ChemPubSoc Europe

DOI: 10.1002/cmdc.201800401

CHEMMEDCHEM Minireviews

Progress in the Development of Platelet-Activating Factor Receptor (PAFr) Antagonists and Applications in the Treatment of Inflammatory Diseases

Isabel K. Hyland,[a] Ronan F. O'Toole,[b] Jason A. Smith,*[a] and Alex C. Bissember*[a]

Platelet-activating factor (PAF) and its receptor (PAFr) have been implicated in a wide range of diseases and disorders that originate from the activation of inflammatory pathways. Although the exact structure of the binding site on the PAFr remains unknown, the PAFr is a well-established therapeutic target, and an array of structurally diverse PAFr antagonists have been identified. These include compounds that are structurally similar to the natural PAF ligand, synthetic heterocycles, complex polycyclic natural products, and various metal complexes. This review provides an update on more than 20 years of progress in this area. The development and synthesis of new PAFr antagonists, structure–activity relationship studies, the biological activity of these molecules, and their therapeutic potential are discussed.

Introduction

■■Academic titles (Dr.) added for authors; OK?■■ Inflammation is a biological response to injury that provides protection against infection. However, if inflammation is left unregulated, as can occur in disease, it can lead to severe tissue injury and dysfunction.[1,2] Ameliorating inflammation in disease states depends on the active control of inflammatory mediators such as the platelet-activating factor (PAF, **1**; Figure 1).

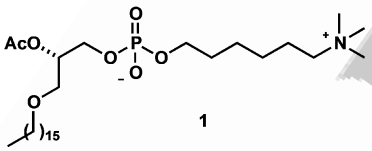


Figure 1. Platelet-activating factor (PAF).

Phospholipid **1**, first reported over 45 years ago, is one member of a family of structurally related phospholipid signaling molecules.[3–5] PAF mediates inflammatory pathways, and can exert its effects at concentrations as low as 10^{-12} M, which makes it the most potent member of its family.[6,7] Other biomolecules can also exert PAF-like activity, acting as agonists to the PAF receptor (PAFr), including oxidized low-density lipoprotein, bacterial lipopolysaccharide, and lipotechoic acid moieties in the cell walls of *Streptococcus* species.[8]

[a] *I. K. Hyland, Dr. J. A. Smith, Dr. A. C. Bissember*
School of Natural Sciences Chemistry, University of Tasmania, Hobart (Australia)
E-mail: Jason.Smith@utas.edu.au
Alex.Bissember@utas.edu.au

[b] *Dr. R. F. O'Toole*
School of Medicine, University of Tasmania, Hobart (Australia)

The ORCID identification number(s) for the author(s) of this article can be found under:
https://doi.org/10.1002/cmdc.201800401.

The PAFr is a G-protein-coupled receptor that has been a therapeutic target for many years.[3,4,9] PAF is an agonist of PAFr. When PAF binds to PAFr, it induces the mitogen-activated protein kinase (MAPK) pathway, leading to a pro-inflammatory cascade. PAF receptors are present throughout the body, in most major organ tissues, the central nervous system (CNS), muscles and inflammatory cells.[1,10–13] In 2002, the PAFr gene was localized to chromosome 1p35-1p34.4.[14] Although there have been a number of attempts to determine the structure of the PAFr and its binding site using molecular modeling methods, both still remain unknown.[15,16] However, work has focused on elucidating PAF binding by using radiolabeled species such as [^{3}H]PAF and [^{3}H]WEB 2086, a PAFr antagonist.[9,17] Melnikova and Bar-Eli[8] and Peplow[18] have provided detailed accounts of the mechanisms involved in PAF production and receptor binding.

The production and biosynthesis of PAF have been extensively studied, and a large range of cells, tissues, and signaling pathways have been implicated in PAF-mediated inflammation. PAF-producing cells are often white blood cells or other cells involved in inflammation. However, hepatocytes, keratinocytes, osteoclasts, renal mesangial cells, and vascular smooth muscle are also reported to provide molecule **1**.[1,9,19,20] A large number of diseases and disorders are associated with the inflammatory actions of PAF and its receptor, including cancers, respiratory and neurodegenerative diseases. However, PAF has been implicated in over 40 disease states.[9,21,22]

This review discusses PAFr antagonists that demonstrate potential for the treatment of inflammatory diseases, their activity against a range of disease states, progress in the synthesis of PAFr antagonists, and structure–activity relationships that have been established. The last comprehensive review of PAF antagonists was reported in 1994.[9] For this reason, this review focuses on providing an overview of progress in this area over the preceding 20 years.

Inflammatory Diseases

Cancer remains one of the leading causes of death worldwide.[23] It is accepted that an inflammatory microenvironment and angiogenesis (the formation of new blood vessels), are both important factors in cancer initiation and progression.[8,24–29] Considerable research has focused on investigating the role that PAF and its receptor play in cancer, and both have been specifically implicated in breast, prostate, and skin cancer, in addition to Kaposi's sarcoma and melanoma metastasis.[8,24,30] Consequently, this suggests that some anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and PAFr antagonists could be used to treat certain types of cancer.[24] In addition, the PAFr has been implicated in modulating radiation and cisplatin sensitivity in various cancers, including prostate, cervical, and ovarian cancers.[31–33]

Inflammatory diseases of the respiratory system include chronic obstructive pulmonary disease (COPD), which is defined as chronic airflow obstruction that is progressive and only partly reversible and which comprises emphysema and chronic bronchitis.[34] This disease usually develops over a number of years after routine exposure to oxidative stress, often from cigarette smoke. However, genetic triggers and air pollution are other possible factors.[24] Typically, irritation by fine particles in the air elicits an inflammatory immune response.[35,36] Bacterial and viral infections of the respiratory tract can also trigger these immune responses; in particular, *Streptococcus*, *Haemophilus,* and *Pseudomonas* species have the capacity to directly interact with the PAFr.[37–39,8] Consequently, the upregulation of PAFr expression on airway epithelial and alveolar cells in smokers and COPD patients could provide a gateway to respiratory infections.[40,41]

Mallia and co-workers provide a detailed account of the respiratory microbiome and the bacterial exacerbations that can occur.[42] Current treatments include short-acting bronchodilators and inhaled corticosteroids, with antibiotics often used in conjunction to treat cases of bacterial exacerbation.[43,44] These treatment methods mainly focus on alleviation of symptoms, and there are obvious limitations associated with the use of steroids and antibiotics for long-term treatment.[42,44] PAFr antagonists could be used to decrease the incidence of bacterial infection and reduce exacerbations.[15]

The role of PAF in asthma is well established. Specifically, PAF has been implicated in the pathogenesis of asthma by inducing bronchospasms, increasing vascular permeability, and activating inflammatory cells in the lower airways.[45] The function of PAF in these biological pathways and disease progression is well documented.[45–47] The inflammatory process associated with bronchial asthma is complex and requires treatments that target various steps in the inflammatory cycle, including binding of the PAFr. For example, several antagonists of the PAFr have been tested for asthma in various assays with mixed results.[45–47] ■■Refs. [45–47] OK?■■ PAF has been implicated in other allergic disorders, such as anaphylaxis and allergic rhinitis. Although the relevance of PAF is well established in the pathophysiology of these diseases, there has been limited research exploring the role that PAFr antagonists might play in treating these diseases.[48] Given the increasing incidence of allergic disorders, further studies are required in this area.[49]

PAF has been implicated in tissue injuries and cell death in the spinal cord and glial cells, in neuronal apoptosis, synaptic plasticity, and its role in oxidative stress can reportedly lead to neural cell death.[50,51] PAF receptors are present in the brain, on synaptic membranes, and on intracellular membranes in the cerebral cortex. The interplay between glutamate receptors in the CNS and PAF has also been established.[52–54] Interestingly, neurodegenerative disorders such as Alzheimer's disease (AD) have been identified as conditions that are mediated by PAF.[54,55] In the early stages of AD, treatment with the PAFr antagonists ginkgolides A and B has demonstrated a beneficial effect in the prevention of synapse loss and cognitive decline.[55] This work has also been extended to include prion-induced synapse degeneration.[56,57] In addition, it has been suggested that some PAFr antagonists are capable of crossing into the CNS and, thus, could be used in the treatment of Parkinson's disease, which shares some characteristics with AD.[58]

In 2014, researchers succeeded in exploiting the PAFr pathway to facilitate the entry of small interfering RNA oligonucleotides (siRNA) into difficult-to-transfect, well-differentiated airway epithelial cells.[59] The efficient delivery of siRNA is a major challenge in the advancement of RNA interference technology, particularly to the airways. This study took advantage of the existing mechanisms that microorganisms may use to enter epithelial cells, such as the direct interaction of phosphorylcholine (ChoP) molecules on the surface of *Streptococcus* species or non-typeable *Haemophilus influenzae* (NTHi) with the PAFr. In this way, these often-detrimental invasion mechanisms were used to facilitate entry of siRNA oligonucleotides. This novel work demonstrates that binding of the PAFr may be used to exploit RNA interference technology in the treatment of lung disease or to protect against pathogens.

Besides the well-established pro-inflammatory effects of PAF, in select cases, PAFr has also been implicated in damping the immune response, inducing immune suppression.[60] Several in vitro and in vivo studies have reported that the PAFr activation in macrophages and dendritic cells is able to shift these cells toward an anti-inflammatory or tolerogenic phenotype.[61–63] Thus, PAFr antagonists may have the ability to fine-tune the innate and adaptive immunity with a potential role in immunization.[62]

PAFr Antagonists

PAFr antagonists are molecules that are capable of binding to the PAF receptor. These antagonists may competitively or non-competitively displace PAF, the naturally occurring agonist, from its binding site on the PAFr.[24] A wide variety of molecules exhibit PAFr antagonistic activity, which can be grouped into four broad categories: structurally related synthetic PAF derivatives, synthetic compounds without structural similarity to PAF, natural products, and metal complexes.[64] The majority of the most important antagonists were synthesized prior to 1995.[9] Subsequent research has generally focused on refining the activity of these lead compounds and testing them in vari-

ous animal and human assays. More recent studies have primarily sought to clarify and improve the synthetic routes leading to these antagonists. With this in mind, only a brief summary of key PAFr antagonists is provided below. Reviews by Koltai and Braquet[9] and Whittaker,[65] published over 20 years ago, already provide detailed information on many PAFr antagonists.

Structurally related synthetic PAF derivatives

PAF (**1**) contains a long alkyl tail and a quaternary ammonium head (Figure 1). In 1983, thiazolium derivative CV-3988 (**2**), a zwitterionic species similar in structure to PAF, was the first synthetic substance identified as a PAFr antagonist, (Figure 2 A).[66] This led to the preparation of a range of related derivatives.[9,65] Most examples feature a carbamate group as the ether isostere and the lipophile is often a C_{16} to C_{18} alkyl chain.[67] The key structural elements of this family of compounds are depicted in Figure 2 B. These antagonists are generally not orally active, and some suffer from toxicity issues,[68,69] which have limited their therapeutic utility.[65,70]

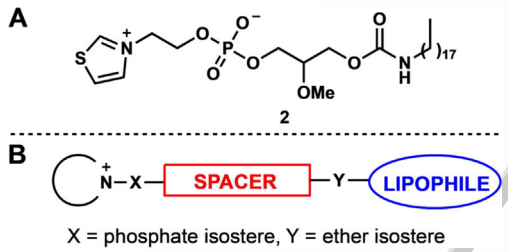


Figure 2. A) CV-3988 (**2**). B) Key structural elements present in structurally related synthetic PAF derivatives.

Other synthetic molecules

Since the discovery of benzodiazepines as bioactive molecules, many similar compounds have been developed. Within this category are the hetrazepine antagonists; much attention has been directed to replacing the fused benzene ring in the benzodiazepines with other fundamental heterocyclic motifs such as pyrazole, imidazole, pyrrole, or indole.[66] One thienodiazepine, brotizolam (**3**), was found to inhibit PAF-induced platelet aggregation selectively and at relatively low concentrations when compared with other antagonists (Figure 3).[71] Further work led to the development of WEB 2086 (apafant, **4**) and WEB 2170 (bepafant, **5**), which are even more potent than brotizolam.[17,72] Both compounds **4** and **5** exhibit good in vitro activity and excellent bioavailability, with long-lasting pharmacological effects.[9] WEB 2086 is an effective PAFr antagonist in humans, and no significant side effects have been identified that may prevent clinical trials.[71] Indeed, molecule **5** reportedly featured in a number of clinical trials that were later discontinued.[65,73–75] The reasons for this are unclear. The synthetic accessibility of the requisite fused diazepine ring system has complicated the large-scale synthesis of lead compound **5**.

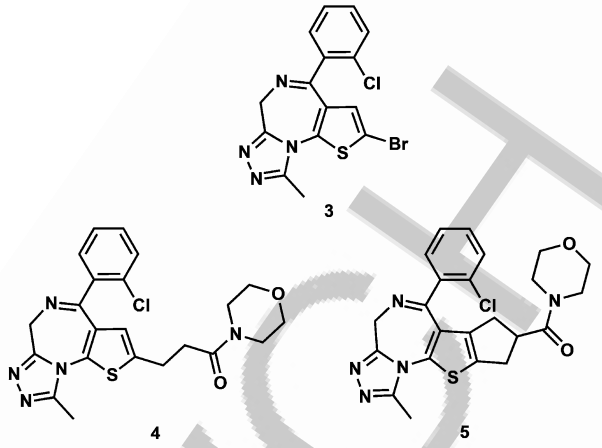


Figure 3. Brotizolam (**3**), WEB 2086 (apafant, **4**), and WEB 2170 (bepafant, **5**).

In 1991, three more potent analogues of WEB 2086 were identified; however, their potencies still do not compare well with that of the natural ligand PAF.[76,77] Interestingly, while patents exist for WEB 2086 and brotizolam, there are limited spectroscopic data for either compounds **3** or **4** (or their known analogues) in the peer-reviewed scientific literature. Indeed, the ^{1}H and ^{13}C NMR spectroscopic data for brotizolam were only published in 2013,[78] and, to our knowledge, extensive spectroscopic data for WEB 2086 are not available.

While the diazepine core structure provides potency and activity for PAFr antagonists, other heterocyclic scaffolds are more synthetically accessible. The imidazo[4,5-*c*]pyridyl framework is a particularly important motif, with key substituents present at either the 1- or 5-positions of this subunit. An important example is UK-74,505 (modipafant, **6**; Figure 4). Ana-

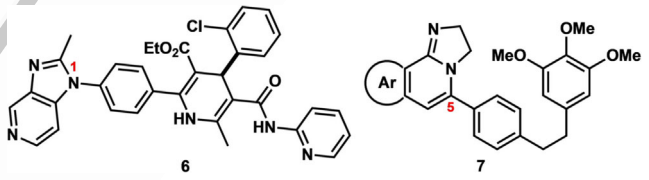


Figure 4. UK-74,505 (modipafant, **6**) and SDZ compounds **7**.

logues of modipafant have been constructed by replacing the dihydropyridine structure with benzodiazepine, benzazepine, and other more lipophilic moieties.[9] Various benzimidazole structures have also shown activity, and modifying the lipophilicity of the substituents on these compounds provides a marked effect on antagonist activity. Interestingly, some of the most active benzimidazole-containing antagonists, such as SDZ series **7**, bear a close resemblance to the diazepine core of the WEB compounds, with three fused rings comprising the core structure.[9]

Natural products

The ginkgolides, isolated from *Gingko biloba*, are the most well-studied natural products that have been identified as PAFr

antagonists.[9] As a representative example, ginkgolide B (BN 52021, **8**) shows particular potency for the PAFr (Figure 5).[79] Unfortunately, the ginkgolides are not selective antagonists of the PAFr and have been shown to influence

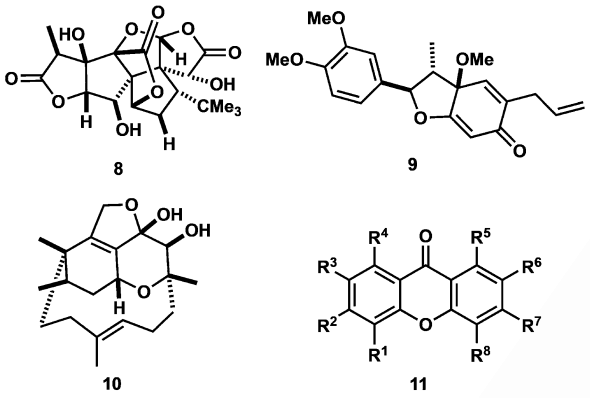


Figure 5. Ginkgolide B (BN 52021, **8**), kadsurenone (**9**), phomactin A (**10**), and xanthones **11**.

other biological functions.[65] Furthermore, the generally low natural abundance and complex structures of the ginkgolides has limited their accessibility, and this has hindered efforts to extensively evaluate their therapeutic potential. In addition, a number of marine natural products, compounds of microbial origin, and Chinese medicinal herbs have shown PAFr activity. These include kadsurenone (**9**), phomactin A (**10**), and a range of xanthones **11**.[9,80–82] Some other natural substances (mixtures or extracts) also display PAFr antagonism, including olive oil and fish oil.[24]

Metal complexes

Recently, various rhodium complexes have been synthesized that display activity against the PAFr.[16,83] Specifically, square planar rhodium(I) complexes **12** and **13** and a series of rhodium(III) complexes, including representative compounds **14**, were evaluated as PAFr antagonists (Figure 6). It was determined that their activities were similar to that of WEB 2170 (**5**), one of the more potent antagonists.[83] Rhenium complex **15**

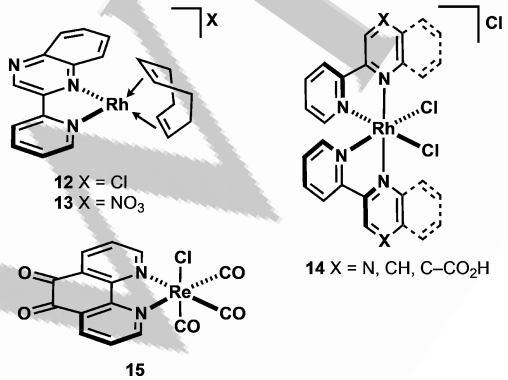


Figure 6. Metal complexes **12**–**15**.

was also found to be active against the PAFr. Interestingly, this molecule was also evaluated for antitumor activity, and found to be more effective than cisplatin.[84] A range of other metal complexes have also been evaluated for their PAFr activity.[16]

Structure–Activity Relationship (SAR) Studies

Structure–activity relationship (SAR) studies are central to the development of potent and selective PAFr antagonists, particularly as the structure of the PAFr active site remains unknown. In silico modeling studies have identified some key features of the PAFr binding site, but crystallographic data and protein structural modeling are required to accurately elucidate the binding properties of the PAFr.[15,85–88] It has been proposed that the binding site of the PAFr consists of a bipolarized cylinder ≈10–12 Å in diameter, and a second, shorter domain ≈6–7 Å in diameter.[89,90] Recent reports even suggest that the binding site may consist of a tetrapolarized cylinder.[90] Work has also been undertaken toward the design of a pseudo-receptor.[91] It appears that the PAFr contains a large lipophilic binding pocket that can accommodate considerable steric bulk, a hydrogen bond donor that can interact with either a carbonyl or oxygen atom, and a functional group capable of interactions with pyridine-like moieties.[89] However, these data were obtained through a variety of different (human and animal) assays and may not translate directly to the viability of these compounds for the treatment of human disease.[92]

Organic small molecules

Significant research over the past decade has generated SAR data for non-diazepine-based synthetic molecules and some natural products.[89,90,93–96] In contrast, seminal SAR studies relating to diazepine-type compounds were reported over 25 years ago, and there have been few major developments since this time.[9,65] Heterocyclic N-substituents (possible hydrogen bond acceptors) have been identified as crucial to providing active PAFr antagonists. The spatial orientation of this sp^2-hybridized nitrogen atom appears to influence activity. Three common heterocyclic scaffolds present in PAFr antagonists that contain sp^2-hybridized N-substituents are fused triazole moieties (e.g., WEB 2086), 3-pyridyl groups, and fused imidazole derivatives.[65]

More recently, 24 conformationally strained PAFr analogues **16** were synthesized (Figure 7 A).[89] These molecules share some structural similarities with PAF. Specifically, these compounds consist of various combinations of lipophiles and *N*-heterocycles joined via a hydrogen bond acceptor such as an ether or a carbamate group, which were linked to cyclic frameworks such as 1,1-bis(hydroxymethyl)cycloalkanes or 3,3-bis(hydroxymethyl)oxetanes, -thietanes, and -azetanes. All of these analogues were found to be potent antagonists of PAFr, with greater activity than CV-6209 (**17**) and ginkgolide B (**8**).

Although thietanes **16** provided superior activity in some instances, in the majority of cases the presence of a heteroatom in the four-membered ring made little difference. Ring size did affect potency, but a discernable trend was not evident. The

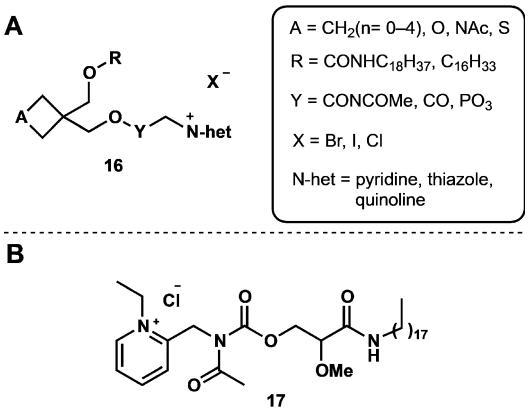


Figure 7. A) Conformationally strained PAFr analogues **16**. B) CV-6209 (**17**).

identity of the counter-anion was not significant; however, carbamates did appear slightly more active than ethers. The nature of the heterocyclic nitrogen atom provided the greatest influence on potency, with quinolones having been found to be the most potent, followed by thiazoles and pyridines. Quaternization of compounds appeared crucial, as neutral synthetic precursors displayed much lower activities.[89]

A number of new trisubstituted 4-aminopiperidines **18** were prepared as alternatives to previously reported piperazine antagonists (Figure 8).[65,90] These heterocycles featured improved

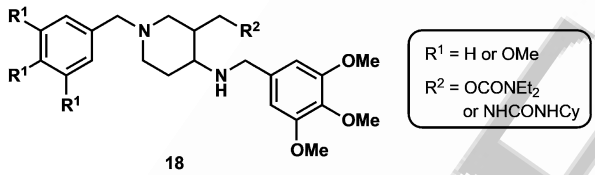


Figure 8. Examples of 4-aminopiperidine-based PAFr antagonists **18**.

conformational flexibility and greater distances between the key N-substituents. All of the molecules in this series contained a trimethoxybenzyl group (TMB), which is present in known PAFr antagonists (e.g., SDZ series **7**).[65] SAR data revealed that the replacement of the piperazine core with the aminopiperidine motif did not change the ability of molecules to bind the PAFr. Although there were marked differences in PAFr binding activity associated with various diastereomers used in this study, a clear trend was not evident.[90]

Novel ginkgolide derivatives have been primarily accessed via semi-synthesis, and this has facilitated SAR studies of this class of compounds. In the ginkgolide series, a *tert*-butyl group appears to be critical for providing notable biological activity (Figure 9).[9] Rings E and F are also essential for the retention of activity.[94,95] However, substitution of the hydroxy group on lactone ring F with lipophilic moieties, such as substituted benzyl groups, does not have a significant effect.[95] The presence of B ring-substituents typically increases the ability of the molecules to bind the PAFr.[95] Furthermore, 7-α-substituted ginkgolides generally feature higher affinity for the PAFr than the corresponding 7-β-derivatives; 7-α-chloroginkgolide B is

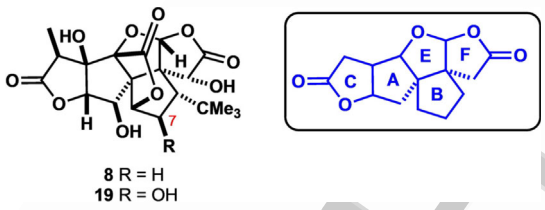


Figure 9. Ginkgolide B (**8**) and ginkgolide C (**19**).

the most potent derivative described.[95] Interestingly, ginkgolide C (**19**), which differs from ginkgolide B (**8**) by the presence of a 7-hydroxy group, is 25-fold less potent than the latter.[95]

Various xanthones **11** were assessed for their ability to inhibit the effects of PAF (Figure 10). It was determined that a R^6-

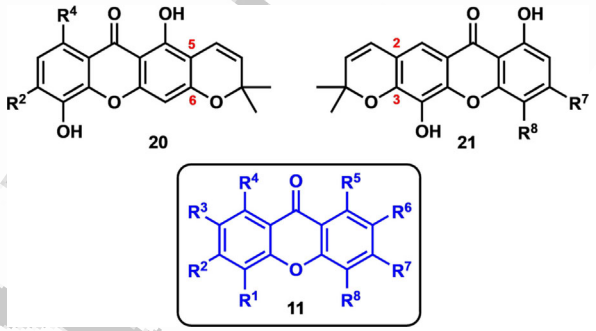


Figure 10. Xanthones **11** and annulated chromene derivatives **20** and **21**.

prenyl group features strong binding affinity for the PAFr, but when this group is hydroxylated, activity is lost. Cyclization of the R^6-prenyl group to deliver chromene **20**, which is annulated at the 5- and 6-positions, provided only slightly lowered potency (as did the presence of a R^8-dimethylprop-2-enyl substituent; Figure 10). However, chromene **21**, annulated at the 2,3-positions provided a small loss in binding affinity. The presence of a R^1-phenolic substituent increased potency, while the presence of a R^2-hydroxy group had the opposite effect.[82]

Metal complexes

Some metal-based complexes have been assessed for their anti-inflammatory activity.[97] However, limited research has investigated applications of metal-based compounds as PAFr antagonists. Some studies have provided preliminary SAR data regarding coordination geometry, ligand substitution, the effects of the counter-anions and the overall size and charge of the complex. A range of rhodium(III) complexes featuring bidentate ligands have been prepared (e.g., complexes **14**; Figure 6).[16,83] Molecular modeling studies illustrated that octahedral complexes containing two aromatic ligands are unable to fit into the PAFr binding site and, hence, any observed activity occurs via nonspecific binding.[16] Various square planar rhodium(I) complexes were also prepared (e.g., complexes **12** and **13**; Figure 6).[83] Biological assays evaluating the inhibition of PAF-induced platelet aggregation and theoretical docking

studies illustrate that these compounds are selective and potent inhibitors.[83] All of the square planar rhodium complexes prepared as part of this study were more active than rhodium analogues featuring octahedral coordination geometries.[16,83]

Copper(II), zinc(II), cobalt(II), nickel(II), and gallium(II) complexes containing chalcogenated imidodiphosphinato ligands are PAFr antagonists (Figure 11).[98,99] These complexes feature

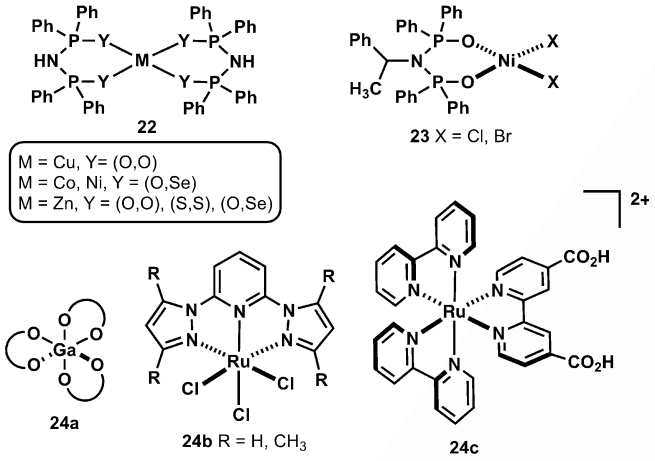


Figure 11. Copper(II), cobalt(II), zinc(II), nickel(II), gallium(II), and ruthenium complexes **22**–**24**.

a range of fundamental metal coordination geometries, with the bulkiest gallium complex **24a** identified as the most active of these molecules. A range of ruthenium(II/III) complexes, including compounds **24b** and **24c**, were also synthesized and evaluated as PAFr antagonists.[16] The activity of these complexes increased dramatically upon exchanging the chloride for a hexafluorophosphate counter-anion. In addition, ionic compounds were typically found to be more active than their neutral analogues.[16]

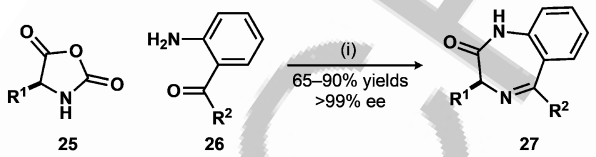
Synthesis of PAFr Antagonists

A variety of structurally diverse molecules have been identified as PAFr antagonists, and an array of approaches have been developed to prepare them. However, over the past 20 years, the focus of research in this area has primarily centered on improving the synthetic accessibility of established diazepine-based antagonists. During this period, the synthesis of original metal-based antagonists was also notable.

Synthetic heterocycles

Compounds containing the 1,4-diazepine motif exhibit a broad range of biological activity, including PAFr antagonism, and are traditionally used as sedatives.[100] Examples include alprazolam, clonazepam, diazepam or lorazepam, among others. The most common approaches to the construction of these benzodiazepine drugs involve multistep pathways in which protected α-amino acids are coupled with an *o*-ketoaniline, deprotected, and then cyclized. These methods feature poor atom economy and can be complicated by racemization at the α-stereogenic center.

In 2017, a direct method enabling stereospecific, single-step syntheses of benzodiazepine core structures was reported (Scheme 1).[101] This method featured the reaction of chiral *N*-



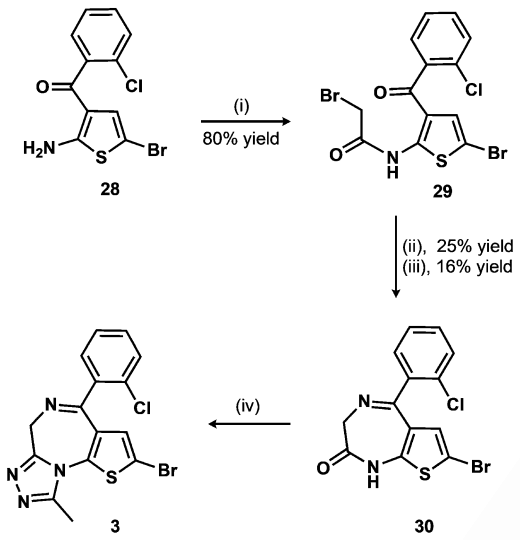
Scheme 1. Synthesis of diazepinones **27**: (i) 2 equiv TFA, PhMe, 60 °C, then 2 equiv NEt_3, 80 °C.

carboxyanhydrides (NCAs, **25**) with *o*-ketoanilines **26** to efficiently provide enantiopure diazepinones **27** (with water and carbon dioxide as the only byproducts). Notably, products **27** did not require purification using this approach. This strategy avoids issues associated with earlier routes, which were complicated by lengthy and low-yielding syntheses, low atom economy, and difficulties constructing the key seven-membered heterocyclic core.[102–105] Unfortunately, the synthesis of NCAs **25** typically requires triphosgene or phosphorus trichloride and, consequently, this method is perhaps not well suited to synthesis on industrial scales.[101] Although the transformation shown in Scheme 1 has enabled the synthesis of compounds related to WEB 2086 and WEB 2170, these specific molecules have not been prepared by this approach.[101]

Thiophenes can act as effective bioisosteres for benzene, (e.g., in benzodiazepine drugs).[106] The thiophene analogues are generally less well studied than traditional benzodiazepine congeners. The early PAFr antagonists WEB 2086 (**4**) and WEB 2170 (**5**) contain a thienodiazepine core (Figure 3), as do other related drugs already on the market, such as etizolam or clotiazepam.[66,107] Notably, the use of a thiophene in place of a benzene could serve to overcome the selectivity issues that have been associated with the benzodiazepine drugs.

The benzodiazepine scaffold is known to interact with various receptors in the body, making it an excellent structure to use in drug development; however, this also means that there are risks of off-target effects.[106] Consequently, efforts have been directed at improving the synthesis of thienodiazepine scaffolds to investigate their therapeutic potential.[100] To this end, recent work has strived to establish more viable synthetic approaches that are shorter, more efficient, and are amenable to scale-up.[108]

The synthetic accessibility of thienodiazepines, such as WEB 2086 (**4**), has improved. Specifically, a number of patents concerning the synthesis of brotizolam (**3**, a precursor of WEB 2086) and its analogues have been filed, most recently in 2012.[103,109–112] Brotizolam can be synthesized in six to eight steps (Scheme 2).[113] The majority of these processes require relatively hazardous reagents and are complicated by rather time-intensive procedures and typically low-yielding transformations.

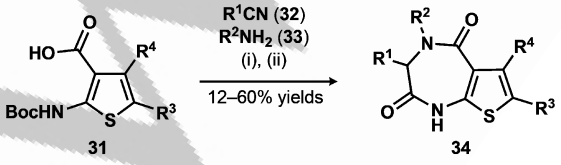


Scheme 2. Synthesis of brotizolam (**3**): (i) 1.1 equiv bromoacetyl bromide, 1 equiv pyridine, Et_2O, RT; (ii) 1:1 CH_2Cl_2/ $NH_{3(l)}$, reflux, then DMSO, 100 °C; (iii) 1.4 equiv NaN_3, DMSO, RT, then H_2, Pd/C, AcOH; (iv) 1 equiv P_2S_5, pyridine, reflux, then AcOH, 3 equiv methylhydrazine, *n*BuOH, reflux.

Naik et al. reported an improved 75–80% yield over the last three synthetic steps by slightly modifying established chemistry.[103] Many of the intermediate compounds also appear difficult to purify.[113] The four steps, shown in Scheme 2, are representative of general approaches. In this case, the reaction of 2-aminothiophene **28** with a 2-halogenated acetyl halide, such as bromoacetyl bromide, afforded amide **29**. Subsequent cyclization formed heterocycle **30**, and the installation of the triazole was then completed over two steps to furnish brotizolam (**3**).

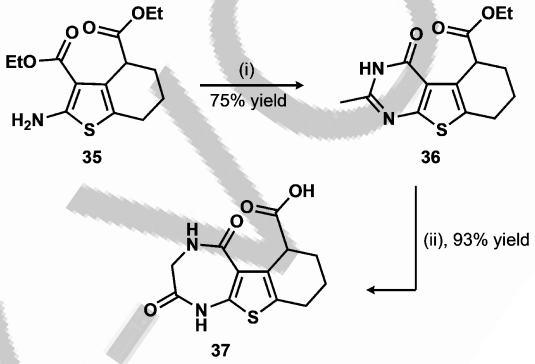
It appears that the order of the steps in the above-mentioned reaction sequence leading to the key seven-membered ring can be varied.[110,113] Although most reports illustrate that acid catalysts and high temperatures are necessary,[71,105,110,114,115] a patent suggests that bases, such as sodium hydroxide or triethylamine, can also catalyze these transformations.[116] In addition, it has been noted that cyclizations can occur spontaneously.[117] Installation of the triazole ring has been effected using a number of different reagents, usually by treatment of amide **30** with a chlorination or thionation agent, and then reaction with a hydrazide or hydrazine.[103,105,110,112,114]

A series of novel 1,4-thienodiazepine-2,5-diones **34**, 18 in total, were synthesized via four-component Ugi reaction/ deprotection sequences from thiophenes **31** (Scheme 3).[100,118] This two-step Gewald process has been used for preparing



Scheme 3. Synthesis of 1,4-thienodiazepine-2,5-diones **34**: (i) 1 equiv ethyl glyoxylate, MeOH, RT; (ii) TFA, RT, then 1,5,7-triazabicyclo[4,4,0]dec-5-ene, NEt_3, THF, 40 °C.

these heterocycles for many years,[110] and has been used in the synthesis of WEB 2086 (**4**) and analogues.[71,119] The four-component Ugi condensation reaction has not been used previously to access this manifold and offers an efficient route to thienodiazepines and the ability to introduce up to six points of diversity into the products.[100,118] By using this strategy, the successful synthesis of thieno[2,3-*e*][1,4]diazepines was established by a sequence commencing from proline derivatives and 2-aminobenzenethiols or dithiandiol.[95,120] For example, a Gewald reaction provided heterocycle **35** (Scheme 4), which



Scheme 4. Synthesis of thieno[2,3-*e*][1,4]diazepines **37**: (i) 2 equiv MeCN, dioxane/HCl, 40 °C; (ii) 3 equiv K_2CO_3, 1.1 equiv ethyl bromoacetate, DMF, RT.

was then transformed into a thienopyrimidinone and alkylated to deliver tricycle **36**. The seven-membered ring was formed via a one-pot base-mediated hydrolysis/N-alkylation/cyclization transformation to afford product **37**.[121] The biological activity of compound **37** has yet to be evaluated.

The majority of thienodiazepine-based PAFr antagonists such as WEB 2086 (**4**) feature 2-amino-fused thiopheno-1,4-diazepines **38** (Figure 12). In contrast, 3-amino-fused congener **39**

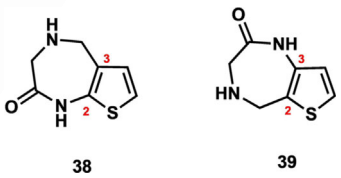
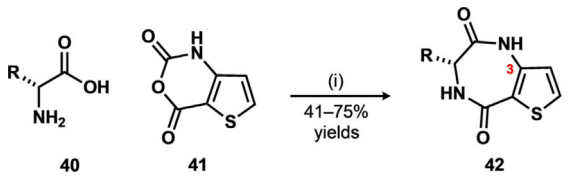


Figure 12. 2-Amino-fused thiopheno-1,4-diazepinone **38** and 3-amino-fused 1,4-diazepinone **39**.

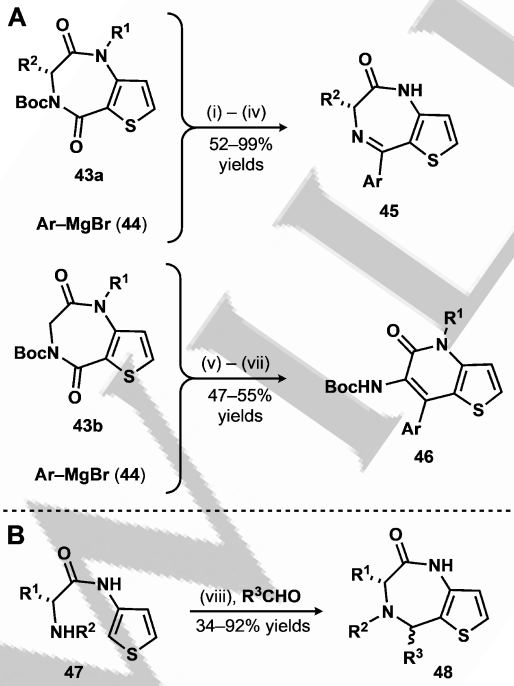
has not been the subject of significant interest. Heterocycles of this type were first prepared via the condensation of 3-thiaisatoic anhydride with L-proline in 1998.[122] These frameworks have been constructed by the one-pot regioselective ring opening of 3-thiaisatoic anhydride **41** using chiral α-amino acids **40**, followed by intramolecular condensation to afford heterocycles **42** (Scheme 5).[120,123] This method is also amenable to solid-phase synthesis using Wang resin, and in this way, eight analogues were prepared in 71–95% yields.[124] While these regioisomeric thienodiazepines have not been tested as PAFr antagonists, these structures have demonstrated activity



Scheme 5. Synthesis of 3,4-substituted thieno[2,3-*e*][1,4]diazepine-2,5-diones **42**: (i) H_2O, 40 °C, then AcOH, reflux.

against other receptors. As previously noted, the spatial orientation of molecules in PAFr antagonists does influence receptor binding.[65] Thus, it is possible that regioisomeric thienodiazepines, such as molecules **42**, may represent potential PAFr antagonists.

A series of thienodiazepinones **45** and various thienopyridinones **46** were prepared from precursors **43** (Scheme 6 A).[107] Heterocycles **45**, which are structurally similar to PAFr antagonists WEB 2086 (**4**) and WEB 2170 (**5**), were synthesized by a sequence featuring the addition of aryl Grignard nucleophiles **44** to substrates **43 a**, Boc-group deprotection, Schiff base condensation, and oxidative deprotection. In contrast, compounds **46** were efficiently prepared by the nucleophilic addition of reactants **44** to heterocycles **43 b** followed by a two-step intramolecular aldol condensation. In 2015, Pictet–Spengler reactions were used to construct a library of 24 novel thienodiazepines **48** (Scheme 6 B).[67] In this work, α-amino acids were coupled with 3-aminothiophenes to provide substrates **47**, and Pictet–Spengler reactions furnished heterocycles **48**.[67] By this approach, a series of phenylalanine-, alanine-, and proline-based analogues were prepared.[67]



Scheme 6. A) Synthesis of thienodiazepinones **45** and thienopyridinones **46**: (i) THF, RT; (ii) 1:1 TFA/CH_2Cl_2, RT; (iii) NEt_3; (iv) 5 equiv CAN, 3:1 MeCN/H_2O, RT; (v) THF, RT; (vi) 2.2 equiv KO*t*Bu, THF; (vii) 4.4 equiv *p*-TsOH. B) Synthesis of thienodiazepine compounds **48**: (viii) PhMe, 4 Å molecular sieves, 110 °C.

A range of thienopyrimidines **49**, thienotriazones **50**, and thienodiazepinones **51** have been prepared from 2-aminothiophenes by standard methods (Figure 13).[125] These compounds featured potent antitumor properties, but have not been specifically tested for activity toward the PAFr. In addition, the Beckmann rearrangement was used to construct various thienoazepines **52**, furanoazepines **53**, pyrroloazepines **54**, and thienodiazepines **55**.[126] Many of these molecules were identified as vasopressin antagonists and antitumor agents, but have not been specifically tested for PAFr activity. These previously unreported compounds all contain clear structural similarities to existing PAFr antagonists and guide the development of new PAFr antagonists.

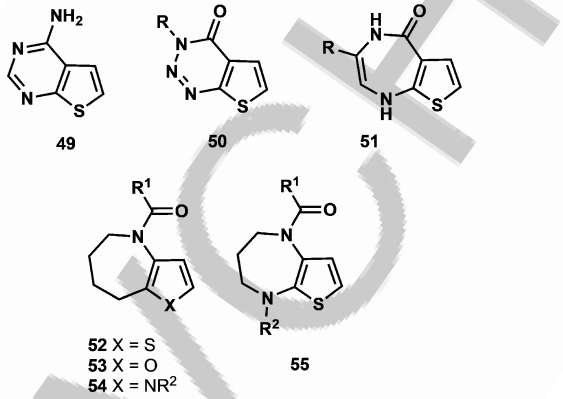
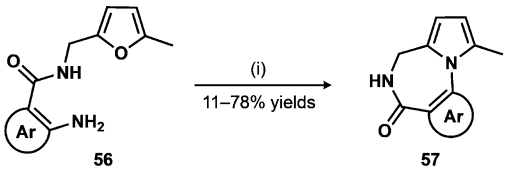


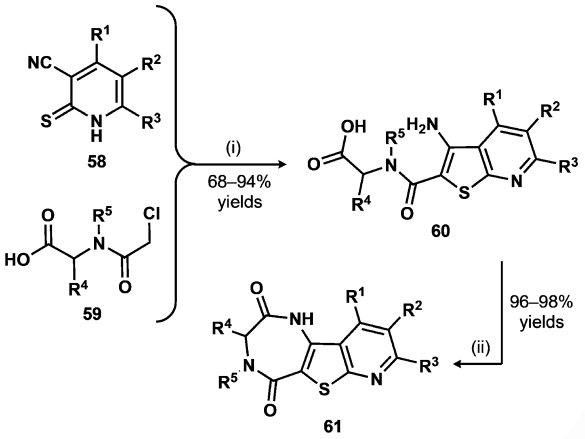
Figure 13. Representative thienopyrimidines **49**, thienotriazinones **50**, thienodiazepinones **51**, thienoazepines **52**, furanoazepines **53**, pyrroloazepines **54**, and thienodiazepines **55** that have been synthesized.

Pyrrolodiazepines represent alternative scaffolds to both benzodiazepines and thienodiazepines. The majority of established methods to these systems use Pictet–Spengler reactions to convert 1-[2-(α-aminoalkyl)phenyl]pyrroles into these targets.[127] However, the arylpyrrole starting materials featured in these transformations are susceptible to decomposition in the presence of acids and other electrophilic agents, which potentially limits their utility.[127] In 2010, a novel approach was developed that obviates the need for using arylpyrrole substrates (Scheme 7).[127] The method converts furans **56** directly into heterocycles **57** in a single step.

Pyridine-fused compounds **61** were synthesized from pyridines **58** and amino acids **59** (Scheme 8).[128] Importantly, intermediates **60** do not require further functionalization prior to conversion into cyclized products **61**. In contrast, germane conventional approaches do require additional functional



Scheme 7. Synthesis of pyrrolodiazepines **57**: (i) 7:1 AcOH/HCl, RT.



Scheme 8. Synthesis of heterocycles **61**: (i) 10% KOH/H_2O, DMF, 50 °C; (ii) neat, 220 °C.

group manipulations prior to construction of the diazepine ring. For example, transformation of amines **59** into 1,3-oxazine-2,6-dione derivatives or the Boc-protection/acylation of amines **59**.[117,128] It is unknown whether these pyrrolo- and pyridine-fused compounds act as PAFr antagonists similar to thieno- and benzodiazepine analogues.

Other PAFr antagonists

The first total syntheses of (±)-ginkgolides A and B were completed in 1988 by Corey et al.[129] Over a decade later, a 25-step total synthesis of (±)-ginkgolide B (**8**) was disclosed, which showcased an original stereoselective intramolecular photocycloaddition reaction.[79]

Total syntheses of phomactin A (**10**), phomactin D (**62**), phomactin B2 (**63**), and phomactin G (**64**) have been completed (Figures 5 and Figure 14).[130,131] Phomactin A has been prepared a number of times. This natural product has been accessed via an intramolecular Suzuki–Miyaura cross-coupling and Nozaki–Hiyama–Kishi-type macrocyclizations.[130a–c] The centerpiece of another approach to molecule **10** featured an original intramolecular *oxa*-[3+3] annulation.[130d,e] Compound **62** was prepared stereoselectively from ascorbic acid, phomactin B2 (**63**) was synthesized via the intramolecular annulation of a chromium carbene, and target **64** was realized by an intramolecular Nozaki–Hiyama–Kishi reaction.[131]

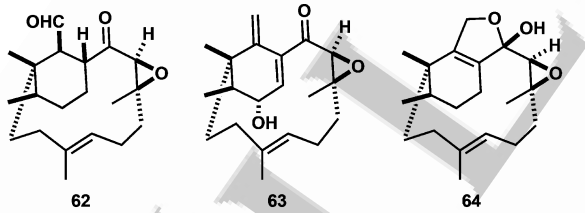


Figure 14. Phomactin D (**62**), phomactin B2 (**63**), and phomactin D (**64**).

A number of xanthone natural products have been prepared, including elliptoxanthone A, tovophyllin B, the aglycone analogue of IB-00208, and α- and β-mangostin.[132] Total syntheses of kadsurenone (**9**) have been reported (Figure 5).[133] Notably, Popinom et al. prepared molecule **9** in only three steps from allyloxyphenol and 3,4-dimethoxycinnamyl alcohol by developing a novel pericyclic cascade reaction.[133a] Over a decade later, a biomimetic synthesis of this natural product was reported.[133b]

Finally, several patents have been published on the synthesis and use of PAFr antagonists. These include the synthesis of ginkgolide analogues, 2,5-diaryltetrahydrothiophenes, tetrahydrofurans, and 1,3-diarylcyclopentanes and their applications in the treatment of PAF-associated conditions including cardiovascular, inflammatory, and immune disorders.[134–138] In addition, a range of sulfamoylheteroaryl pyrazole anti-inflammatory agents have been prepared,[139] and various cyclohexylsulfonamide compounds have also been developed for use as PAFr antagonists.[140]

A number of molecules have also been synthesized that are structurally similar to existing PAFr antagonists; however, these compounds have not been tested for PAFr activity. These include pyrrolo[1,4]thienodiazepines, furano-, thieno-, and pyrrolazepines, pyrrolo-, indolo, benzo-, and thieno[1,4]diazepines, thineodiazepinones, thienotriazolo[1,4]diazepines, substituted triazolodiazepines, thienopyrimidine, thienopyridine, thienothiazine, and thienoxazine and 2-amino-3-aroyl-4,5-alkylthiophenes. Among these classes of compounds, various analogues have been identified as human adenosine receptor inhibitors and bromodomain inhibitors.[141–152] Metal-based PAFr antagonists have typically been prepared via standard methods.[83,103,153]

Conclusions and Outlook

PAF is implicated in over 40 different disease states. Specifically, PAF and its receptor are involved in many biological functions involving inflammatory processes, including cancer, lung diseases and neurodegeneration. Despite this, specific therapeutic applications of PAFr antagonists are somewhat limited.

A range of structurally diverse classes of molecules have been demonstrated to serve as PAFr antagonists. Synthetic strategies for construction of the more complex antagonists have evolved, and new efficient methods have been developed over the past 20 years, particularly in the case of diazepine-based antagonists. SAR studies have been conducted on many of these molecules, and some key properties have been identified. Indeed, many PAFr antagonists feature sp^2-hybridized nitrogen substituents with appropriate spatial orientations in addition to lipophilic groups. A number of metal-based complexes have recently demonstrated potent PAFr antagonistic activity. Indeed, some are more effective than cisplatin in the treatment of tumors, which suggests that these compounds may feature other interesting biological activities.

While detailed SAR data can assist in designing and identifying lead compounds, crystallographic and protein structural modeling are crucial to pinpointing the key PAFr amino acid residues involved in ligand binding, and the development of

highly specific and potent antagonists. Consequently, advances in the latter areas are crucial moving forward.

The structurally related synthetic PAF derivatives show good potency and selectivity but suffer from issues of toxicity and bioavailability. Hence, subsequent studies have shifted away from these molecules to focus on heterocyclic structures. While a number of these diazepine-based compounds are very potent and selective antagonists, there are concerns regarding their potential side effects due to the presence of the diazepine motif, in addition to their somewhat limited synthetic accessibility. More recently, heterocyclic compounds that do not contain diazepine cores have emerged as effective antagonists. Natural products that exhibit PAFr antagonism are often not selective and arguably feature limited therapeutic potential.

PAF and the PAFr and their role in biological functions have been investigated in detail, and there is now a solid basis for exploring therapeutic strategies involving these molecules. Over the past 20 years, a range of new compounds have been prepared, which represent potent and specific antagonists, such as the regioisomeric thienodiazepines. In the future, existing (and new) PAFr antagonists which show promise in in vitro cell-based assays and in vivo animal models should be further investigated with regard to their therapeutic potential and candidacy for advancement to clinical trials.

Acknowledgements

The authors thank Mr. Rajendra K.C. for helpful discussions. I.K.H. thanks the Australian Government for a Research Training Program Scholarship.

Conflict of interest

The authors declare no conflict of interest.

Keywords: ginkgolides · inflammatory diseases · platelet-activating factor · thienodiazepines · xanthones

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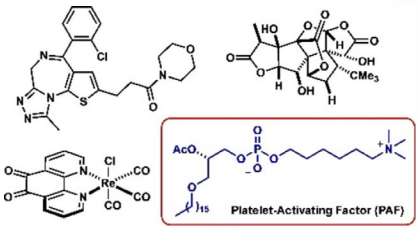
Manuscript received: June 14, 2018
Revised manuscript received: July 8, 2018
Accepted manuscript online: July 15, 2018
Version of record online: ■■ ■■, 0000

Many structures, one target: Platelet-activating factor (PAF) and its receptor (PAFr) have been implicated in a wide range of diseases and disorders that originate from the activation of inflammatory pathways. The PAFr is a well-established therapeutic target, and an array of structurally diverse PAFr antagonists have been identified. These include compounds that are structurally similar to the natural PAF ligand, synthetic heterocycles, complex polycyclic natural products, and various metal complexes.



I. K. Hyland, R. F. O'Toole, J. A. Smith, A. C. Bissember**

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Progress in the Development of Platelet-Activating Factor Receptor (PAFr) Antagonists and Applications in the Treatment of Inflammatory Diseases

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Isabel K. Hyland
Dr. Ronan F. O'Toole
Dr. Jason A. Smith http://orcid.org/0000-0001-6313-3298
Dr. Alex C. Bissember http://orcid.org/0000-0001-5515-2878

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