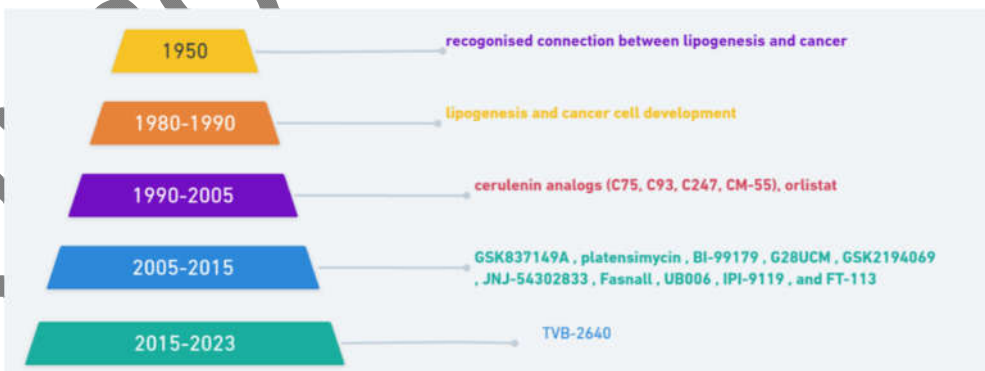
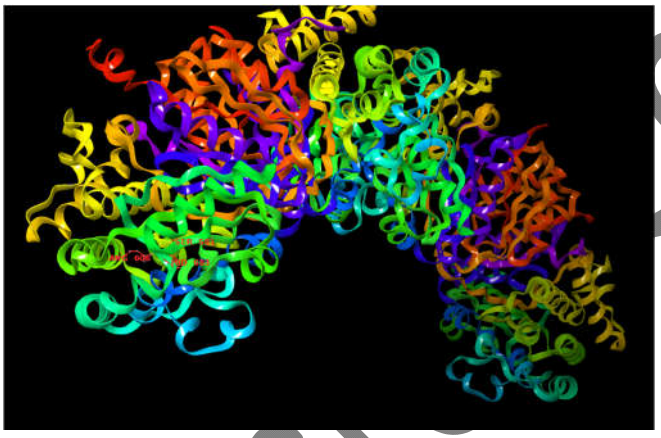


Fig. 7: Timeline of key milestones in understanding lipogenesis and the development of fatty acid synthase inhibitors highlighting the transition from first-generation natural products like cerulenin and C75 to second-generation, domain-specific synthetic inhibitors and culminating in TVB-2640, the first to reach clinical trials (created by author)

Domains of fatty acid synthase

MAT domain

The synthesis of long chain fatty acids is initiated in the malonyl acetyltransferase domainwhere acyl moiety from acetyl CoA binds to the 4′-phosphopantetheine arm, which is covalently attached to an acyl carrier protein [34]. The MAT domain plays a pivotal role in both the initiation and elongation phases of fatty acid biosynthesis. It facilitates the transfer of the acetyl moiety from acetyl-CoA during initiation and mediates substrate delivery to the β-ketoacyl synthase (KS) domain during chain elongation [35]. Both FAS I and II utilize the ACP system to transfer carbon atoms, primarily from Acetyl CoA for initiation and from malonyl-CoA for chain extension. MAT is made up of two distinct subdomains, where the larger domain demonstrates a characteristic alpha and beta hydrolase fold, featuring a four-stranded parallel beta-sheet surrounded by alpha helices. In contrast, the smaller domain adopts a long, symmetric hairpin-like fold (ferredoxin-like fold) which includes an antiparallel multistranded beta-sheet that is packed against two distant alpha helices. Like other protein, MAT domain comprises well-structured amino and carboxy-terminal that connect to the adjacent domains of FAS-I. The key active-site residues involved in substrate binding include Ser581 (amino-terminal), His683, and Arg606 (fig. 8a) Malonyl CoA forms an ionic interaction with arginine residue, and the acetyl group forms hydrophobic bonding with phe553, phe682 and Met499. Met499 is essential for maintaining the hydrophobic environment of the active site, Arg606 forms ionic interactions with substrate malonyl CoA and His683 enhances the nucleophilicity of the thiol group (SH) by abstracting hydrogen and facilitating the binding of the acyl moiety to the sulfur atom, Leu 582 interacts with oxygen atom of the carbonyl group in the acetyl moiety. Leu582 and Met499 together are considered oxyanion hole, the amine group present in these amino acids is crucial for stabilizing the substrates. MAT domain of human FAS has a shorter binding pocket compared to the fungal FAS, allowing it to accommodate acyl moieties with up to 10 carbon atoms. Structurally, the MAT domain contains extensions at both the amino-and carboxyl-termini, which form parts of the linker regions connecting MAT to the adjacent β-ketoacyl synthase and dehydratase domains, respectively [33-36].



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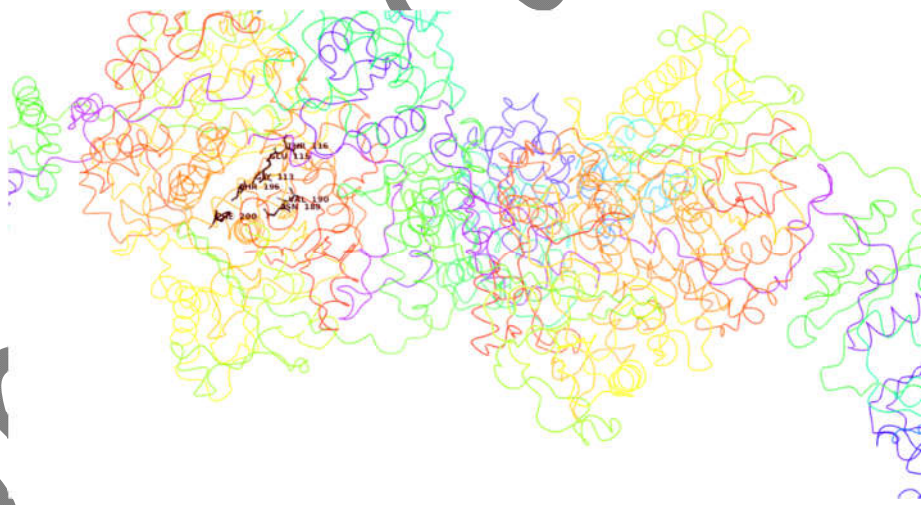

b)

Fig. 8: Structural features of the human FAS MAT and KS domains relevant to inhibitor binding. (a) Close-up view of the MAT domain (PDB ID: 2JFD) highlighting key catalytic residues-Ser581, His683, and Arg606-that facilitate malonyl/acetyl transfer and contribute to substrate specificity. (b) Overall architecture of the ketoacyl synthase domain (PDB ID: 3HHD), showing the substrate-binding and acetyl-binding pockets that accommodate both natural substrates and small-molecule inhibitors (created by author)

Table 2: Key catalytic and binding residues, hydrogen-bonding residues, and hydrophobic pocket residues identified across different domains, along with the corresponding PDB IDs (organism source) and reference types.

Domain	Key catalytic/binding residues	Hydrogen-bonding residues	Hydrophobic pocket residues	PDB ID (Organism)	Reference type	Ref
KS (Ketoacyl Synthase)	Cys161 (catalytic), His293	Ser112, Gly113, Thr116, Glu115,	Val190, Phe200, Thr196	3HHD (Human FASN)	Structural (X-ray crystallography)	[36-37]

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		Asn189				
KR (Ketoacyl Reductase)	Tyr2034, Ser2021 (NADPH-dependent catalysis)	Ser2021, Gln2031, Tyr2034	Phe2019, Leu1265, Val2022, Ile2068	3TJM, 4PIV, 6NNA (Human FASN)	Structural and SAR	[42-43]
TE (Thioesterase)	Ser2308, Asp2338, His2481 (catalytic triad)	Ser2308, His2481, Tyr2462	Phe2370, Phe2371, Leu2222, Leu2223, Val2224, Ile2250	1XKT, 4Z49, 7MHE (Human FASN)	Structural	[44, 48-49]
ER (Enoyl Reductase)	Tyr1563, Lys1771, Asp1797	Tyr1563, Lys1771	Leu1556, Val1567, Ile1801	4W9N, 4W82 (Human FASN)	Structural	[44]
MAT (Malonyl/Acetyl Transferase)	Ser581 (catalytic nucleophile), His683	Ser581, His683, Arg606	Val604, Leu608	2JFK, 2JFD (Human FASN) S	Structural	[33-36]

KS domain

Ketoacyl synthase is the second catalytic domain located at the amino terminus of fatty acid synthase and is responsible for the decarboxylation of malonyl-ACP followed by Claisen condensation with the acetyl moiety during fatty acid chain elongation. Structurally, the KS domain comprises substrate-binding and acetyl-binding pockets, spanning amino acid residues 1–409 and 825–852 of the FASN polypeptide. Zeng *et al.* identified key pharmacophoric features within the KS active site, including hydrogen bond acceptors (Val190, Thr116, Glu115), a hydrogen bond donor (Ser112), and hydrophobic residues (Asn189, Phe200, Gly113, Ser115, and Thr196) that collectively govern ligand recognition and binding affinity (fig. 8b).

Among early KS-directed inhibitors, a thiazole derivative (compound 24) exhibited moderate FASN inhibition with an IC_{50} of 12.7μM, alongside cytotoxic activity in MDA-MB-468 (8.3μM) and SW480 (1.5μM) cell lines, indicating improved cellular potency relative to enzymatic inhibition [37] table 2. In a related effort, Wang *et al.* synthesized a series of C75 analogues by modifying the C5 position of the lactone core with dimethyl, isopropylidene, and phenyl substituents. While these substitutions resulted in reduced activity compared to C75, incorporation of a long-saturated alkyl chain at the C2 position (compound 25) significantly enhanced KS-domain engagement by occupying the hydrophobic pocket formed by Ala162, Tyr224, Val263, Phe202, Phe258, and Glu335. This modification yielded IC_{50} values ranging from 0.79 to 16.73 μM in HL-60 and HeLa cells, representing a ~2–10-fold improvement over C75, depending on the cell line [38]. Conversely, increasing steric bulk and hydrophilicity of the lactone ring through methyl or phenyl substitution diminished biological activity, underscoring the sensitivity of KS inhibition to steric constraints (fig. 9). Structure-based virtual screening by Nisthul *et al.* identified two KS inhibitors, BDD27845077 (compound 26) and BDD27845082 (compound 27), which share a common scaffold. Notably, compound 27 contains an additional methylene spacer between the phenyl ring and the carbonyl-linked pyrrolidine, conferring improved conformational flexibility and stability relative to compound 26. Compound 27 demonstrated cytotoxic activity in HCT-116 colon cancer cells with an IC_{50} of ~25μM, whereas compound 26 showed reduced potency [39]. Further optimization led to the identification of LEG-17649942 (compound 28), a close analogue of compound 27. SAR analysis revealed that a pyrimidine moiety bearing a carbonyl group is essential for forming hydrogen bond interactions with KS residues D254, H293, H331, and F393, resulting in measurable KS inhibition with an IC_{50} of ~50μM [40].

Structurally, the KS active site is characterized by a narrow catalytic tunnel terminating at the conserved Cys161 residue, which acts as a nucleophile during fatty acid chain elongation. This confined geometry exerts a dominant influence on inhibitor binding and selectivity. Covalent inhibitors such as cerulenin irreversibly block KS activity by forming an adduct with Cys161; however, the high intrinsic reactivity of the epoxide moiety leads to poor selectivity, as similar nucleophilic residues are present in unrelated enzymes. Lack of stabilizing noncovalent interactions beyond Cys161 engagement contributes to extensive off-target reactivity, explaining the limited translational success of early KS-directed covalent inhibitors. Moreover, the restricted size and rigidity of the KS tunnel disfavour bulky, drug-like molecules, rendering the development of selective, reversible KS inhibitors particularly challenging [36-40, 56].

The KS domain imposes stringent structural constraints on inhibitor design due to its narrow catalytic tunnel terminating at the nucleophilic Cys161. Potent inhibition observed with covalent modifiers such as cerulenin arises from irreversible Cys161 acylation; however, this mechanism inherently sacrifices selectivity, as comparable nucleophilic residues are present in unrelated enzymes. SAR studies reveal that noncovalent inhibitors require elongated, conformationally restrained scaffolds to access the tunnel while simultaneously engaging peripheral hydrophobic residues, explaining why moderate cellular potency is achieved only with linear or flexible alkyl chains. Bulky or highly polar substituents are poorly tolerated, resulting in rapid potency loss. Thus, KS structural geometry explains both the limited success of reversible inhibitors and the translational failure of covalent KS inhibitors, despite favourable enzymatic inhibition.

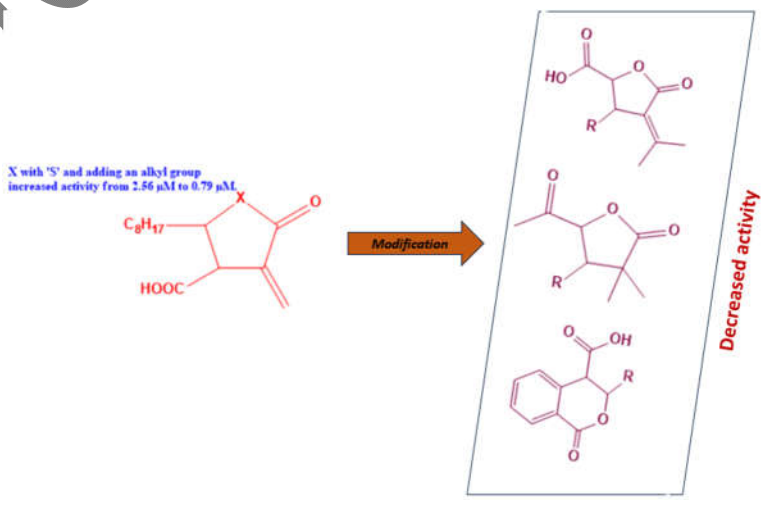

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Fig. 9: Structure–activity relationship of β-lactone–based FASN inhibitors targeting the ketoacyl reductase domain. The panel illustrates representative β-lactone scaffolds and key chemical modifications, highlighting structural features that influence binding affinity, selectivity, and inhibitory potency (created by author)

KR domain

The β-ketoacyl-ACP reductase domain catalyses the NADPH-dependent reduction of β-ketoacyl intermediates during fatty acid chain elongation. Early KR-directed inhibition strategies were derived from structural modification of C75, in which substitutions were introduced at the C-3 position of the pantolactone ring using aromatic and aliphatic hydrocarbon chains. Structure activity relationship studies demonstrated a clear chain-length dependence, wherein aliphatic substituents longer than 14 carbon atoms led to a progressive loss of activity, and 18-carbon chains completely abolished KR inhibition, highlighting steric constraints within the KR binding pocket. At the C-4 position, phenyl ring substitution with halogen, alkyl, or alkoxy groups was explored. Among these analogues, the alkoxy-substituted phenyl derivative (compound 29) exhibited optimal KR engagement by forming hydrogen-bond interactions with Thr2083 and Arg1462, resulting in an IC_{50} of 13.68±1.52μM (fig. 10a) [41]. These results established that moderate steric bulk combined with hydrogen-bonding capability is essential for effective KR inhibition.

Martin *et al.* subsequently reported a series of alkyl and cyclo alkyl substituted piperidine derivatives incorporating a biaryl pharmacophore. Introduction of a hydroxy-cyclopropyl moiety produced a potent FASN inhibitor (compound 30) with an IC_{50} of 350nM, representing a ~40-fold potency enhancement relative to early C75 derived KR inhibitors. Further scaffold refinement demonstrated that replacement of the biaryl system with a benzoxazole moiety resulted in pronounced antiproliferative activity against PC-3 prostate cancer cells, achieving an IC_{50} of 15nM (fig. 10b) [42]. Singh *et al.* developed carboxylate-linked piperidine derivatives tethered to a 2-(2-hydroxyphenyl)-1H-benzimidazole core, designed to enhance hydrogen bonding interactions within the KR domain. In this series, both the phenolic hydroxyl group and benzimidazole NH of (compound 31) formed stabilizing hydrogen bonds with Gly2027, facilitating deep occupation of the hydrophobic KR pocket defined by His1263, Arg1462, Arg2026, Phe2019, Pro1264, Leu1265, and Val2022 (fig. 10c, fig. 11, table 2). Notably, chlorine substitution at the 5-position of the phenyl ring improved cellular potency by ~3-fold, yielding an IC_{50} of approximately 2.5μM in MCF-7 breast cancer cells [43].

Crystallographic studies reveal that the KR active site comprises a deep hydrophobic cavity adjacent to the NADPH-binding groove, flanked by conserved polar residues essential for cofactor recognition and catalysis. High affinity KR inhibitors such as GSK2194069 exploit this architecture through biaryl or cyclopropyl-containing scaffolds that optimally occupy the hydrophobic pocket, maximizing van der Waals interactions and conferring high selectivity for human FASN over related reductases. In contrast, substitution of biaryl motifs with polar heteroaryl systems disrupts hydrophobic complementarity, resulting in reduced potency. In addition to hydrophobic interactions, potent inhibitors establish key hydrogen bonds with conserved catalytic residues, notably Ser2021 and Tyr2034, stabilizing reversible binding without covalent modification. This binding mode enables effective competition with NADPH while maintaining favourable pharmacokinetic properties, explaining the nanomolar potency and clinical advancement of KR inhibitors such as GSK2194069 and TVB-2640. Collectively, these structural features underpin the KR domain's dominance as the most drug gable and clinically validated FASN target [41-43, 53].

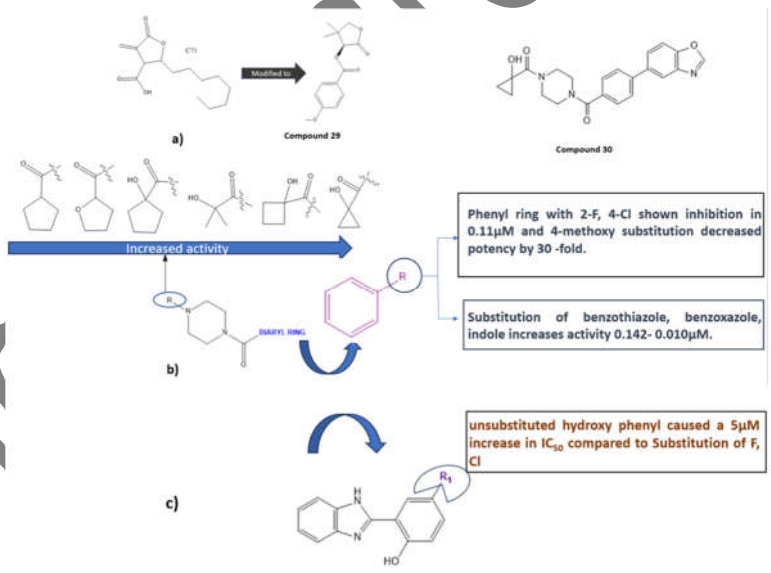

Fig. 10: Structure–activity relationship studies of C75 analogues and biaryl-containing FASN inhibitors. (a) Modification of C75 at the C-3 or C-4 position with methoxy-substituted phenyl groups, illustrating the effect of aromatic substitutions on KR domain binding and inhibitory potency. (b) SAR analysis of piperidine derivatives incorporating a biaryl ring system, showing how variations in the biaryl scaffold modulate hydrophobic pocket occupancy, van der Waals interactions, and overall FASN inhibitory activity. (c) Structural modification of the biaryl ring with benzimidazole substituents, demonstrating the impact of heteroaryl incorporation on potency, hydrogen-bonding interactions, and selectivity for the KR domain (created by author)

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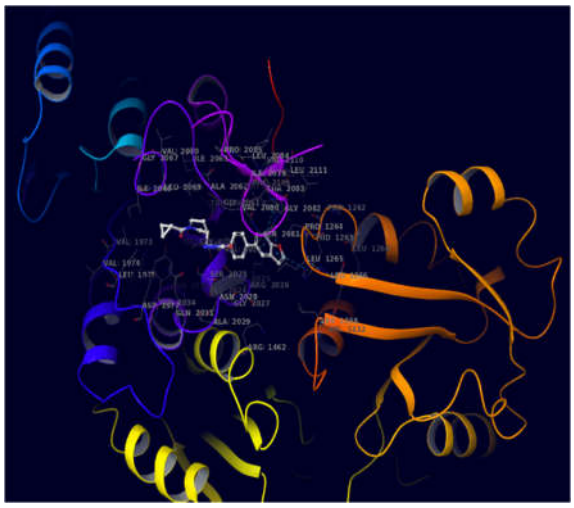

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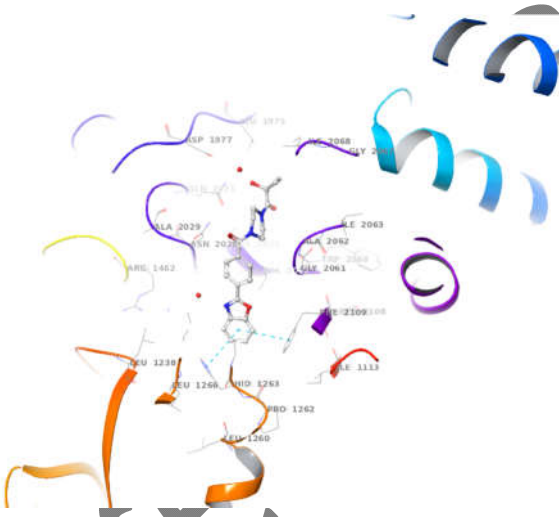

b)

Fig. 11: Binding mode of GSK2194069 within the ketoacyl reductase domain of human fatty acid synthase. (a) Predicted or experimentally determined binding pose of GSK2194069 in the KR domain (PDB ID: 4PIV), showing deep occupation of the hydrophobic pocket and key van der Waals and hydrogen-bonding interactions that stabilize inhibitor binding. (b) Binding orientation of GSK2194069 in the KR domain (PDB ID: 6NNA), highlighting conserved contacts with active-site residues (e. g., Ser2021, Tyr2034) and the adjacent NADPH-binding groove, which contribute to its nanomolar potency, high selectivity, and reversible inhibition. This structural depiction illustrates the molecular basis for KR-domain-targeted FASN inhibition and provides insights for rational inhibitor optimization (created by author)

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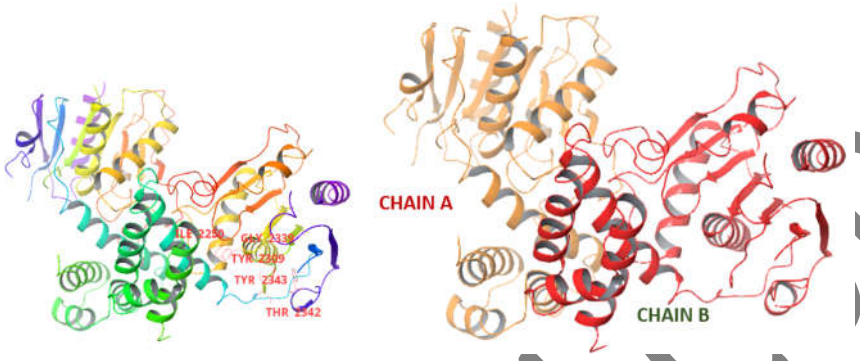
Thioesterase domain

The thioesterase domain regulates fatty acid chain termination and product release from fatty acid synthase through hydrolytic cleavage of the acyl–ACP intermediate. Structurally, the TE domain comprises α-helices and β-sheet motifs organized into two subdomains (A and B) (fig. 12a). A defining feature of this domain is the presence of a conserved catalytic triad consisting of Ser2308, His2481, and Asp2338, which governs ester hydrolysis and substrate specificity (fig. 12b, table 2) [44]. Structural analysis of orlistat binding to the TE domain reveals that the β-lactone ring forms a covalent interaction with the catalytic Ser2308, mimicking the natural acyl–enzyme intermediate. The C16 alkyl chain of orlistat occupies the hydrophobic fatty acyl binding channel, while Ile2250 and Tyr2309 establish weak hydrogen-bond interactions with the C1 carbonyl oxygen. Additionally, the six-carbon substituent at the C2 position extends toward Gly2339, Thr2342, Tyr2343, and Tyr2462, further stabilizing the inhibitor enzyme complex (fig. 12a). These interactions explain the potent enzymatic inhibition observed for orlistat despite its limited cellular translation.

Mechanistically, TE inhibition is achieved through irreversible acylation of the active-site serine, a process facilitated by optimal positioning of the electrophilic carbonyl within the lactone ring and stabilization of the lipophilic tail within the fatty acyl channel. Charles *et al.* demonstrated that effective TE inhibitors require a minimum alkyl chain length of ~16 carbon atoms to achieve sufficient hydrophobic engagement within the binding pocket [45]. Supporting this observation, Zhang *et al.* employed molecular docking studies of long-chain polyunsaturated fatty acids, showing that methyl γ-linolenyl phosphonate (MGLP; C18) fits snugly into the hydrophobic cavity formed by Val2344, Tyr2347, Arg2352, Tyr2351, Glu2366, Ala2367, Phe2370, Tyr2424, Leu2427, Arg2428, and Glu2431, while Ile2250 and Tyr2309 participate in stabilizing hydrogen-bond interactions (table 2) [46]. Further SAR-driven optimization was reported by Lupien *et al.*, who developed N,N′-disubstituted imidazole-4,5-dicarboxamide derivatives bearing benzylamine or cyclohexylamine substituents for TE targeting. These compounds exhibited moderate inhibitory activity (IC_{50} = 8.9–19.5μM and 8.9–20μM, respectively). Notably, compound 32 demonstrated a marked improvement in potency (IC_{50}<0.16μM) along with enhanced plasma stability, representing a>50-fold enhancement relative to earlier analogues [47]. Consistent with these findings, Jubie *et al.*

confirmed that long-chain fatty acid substituents are critical for TE domain engagement. In their study, 1,3,4-oxadiazole derivatives conjugated with long chain fatty acids (compound 33) displayed cytotoxic activity against A549 lung carcinoma cells with IC_{50} values ranging from 1.75 to 2.56μM, reinforcing the importance of alkyl chain length in determining anticancer efficacy [48, 49].

TE inhibition is mechanistically driven by covalent acylation of the catalytic Ser2308, mimicking the natural acyl–enzyme intermediate. Structural studies demonstrate that long alkyl chains (≥C16) are essential to occupy the fatty acyl channel and stabilize binding, directly correlating chain length with enzymatic potency. However, the open and solvent-accessible architecture of the TE active site reduces selectivity, as electrophilic inhibitors readily interact with off target serine hydrolases. Additionally, irreversible binding and high lipophilicity negatively impact pharmacokinetics. These structural characteristics explain why TE inhibitors display strong biochemical inhibition but limited clinical progression, highlighting a fundamental trade-off between potency and drug ability.



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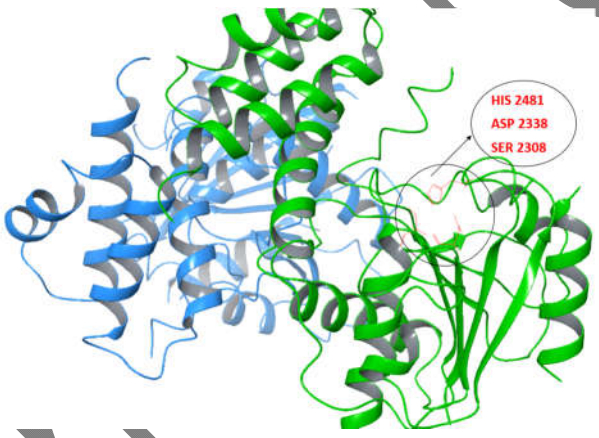

12b)

Fig. 12: a) Structural features of the thioesterase domain of human fatty acid synthase. (a) Overall representation of the TE domain showing subdomain A (green) and subdomain B (blue), with key amino acid residues involved in substrate and inhibitor interaction (b) Close-up view of the catalytic triad-Ser2308, His2481, and Asp2338-within the TE active site of human FASN (PDB ID: 1XKT), This representation emphasizes the structural basis for substrate specificity and covalent inhibitor interactions, providing insights into rational TE-targeted inhibitor design (created by author)

Enoyl-ACP reductase domain

The human enoyl-ACP reductase (hER) domain of fatty acid synthase remains one of the least explored catalytic domains, despite its essential role in fatty acid biosynthesis. This domain catalyses the NADPH-dependent reduction of trans-2-enoyl-ACP intermediates to saturated fatty acyl chains, completing one cycle of chain elongation. Structurally, hER adopts a Rossmann-like fold, with NADPH binding occurring in a relatively shallow and solvent-exposed pocket adjacent to the substrate channel, a feature that significantly influences inhibitor binding and selectivity. Triclosan (compound 34), a widely used antibacterial agent, was among the first small molecules identified as hER inhibitor. It exhibited moderate antiproliferative activity with IC_{50} values of 4.5–7.8μM in prostate cancer cell lines (LNCaP and PC-3) and 2.5–7.5μM in HepG2 hepatocellular carcinoma cells. Subsequent SAR studies by Sadowski *et al.* demonstrated that replacement or modification of the phenolic hydroxyl group of triclosan resulted in a significant loss of inhibitory activity, confirming the critical role of this hydroxyl moiety in hER binding (fig. 13) [50,51]. Beebe *et al.* further expanded hER-targeted inhibition by identifying 5-hydroxylansoprazole sulfide (5HLS; compound 35) as a structurally distinct hER inhibitor. Molecular docking and biochemical studies revealed that compound 35 forms stabilizing hydrogen-bond interactions with Gly1850, Asn1572, and Lys1771, leading to induction of apoptosis in MDA-MB-231 breast cancer cells at a concentration of 10μM. Importantly, comparative analyses showed that the presence of the hydroxyl group is essential for hER inhibition, as the parent lansoprazole scaffold lacking this functionality exhibited markedly reduced activity [52].

Despite validated cytotoxic effects observed upon ER inhibition, the drug ability of her domain remains intrinsically limited by its structural and mechanistic features. The ER active site adopts a shallow, solvent exposed NADPH-binding pocket, which restricts the formation of high affinity, drug like interactions and makes selective inhibition challenging. Most reported ER inhibitors, including triclosan and its analogues, rely heavily on single hydrogen bond interactions with conserved residues, resulting in micromolar potency and poor selectivity due to structural similarity with

other cellular reductases. Unlike the KR domain, ER lacks a deep hydrophobic pocket capable of stabilizing bulky scaffolds, thereby constraining SAR optimization. However, emerging opportunities lie in allosteric modulation, dual-site inhibitors targeting both the cofactor and substrate channels, and fragment-based approaches that exploit transient pockets formed during catalytic cycling. Additionally, ER inhibition may be best leveraged through poly pharmacological strategies, where moderate ER inhibition complements KR or TE directed agents to enhance metabolic stress in cancer cells. Collectively, while the ER domain is unlikely to yield standalone high-affinity inhibitors, it represents a promising adjunct target within multidomain FASN inhibition strategies.

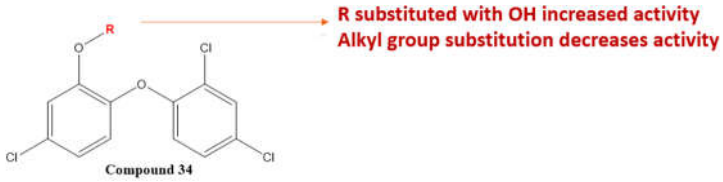

Fig. 13: Triclosan and structurally modified derivatives as inhibitors of the ER domain of human fatty acid synthase. The panel illustrates chemical structures of triclosan and representative analogues, highlighting functional groups critical for ER inhibition (created by author)

Future perspectives

The development of fatty acid synthase inhibitors has progressed from non-specific, highly reactive compounds toward rationally designed, domain-selective agents with improved translational potential. Early clinical failures, including severe weight loss associated with C75 and the poor bioavailability and off-target effects of orlistat and epigallocatechin gal late, provided critical insights into the systemic consequences of indiscriminate FASN inhibition and underscored the necessity for selective and reversible targeting strategies. In this context, selective inhibition of the ketoacyl reductase domain has emerged as the most clinically viable approach, as exemplified by the advancement of TVB-2640 into Phase I and II clinical trials [53, 54]. Although TVB-2640 remains the only FASN inhibitor to reach clinical evaluation, preclinical studies of compounds such as TVB-3166 (compound 36) and PF-109 (compound 37) have demonstrated significant antitumor activity across multiple cancer models, including breast, prostate, colorectal, and lung cancers.

Despite these advances, resistance mechanisms and treatment-limiting toxicities remain major obstacles to the long-term efficacy of FASN-directed therapies. Cancer cells exhibit pronounced metabolic plasticity and can rapidly adapt to FASN blockade by activating compensatory lipid acquisition pathways that bypass de novo lipogenesis. Upregulation of fatty acid uptake via CD36, increased lipoprotein lipase (LPL) activity, and enhanced fatty acid binding protein mediated intracellular transport collectively sustain membrane biogenesis, β-oxidation, and lipid-dependent oncogenic signalling pathways such as PI3K/AKT. These adaptive responses significantly blunt the antitumor effects of FASN inhibition and represent a dominant mechanism of on-target resistance. In parallel, on-target toxicity arising from systemic lipid deprivation, particularly in metabolically active tissues, and off-target inhibition of related enzymes continue to constrain dose escalation and therapeutic windows. These liabilities are especially pronounced for pan-FASN and covalent inhibitors, reinforcing the importance of domain-selective and reversible inhibition. Consequently, future therapeutic strategies are increasingly shifting toward combination approaches that simultaneously target lipid synthesis and lipid uptake or oxidation pathways, as well as transcriptional regulators such as sterol regulatory element-binding protein-1. Integration of FASN inhibitors with agents targeting CD36, FABPs, or fatty acid oxidation, potentially augmented by dietary lipid modulation, may be required to achieve durable clinical responses. Collectively, while KR-selective FASN inhibitors represent a significant translational advance, overcoming compensatory metabolic reprogramming and minimizing systemic toxicity will be essential for realizing the full therapeutic potential of FASN-targeted cancer therapy [55-57].

CONCLUSION

FASN remains a compelling therapeutic target in cancer due to its central role in lipid biosynthesis and tumour metabolic reprogramming. While early generations of FASN inhibitors were limited by toxicity and poor pharmacokinetic properties, recent advances in domain-selective inhibitor design particularly targeting the KR domain have significantly improved clinical feasibility. However, the ability of cancer cells to adapt by exploiting alternative lipid acquisition mechanisms continues to restrict the efficacy of monotherapies. Therefore, successful translation of FASN-targeted therapies will likely depend on integrated approaches that combine selective FASN inhibition with blockade of lipid uptake, transport, and regulatory pathways. Such multifaceted strategies may overcome metabolic resistance mechanisms and enhance therapeutic durability, ultimately improving clinical outcomes in FASN driven malignancies.

LIST OF ABBREVIATIONS

FAS-Fatty Acid Synthase

ACSs-Acyl-CoA Synthetases

CPT-Carnitine Palmitoyl Transferase

DH-Dehydratase,

KR-Ketoacyl Reductase,

KS-Ketoacyl Synthase,

MAT-Malonyl Acyl Transacylase,

MAPK-Mitogen-Activated Protein Kinase

PI3K-Phosphatidylinositol-3 Kinase

SCD1-Stearoyl-CoA Desaturase-1

SREBP-1-Sterol Regulatory Element Binding Protein-1

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SAR-Structural Activity Relationship

TCL-Triclosan

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AUTHORS CONTRIBUTIONS

First Author-Conducted literature search, analysed the published studies, and drafted the original manuscript, Corresponding author-Provided overall supervision and guidance, refined the review framework, critically revised the manuscript for important intellectual content

CONFLICT OF INTERESTS

The authors confirms that there is no conflict of interest related to the manuscript.

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