Major structure activity relationships of resolvins, protectins, maresins and their analogues.

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ABSTRACT

Resolvins, protectins and maresins are a series of polyunsaturated fatty acid-derived molecules which play important roles in the resolution of inflammation. They are termed specialised pro-resolving mediators (SPMs) and faciliate a return to homeostasis following an inflammatory response. These molecules are currently the focus of intensive investigation, primarily for their ability to suppress inflammation in chronic disease states. Researchers have employed different synthetic approaches to assess whether various structural modifications of these compound could provide access to future therapeutics. This review summarises the modifications made thus far and focuses on the key structure activity relationships which have been uncovered for resolvins, protectins, maresins and their analogues.

Keywords: Resolvins, protectins, maresins, polyunsaturated fatty acids, drug design, inflammation.

Introduction

Inflammation is a reaction of the body to tissue damage or infection [1]. Ordinarily, the process should lead to the restoration of tissue homeostasis. Under certain conditions, however, inflammation is not resolved and this can ultimately result in tissue damage and chronic inflammatory diseases. Excessive inflammation is intrinsically linked with many chronic diseases, including neurological (e.g. Alzheimer's disease) and vascular conditions (e.g. atherosclerosis)[2, 3]. It has been long appreciated that lipid mediators play a critical role in the resolution of inflammation [4]. For this reason, considerable effort and resources have been expended in identifying individual mediators and fully elucidating their biological properties.

Lipoxins, resolvins, protectins and maresins are a series of polyunsaturated fatty acid-derived molecules which play important roles in the resolution of inflammation. They are termed specialised pro-resolving mediators (SPMs) and enable a return to homeostasis following an inflammatory response [5]. The SPMs typically display very high potency and are often active at nano- or even picomolar concentrations [6, 7]. However, the active SPMs are usually short lived *in vivo* and are instead rapidly transformed enzymatically into less active metabolites. By firstly characterising these metabolites, researchers have been able to identify the structural features which are important for activity. More recently, chemists have created synthetic analogues with the aim of increasing the duration of action of the SPMs while retaining, or improving, their potent biological effects. Such structural modifications could provide access to future therapeutics for a wide range of conditions (Table 1).

Table 1. Potential applications of different SPM classes

SPM class	Condition
Resolvins D series	Lung injury; Sepsis; Peritonitis; Skin inflammation; *E. coli* infection
Resolvin E series	Colitis; *C. albicans* infection; Lung injury; Dermal inflammation
Protectins	Lung inflammation; *E. coli* infection; Neuropathic pain
Maresins	Neuropathic pain; Lung inflammation

Several articles have summarised the wide array of different biological effects elicited by these molecules [5, 6, 8-10]. In particular, the biology and chemistry of the lipoxins and their derivatives have been the subject of numerous in-depth surveys and analyses [11-19]. By contrast, reviews of the related resolvins, protectins and maresins are much more limited, primarily as a result of their more recent discovery [7, 20, 21]. Accordingly, this article focuses on resolvins, protectins, maresins and their

analogues to identify the key structure–activity relationships observed for the range of natural and synthetic molecules which have been previously evaluated. Across the wide array of biological effects studied, particular attention is paid to anti-inflammatory activity. Where possible, the main structure–activity relationships of each compound class are also summarized. By capturing the successful modifications which have been accomplished thus far, this review should serve as a useful guide for the design of future therapeutics. The structure of the review is as follows:

Resolvins – D series and E series

- Protectins – Protectin D1
- Maresins – Maresin 1 and Maresin 2

Resolvins

The resolvins are a family of compounds derived from three polyunsaturated fatty acids (PUFA) (Figure 1) [22]. This family is divided into three subgroupings based on the PUFA from which they are biosynthesised. Originally, Serhan *et al.* identified two series of resolvins, the D-series and the E-series, which are generated in cells from docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) respectively [23, 24]. The E-series is comprised of three molecules and the D-series is made up of six [25]. Another series of resolvins was later discovered in people with a single nucleotide polymorphism which causes a change in the enzyme fatty acid elongase-2 [26]. This change allows for the production of four more resolvins from docosapentaenoic acid (DPA) as it accumulates in the body. DPA is an intermediate in the synthesis of DHA from EPA. These resolvins were termed the 13-series resolvins based on the alcohol group on carbon 13. Due to the limited data available, the 13-series resolvins are not discussed in detail in this review.

The D-series resolvins are biosynthesised from the omega-3 fatty acid DHA (**1**) for which they are named after (Figure 1) [24, 25]. This category of resolvins is made up of RvD1 (**1**), RvD2 (**2**), RvD3 (**3**), RvD4 (**4**), RvD5 (**5**), and RvD6 (**6**). Administration of aspirin may result in the production of their corresponding epimers. These so-called aspirin-triggered (AT) epimers possess beneficial therapeutic effects and may, in part, account for aspirin's anticancer activity [27]. Insufficient data currently exists to make meaningful conclusions on the structure activity relationships of RvD3 (**7**), RvD5 (**8**) or RvD6 (**9**).

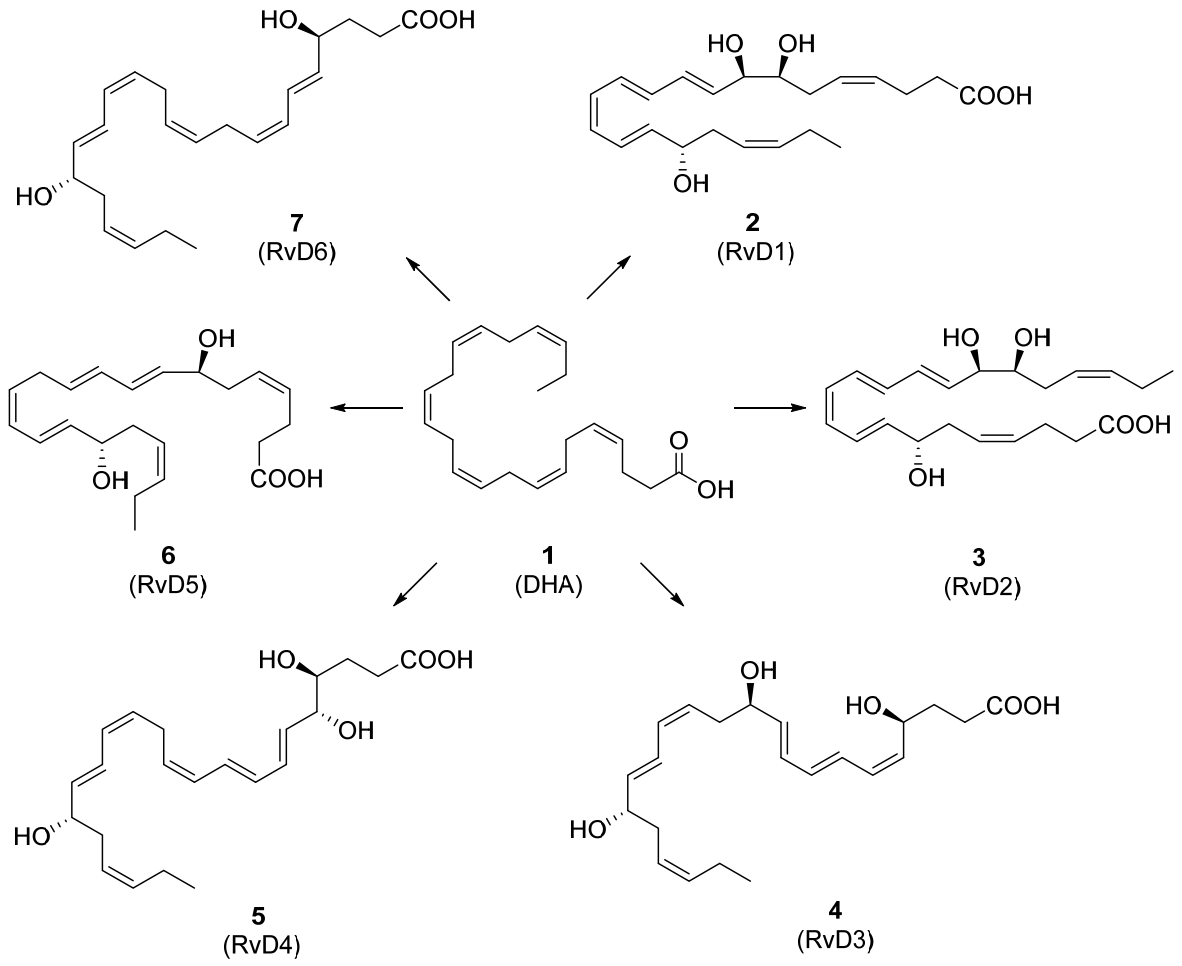


Figure 1. Resolvin D series.

RvD1 (**2**) is a potent pro-resolving mediator which limits PMN (polymorphonuclear neutrophil) infiltration on a par with indomethacin and also plays a role in regulating cytokine expression [28]. Additionally, RvD1 (**2**) has demonstrated a protective effect on lung tissue from infection by *P. aeruginosa* [29].

Aspirin-triggered RvD1 (AT-RvD1) (**8**) differs from RvD1 (**2**) with an (*R*)-configuration at the C17-hydroxyl group (Figure 2) [24, 25, 30]. Interestingly, AT-RvD1 (**8**) is highly resistant to *in vivo* oxidation, in contrast to the endogenous 17*S*-epimer (**2**) [30]. AT-RvD1 (**8**) inhibits leukocyte infiltration with similar efficacy to RvD1 (**2**) and both were found to reduce PMN infiltration by approximately 35%. Additionally, AT-RvD1 (**8**) enhances the removal of both sickle red cells and PMN leukocytes by macrophages while also preventing the adhesion of neutrophils to vascular endothelium [31].

RvD1 (**2**) is metabolised in the body by eicosanoid oxidoreductases (EOR) [30]. The EORs oxidise specific alcohol groups in RvD1 to form 8-oxo-RvD1 (**9**) and 17-oxo-RvD1 (**10**). In the case of 8-oxo-RvD1 (**9**), the metabolite mostly retains its activity, reducing PMN transmigration by 41%, whereas 17-oxo-RvD1 (**10**) appears inactive. In related work, 8-oxo-RvD1 (**9**) was found to inhibit monocyte adhesion to adipocytes whereas 17-oxo-RvD1 (**10**) was essentially inactive [32]. These findings highlight the importance of the 17-hydroxy group for biology activity.

In an effort to limit C17 oxidation, Serhan and colleagues introduced a methyl group to RvD1 (**2**) at C17 [33]. The epimeric mixture was converted to methyl ester **11** prior to intravenous administration. Both RvD1 and its analogue were found to significantly reduce myeloperoxidase levels in lung tissue, by ~25% for RvD1 methyl ester and by ~30% for tertiary alcohol **11**. In a similar vein, Gao *et al.* incorporated an *p*-fluorophenyl ether into AT-RvD1 (**8**) [34]. As before, the compound was converted to its methyl ester prior to administration *in vivo*. *p*-Fluorophenyl ether **12** was shown to be effective in protecting against IgG immune complex development which is normally associated with lung injury. **12** also inhibited transcription factors, such as nuclear factor-κβ (NF-κβ), which regulate genes involved in inflammation. In these studies, analogue **12** reduced the infiltration of neutrophils by 46% in mice BALFs (Bronchoalveolar Lavage Fluids) while AT-RvD1 (**8**) caused an 81% reduction by comparison.

Specialised pro-resolving mediators, such as lipoxins and resolvins, are rapidly metabolised *in vivo* in part due to the presence of the reactive triene system [35, 36]. In 2015, Serhan and co-workers prepared and evaluated aromatic analogues **13** and **14** [37]. By replacing the triene backbone with a benzene ring, the essential 10*Z*-geometry was retained. Benzene-containing derivative **13** was synthesised as an epimeric mixture at the 17-position, thereby mimicking a mixture of RvD1 (**2**) and AT-RvD1 (**8**). The two *Z*-alkenes in **13** were replaced with alkynes in **14**. Dialkyne **14** displayed an ability to activate the ligand-receptor GPR32 which is triggered by RvD1 (**1**), suggesting that these molecules share the same activation pathways for resolution. Dialkyne **14** was significantly more potent than RvD1 in lung tissue, reducing PMN infiltration by 58% versus an 18% reduction by RvD1 as measured by myeloperoxidase levels. Accordingly, **14** should provide better protection against second-organ ischemia-reperfusion-induced lung injury following surgery than the natural resolvin. Benzo-derivative **14** resisted enzymatic oxidation and deactivation by the EORs unlike RvD1. This potentially accounts for the higher potency of **14** considering the elevated local expression of EORs in the lungs. Benzene-containing derivatives **13** and **14** were both found to decrease leukocyte activation to a similar extent while also promoting resolution on a par with RvD1 (**2**).

More recent studies have demonstrated that endogenous peptide-containing conjugates of RvD1 play a role in the resolution of inflammation and tissue regeneration [38, 39]. These conjugates contain a small peptide in place of the 8-OH group. A glutathione sidechain is incorporated into RCTR1 (**15**) at C8. Enzymatic removal of glutamic acid from RCTR1 (**15**) generates RCTR2 (**16**). Subsequent cleavage of the glycine component from RCTR2 (**16**) affords RCTR3 (**17**). These cysteinyl conjugates were found to reduce leukotriene B4-initiated chemotaxis by 37%, 42% and 47% respectively. RCTR1 (**15**), RCTR2 (**16**) and RCTR3 (**17**) restricted PMN infiltration in lung tissue by ~30%, ~40% and ~50% respectively, making RCTR3 (**17**) the most potent of these conjugates.

The structure-activity relationships for RvD1 (**2**) are summarised in Figure 2. The 17-OH group and the 10*Z*-configuration are important for activity. Modifications to the aliphatic backbone can improve metabolic resistance. Lastly, the 8-OH has been shown to be a potential site for modification.

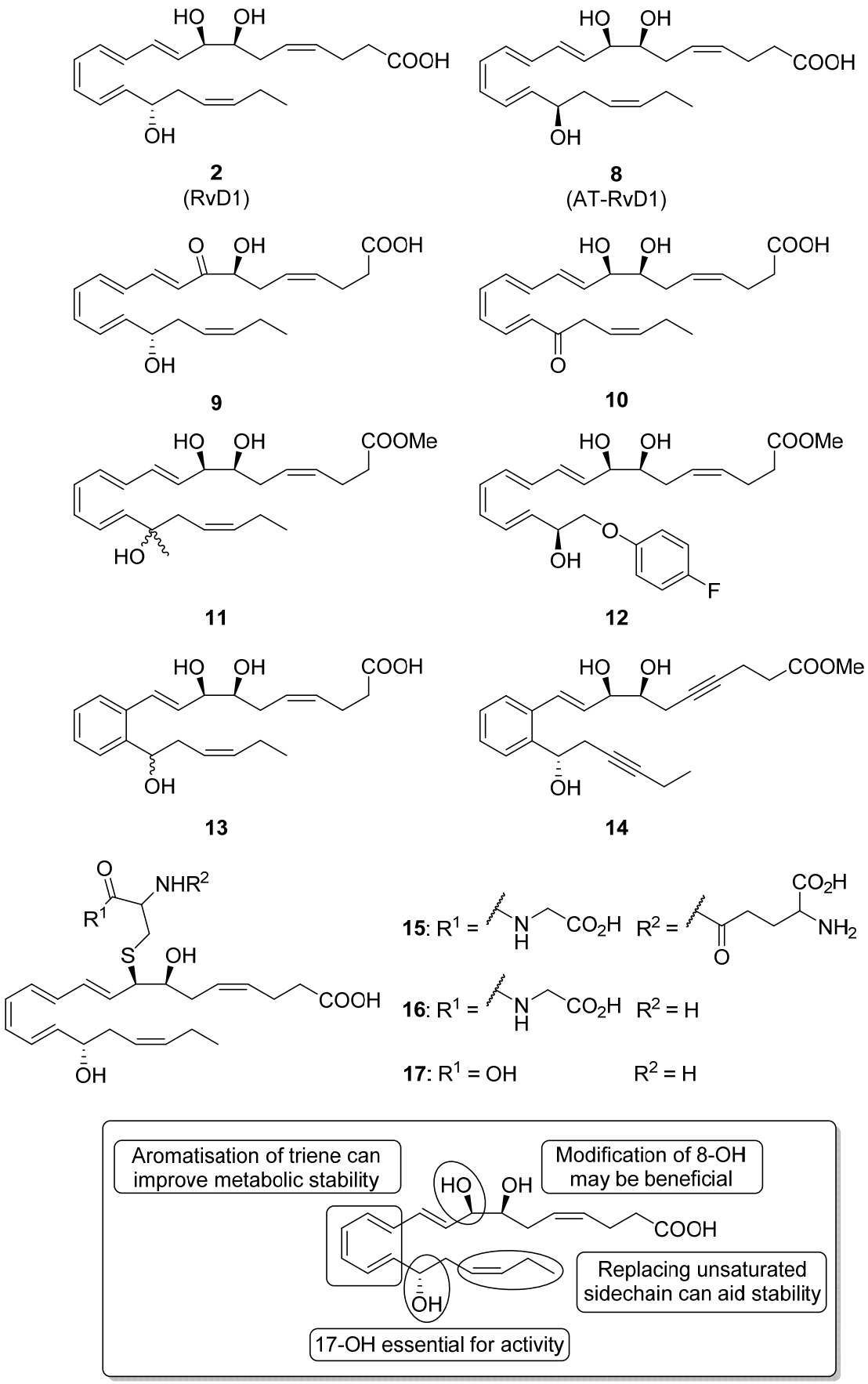


Figure 2. RvD1 and analogues – main structure activity relationships.

Like all other D-resolvins, RvD2 (**3**) is synthesised *in vivo* from DHA (Figure 3). Among its main effects are the reduction of bacterial burden, cytokine production and neutrophil recruitment [40]. Along with RvD1 (**2**), it can also reduce obesity-induced adipose inflammation [32]. Its aspirin-triggered 17*R*-epimer, namely AT-RvD2 (**18**), was found to significantly reduce edema and neutrophil accumulation in lung inflammation injury after sepsis in mice [41]. The 10*Z*-configuration is significant as the corresponding *E*-isomer (**19**) is essentially inactive [40].

RvD2 (**3**) undergoes metabolic oxidation at C7 or C16 by EOR enzymes, generating the corresponding conjugated ketones. 16-oxo-RvD2 (**20**) exhibits negligible anti-inflammatory effects whereas 7-oxo-RvD2 (**21**) is almost as active as RvD2 (**3**) [32].

Although synthetic analogues have yet to be reported, some important structure activity relationships are already apparent for RvD2 (**3**) (Figure 3). These may help in the design of future RVD2 derivatives.

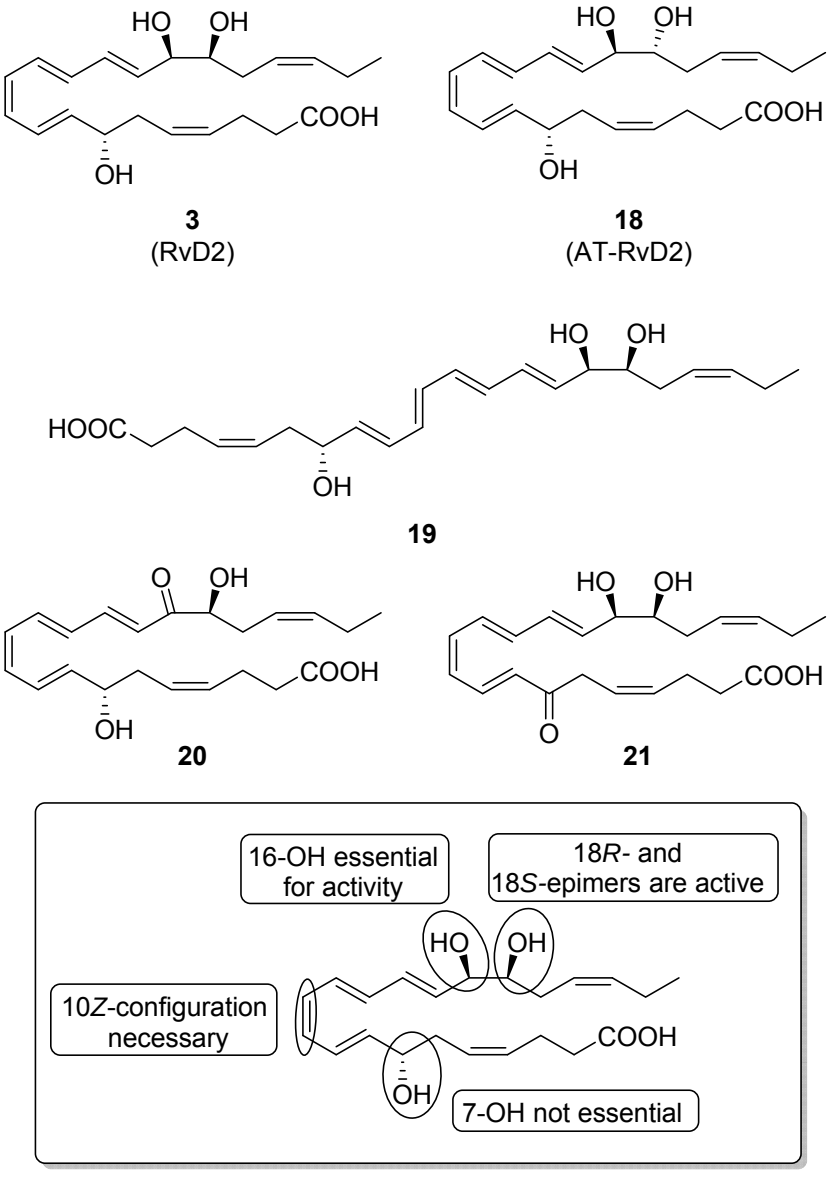
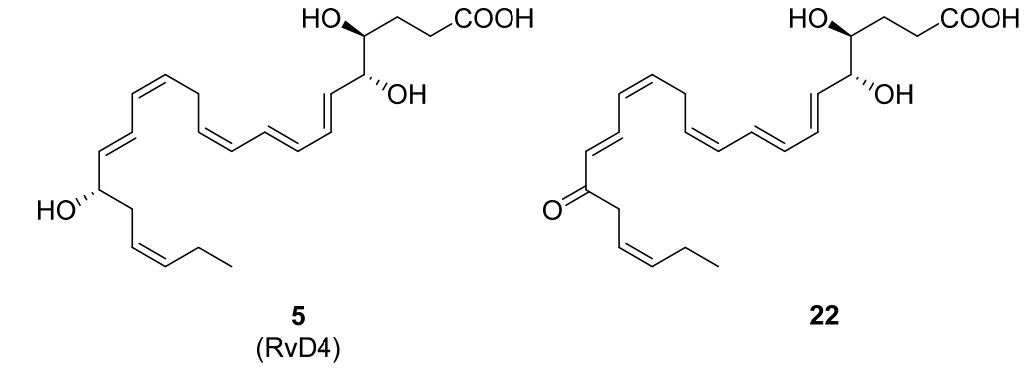


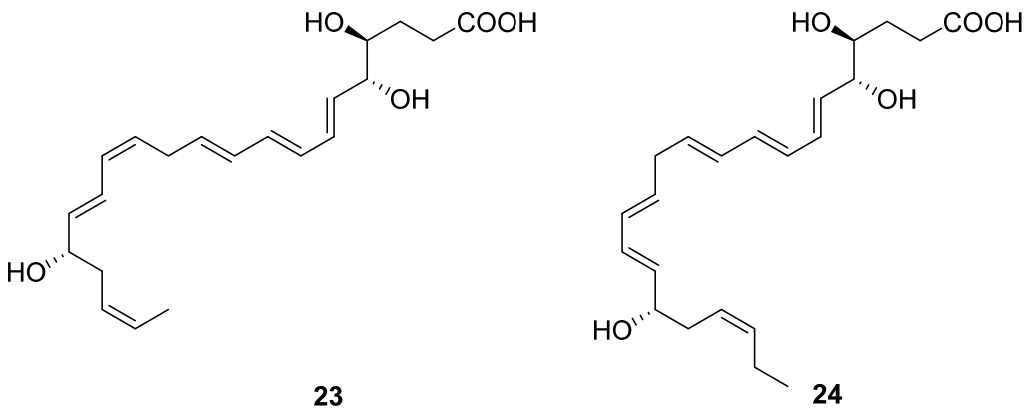
Figure 3. RvD2 and analogues – main structure activity relationships.

RvD4 (**5**) plays a beneficial role in both host protection and bacterial clearance of *S. aureus* infections (Figure 4) [24, 42]. RvD4 (**5**) is effective at very low levels, reducing neutrophil infiltration and enhancing uptake of apoptotic PMNs at 0.1 nM concentration [42]. The principal metabolite of RvD4 (**5**) is 17-oxo-RvD4 (**22**), which is essentially inactive as measured by its ability to evoke chemotaxis [43].

Two isomers of RvD4 were synthesised in order to examine the importance of RvD4's double bond geometry for its pro-resolving effects. Subsequent evaluation of the two geometric isomers, 10*E*-RvD4 (**23**) and 10*E*,13*E*-RvD4 (**24**), confirmed that they did not significantly stimulate *E. coli* phagocytosis in human peripheral blood neutrophils or monocytes. By contrast, RvD4 (**5**) was found to increase phagocytosis by up to 50% in both neutrophils and monocytes at 10 nM concentration.

Taken together, these findings highlight the importance of the C17 hydroxyl and the 10*Z*,13*Z* double bond geometry for activity (Figure 4).





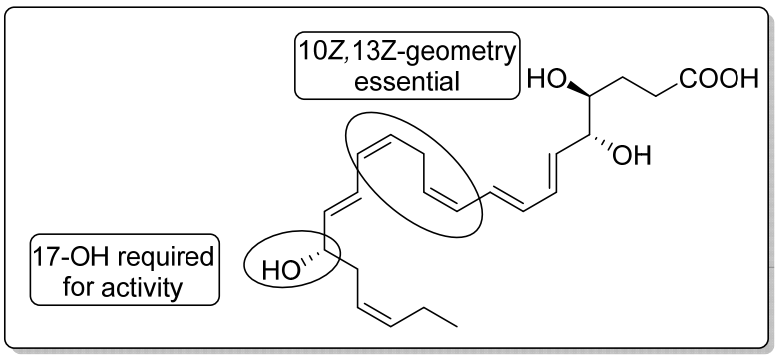


Figure 4. RvD4 and analogues - main structure activity relationships.

The E-series resolvins (RvE) are biosynthesised from EPA (**25**) *in vivo* and include RvE1 (**26**), RvE2 (**27**), RvE3 (**28**) and the newly discovered RvE4 (**29**) (Figure 5) [24, 44]. As no data currently exists on metabolites or derivatives of RvE4 (**29**), it is not possible to draw any conclusions about its structure activity relationships at this time.

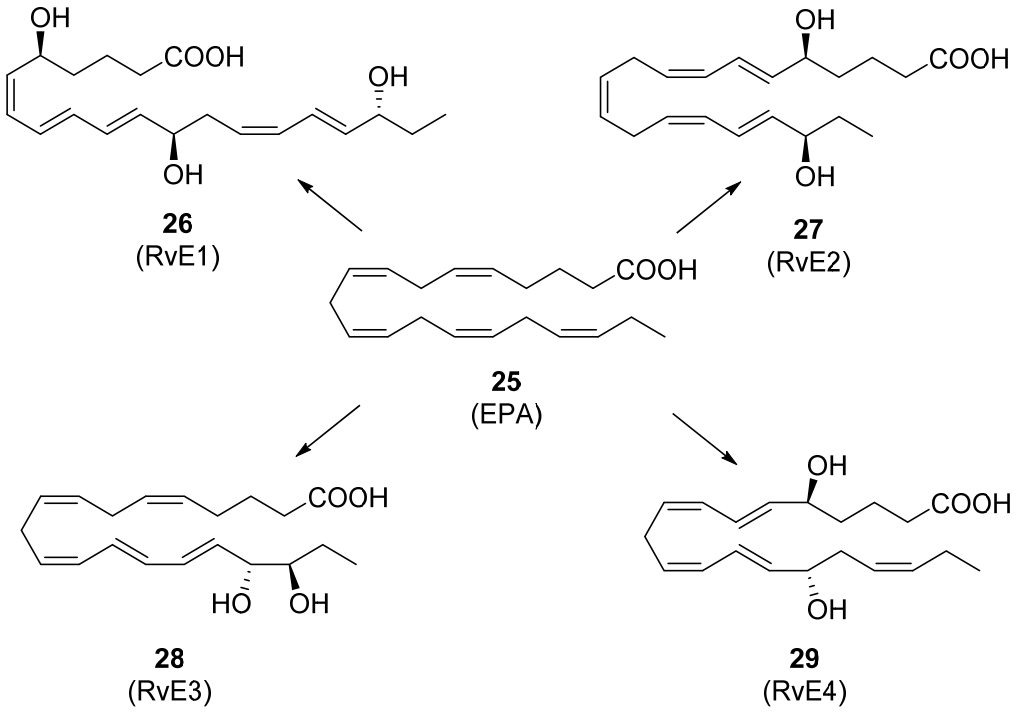


Figure 5. Resolvin E series.

The principal physiological effect of RvE1 (**26**) is to reduce PMN infiltration and tumor necrosis factor (TNF-α) exacerbation [45]. Nanomolar concentrations of RvE1 (**26**) have proven to be as effective as much higher doses of dexamethasone and aspirin in live mouse studies. Dexamethasone resulted in 60% inhibition of leukocyte recruitment at 10 μg/mouse, while aspirin caused 70% inhibition at 1.0 mg/mouse. A low dose of only 100 ng/mouse was sufficient for RvE1 to inhibit leukocyte recruitment by 50%-70%. Figure 6 contains the primary metabolites of RvE1 (**26**) [46]. Reduction of the 10,11-alkene into 10,11-dihydro-RvE1 (**30**) or oxidation of the 18-OH into 18-oxo-RvE1 (**31**) results in complete loss of activity [47]. Oxidation of C19 produces 19-hydroxy-RvE1 (**32**), which retains some activity, reducing PMN infiltration by ~15%. However, **32** is significantly less potent than RvE1 (**26**) which suppresses neutrophil recruitment by ~40%. 20-Hydroxy-RvE1 (**33**) is almost equipotent (~35%) with RvE1 (**26**) until it is further oxidised *in vivo* to 20-carboxy-RvE1 (**34**) which is inactive. Blocking these metabolic pathways by modifying the structure of RvE1 (**26**) in order to resist ω-oxidation is essential for

developing more effective anti-inflammatories. Oh and co-workers examined the aspirin-triggered epimer 18*S*-RvE1 (**35**) and determined that it is significantly less potent than endogenous RvE1 (**26**) [48]. 18*S*-RvE1 (**35**) was found to reduce PMN infiltration by only 10% after 12 hours, whereas RvE1 (**26**) caused a ~78% reduction over the same time period. Additionally, the *S*-isomer undergoes more rapid *in vivo* oxidation to 18-oxo-RvE1 (**31**) compared to epimeric RvE1 (**26**). This finding underlines the importance of stereochemical configuration for both biological activity, metabolic stability and duration of action.

In addition to these naturally-occurring RvE1 derivatives, several synthetic analogues of RvE1 have been prepared for biological evaluation. In an effort to improve metabolic stability, Artia and colleagues introduced a *para*-fluorophenoxy at C19. Incorporation of a bulky substituent at this position should both hinder oxidation of the 18-hydroxyl group and block ω-oxidation [47]. Subsequent esterification of the resulting carboxylic acid afforded methyl ester prodrug **36**. Phenoxyether **36** was as potent (~31% inhibition) as endogenous RvE1 (**26**) (~35%) in preventing PMN infiltration. Importantly, phenoxyether **36** proved resistant to oxidation by recombinant human dehydrogenase. Subsequent analysis confirmed that this modification also prevents ω1-oxidation.

Resolvyx Pharmaceuticals have progressed their dialkyne-containing analogue RX-10045 into phase II clinical trials as a treatment against dry eye and goblet cell loss [49]. This compound successfully reduces corneal inflammation and accelerates tissue repair in the eye. Formulation of the active compound as its isopropyl ester prodrug **37** increases permeability through the cornea where it undergoes *in vivo* hydrolysis to the original carboxylic acid [50, 51]. As the olefinic bonds in the native RvE1 (**26**) are susceptible to radical hydrogen abstraction [52, 53], these are replaced by alkynes in **37**, enhancing its metabolic stability. Interestingly, **37** features a truncated carbon backbone, with the deletion of five carbons (i.e. C16-C20), yet retains biological activity.

The C1-C4 segment of RvE1 (**26**) is conformationally flexible and the arrangement of the carboxyl group relative to the other conformationally restricted, unsaturated segments is considered important for activity. Ishimura and colleagues introduced a cyclopropane ring adjacent to the carboxylic acid group in order to restrict relative rotation of the carboxyl group [54]. Of the four variations investigated, **38** and **41** were significantly more effective at reducing PMN infiltration *in vivo*. A reduction of ~50% was recorded with stereoisomers **38** and **41**, thereby surpassing the native resolvin RvE1 (~30% reduction). Conversely, the remaining isomers **39** and **40** proved inferior to RvE1 with a ~25% reduction observed.

Comparing the activity of these different molecules provides an insight into the essential features of RvE1 (**26**) (Figure 6). The reduced activity of specific metabolites of RvE1 (**26**) highlights the importance of the conjugated triene unit and the stereochemical configuration of the 18*R*-OH. Alteration of the structural backbone can improve metabolic stability while retaining activity e.g. modification of C19/20 to block unwanted ω/ω1-oxidation. Restricting rotation of the carboxylic acid group sidechain may afford more potent analogues.

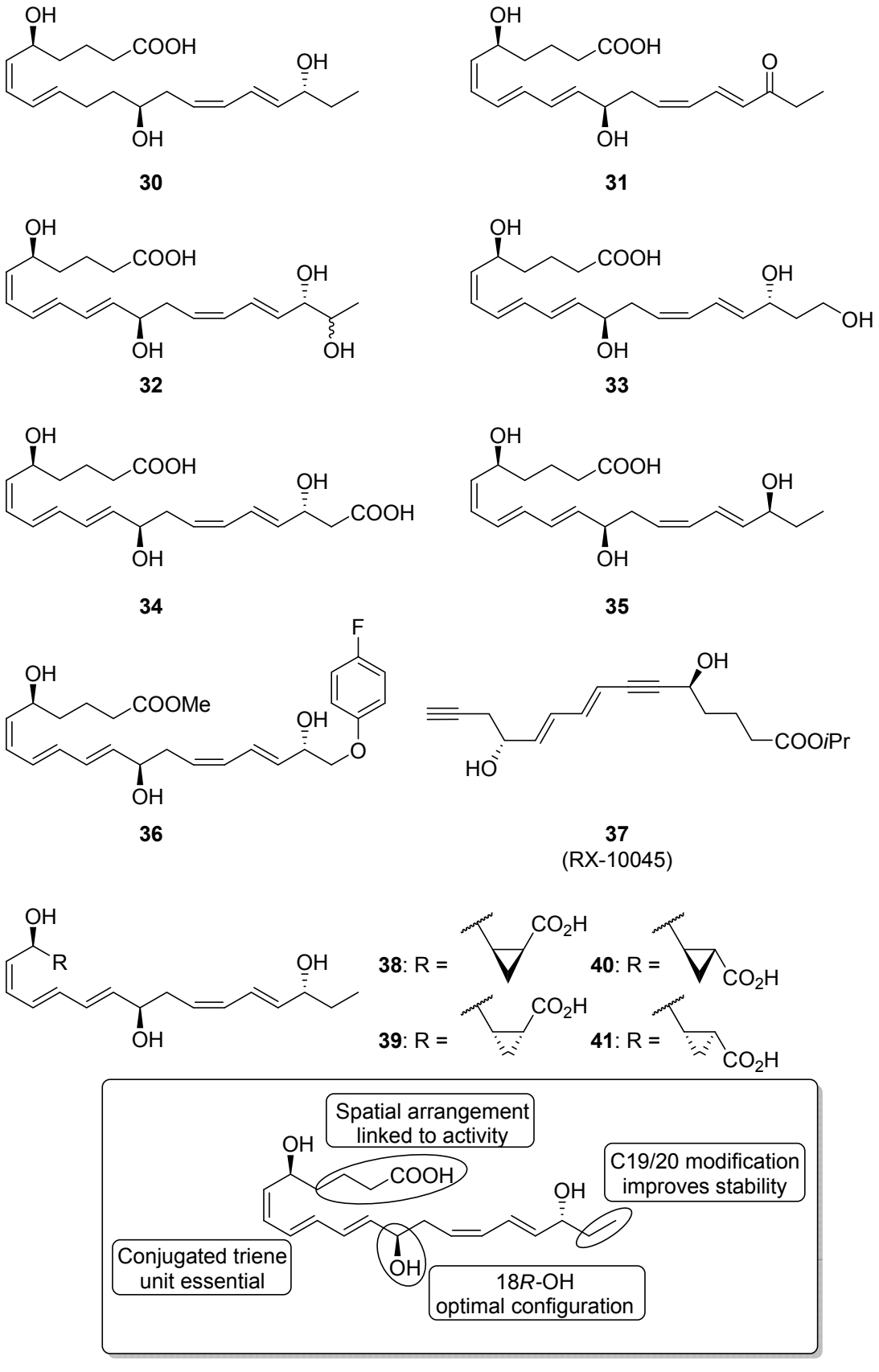


Figure 6. RvE1 and analogues – main structure activity relationships.

The efficacy of RvE2 (**27**) is comparable to that of RvE1 (**26**) in terms its ability to reduce neutrophil infiltration (Figure 7) [55]. RvE2 (**27**) is built upon a central 1,4-diene scaffold which makes it highly

susceptible to autoxidation [52]. With this in mind, cyclopropane-containing derivatives **42** and **43** were synthesised by Fukuda and co-workers *via* cyclopropanation of the C11-C12 *cis*-olefin in RvE2 (**27**). **42** and **43** were discovered to have a 35-hour half-life in air which compares very favourably to the 1.5 hour half-life of RvE2 (**27**) under the same conditions. At a 30 pg dose, cyclopropane **43** had a similar effect to RvE2 (**27**), reducing the number of peritoneal exudate cells (PECs) by ~30% after 24 hours. Isomer **42** proved to be even more potent than the native resolvin, and was found to reduce PECs by ~50%.

In a similar vein, Murakami and colleagues installed an aromatic ring in place of the unsaturated C9-C15 backbone of RvE2 (**27**) [56]. *Ortho-, meta-* and *para*-substitution patterns were systematically investigated. The metabolism of the *para*-substituted isomer was similar to that of the original resolvin, with concentrations of both being reduced to less than 40% of the original dose after exposure to human liver microsomes for six hours. The *ortho-* and *meta*-substituted analogues proved significantly more stable, with 90% and 80% of the original dose present after six hours respectively. The *meta-* and *para*-substituted isomers were discovered to be inactive compared to the control group. By contrast, *ortho*-substituted **44** was more efficacious than RvE2 (**27**) even at very low doses, reducing infiltration by ~35% in comparison to ~20% reduction by RvE2 (**27**) at the same 300 fg concentration. With a combination of higher potency and improved metabolic stability, *ortho*-substituted analogue **44** was identified as a valuable lead compound for future studies.

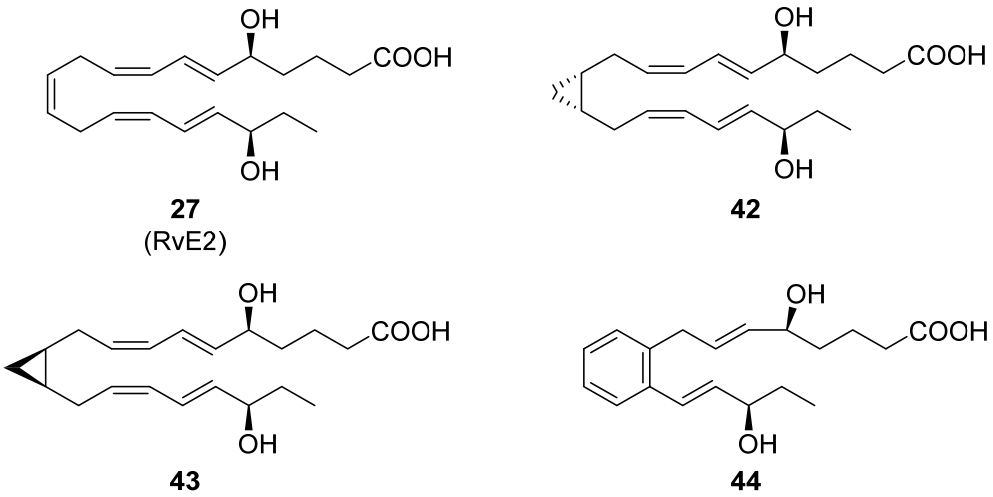


Figure 7. RvE2 and its analogues.

Research into the four stereoisomers of RvE3 confirmed that two isomers are present in nature, namely 17*R,*18*R*-RvE3 (**28**) and its epimer 17*R,*18*S*-RvE3 (**45**) (Figure 8) [57-59]. The stereochemical configuration of the 17-OH was determined to be important for activity. Both RvE3 (**28**) and 17*R,*18*S*-RvE3 (**45**) hinder migration of human PMN leukocytes. RvE3 (**28**) was the more potent molecule, reducing PMN migration by 12% in comparison to a smaller 8% reduction for 17*R,*18*S*-RvE3 (**45**). Inverting the stereochemistry at C17 to furnish 17*S,*18*S*-RvE3 (**46**) or 17*S,*18*R*-RvE3 (**47**) proved detrimental overall. A more recent study by Sato *et al.* has revealed how RvE3 (**28**) inhibits airway

inflammation in murine models [60]. RvE3 causes a major reduction in both eosinophil recruitment and mucus cell hyperplasia. The anti-inflammatory effect is achieved by down-regulating cytokines, such as IL-5 and IL-4, in addition to other key mediators. This is a noteworthy discovery as many current non-steroidal anti-inflammatories (NSAIDs) cause bronchoconstriction and are, therefore, not compatible with chronic airway inflammatory diseases such as asthma.

The impact of removing the C17 or C18 hydroxyl groups from RvE3 (**28**) was investigated by Fukuda and co-workers [61]. 17-Deoxy-RvE3 (**48**) was effectively inactive and did not significantly reduce PMN infiltration at a 300 ng dose in comparison to a 33% reduction recorded for RvE3 (**28**). In sharp contrast, 18-deoxy-RvE3 (**49**) proved more effective than RvE3 (**28**) and reduced PMN infiltration by ~50% under the same conditions. 18-Deoxy-RvE3 (**49**) was still active at 300 fg concentration with 30% inhibition observed. Neither **48** nor **49** was more metabolically stable than the lead compound.

While the number of analogues of RvE3 reported to date remains limited, the importance of the C17 hydroxyl is apparent. Interestingly, the adjacent C18 hydroxyl is not considered essential and its deletion may actually improve overall efficacy.

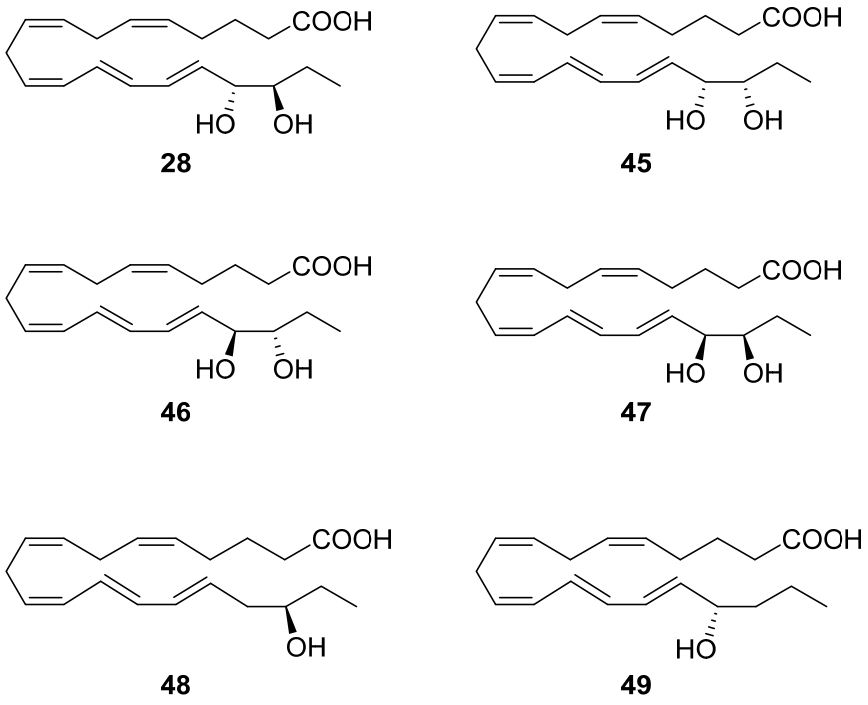


Figure 8. RvE3 and its analogues.

Although much of the literature focuses on the structure activity relationships between a particular resolvin and its immediate natural and non-natural analogues, a small number of studies described above also compare the effects within and across the different resolvins series. These studies are summarised in Table 2.

Table 2. Comparison of bioactive resolvins within and across series.

Resolvins compared	Effect Evaluated	Reference
RvD1 & RvD2	Attenuation of monocyte adhesion & transadipose migration	[32]
RvD1 & RvE1	PMN infiltration	[33]
RvD3 & RvD4	PMN infiltration & macrophage phagocytosis	[42]
RvD3 & RvD4	PMN infiltration	[43]
RvE1 & RvE2	Murine peritonitis & PMN infiltration	[48]
RvE1 & RvE2	PMN infiltration	[55]
RvE1 & RvE2 & RvE3	Interleukin 23 release & inhibition of leukotriene B4 receptor 1	[60]

Protectins

Protectins are structurally similar to the resolvins and act in a coordinated manner in the resolution of inflammation. Protectins are derived from DHA and are so named for their protective/neuroprotective effects [62].

Protectin D1 (PD1) is reported to be therapeutically effective for respiratory homeostasis [63]. Administration of PD1 results in decreased eosinophil and T-cell recruitment and mucus hypersecretion. As a result, PD1 was seen to decrease allergic airway inflammation and block airway hyperresponsiveness which confirms its underlying therapeutic potential particularly for the treatment of asthma. All four stereoisomers of PD1 (**50**) have been identified *in vivo* and assessed for anti-inflammatory activity (Figure 9) [64, 65]. Similar to the resolvins, it was found that the administration of aspirin promotes the synthesis of an endogenous 17*R*-epimer of PD1 (**50**), namely AT-PD1 (**51**). This epimer displayed reduced anti-inflammatory activity. **51** attenuated PMN migration by roughly 30%, whereas PD1 (**50**) had an effect of around 50%. The alternative 10*S*-epimer **52** was significantly more potent when compared to PD1 (**50**) despite being detected in only trace amounts in cells. At a dosage of 10 ng, epimer **52** inhibited PMN infiltration by ~40% while PD1 (**50**) effected ~30% inhibition at the same concentration. The difference in activity was even more pronounced at lower concentrations, with ~30% inhibition recorded for **52** versus ~5% for **50** at 0.1 ng levels. On the other hand, isomer **53**, which is an enantiomer of **50**, was essentially inactive. As such, the order of potency of these diastereomers may be ranked as follows: 10*S,*17*S*-PD1 (**52**) > PD1 (**50**) > 10*R,*17*R*-PD1 (**51**) >> 10*S,*17*R*-PD1 (**53**).

Several geometric isomers of PD1 (**50**) have been evaluated in an effort to elucidate the significance of alkene bond configuration [64, 65]. Δ15-*trans*-PD1 (**54**) showed little activity. Its 17*R*-epimer **55** was likewise essentially inactive. The usual 13*E*-,15*Z*-configuration of PD1 (**50**) is reversed in isomer **56**. Unsurprisingly, **56** and its epimer **57** displayed significantly reduced activity, blocking PMN infiltration by ~20% in comparison to a 40% effect observed with PD1 (**50**).

22-Hydroxy-PD1 (**58**), which incorporates a terminal hydroxyl group, is the ω-hydroxylation metabolite of PD1 (**50**) [66]. **58** was found to be equipotent to PD1 (**50**) *in vivo*. PD1 is also subject to β-oxidation, generating two metabolites, 20-carbon metabolite **59** and subsequently 18-carbon **60**, which represents its main metabolic route [67]. Interestingly, **59** is inactive whereas **60** retains PD1's anti-inflammatory properties. It has been postulated that this unexpected effect is due to the critical distance of the alkene bond from the carboxyl group.

Fluorinated analogue **61** was created with the aim of conducting PET (positron emission tomography) studies using an ^{18}F-label [68]. Unfortunately, it proved impossible to synthesise ^{18}F-**61** due to stability issues. However, administration of **61** significantly reduced neutrophil recruitment on a par with PD1 (**50**). This compound may be worthy of further investigation for its resistance to ω-oxidation. Nesman *et al.* designed analogue **62** in the hope of resisting β-oxidation [69]. By removing a double bond and introducing an ether functionality into the carbon backbone, **62** should serve as a more robust mimic of PD1 (**50**). **62** was equipotent with PD1 (**50**) in reducing neuropathic pain in mice at pmol levels. At the lowest possible dose of 30 pmol, **62** was the sole compound to exhibit an analgesic effect.

Based on these studies. it is possible to determine the essential structural features of PD1 (**50**) (Figure 9). The *E,E,Z*-triene motif and the hydroxyl stereochemistry significantly impact biological activity. The length of the upper chain can be modified but the distance between the alkene bonds and the carboxylic acid is significant. Substitution of C22 could be a useful avenue for creating analogues of PD1 (**50**) which are more resistant to ω-hydroxylation.

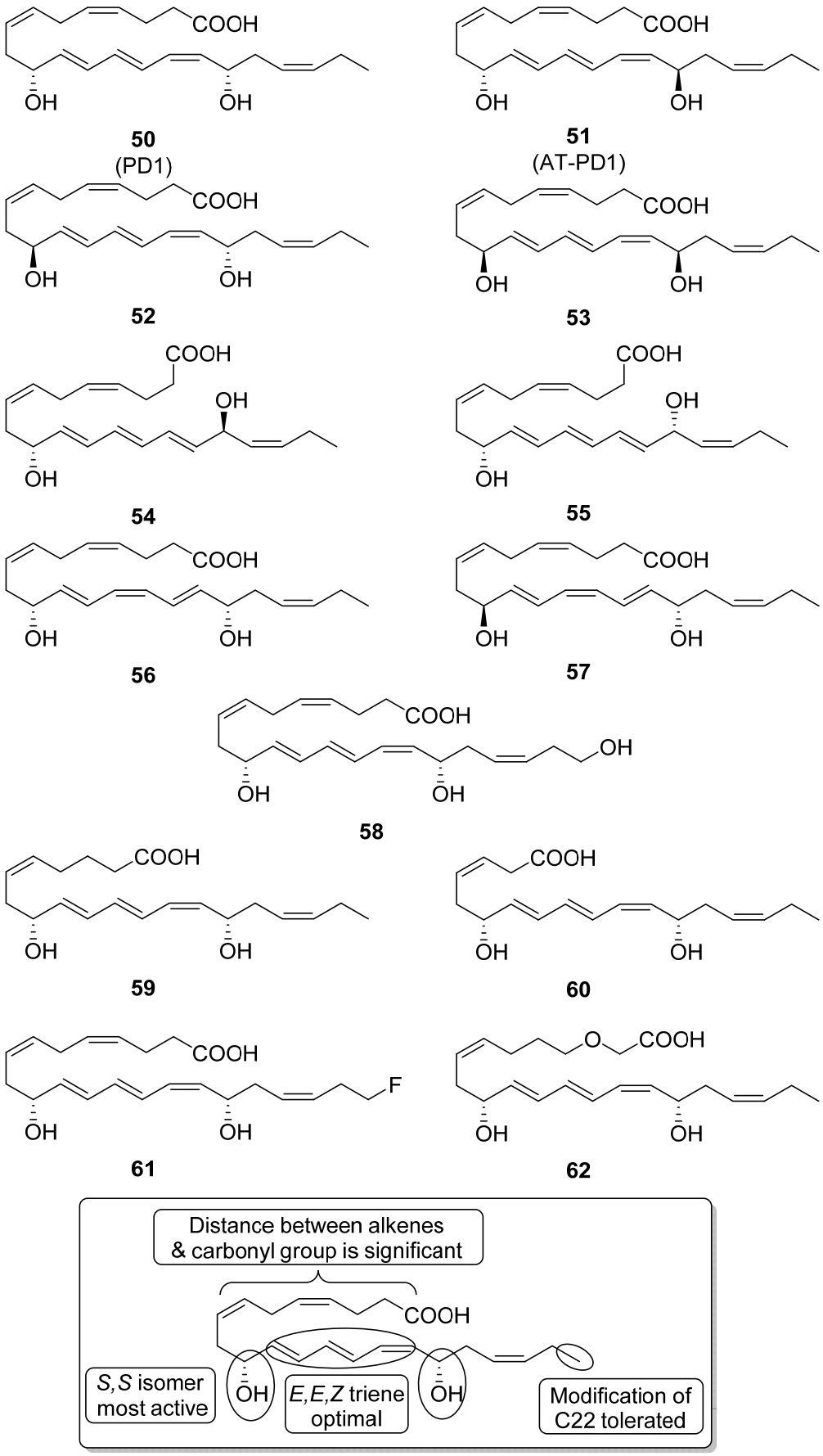


Figure 9. PD1 and analogues – main structure activity relationships.

Maresins

The maresins are derived from DHA, and comprise Maresin 1 (MaR1) and Maresin 2 (MaR2) [70, 71]. Three maresin conjugates, which incorporate peptide moieties (i.e. glutathione, cysteine and glycine) in their structures, have also been identified.

The first maresin discovered was naturally-occurring MaR1 (**63**) [71] (Figure 10). MaR1 (**63**) is a potent anti-inflammatory agent and reduces PMN infiltration in mice even at 0.1 ng dose. Additionally, MaR1 (**63**) proved to be a more effective agonist of efferocytosis than RvD1 (**2**) at 1 nM concentration. This was also true when compared to its 7*S*-epimer **64** or 12*E*-isomer **65**, although both molecules were partially active.

Investigations into the metabolome of MaR1 (**63**) saw the identification of two main metabolites [72]. The 14-oxo-MaR1 metabolite (**66**) retained partial activity and increased phagocytosis by ~30% in comparison to MaR1 (**63**) which caused a ~93% increase at 10 pM. The hydroxylated 22-OH-MaR1 metabolite (**67**) was discovered to have a biphasic dose-response curve. At 10 pM, **67** displayed similar efficacy to **66**. At 100 pM, however, both MaR1 (**63**) and 22-OH MaR1 (**67**) increased phagocytosis by ~75% each.

The major structure activity relationships are outlined in Figure 10 and highlight the importance of the *E*,*Z*-configuration and hydroxyl group stereochemistry.

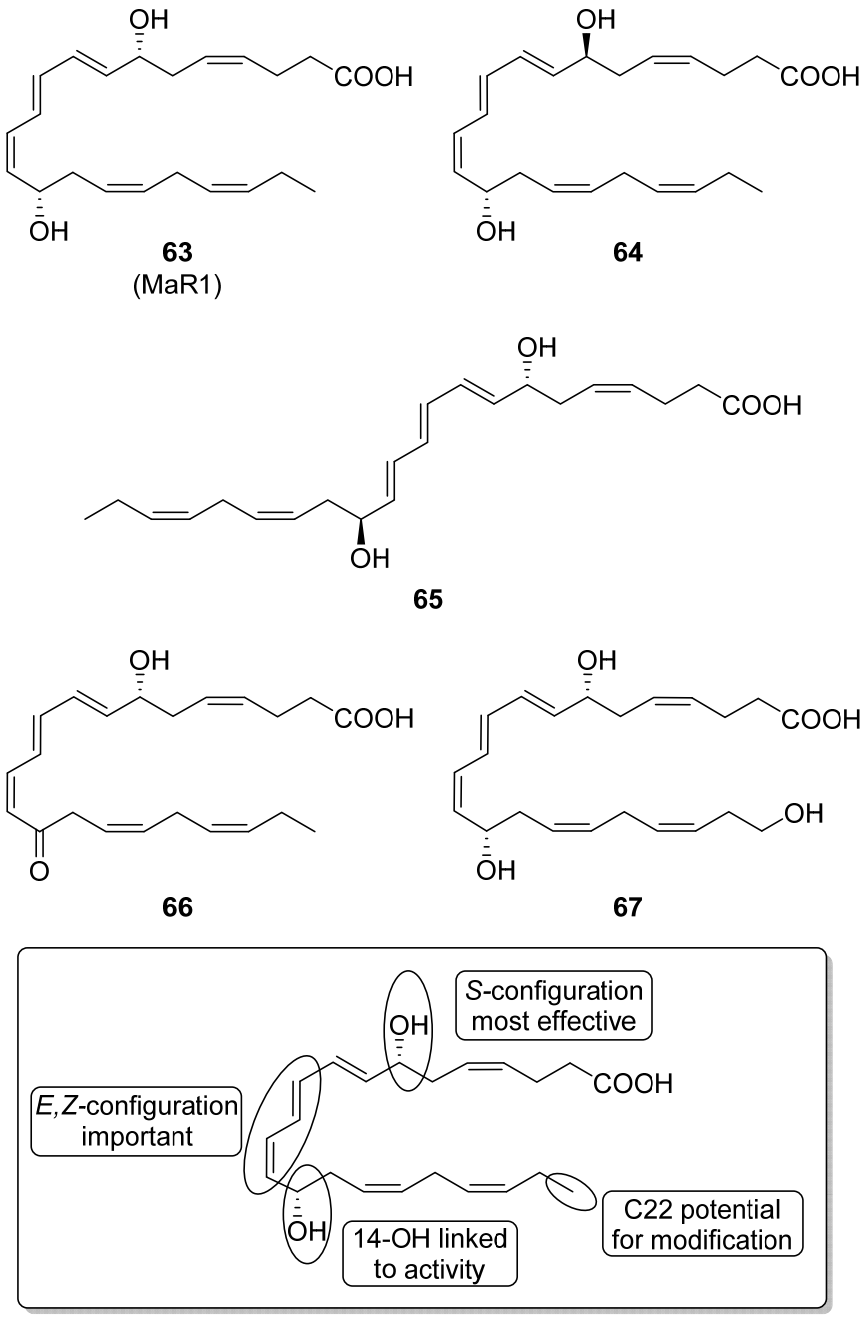


Figure 10. MaR1 and analogues – main structure activity relationships.

MaR2 (**68**) is characterised by its anti-inflammatory and pro-resolving actions (Figure 11). It enhances macrophage phagocytosis by ~90% at 1 ng dose and reduces PMN infiltration by ~40% at 10 pM concentration [70]. The maresin conjugates in tissue regeneration (MCTR) are potent pro-resolving mediators. They are effectively derivatives of MaR2 (**68**) with the replacement of the 13-OH functional group by a peptide [39, 73, 74]. In MCTR1 (**69**), the peptide is a glutathione unit connected by a sulfide bond. Enzymatic cleavage of the peptide to remove the glutamic acid component, produces MCTR2 (**70**). Subsequent cleavage of the glycine unit generates MCTR3 (**71**). These cysteinyl maresins have been found to act as cysteinyl leukotriene 1 (CysLT1) receptor antagonists by blocking the effects of the inflammatory sulfide-conjugated leukotrienes, aiding in phagocytosis, reducing neutrophil infiltration and shortening the resolution interval. MCTR1 (**69**) and MCTR2 (**70**) proved less potent in humans and

promoted phagocytosis by ~36% and ~25% respectively. By contrast, MCTR3 (**71**) promoted phagocytosis by ~60%. As such, MCTR3 elicited the largest human macrophage response.

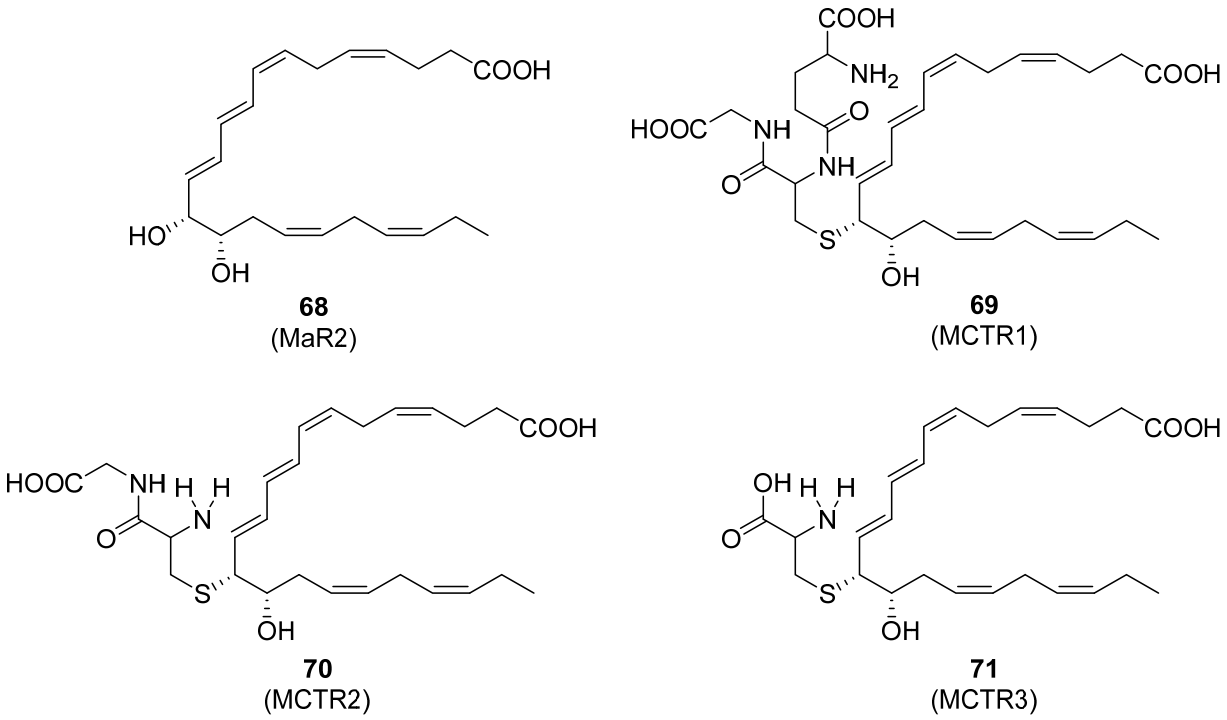


Figure 11. MaR2 and its analogues.

Conclusions

Over the last two decades, a large number of structurally diverse resolvins, protectins and maresins have been isolated and evaluated for their myriad biological effects. In addition, modification of the endogenous mediators can result in synthetic analogues which are as potent as the lead compound but are more resistant to metabolic decactivation. We have drawn together these different structures and summarised their major structure activity relationships. This review should serve as a useful platform to guide chemists and biologists on the development of metabolically stable therapeutics in the years to come.

Future Perspective

It has been estimated that chronic diseases account for almost 90% of all deaths in the European Union [75]. Chronic diseases place a significant burden on societies and this strain is likely to increase further with our aging populations [76-78]. A chronic pro-inflammatory status is characteristic of older individuals and represents a risk factor for a variety of diseases including atherosclerosis, diabetes, hypertension, and cancer [79]. While some of these conditions may be partly preventable by lifestyle changes, access to effective and safe therapies for controlling chronic inflammation remains a concern.

NSAIDs are widely used in the management of inflammatory conditions. The majority of NSAIDs are non-natural molecules that typically disrupt prostaglandin synthesis by inhibition of the normal functioning of the cyclooxygenase enzymes [4]. Long-term usage of traditional NSAIDs is associated with various side effects including gastrointestinal irritation, ulceration, hypercalemia, and acute renal dysfunction among others [80]. The resolvins, protectins and maresins are, by contrast, endogenous pro-resolving mediators and might be expected to possess superior safety profiles. Increased stability and longer duration of action will be key to their future clinical application. This may be accomplished by modification of the lead structure to develop stable analogues (e.g. prodrugs) as highlighted by several examples in this review. Alternatively, innovative formulations of the endogenous SPMs using high-tech liposomes may constitute a worthwhile, alternative strategy [81].

Patents on the use of resolvins for the treatment of asthma and diseases linked to angiogenesis have been filed [78, 82, 83]. A small number of molecules have proceeded to clinical trials. RvE1 (**26**) was one of the earliest contenders for preclinical and clinical development and entered Phase I clinical trials in 2009 but no results are available [84, 85]. The more lipophilic methyl ester of RvE1 was subsequently investigated as a remedy for dry eye [51, 86, 87]. Resolvyx entered RX-10045 (**37**) into Phase I and subsequently Phase II trials as a topical treatment for dry eye [49]. Although **37** was deemed safe and was well tolerated, the efficacy results were not promising enough for it to progress to Phase III. More recently, PD1 (**50**) has undergone clinical trial development for neurological conditions including Parkinson and Alzheimer's disease [68]. As is clear, we are still some distance from resolvin-, protectin- or maresin-based therapeutics reaching the market. In the meantime, further research and development will be necessary before the full potential of these exciting compounds can be unlocked.

Executive Summary

Background

-Resolvin, protectins and maresins are naturally-occurring, specialised pro-resolving mediators with potent anti-inflammatory effects.

-Rapid metabolism *in vivo* limits their potential as effective therapeutics.

Resolvins

-A wide range of resolvin structures, from metabolites to synthetic analogues, have been evaluated.

-The resolvins are sensitive to changes in stereochemical configuration of the main alcohol groups, as well as the geometry of their alkene bonds.

-Anti-inflammatory effects are often retained on incorporation of an aromatic ring in place the reactive triene.

Protectins

-The *S*,*S*-isomer of PD1 represents the most potent configuration.

-Shortening the carbon backbone does not necessarily eliminate biological activity.

Maresins

-Relative to the resolvins and protectins, a smaller number of maresin derivatives have been characterised and evaluated to date.

-It is possible to draw meaningful conclusions on structure activity relationships for MaR1 but not yet for MaR2.

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