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ABSTRACT

Mucosal-associated invariant T cells (MAIT cells) represent a potential therapeutic target as they can tune or enhance immune responses. They recognise and become activated by antigens, presented by the monomorphic MHC-I related molecule, MR1. To assess the significance of MAIT cells in human diseases, a better understanding of the MAIT cell-MR1-antigen interaction is imperative. Easy access to MR1 ligands and MAIT cells activators can help achieve this. In this review, we summarise current literature that has identified the natural ligands and drug-like molecules that activate MAIT cells and provide insight into their key molecular interactions with MR1 and MAIT T cell receptors (TCRs). We focus on the progress made in synthesizing and isolating 5-amino-6-D-ribityl-laminouracil (5-A-RU), a key precursor in the synthesis of the known natural ligands, 5-(2-oxopropylidenea-mino)-6-D-ribitylaminouracil(5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), and also on the stabilisation and optimisation of the latter compounds.

1. Introduction

Non-conventional T cells include the invariable natural killer T (iNKT) cells and mucosal-associated invariant T (MAIT) cells, which recognize non–peptide antigens, presented to them by MHC class I like molecules (Mohan and Unanue, 2012; Neefjes et al., 2011; Garner et al., 2018, Ussher et al., 2014). MAIT cells are abundant in the blood, liver and gut mucosa of humans and somewhat less abundant in mice (Martin et al., 2009; Serriari et al., 2014; Tilloy et al., 1999; Treiner et al., 2003), where they are believed to be involved in protective immunity possibly through modulation of innate and adaptive immune responses (Godfrey et al., 2019; Kawachi et al., 2006). They can be activated by TCR signals, cytokine signals independent of the TCR, or by a combination of both (van der Merwe and Dushek, 2011, Garner et al., 2018, Usshe et al., 2014). TCR-dependent activation occurs when the MAIT cell semi-invariant TCR recognizes antigens presented by the MHC-related protein, MR1 (Huang et al., 2005; Kjer-Nielsen et al., 2012). This review will summarise the research that has identified the ligands and key functional groups required for MR1-dependent MAIT cell activation and also probe other structural changes that could modulate their stimulatory or inhibitory activities.

2. MR1-dependent MAIT cell activation

The monomorphic MHC-related 1 (MR1) protein belongs to the family of non-classical MHC-I proteins (MHC-Ib) and consists of three main domains: the α1 and α2 domains, which create the antigen (Ag) binding cleft, known as the A' pocket and the α3 domain, which interacts with β-2-microglobulin (β2m) (Hashimoto et al., 1995; Kjer-Nielsen et al., 2012). MR1 is expressed extensively in various tissues and cell types in mammals where it also tends to display a high degree of conservation (Treiner et al., 2003; Yamaguchi et al., 1997). Despite its abundance at the transcript level, MR1 protein expression is not prevalent on the cell surface (Harriff et al., 2016; Huang et al., 2008). Rather, MR1 exists in a ligand receptive unfolded conformation as an immature protein in the endoplasmic reticulum (ER) and its egression to the cell surface is facilitated when small molecules bind in its A' pocket (McWilliam et al., 2016). The bound ligand or antigen triggers a conformational change and causes the MR1 protein to refold. The MR1/antigen complex then moves to the surface of the antigen-presenting cell (APC) where the ligand is presented to MAIT cells, before the molecules are internalized and degraded in the endocyctic pathway (Huang et al., 2008; Kjer-Nielsen et al., 2012; 2013; Miley et al., 2003).

2.1. Natural MR1 ligands: inhibitors and activators of MAIT cells

The exact nature of the molecules, capable of both binding to MR1 and stimulating MAIT cells, remained elusive until recently. In 2010, two groups reported that MAIT cells were activated in a MR1-dependent manner by certain bacteria and yeasts but not viruses (Gold et al., 2010; Le Bourhis et al., 2010). This was followed by the identification of metabolites that were responsible for activation or inhibition of MAIT cells, derived from vitamin B2 and B9 biosynthetic pathways (Kjer-Nielsen et al., 2012). The first ligand, identified was attributed to 6-formylpterin (6-FP) (Fig. 1a), which is a degradation product of folic acid. The crystal structure of the MR1−6-FP complex showed that 6-FP was sequestered deep within the binding groove of MR1 (the A' pocket) and, importantly,

was also covalently bonded to MR1 through a Schiff base linkage between its carbonyl group and the side chain ε-amine of lysine 43 of MR1 (Kjer-Nielsen et al., 2012). It was postulated that the formation of this covalent bond allowed MR1 to refold, egress from the ER and translocate to the cell surface. The acylated form of 6-FP (Ac-6-FP) also showed the same MR1 up-regulating properties (Eckle et al., 2014). However, neither compound was able to stimulate MAIT cells due to a lack of interaction with the MAIT cells' TCRs. Instead, they were found to inhibit the activation of MAIT cells by competitively binding to MR1 (Eckle et al., 2014).

A second ligand capable of stimulating MAIT cells was also identified and believed to be 6-hydroxymethyl-8-D-ribityl-lumazine (rRL-6−CH2OH) (Fig.1b), which was later synthesized along with the related
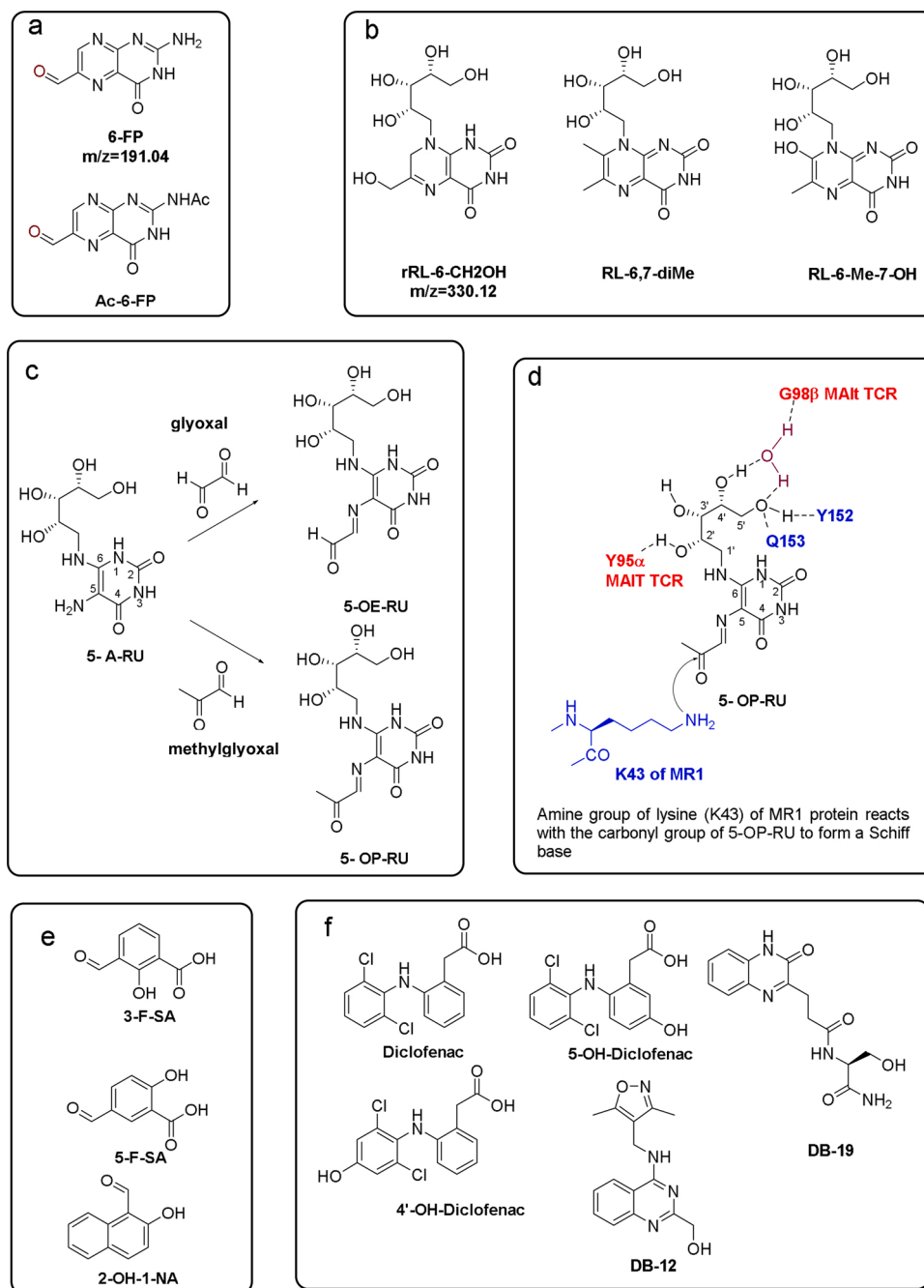

Fig. 1. MR1 ligands. (**a**) Pterins, MAIT Cell Inhibitors; (**b**) Lumazines, MAIT cell activators; (**c**) Formation of vitamin B metabolites 5-OP-RU and 5-OE-RU through interactions with glyoxal and methylglyoxal; (**d**) Important interactions between 5-OP-RU, MR1 and MAIT TCR; (**e**) Drug-like MAIT cell inhibitors; (**f**) Drug-like MAIT cell activators.

ribityl lumazines; 7-hydroxy-6-methyl-8-ᴅ-ribityllumazine (RL-6-Me-7−OH) and 6, 7-dimethyl-8-ᴅ-ribityllumazine (RL-6, 7-diMe) (Fig.1b), to test their MAIT stimulatory properties (Kjer-Nielsen et al., 2012; Patel et al., 2013). All three synthetic lumazines were found to activate MAIT cells, with rRL−6−CH2OH being the more potent activator (Kjer-Nielsen et al., 2012; Patel et al., 2013). Analysis of the MAIT TCR–MR1–RL-6-Me-7−OH complex allowed Kjer-Nielsen and colleagues to establish that RL-6-Me-7−OH is able to activate MAIT cells through direct interaction of its ribityl chain with the MAIT TCR and that therefore both rRL-6−CH2OH and RL-6, 7-diMe can activate MAIT cells in a similar manner. However, while RL-6, 7-diMe is a known intermediate in the riboflavin synthetic pathway, the origin of the potent activator rRL−6−CH2OH was unexplained. After further investigation, it was shown that the molecule responsible for strongly stimulating MAIT cells was actually pyrimidine-5-(2-oxopropylideneamino)-6--ᴅ-ribitylaminouracil (5-OP-RU) (Fig.1c), which exhibited the same *m/z* value of 329.11 as rRL−6−CH2OH (Corbett et al., 2014). Another pyrimidine, 5-(2-oxoethylideneamino)-6-ᴅ-ribitylaminouracil (5-OE-RU) (Fig.1c), was also identified and shown to activate MAIT cells, albeit to a slightly lesser extent (Corbett et al., 2014).

2.1.1. MAIT cell activation by 5-OP-RU

The α-ketoimine adducts 5-OP-RU and 5-OE-RU are formed when 5-amino-6-ᴅ-ribitylaminouracil (5-A-RU), a key intermediate in microbial and fungal riboflavin biosynthesis, reacts with α-dicarbonyl compounds, such as glyoxal and methylglyoxal (Fig.1c), formed from mammalian glycolysis or bacterial metabolism (Corbett et al., 2014; Mak et al., 2017). Both adducts activate MAIT cells more potently than the lumazines because their carbonyl moiety forms a Schiff base with lysine 43 of MR1 in the binding groove, alike to 6-FP (Corbett et al., 2014). The formation of this intermolecular covalent bond allows the upregulation of MR1 and subsequent presentation to the MAIT TCR. Crystal structures (Patel et al., 2013; Corbett et al., 2014; Gherardin et al., 2016; Keller et al.;, 2017; Mak et al., 2017) of the bound pyrimidines in complex with MR1 have shown that the ribityl chains 5-OP-RU and 5-OE-RU are presented to the MAIT TCR for binding. 5-OP-RU orients itself within the MR1 A' pocket through a network of intermolecular polar contacts, which include hydrogen bonding interactions between its 5′−OH group and Tyr152 with the Gln153 of MR1 and between the 2′−OH group and the Tyr95α of the MAIT TCR (Fig.1d). Additionally, Tyr95α of the MAIT TCR also forms hydrogen-bonding interactions with Tyr152 from MR1. This hydrogen-bonding network between Tyr95α, Tyr152 and 5-OP-RU is conserved among all published ternary structures of TRAV1−2+ MAIT TCRs in complex with MR1 presenting 5-OP-RU and has been coined as the 'interaction triad' by Awad et al. (2020). Other interactions of interest are the water-mediated hydrogen bonding between both the 5′−OH and 4′−OH of 5-OP-RU and G98β of the MAIT TCR (Awad et al., 2020).

2.2. Drug and drug-like MR1 ligands, inhibitors and activators of MAIT cells

A combination of *in silico* screening of various chemical libraries for molecules containing structural motifs reminiscent of 6-FP and 5-OP-RU and *in vitro* functional assays revealed that other non-microbial ligands were able to bind to MR1 and either inhibit or activate MAIT cells (Keller et al., 2017). Among these ligands are 3-formylsalicylic acid (3-F-SA), 5-formylsalicylic acid (5-F-SA) and 2-hydroxy-1-naphthaldehyde (2−OH-1-NA) (Fig. 1e), which are able to upregulate MR1 and inhibit the activation of MAIT cells to the same extent as Ac-6-FP. The MAIT activating ligands, include the aromatic molecules diclofenac (DCF) and its analogues 4'−OH-DCF and 5−OH-DCF, along with DB-12 and DB-19 (Fig. 1f). Though less potent than 5-OP-RU, they exhibited non-negligible MAIT stimulatory properties comparable to or surpassing those of RL-6-Me-7−OH and RL-6, 7-diMe. Interestingly, despite binding in the MR1 binding cleft, none of the activating drug like molecules

induced detectable up-regulation of cell surface MR1 (Keller et al., 2017). Nonetheless, these results highlighted the plasticity of the MR1 binding cleft towards other small molecules.

3. Synthetic tools used to probe the ligand-MR1-MAIT TCR interactions

To date, 5-OP-RU remains the most potent MAIT cell activator because of its ability to both up-regulate MR1 and interact with the MAIT TCR (Patel et al., 2013; Corbett et al., 2014; Gherardin et al., 2016; Keller et al.;, 2017; Mak et al., 2017). During infection, 5-OP-RU and related compounds are thought to act locally and rapidly. However, 5-OP-RU is an unstable compound and not isolable. Pharmacological agents require storage and the chemical stability necessary to act on a systemic basis, so overcoming the chemical instability of natural compounds has emerged as a major problem in the field. Consequently, most of the research, including synthetic efforts, has focused on 5-OP-RU, in order to: (1) facilitate its synthesis and increase its stability; and (2) improve its stimulatory activities by identifying the specific interactions essential for MR1 binding and upregulation and recognition by the MAIT TCR. The next section summarises the work carried out to address some of these key issues.

3.1. Chemical synthesis and stabilisation of 5-ARU

5-OP-RU and several other analogues are derived from the benchmark scaffold 5-A-RU. 5-A-RU is relatively unstable, being prone to oxidation and degradation by light (Cushman et al., 1991.; Cushman et al., 2001). We, along with others, have reported that 5-A-RU is more stable when the 5-amino group is trapped in the ammonium chloride form (Li et al., 2018; Lange et al., 2020; Salio et al., 2020). The most common methodology for preparing 5-A-RU involves a nucleophilic aromatic substitution reaction between ribitylamine and either 6-chloropyrimidine-2,4-dione or the more reactive nitrouracil (Fig. 2a, Scheme 1) (Al-Hassan et al., 1980; Cushman et al., 1991, 1997; Cushman et al., 2001; Romisch et al., 2002; Talukdar et al., 2012; Philmus et al., 2015; Li et al., 2018; Lange et al., 2020; Salio et al., 2020). The main differences between the reported syntheses in the literature relate to the yields of formation and the appearance of the isolated 5-A-RU.HCl salt. Typically, the coupling reaction between ribityl amine and the chlorinated nitrouracil in basic conditions is higher yielding than with 6-chloropyrimidine-2,4-dione. With regards to the appearance, Li et al. (2018) reported isolating a red tar after purification and isolation. We have isolated 5-A-RU.HCl both as a pink solid and as an off white solid (Salio et al., 2020). We observed that the pink colour caused the compound to fluoresce making it unsuitable for use in certain immunological-based experiments, such as the preparation of MR1-loaded tetramers. Other groups (Li et al., 2018; Constantinides et al., 2019) have reported the successful preparation of MR1-loaded tetramers with the colored 5-A-RU.

As reported, the HCl form of 5-A-RU is more stable as a solid (Li et al., 2018; Lange et al., 2020; Salio et al., 2020), but in solution it still gradually degrades upon standing with or without exposure to light, going from colourless to pink and finally yellow. These observations were corroborated by Lange et al. (2020) who recently shed some light on the nature of the degradation products. They followed the degradation of an aqueous solution of 5-A-RU by LCMS and suggested that the latter is first oxidised to an azaquinone, which subsequently undergoes hydrolysis (Fig.2b, Scheme 2). Minimising manipulations in the purification stage in the synthesis of 5-A-RU is crucial for retarding the degradation process. We found that using the stable protected form of 5-A-RU removes the need for lengthy purification in the final deprotection step, as the side-products (toluene and tertiary-butylsilyl alcohol) are volatile and can easily be removed under reduced pressure to afford pure 5-A-RU (Fig.2, Scheme 1).

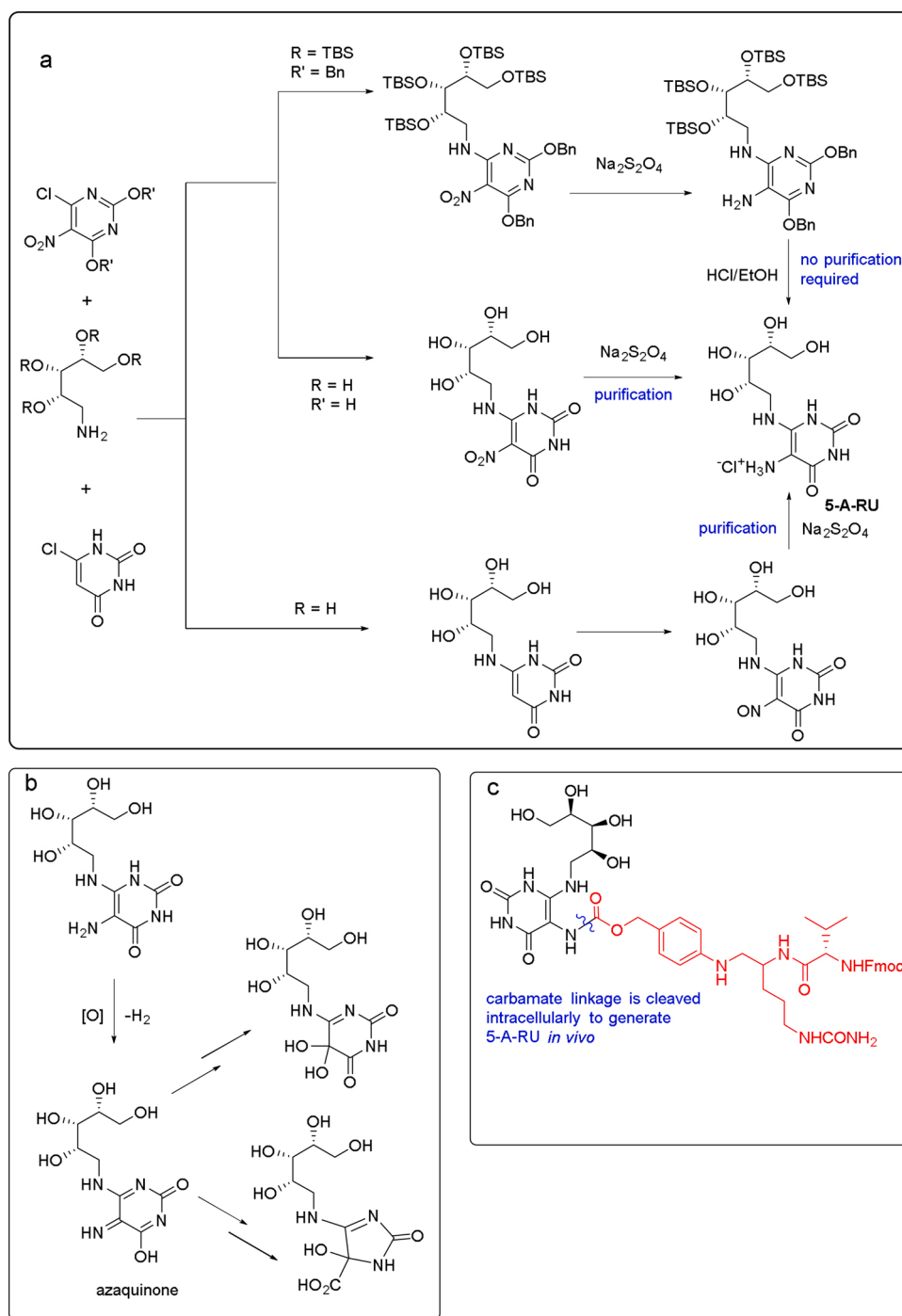Despite the progress made in isolating 5-A-RU, it remains that 5-A-

Fig. 2. Synthetic route to 5-A-RU and degradation products. (a) Scheme 1: Synthesis of 5-A-RU; (**b**) Scheme 2: Proposed degradation pathway of 5-A-RU; (**c**) 5-A-RU pro-drug.

RU used in biological and immunological studies has to be handled carefully. In light of the information at hand, it is recommended to prepare a solution of 5-A-RU in degassed solvent, to exclude oxygen, and wherever possible to use non-protic solvents to limit hydrolysis and maintain the reproducibility of 5-A-RU activity between experiments. However, the total exclusion of oxygen is difficult for *in vivo* experiments because of the presence of dissolved oxygen in blood or tissues. To address this issue, Lange et al. (2020) designed and synthesised a stable form of 5-A-RU, which can be cleaved to generate 5-A-RU intracellularly. To this effect, the authors introduced a carbamate group (valine-citrulline-p-aminobenzyl carbamate) on the 5-amino group of 5-A-RU, which upon enzymatic cleavage by proteases, such as

cathepsin B (Dubowchik et al., 2002), generates 5-A-RU intracellularly. The carbamate group, being electron withdrawing, also offers the benefit of stabilising the uracil ring towards oxidation and hence facilitating storage. The stable pro-drug 5-A-RU (Fig.2c) was found to induce higher levels of MAIT cell activation than 5-A-RU alone in MAIT cell cultures and in the lung. Of note, the authors commented on the altered mode of presentation of the 5-A-RU pro-drug, where the antigen MR1-loading possibly occurs through access to MR1 in recycling endosomes. This is based on the fact that the pro-drug requires prior cleavage from the enzymes mainly present in the endo-lysosomal compartments to release 5-A-RU. This endosomal MR1 loading differs from the predominant ER loading observed with 5-A-RU and 5-OP-RU (Harriff et al.,

2016; McWilliam et al., 2016). These recent findings will likely instigate further investigations into how ER and endosomal loading pathways of MR1 affect MAIT cell responses and also encourage other chemical modifications on 5-A-RU to explore MAIT cell biology *in vivo*.

3.2. Increasing the stability of 5-OP-RU

5-OP-RU is a fleeting intermediate ($t^{1/2}$ = 2 h at 37 °C, pH 6.8, or 14 h at 15 °C, pH 6.8) as it is prone to both cyclisation and hydrolysis of its imine functionality in mildly acidic medium (Fig. 3a) (Corbett et al., 2014). The aromaticity of the bicyclic lumazine product conveys thermodynamic stability and is the driving force behind cyclisation. Initial attempts by Mak et al. (2017) at stabilising 5-OP-RU included adding steric bulk to the amino group at C-6 to discourage cyclisation (Fig. 3b). However, the effect of the methyl substituent is two-fold; steric and electronic. The increased nucleophilicity of the nitrogen, imparted by the positive inductive effect of the methyl group, made the additional steric bulk inconsequential, resulting in those compounds being less

stable than the parent compound 5-OP-RU and still prone to cyclisation (Mak et al., 2017). A screening of various substituents, such as tertiary butyl and phenyl, with both positive and negative inductive effects and different sizes, would be useful to determine the optimum balance between electronic and steric effects and would aid in finding a stable open chain form of 5-OP-RU.

Mak et al. (2017) were more successful when they replaced the nucleophilic nitrogen substituent on the ring at position 6 with a non-nucleophilic carbon (Fig. 3c) as ring cyclisation onto the carbonyl moiety was no longer possible. These analogues, which also lacked the imine functionality at C-5, were found to be resistant to hydrolysis and have longer half-lives. Hence, both compounds were able to achieve sustained MR1 upregulation, with JYM72 being superior to Ac-6-FP (Mak et al., 2017). However, only JYM72 was able to activate MAIT cells through interaction of its ribityl chain with the MAIT TCR.
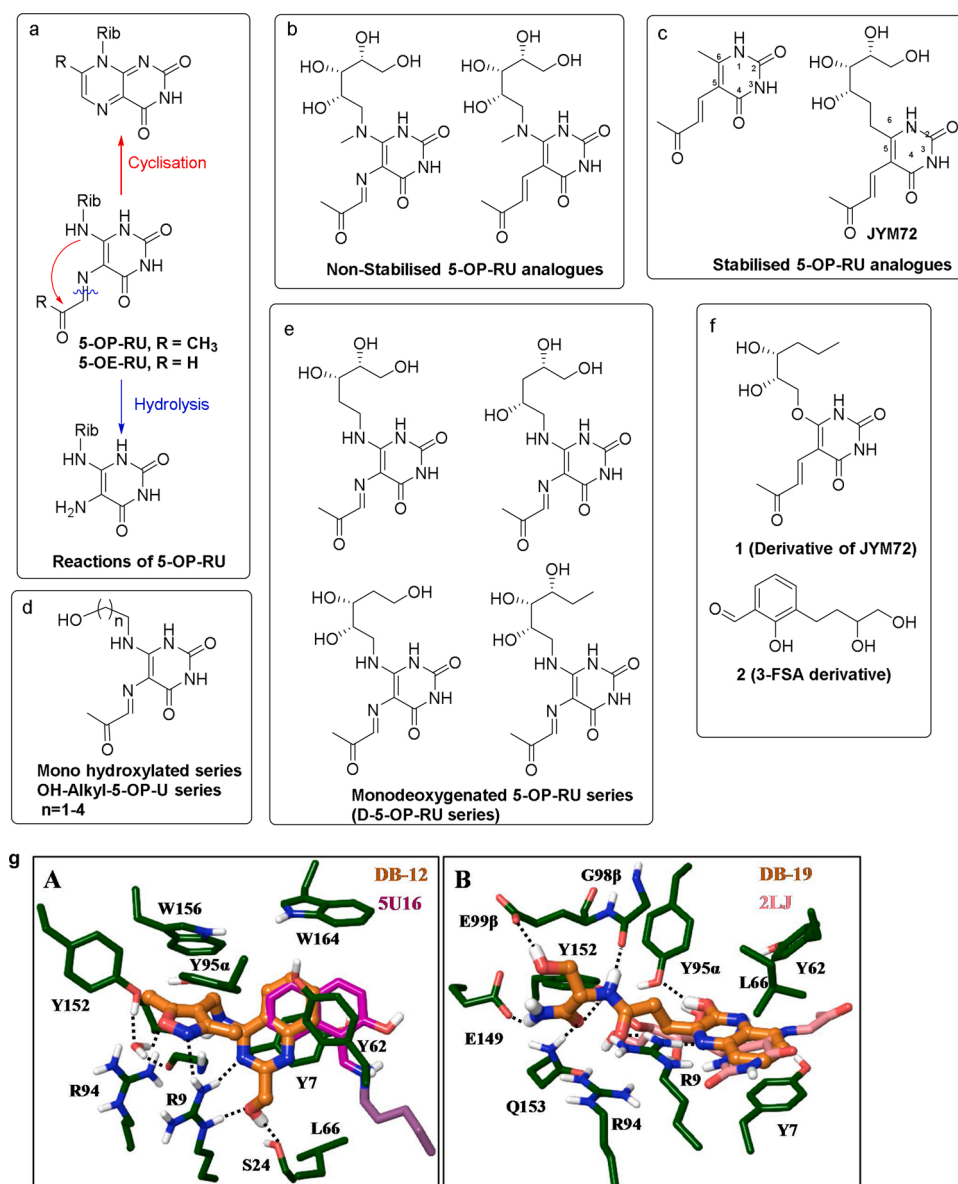
Fig. 3. (**a**) Reactions of 5-OP-RU; Rib, ribityl tail; (**b**) Non-Stabilised 5-OP-RU analogues; (**c**) Stabilised 5-OP-RU analogues; (**d**) and (**e**) 5-OP-RU analogues with modified ribityl chains; (**f**) Proposed modified molecules for optimum MAIT TCR interaction; (**g**) Induced Fit docked poses of DB-12 (A) and DB-19 (B). Residues in close proximity of the ligands are shown with dark green colored carbons; carbon atoms in docked hits are colored light brown.

3.3. Modification of the ribityl chain of 5-OP-RU for MAIT TCR interaction studies

The ribityl chain of 5-OP-RU is essential for MAIT activation through direct TCR contacts (Kjer-Nielsen et al., 2012; Corbett et al., 2014; Awad et al., 2020). However, 5-OP-RU only contributes 0.6 % of the MR1-ligand complex for MAIT TCR binding *via* hydrogen bonding interactions between its $2'-OH$ and the CDR3α loop of TRAV1-2^+ and a water-mediated interaction between its $4'-OH$ and $5'-OH$ group and the CDR3β (Awad et al., 2020). Two groups (Awad et al., 2020; Braganza et al., 2019, 2020) have investigated how 5-OP-RU achieves MAIT activation and evaluated which interactions with the MAIT TCR are crucial by modifying the ribityl chain of 5-OP-RU. The authors of these studies (Awad et al., 2020; Braganza et al., 2019, 2020) independently synthesised a series of monodeoxy (D-5-OP-RU) (Fig. 3d) and mono-hydroxylated analogues of 5-OP-RU (OH-alkyl-5-OP-U) (Fig.3e) and assessed the compounds based on the following criteria: their stability, ability to upregulate MR1, and finally, capacity to stimulate MAIT cells. Because modifications were on the ribityl chain, both series of compounds were as unstable as 5-OP-RU, irrespective of where the deoxygenation was introduced in the ribityl chain. The main differences were in terms of MR1 upregulation and intrinsic MAIT activation. Both the D-5-OP-RU and the OH-alkyl-5-OPU series of compounds demonstrated that the modifications at the $2'-$ and $3'-OH$ positions of the ribityl moiety resulted in less potent MAIT cell activation, while modifications at the $4'-OH$ and $5'-OH$ had negligible impact on MAIT cell activation. The formation of a direct hydrogen bond with Tyr95α of the MAIT TCR was prevented in $2'$-D-5-OP-RU while it was maintained in $3'$-D-5- OPRU, $2'-$—OH-ethyl-5-OP-U and $3'-OH$-propyl-5-OP-U. However, in the latter compounds, any direct or water-mediated interaction between the ribityl chain and Tyr152 from MR1 was absent. Therefore, it seems that for optimum MAIT cell activation, the interaction triad of hydrogen bonding between MAIT TCR Tyr95α, MR1 Tyr152 and ligand (Awad et al., 2020) is essential.

While it was previously recognised that the ribityl chain is not necessary for potent MR1 upregulation, as evidenced by 6-Ac-FP and other compounds (Corbett et al., 2014), Awad et al. (2020) observed that as the number of strong interactions (*e.g.* hydrogen bonding interactions) between the ligand and the residues in the MR1 binding cleft increased, the cell surface MR1 upregulation decreased. This was exemplified by the monohydroxylated analogues (OH-alkyl-5-OP-U, Fig. 3d), which upregulated MR1 to a larger extent than the mono-deoxygenated analogues (D-5-OP-RU, Fig. 3e). In summary, these latest findings indicate that the hydroxyl groups on the ribityl chain are essential for TCR recognition but that MR1 upregulation is favoured by weaker Van der Waals forces with the aromatic residues within the cleft over stronger hydrogen bonding interactions.

4. Future directions

All the present data indicates that the ideal MR1 ligand, suitable for MAIT activation, should fit into the aromatic cradle with limited polar interactions in the MR1 binding cleft and possess a flexible chain capable of interacting with MAIT TCR Tyr95α. It has also been established that the Schiff base formation between the carbonyl moiety of the ligand and Lys 43 of MR1 and the ensuing conformational change is the trigger that causes MR1 to "mature" (McWilliam et al., 2016). However, the presence of a carbonyl group in a ligand does not necessarily translate into MR1 cell surface expression (Keller et al., 2017). The egression of MR1 to the cell surface seems to be hindered by strong interactions, such as hydrogen bonding (Awad et al., 2020). This might explain why the less polar ligands, such as the ribityl-less compounds (e.g Ac-6-FP), and the monohydroxylated 5-OP-U compounds exhibit higher percentages of MR1 upregulation than their highly hydroxylated counterparts (e.g 5-OP-RU and the monodeoxygenated 5-OP-RU) (Awad et al., 2020; Braganza et al., 2019, 2020).

JYM72 is a more stable analogue of 5-OP-RU and demonstrates that modifications on the uracil ring at C5 and C6 are well tolerated (Mak et al., 2017). However, relative to 5-OP-RU, JYM72 is slightly less activating. This was attributed by Awad et al. (2020) to the extra flexibility of the C—C bond compared to the C—N bond, which affects the orientation of the ribityl chain for TCR recognition. A C—O bond is shorter than a C—C bond and slightly less flexible. Derivatives of JYM72, such as molecule 1 (Fig. 3f), are therefore worth investigating. Both Stocker's and Rossjohn's groups observed that the progressive removal of hydroxyl groups from 5-OP-RU induced less and less activation of MAIT cell and eventually converted the agonists into antagonists (Awad et al., 2020; Braganza et al., 2020). It would be interesting to see if it is possible to convert inhibitors, such as 3-FSA, into activators by introducing hydroxylated moieties. The simple, stable small aldehydes 3-F-SA and 5-F-SA strongly upregulate MR1 surface cell expression, but fail to stimulate MAIT cells, because they lack a ribityl chain. Replacing the carboxylic acid group with a hydroxylated alkyl chain such as the compound in Fig. 3f, mimicking the ribityl chain, is another option to explore.

Despite identifying drug-like MAIT activating molecules (Keller et al., 2017), little effort has been dedicated to modifying and optimising these molecules. Characteristically, the drug- like molecules differ from 5-OP-RU by inducing limited or no upregulation of MR1 because of their inability to form a Schiff base with MR1. Since, upregulation of MR1 contributes to better activation of MAIT cells, it might be useful to introduce functionalities in these ligands that allow them to strongly bind, either permanently or temporarily, to Lys 43.

We recently identified DB-12 and DB-19 as weakly activating compounds (Salio et al., 2020). To understand the structure-activity relationship of DB-12 and DB-19, we re-docked them, using $2-OH$-1-NA (5U16) and 5-OP-RU (2 L J) respectively as references for comparison (Fig. 3g). DB-12 interacts efficiently in the MR1 binding cleft through two hydrogen bonding interactions with R9, and an interaction with S24, though no favourable contacts are formed with K43. Hydrogen bonding with K43 is possible for example, if the quinazoline ring were replaced by pyrido[3,2-*d*]pyrimidine. DB-12 forms hydrogen bonds with Gly98 (TCRβ chain) and Y152, with interactions mediated through the same water molecule. Replacement of the 3-methyl by a polar substituent may lead to additional hydrogen bonding interactions with the all important, Tyr95α. DB-19 contains a propanoyl-serine amide group that can form extensive interactions in the ribityl region. This group is longer than ribitylamine, and extends further, outside of the 2 L J occupied region of the binding site, where it forms interactions with M72, E149 and E99 (TCRβ). In the MR1 cleft DB-19 only forms the key π-stacking interaction with Y7 and a hydrogen bond with R9. For more efficient binding, the benzene ring within the quinoxaline may be replaced by heterocycles or polar substituents may be introduced. Substituents may potentially form interactions with R9, S24, T34 or K43.

The search for new, optimised MR1 ligands and MAIT cell activators continues. However, armed with the continuous emerging information, finding one with the right balance between upregulation of MR1 and interaction with MAIT TCR is within reach.

Ethics statement

All experiments were approved by the University of Birmingham ethical committee where required and there are no ethical issues to report.

CRediT authorship contribution statement

Natacha Veerapen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Judith Hobrath:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Amareeta K. Besra:** Methodology, Formal analysis,

Investigation, Writing - original draft, Writing - review & editing. **Gurdyal S. Besra:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

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