ELSEVIER

Coordination Chemistry Reviews 232 (2002) 95–126

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized

Jane E. Weder [a], Carolyn T. Dillon [a], Trevor W. Hambley [a,*], Brendan J. Kennedy [a,*], Peter A. Lay [a,*], J. Ray Biffin [b], Hubert L. Regtop [b], Neal M. Davies [c]

[a] *Centre for Heavy Metals Research, School of Chemistry, University of Sydney, Sydney NSW 2006, Australia*
[b] *Biochemical Veterinary Research Pty. Ltd., Braemar NSW 2575, Australia*
[c] *Faculty of Pharmacy, University of Sydney, Sydney NSW 2006, Australia*

Received 31 October 2001; accepted 8 March 2002

Contents

Abstract

The proposed curative properties of Cu-based non-steroidal anti-inflammatory drugs (NSAIDs) have led to the development of numerous Cu(II) complexes of NSAIDs with enhanced anti-inflammatory activity and reduced gastrointestinal (GI) toxicity

* Corresponding authors. Tel.: +61-2-9351-2830x23490; fax: +61-2-9351-3329
E-mail address: p.lay@chem.usyd.edu.au (P.A. Lay).

PII: S0010-8545(02)00086-3

compared with their uncomplexed parent drug. These low toxicity Cu drugs have yet to reach an extended human market, but are of enormous interest, because many of today's anti-inflammatory drug therapies, including those based on the NSAIDs, remain either largely inadequate and/or are associated with problematic renal, GI and cardiovascular side effects. The origins of the anti-inflammatory and gastric-sparing actions of Cu-NSAIDs, however, remain uncertain. Their ability to influence copper metabolism has been a matter of debate and, apart from their frequently reported superoxide dismutase (SOD)-like activity in vitro, relatively little is known about how they ultimately regulate the inflammatory process and/or immune system. Furthermore, little is known of their pharmacokinetic and biodistribution profile in both humans and animals, stability in biological media and pharmaceutical formulations, or the relative potency/efficacy of the Cu(II) monomeric versus Cu(II) dimeric complexes. The following review will not only discuss the etiology of inflammation, factors influencing the metabolism of copper and historical overview of the development of the Cu-NSAIDs, but also outline the structural characteristics, medicinal and veterinary properties, and proposed modes of action of the Cu-NSAIDs. It will also compare the SOD, anti-inflammatory and ulcerogenic effects of various Cu-NSAIDs. If the potential opportunities of the Cu-NSAIDs are to be completely realized, a mechanistic understanding and delineation of their in vivo and in vitro pharmacological activity is fundamental, along with further characterization of their pharmacokinetic/pharmacodynamic disposition.

Keywords: Copper; NSAID; Inflammation; Gastric sparing; Veterinary pharmaceuticals; Human pharmaceuticals; Pharmacology; SOD activity

1. Introduction

The medicinal properties of both Cu and the anti-inflammatory agent salicin, a glycoside of salicylic acid (and prototype of many of today's non-steroidal anti-inflammatory drugs (NSAIDs)) (Fig. 1) have been known for thousands of years [1–4]. Extracts of willow, a source of salicin, were used for the relief of pain and fever by the physicians Hippocrates (~ 460–377 BC) and Dioscorides (~ 40–90 AD) [1,3,5,6]. Chewing willow leaves was recommended for analgesia in childbirth and a decoction of myrtle or willow leaves was a therapeutic treatment for joint pain [5]. Salicylic acid was first purified from salicin in 1838 [7]. Aspirin (AspH = acetylsalicylic acid), the first commercially available NSAID, was introduced into medicine by Frederick Bayer & Company in 1889 [8,9].

As early as 3000 BC, the Egyptians used Cu as an antiseptic to sterilize drinking water [10]. The *Papyus Ebers* (~ 1550 BC) describes the medicinal use of Cu-containing ointments [11,12] and the Cu bracelet has long been used as a folk remedy for the treatment of arthritis [13]. The curative properties of Cu-based NSAIDs is not, therefore, without historical reference and has led to the development of numerous Cu(II) complexes of NSAIDs [14–18]. These are reported to have enhanced anti-inflammatory activity and reduced gastrointestinal (GI) toxicity compared with the parent constituents [10,19,20].

Despite the long history of the Cu-NSAIDs, they are yet to make a significant impact on the human market. Notwithstanding such intense interest, little is known about their mechanism(s) of action, pharmacology and disposition in biological matrices. The following review will, therefore, not only give an overview of the development of the Cu-NSAIDs, including a brief description of the etiology of inflammation, major shortcomings with the clinical application of the traditional NSAIDs, e.g. GI and renal toxicity, mode of action of the traditional NSAIDs and the role of Cu in inflammation, but will also discuss the proposed pharmacology and medicinal and veterinary properties of the Cu-NSAIDs. A comparison of the superoxide dismutase (SOD) activity, and the anti-inflammatory and ulcerogenic effects of various Cu-NSAIDs will be described, along with an outline of the solid-state structural characteristics of the Cu-NSAIDs.

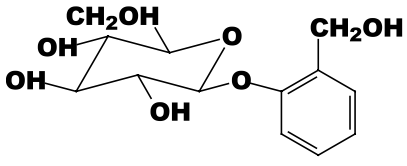


Fig. 1. Salicin.

2. Background

2.1. NSAIDs

'Non-selective' cyclooxygenase (COX) inhibitors, of the general arylalkanoic acid formula ArCRHCOOH, (Ar = aryl or heteroaryl; R = H, CH_3, alkyl) make up the largest group of NSAIDs, e.g. salicylates, indoles, propionic acids, and fenamates [8,21–23]. The oxicam NSAIDs (carboxamides–enolic acids), e.g. piroxicam (Pirx = 4-hydroxy-2-methyl-*N*-2-pyridyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide), and tenoxicam (Tenox = 4-hydroxy-2-methyl-*N*-2-pyridinyl-2H-thieno(2,3-e)-1,2-thiazine-3-carboxamide-1,1-dioxide), were developed by Pfizer in the 1980s as non-carboxylic acid NSAIDs designed to reduce the GI toxicity of the NSAIDs [7]. The 'specific' COX-II inhibitor NSAIDs, e.g. celebrex, (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide) and rofecoxib (4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone) [24,25] (Table 1) and 'highly selective' COX-II inhibitor-type NSAIDs, e.g. meloxicam, (2H-1,2-benzothiazine-3-

carboxamide) (Table 1) and etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid) [26,27], have also been shown to reduce GI toxicity compared with traditional non-selective NSAIDs. Details of the pharmacological basis for the action of the COX inhibitors (including a discussion of their GI toxicity) will be presented later in this review. A schematic outline the 'inflammatory cascade', illustrating the major site of action of the NSAIDs, is shown in Fig. 2. References that detail the historical and structural aspects of the development of the NSAIDs, including their mode of action [5,7,8,23,28–31] and structure–activity relationships [7,32–35], have appeared and these aspects will not be discussed in detail here.

There is now a wealth of literature documenting the potential benefits of Cu(II)-NSAIDs and complexes and several patents have also been issued, e.g. Cu(II)-

Table 1
Chemical classes of selected non-steroidal anti-inflammatory drugs

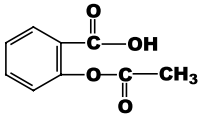
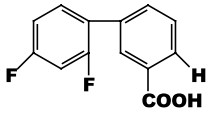
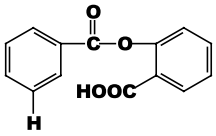
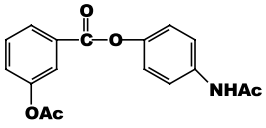
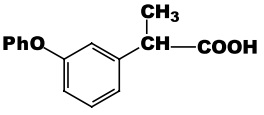
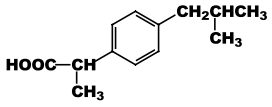
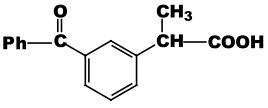
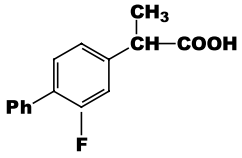
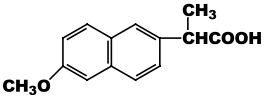
Class	Generic	IUPAC Name	Structure
Salicylates	Aspirin	2-(acetyloxy)benzoic acid	
	Diflunisal	2',4'-difluoro-4-hydroxy-[1,1'-biphenyl]-3-carboxylic acid	
	Salsalate	2-carboxyphenyl ester of salicylate	
	Benorylate	4-(acetylamino)phenyl ester of salicylate	
Phenylalkanoic acids	Fenoprofen	(±) 2-methyl-3-phenoxybenzeneacetic acid	
	Ibuprofen	(±) 2-methyl-4-(2-methylpropyl)-benzeneacetic acid	
	Ketoprofen	(±) 3-benzoyl-2-methylbenzeneacetic acid	
	Flurbiprofen	(±) 2-fluoro-2-methyl-[1,1'-biphenyl]-4-acetic acid	
	Naproxen	(±) 6-methoxy-2-methyl-2-naphthaleneacetic acid	

Table 1 (*Continued*)

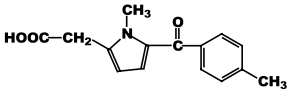
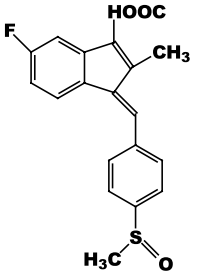
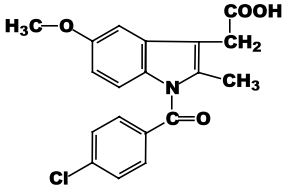
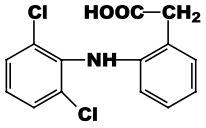
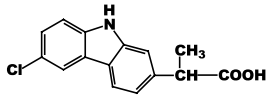
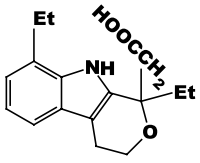
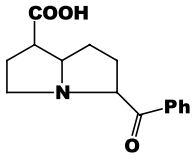
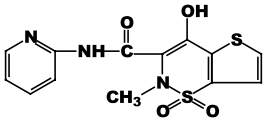
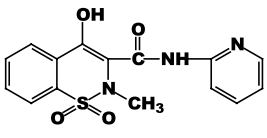
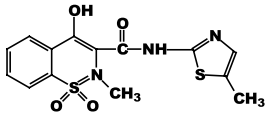
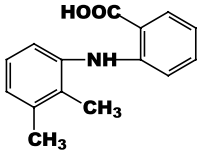
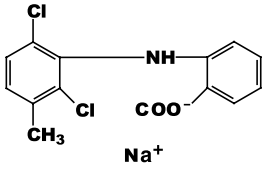
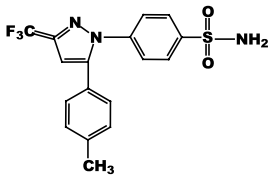
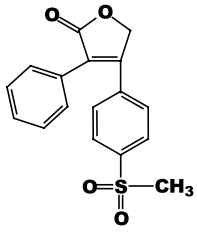
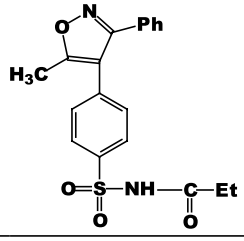
Class	Generic	IUPAC Name	Structure
	Tolmetin	1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetic acid	
	Sulindac	(±) 5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid	
	Indomethacin	1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid	
	Diclofenac	2-[(2,6-dichlorophenyl)amino]-benzeneacetic acid	
	Carprofen	(±)-6-chloro-2-methyl-9H-carbazole-2-acetic acid	
	Etodolac	(±)-1,8-diethyl-1,3,4,9-tetrahydro-pyrano[3,4-b]indole-1-acetic acid	
	Ketorolac	(±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid	
Oxicams	Tenoxicam	2H-thieno[2,3-e]-1,2-thiazine-3-carboxamide	
	Piroxicam	2H-1,2-benzothiazine-3-carboxamide	

Table 1 (*Continued*)

Class	Generic	IUPAC Name	Structure
	Meloxicam	2H-1,2-benzothiazine-3-carboxamide	
Anthranilic acids	Mefenamic acid	2-[(2,3-dimethylphenyl)amino]benzoic acid	
	Meclofenamate sodium	2-[(2,6-dichloro-3-methylphenyl)amino]-benzoic acid, monosodium salt	
Sulfonamides	Celecoxib	4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide	
Furanones	Rofecoxib	4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone	
Propanamide	Parecoxib	N-[[4-(5-methyl-3-phenyl-4-isoxazolyl)phenyl]sulfonyl]-propanamide	

complexes of amino-acids, substituted steroids and NSAIDs [37–41]. No Cu(II) anti-inflammatory drug is currently available for oral human use, although an ethanolic gel-base of Cu-salicylate (Alcusal®) is available for topical temporal relief of pain and inflammation in humans. A Cu(II) dimer of indomethacin (IndoH = 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-Indole-3-acetic acid) with low toxicity is commercially available in Australasia [42], South East Asia and South Africa [43] in a variety of oral pharmaceutical dosage forms for veterinary use. These low toxicity Cu drugs are of enormous interest, because many of today's anti-inflammatory drug therapies, including the NSAIDs, remain either largely inadequate and/or are associated with problematic side effects, e.g. renal insufficiency and failure [44–47], GI ulceration, bleeding or perforation ('NSAID gastropathy') [48–54], exacerbation of hypertension [21] and congestive heart failure (CHF) [55].

The commercial potential of safer NSAIDs is evidenced by the world-wide availability of over 35

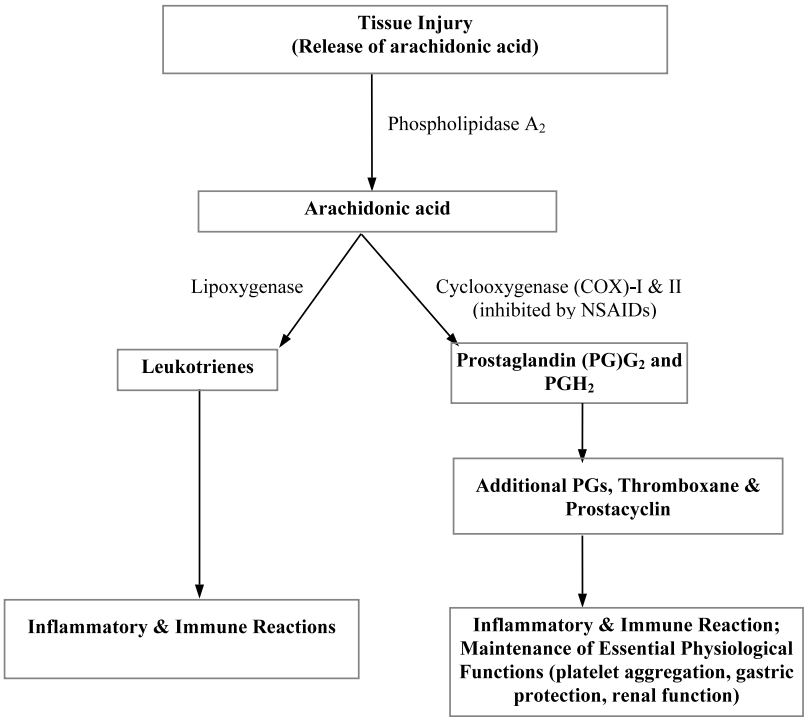


Fig. 2. The 'Inflammatory Cascade'.

different NSAIDs [21]. Additional interest in NSAIDs lies in their possible therapeutic benefits in the prevention of various cancers including colorectal [56–62] and lung cancers [63] and even in the treatment of Alzheimer's disease [64–67]. There is a wide and expanding clinical use of NSAIDs, particularly in chronic diseases of the elderly, in whom the GI [22,68–71], renal [72–75], and cardiovascular [55] side-effects lead to significant morbidity and mortality. The overall cost of NSAID therapy in the US was estimated in 1992 to be increased by ~40% due to the prevention and or treatment of their GI toxicity alone [76,77]; with 20–30% of all hospitalizations and deaths among patients over 65 years due to peptic ulcer disease attributed to the use of NSAIDs [71,73,78]. The burden of illness resulting from NSAID-related CHF, however, may even exceed that resulting from GI damage [55].

Treatment of chronic arthropathies remains largely symptomatic and there is yet no cure for inflammatory diseases such as arthritis [79–81]. The modern management of such ailments relies principally upon the alleviation of the symptoms, with the use of NSAIDs being the mainstay in conjunction with other non-drug treatments [79,80]. Arthritis and associated muscular skeletal diseases do not dominate the mortality statistics, but they account for substantial socioeconomic costs [77,82–88] and are a leading cause of long-term disability [86,87,89]. There are no 'world-wide' figures on the costs of inflammation, although the medical and economic costs associated with arthritis in the USA (1992) [86], Canada (1993) [90] and Australia (1997) [83] are estimated to be ~$(US)64.8B, $(CDN)17.8B and $(AUS)1-5B pa, respectively. The cost of osteoarthritis (OA) accounts for ~1–2.5% of the gross domestic product (GDP) of the USA, Canada, UK, France, and Australia. With an ageing population, the increasing impact of arthritis on public health in the USA is expected to increase to $(US)95B by 2020 and affect ~22% of the population [91]. Interest in developing NSAIDs is therefore related, to their ubiquitous use and hence considerable commercial market and costs associated with treating their various side-effects.

The search for safer and more effective anti-inflammatory drug treatments continues [92–98]. This includes the search for less GI toxic NSAIDs [28,99–104], investigations of the specific COX-II inhibitors [29,105–109], nitric oxide (NO) releasing NSAIDs [110–114], leukotriene pathway inhibitors [115–121], SOD mimetics [122–124], zwitterionic phospholipids [125,126], metal (in particular Cu(II) and Zn(II)) complexes of NSAIDs [10,17,19,38,39,127–140], and sustained release and enteric coating formulations of traditional NSAIDs [141–144]. This review will concentrate only on the medicinal and veterinary properties of Cu(II) complexes of NSAIDs.

2.2. Inflammation

As the pharmacology and pharmacokinetics of Cu-NSAIDs remain currently uncertain, it is appropriate to

review briefly the inflammation process, and the significance and disadvantages of the use of NSAIDs in medical and veterinary practice. This will be followed by a discussion of the pharmacology of some Cu-NSAIDs, including their SOD, anti-inflammatory, and ulcerogenic activities.

Inflammation is a disease condition in which body tissues are affected by heat, redness, swelling and pain [145]. John Hunter (1728–1793), one of the early English physicians to scientifically study the reaction, described inflammation as such [146]:

> This operation of the body termed inflammation requires our greatest attention, for it is one of the most common and most extensive in its effect of any in the animal body.

The etiology of inflammation has been a subject of much research and debate over the years [147–150]. However, the inflammatory response is a normal and essential response of the body to a harmful stimulus and may vary from a localized reaction in an affected tissue or joint to a more generalized whole-body reaction [151]. While inflammation may be a normal response, chronic inflammation results in destruction of normal connective tissue due to the activities of catalytic enzymes and cytokines [149,150,152–158]. This destruction is due to activation of the immune response, the release of hydrolytic enzymes, e.g. collagenases, proteases, gelatinases, matrilysin, and the subsequent degradation of collagen and other extra-cellular components found in body joints and connective tissues [81,151,159–163].

There is a plethora of reviews and textbooks outlining the pathology of inflammation, including the sequence of events, network of mediators, e.g. prostaglandins (PGs), leukotrienes and cytokines, and the complex molecular mechanisms involved [3,95,119,164–171]. This follows the discovery in the mid-1930s of PG [172] and the reporting of its structure in 1962 [173].

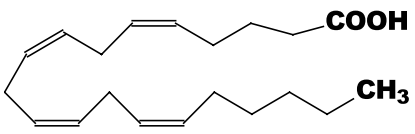
Following the recognition of PGs as an integral component of the body's inflammatory cascade, came the development of immune modulators of inflammation, e.g. matrix metalloproteinase (MMP) inhibitors [121,174,175], anti-leukotriene drugs/5-lipooxygenase inhibitors [95,176], recombinant anti-inflammatory cytokines, proinflammatory cytokine antagonists, and even gene therapy [177]. Inflammation is a key feature of a number of diseases—and the clinical features of these diseases are described in standard medical textbooks [151]. Whilst inhibition of PG synthesis has long been recognized as the mode of action of NSAIDs [150,170,178], it is also suggested that NSAIDs act at additional sites in the inflammatory cascade, e.g. NSAIDs may block tumor necrosis factor-alpha ((TNF)-α) augmentation of PGs [121]. There are significantly higher levels of TNF-α and TNF-α convertase enzyme and an increased expression of TNF-α receptors in OA cartilage compared with normal cartilage [121]. Up-regulation (promotion) by TNF-α and interleukin (IL)-1 of the expression of the MMP gene is also reported [121]. Agents that inhibit TNF-α and IL-1 may, therefore, offer alternate drug strategies for the treatment of inflammatory diseases such as OA [121]. Inhibition of the release of TNF-α and IL-1 (or IL-2) down-regulates (reduces) PG and leukotriene production and, consequently, results in a reduction of the inflammation process [179]. Whilst the mode of action of the NSAIDs is still described as uncertain [22], it is attributed primarily to the inhibition of PG synthesis [21,168,170,178,180], and more specifically inhibition of the COX enzyme system [29,107,169,181–183]. However, there remain a number of other potential sites of action for anti-inflammatory agents.

2.3. Cyclooxygenase (COX) inhibition: proposed mode of action of NSAIDs

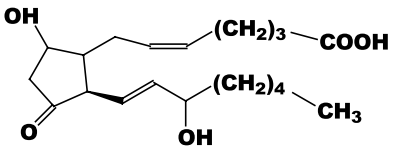
Inhibition of the COX-I and COX-II enzyme systems, and subsequent down-regulation (inhibition) of PG synthesis, is the well-accepted mode of action of NSAIDs [184,185]. COX is a membrane-bound enzyme containing COX and peroxidase catalytic sites responsible for the oxidation of arachidonic acid to PG [186]. In the COX catalytic site, arachidonic acid is converted into the cyclic endoperoxide PGG_2, while in the peroxidase catalytic site, PGG_2 is converted into PGH_2 [170]. The endoperoxides are further metabolized to form additional PGs, e.g. PGI_2 (prostacyclin), PGD_2, PGE_2, $PGF_2\alpha$, and thromboxane with a variety of physiological effects (Fig. 3) [7,167]. PGs, thromboxane and leukotrienes are known collectively as eicosanoids.

COX-I is believed to be expressed constitutively in the body and so regulates 'house-keeping functions', i.e. maintenance of essential physiological functions, such as platelet aggregation, gastric protection and renal function [187]. COX-I also leads to the production of prostacyclin (PGI_2) which, when expressed by the endothelium, is anti-thrombogenic and when expressed by the gastric mucosa is gastric protective [169]. COX-II is inducible at sites of inflammation by, e.g. endotoxins, TNF-α, interferon (INF)-γ, IL-1α, IL-1β, and growth factors, with the anti-inflammatory cytokine IL-10 reportedly down-regulating COX-II [169,186,188,189].

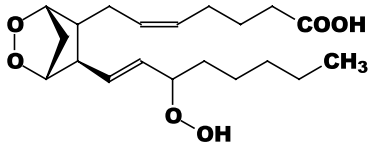
It is not possible to identify all the roles of COX-II, although it is also reportedly expressed constitutively in the brain and kidney [190]. This diverse in roles of COX-II suggests it may play a part in the fine modulation of cellular and organ function and this may help explain the gastric [191] and renal toxicities [46,192] (particularly in sodium-restricted patients) [75,193] of the specific COX-II inhibitor NSAIDs [190].



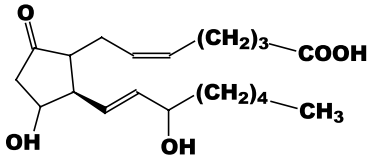
(a) 5,8,11,14-eicosatetraenoic acid (arachidonic acid).



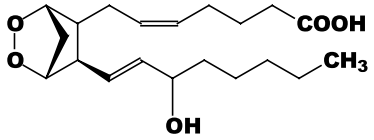
(e) 9,15-dihydroxy-11-oxo-prosta-5,13-dien-1-oic acid (PGD_2).



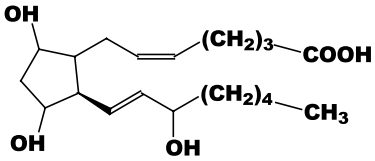
(b) 9,11-epidioxy-15-hydroperoxy-prosta-5,13-dien-1-oic acid (PGG_2).



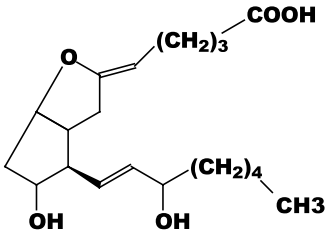
(f) 11,15-dihydroxy-9-oxo-prosta-5,13-dien-1-oic acid (PGE_2).



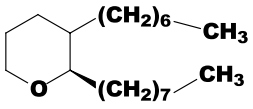
(c) 9,11-epidioxy-15-hydroxy-prosta-5,13-dien-1-oic acid (PGH_2).



(g) 9,11,15-trihydroxy-prosta-5,13-dien-1-oic acid ($PGF_{2\alpha}$).



(d) 6,9-epoxy-11,15-dihydroxy-prosta-5,13-dien-1-oic acid (prostacyclin; PGI_2).



(h) (2R-trans)-3-heptyltetrahydro-2-octyl-2H-pyran (thromboxane).

Fig. 3. The structure of (a) arachidonic acid and the PGs (b) PG_2, (c) PGH_2, (d) PGI_2, (e) PGD_2, (f) PGE_2 and (g) $PGF_2\alpha$ (h) thromboxane. Structures generated by CHEMWINDOW [36].

The structures of COX-I [194] and COX-II [182] are similar, with the COX-II active site accommodating larger substrates than the active site of COX-I [195]. Elevated expression of COX-II is found in synovial tissue of patients suffering from rheumatoid arthritis (RA) [196], with both COX-I and COX-II reportedly expressed by synovial fluid cells of patients suffering acute and chronic arthritis [197].

The COX-I and COX-II active sites are described as hydrophobic channels of amino acids, culminating with serine (Ser) 530, arginine (Arg) 120, and tyrosine (Tyr) 385 at the apex [29]. The known inhibitors of COX can be classified into four types [185]. Aspirin, the first type, reportedly binds irreversibly (to Ser 530 of COX-I and Ser 515 of COX-II) [198] by acetylation, leaving the peroxidase activity unaffected [170] but preventing access of arachidonic acid to the COX site. The second type, e.g. ibuprofen (IbuH = α-methyl-4-(2-methylpropyl)benzeneacetic acid) and mefanamic acid (MefH = 2-[(2,3-dimethylphenyl)amino]benzoic acid), reportedly bind sterically and reversibly to Tyr 385 or Arg 120, competing with arachidonic acid for the COX site, and blocking the COX action of the enzyme [29,185]. Slow and time-dependent binding of NSAIDs by a carboxylate bridge, e.g. IndoH (Fig. 4) and flurbiprofen (FlurH = 2-fluoro-α-methyl-4-biphenylacetic acid), to Arg 120 of both the COX-I and COX-II enzymes is proposed for the third class of NSAIDs [185]. For

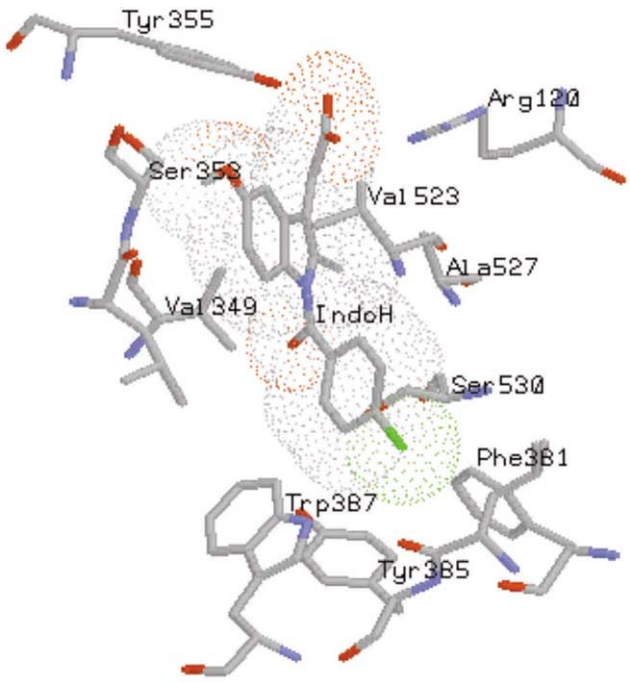


Fig. 4. Binding of the 'non-selective' COX-II inhibitor IndoH.[185] Generated by the molecular visualization program RASMOL [199].

example, *S*-FlurH interacts through its carboxylate group with the Arg 120, which allows its second phenyl ring to come within van der Waals contact of the Tyr 385 [185]. *S*-Enantiomers have much greater activity in blocking PG synthesis than the *R*-enantiomers of NSAIDs [8]. Specific inhibition of COX-II, e.g. by celebrex, represents the fourth class of NSAIDs [185].

It is suggested that there are a number of sub-sites for binding within the narrow COX channel by the selective COX-II NSAIDs [170,195]. These sites include the binding by celecoxib or rofecoxib through a phenysulfonamide group to a side-pocket present in COX-II, but not in COX-I [185], or to the apex of the more flexible COX-II channel, e.g. by the ' selective' COX-II inhibitor meloxicam [185]. It is the presence of a smaller valine (Val 523) in COX-II as opposed to isoleucine (Ile 523) in COX-I [200] that allows access to the side pocket and binding by either a sulfonyl, sulfone or sulfonamide group, which is characteristic of the currently marketed selective COX-II inhibitors [185,195].

Most currently available NSAIDs are preferential inhibitors of COX-I over COX-II [29,187,201]. COX-I is the main isoform throughout the GI tract and maintains mucosal integrity [28,202]. It is the highly selective inhibition of COX-I by the common NSAIDs that is believed to be crucial in the pathology of NSAID induced GI toxicity [28,29,113,202]. IndoH is among the most selective of the NSAIDs for COX-I [109], which provides some understanding of its potential for GI toxicity in animals and humans.

Two specific COX-II inhibitors, celecoxib (by Searle-Monsanto) and rofecoxib (by Merck) have recently been approved by the US Food and Drug Administration (FDA) for human use [24,25], although a number of other compounds, e.g. nimesulide (*N*-(4-nitro-2-phenoxyphenyl)methanesulfonamide) and flosulide (*N*-[6-(2,4-difluorophenoxy)-2,3-dihydro-1-oxo-1H-inden-5-yl]-methanesulfonamide) are in development [195,201]. This includes parecoxib (*N*-[[4-(5-methyl-3-phenyl-4-isoxazolyl)phenyl]sulfonyl]propanamide), which is an injectable COX-II inhibitor in phase 3 clinical trials, valdecoxib (4-[5-methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide), which is a highly specific COX-II inhibitor with an active selective COX-II metabolite [203], and etoricoxib (5-chloro-6′-methyl-3-[4-(methylsulfonyl)phenyl]-2,3′-bipyridine), which is reportedly the most selective coxib in current clinical development (Phase 3 clinical trials) [204].

Celebrex and rofecoxib are as effective anti-inflammatory agents as the non-selective NSAIDs, with less ulcerogenic effects [205]. Following its release on the US market in 1999 and the filling of an initial 2.5 million prescriptions, celecoxib had been linked to ten deaths and 11 cases of GI hemorrhage [206]. It appears to have a markedly safer GI toxicity profile compared with traditional non-selective NSAIDs, reportedly resulting in a 50- to 100-fold lower incidence of serious GI hemorrhaging compared with the traditional NSAIDs [207].

Questions have been raised, not only about the safety of selective COX-II inhibitors in the presence of pre-existing GI inflammation, but also their potential to retard ulcer healing or cause ulcers in patients with pre-existing ulceration [195]. Furthermore, the selective COX-II inhibitors have the ability to increase the incidence of thrombosis in chronic dosing and induce asthma in aspirin-sensitive asthmatics [106], elevate blood pressure and leukocyte adherence [46] and compromise renal function [46,75,192,208]. The commercial success of celecoxib and rofecoxib is evident, none-the-less, by their combined worldwide sales in 1999 of over $US2B [188]. Excellent reviews are available detailing the pharmacology, biochemistry and rationale for the use of selective COX-II inhibitors for the treatment of inflammation [209–211].

Of interest is the selective COX-II inhibitory activity of the IndoH derivative L-748780 [108]. Merck achieved the conversion of IndoH into a selective COX-II inhibitor by an increase in the steric bulk of the 4-chlorobenzoyl group in IndoH with two chlorine substituents. The increase in steric bulk was proposed to take advantage of the larger size of the COX-II compared with the COX-I active site. Furthermore, derivatization of the carboxylato group in IndoH, as well as in fenamate- and phenylalkanoic acid-NSAIDs, into the ester or secondary amide analogue, reportedly

alters specificity of NSAID into a specific COX-II inhibitor [212–214]. Primary and secondary amides of IndoH are more potent COX-II inhibitors than the corresponding tertiary amide [212,213], and the increased length of the methyl ester analogue of IndoH leads to increased potency and COX-II selectivity [212]. Replacement of the 4-chlorobenzoyl group of IndoH in IndoH esters or amides with a 4-bromobenzyl group, or exchange of the 2-methyl group on the indole ring in the ester and amides with a hydrogen atom affords an inactive compound [213]. The selective COX-II activity of these agents is proposed to arise from binding at the opening and apex of the COX-binding site [214,215]. A review of the molecular and structural basis for these selective COX-II inhibitors has been published [216,217]. No studies have, as yet, been reported on the COX-II activity of Cu-NSAIDs.

2.4. Gastrointestinal and renal toxicity

Gastropathy and renal toxicity are major side-effects of NSAIDs [22] and are, consequently, of great interest when developing alternative NSAIDs. The following section discusses some of the proposed mechanisms involved in these toxicities, including the roles of the COX isoenzymes, reactive oxygen species (ROS), SOD and NO. Much work is published on the gastric-sparing effects of Cu-NSAIDs [10,19,218,219], although there is a lack of published studies on their renal (or cardiovascular) side effects.

2.4.1. Gastric toxicity

Damage to the GI mucosa by NSAIDs can occur via several mechanisms. These include direct topical irritation to the GI epithelium, impaired barrier properties of the mucosa, reduced gastric mucosal blood flow, interference with the repair of superficial injury, and suppression of gastric PG synthesis [202,220]—in particular, inhibition of the COX isoenzyme system [169,186]. Whilst COX-1 appears to function as a house-keeping enzyme, COX-II is primarily induced by inflammatory stimuli and mitogens in various cells, including macrophages and synovial cells [77]. Accordingly, the inhibition of COX-II results in anti-inflammatory effects, whereas gastro duodenal ulceration is thought to be related to the inhibition of COX-I [77].

The regulatory relationship between growth factors and PGs in the gastric mucosa is not well characterized [221]. Elevated levels of COX-I and COX-II mRNA occur during ulcer healing [222]. Gastric healing in rats is reportedly associated with up-regulation of mRNA expression of COX-II, along with up-regulation of inducible nitric oxide synthetase (iNOS), cytokine-induced neutrophil chemoattractant (CINC)-1, epithelial growth factor (EGF), hepatocyte growth factor (HGF), epidermal growth factor (ECG), transforming growth factor (TGF)-beta 1, and basic fibroblast growth factor (bFGF)) [223]. Furthermore, the up-regulation of iNOS and these cytokines is attributed to the expression of endogenous IL-1 from the macrophages and fibroblasts in the ulcerated tissue [223]. However, there is a report that delayed healing of chronic gastric ulcers in arthritic rats is unrelated to either NO or PGs [224]. The use of selective COX-II inhibitors may still, therefore, have deleterious GI effects, with recent reports of delayed gastric ulcer healing [225–227] and exacerbation of chronic inflammation [190,228].

COX-II has been implicated in both the early stages of the inflammatory response and the healing phase (~ 48 h after an inflammatory injury) [227]; with both COX-I and COX-II proposed to contribute to the healing of gastric ulcers [222]. Treatment of rats with the selective COX-II inhibitor L-745337 at doses that do not inhibit COX-I reportedly causes significant inhibition of mucosal PG synthesis and a marked exacerbation of colonic damage [229]. Treatment for 1 week resulted in perforation of the bowel wall and death [229]. Furthermore, treatment of mice with the selective COX-II inhibitor NS-398 reportedly results in a reduction in mucosal PG synthesis and significant inhibition of ulcer healing [230], and treatment of rats with the selective COX-II inhibitor L-745337 shows a marked inhibition of gastric ulcer healing [231].

It is proposed that COX-II aids the resolution of inflammation at a mononuclear cell-dominated phase by generating PGs [106], which bind to and activate peroxisome-proliferator-activated receptor PPAR-γ [227,232,233]. The NSAIDs IndoH and IbuH are reported to bind to and activate PPAR-γ [234].

The release of NO from NO-releasing NSAIDs also protects the stomach against damage [110,235] despite inhibiting both COX-I and COX-II [236]. It is proposed that NO-releasing NSAIDs are a new class of NSAID, possessing an anti-inflammatory mechanism independent of COX, with the IL-1β converting enzyme (ICE) a possible target for NO-releasing NSAIDs [237]. Inhibition of ICE reportedly prevents endothelial cell damage induced by pro-inflammatory drugs [238], by causing intracellular NO formation and inhibiting the intracellular release from monocytes of a number of the ILs (including TNF-α, which induces apoptosis) [237].

2.4.2. Renal toxicity

Whilst the use of NSAIDs is associated with a wide range of tubular, interstitial, glomerular and vascular renal lesions [72,239], the long term renal effects of the Cu-NSAIDs are yet to be assessed. Preliminary investigations demonstrate that the Cu(II) complex of Indo is safer than IndoH in animal studies [240]. One potential mechanism is the effect of Cu on PG synthetase [241]. ROS play a role in chronic renal injury and glomerulosclerosis [242], whereas SOD is renal protective [243–

245]. SOD reportedly decreases lipid peroxidation, following renal ischemia and reperfusion, as restoration of oxygen supply to the kidney results in the production of ROS and hence renal damage [245]. ROS [242,246] and decreased catalase gene expression are reported to also play a role in renal injury [242,247].

NO purportedly regulates glomerular ultrafiltration, tubular reabsorption, and intrarenal renin secretion, with defective renal *i*NOS claimed to play a key role in the complex renal hemodynamic and non-hemodynamic disorders associated with renal disease [248]. Drugs capable of enhancing renal NO activity may be renal protective [248]. Somewhat contradictory studies propose that NO plays a role in the progression of renal diseases in animals, but few studies are available in humans [248].

Maintenance of normal renal function reportedly depends mainly upon PGs derived from COX-I [249]. COX-II, however, is also constitutive in renal tubular cells in the cortex, outer medulla, and thick ascending limb, and is proposed to be involved in the handling of electrolytes via the local production of PGs [250]. Targeted disruption of the COX-II allele in mice results in severe renal problems [75]. Renal dysfunction caused by selective COX-II inhibitors has been reported in patients with compromised renal function [192].

3. Biological roles of Cu

Issues pertaining to the absorption, transport, and function of Cu in the body may be relevant to an investigation of the pharmacology and biodistribution of Cu-NSAIDs. The following section briefly discusses the various biological roles of Cu, its biodistribution and its function in inflammation.

Copper was first shown to be an essential biological element in the 1920s when anemia was found to result from Cu-deficient diets in animals [251] and addition of Cu salts corrected this affliction [251,252]. It is now recognized as an essential trace element for many biological functions [253,254]. It serves as a catalytic component in many enzymes, e.g. it is an important constituent of metalloproteins (exhibiting oxidative reductase activity, e.g. oxidases or hydroxylases) [255], and in such enzymes as lysyl oxidase (required for connective tissue) and cytochrome oxidase (electron transport protein) [256].

Copper also influences specific gene expression in mammalian cells [257,258], nerve myelation and endorphin action [259], with Cu deficiency impairing immunity [260–262]. The role of trace metallic elements, such as Cu in inflammation, is of great interest given their function as co-factors in metabolic processes involving articular/connective tissue and the immune system [263] and their effect on PG synthesis [241,264–267].

3.1. Biodistribution of Cu

The general bioavailability and metabolic fate of dietary Cu in humans are well understood [257,268–270]. The daily intake of Cu in humans is ~1.5–3 mg per day [256]. Whilst whole-body Cu metabolism is difficult to study, isotope tracers have enabled direct measurements. The isotopes ^{64}Cu ($t_{1/2} = 12.8$ h) and ^{67}Cu ($t_{1/2} = 58.5$ h) are most commonly used for such metabolic and bio-distribution research [268]. The body of an average healthy adult male (70 kg) contains ~110 mg of Cu, much of which is in the skeleton (46 mg), skeletal muscle (26 mg), liver (10 mg), brain (8.8 mg) and blood (6 mg) [271]. Copper is the third most abundant transition metal element in biological systems [255]. The normal human body contains 80–120 mg of Cu compared with 4–5 g of Fe and 1.4–2.3 g of Zn [255]. Normal human Cu concentrations in various biological media and daily excretion levels are given in Table 2.

In humans, Cu absorption varies inversely with dietary Cu intake [269,273], and occurs from the stomach through to the small intestine [270], predominantly in the stomach and jejunum [274]. The presence of Zn, Fe and Mo reportedly decrease Cu absorption, and high protein diets increase Cu absorption [270,275]. The low pH value of the gastric juices is also claimed to contribute to the freeing of Cu bound to foodstuffs prior to GI absorption [270]. This may be important when investigating the fate of Cu-NSAIDs and the development of veterinary and pharmaceutical formulations of these complexes. Once absorbed, ~70–80% of plasma Cu is in a non-exchangeable form, bound to ceruloplasmin (CP). The remainder is bound to albumin, transcuprien, metallothionein (MT) and low-molar-mass (l.m.m) complexes, e.g. L-His-Cu(II)-L-Ser, L-His-Cu(II)-L-Thr, as the exchangeable Cu fraction in blood [272,276].

Transfer of Cu across the cell membrane of the intestinal mucosa reportedly occurs by diffusion and carrier-mediated systems [272], e.g. Cu-transporting P-type ATPases [277–280]. Little is known about these enzymes, as mammalian genes encoding for Cu-trans-

Table 2
Copper concentrations in various biological fluids and daily excretion in humans

Tissue	Humans	Daily excretion (μg)
Whole blood	800–1300 μg L^{-1} [254]	
Serum/plasma	800–1750 μg L^{-1} [254]	
Urine	12–80 μg L^{-1} [254]	30–70 [272]
Gastric juice	0.39 μg g^{-1} [272]	1000 [272]
Bile	4.0 μg g^{-1} [272]	2500 [272]
Pancreatic fluid	0.13–0.9 μg g^{-1} [272]	400–1300 [272]
Duodenal fluid	0.17 μg g^{-1} [272]	400–2200 [272]
Synovial fluid	0.2–0.5 μg g^{-1} [272]	

porting P-type ATPases have only been identified recently [277,278]. The P-type ATPase enzymes are defective in Menkes disease—an inherited degenerative disease of the central nervous system involving Cu deficiency [254,272,281]. Once in the cytosol of the cell, ~80% of the Cu is bound to MT [272]. The intestinal mucosal cells then transfer Cu into the portal blood, with some of the Cu being released into the portal system with MT [282].

Very little is known, as yet, about the absorption and distribution of Cu-NSAIDs [283,284]; however, it is proposed that the lipophilic nature of the Cu-NSAID may facilitate absorption of the metal complex across the GI tract [285]. A more detailed discussion of the proposed absorption of Cu-NSAIDs will be outlined later in this review.

3.2. Copper transport and binding in the blood and biofluids

Each step of the Cu transport pathway requires specific cell receptors and membranes, and intracellular transport proteins. Recent papers have reviewed these intracellular processes [256,281]. Albumin and CP appear to be the primary Cu carrier proteins in the body—they transfer a large amount of exchangeable Cu in the circulatory system and release it for specific cell uptake [271]. Albumin is the major drug binding protein in the body [286], particularly for acidic xenobiotics such as NSAIDs.

About 40 μg of Cu can bind to the albumin contained in 1 ml of human plasma [271]. The Cu(II) transport site of serum albumin is one of the most extensively studied binding sites of any protein [287] and is well characterized in a number of animal species [276]. There is interspecies variation in the binding of Cu to albumin, e.g. the reported absence of specific Cu(II) binding sites in dog serum albumin (DSA) [288,289] and the increased sensitivity of dogs to Cu intake compared with humans [272,276,290,291]. This may be an important consideration when investigating/developing the pharmacokinetics and pharmaceutical formulations of Cu-NSAIDs.

The proposed structure of the major Cu(II) binding site in HSA involves the *N*-terminus of albumin (L-Asp-L-Ala-L-His), which provides four N ligands: i.e. the terminal amino group, two deprotonated peptide nitrogens (of Ala2, and NH of His3), and a L-His imidazole nitrogen of His3 in a square-planar arrangement (Fig. 5) [287,291–293]. Up to 20 different Cu(II) binding sites in HSA have been reported [294], with X- and S-band electron paramagnetic resonance (EPR) spectroscopy and optical spectroscopy used to help characterize the nature of the major Cu(II) binding site [291,295]. Cu(II) may also form ternary complexes with l.m.w. proteins, or small endogenous ligands, in plasma [296,297]. These ternary complexes of Cu(II) may provide a mechanism

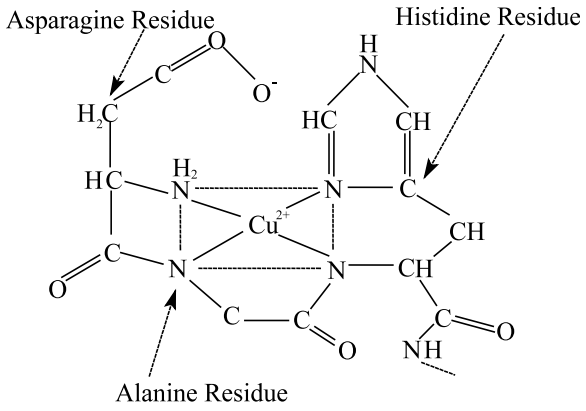


Fig. 5. The proposed Cu binding site of Cu(II) in human serum albumin.

whereby Cu(II) is transferred between the various Cu binding substances in plasma [296,298]. Trace amounts of Cu are also bound to transcuprien [256]. Copper binding to MT does not form a significant proportion of plasma Cu as it has a half-life of only a few minutes [276].

CP contains eight Cu sites (of which two sites are labile) (Fig. 6) [299–302]. CP delivers Cu to specific receptors on the surfaces of cells of target tissues [303]. On binding to the receptor, conformational changes in the protein lead to the release of the Cu in the target cell where its fate will be dependent upon the cell type [303,304]. The reduction of Cu(II) to Cu(I) seems to be a necessary step in the membrane transport of Cu into the cell [304].

The metabolism, turnover and conservation of intracellular Cu are highly organ specific. Most intracellular Cu is recycled, with cells adapting to reduced bioavailability of Cu by reduced cellular release [277]. The brain and heart, for example, are able to conserve Cu following minor decreases in total plasma Cu concentration [277]. The sensors that activate this conservation have not, as yet, been identified [277]. The cellular pools of free Cu are miniscule, with metallochaperone proteins within cells, e.g. Cu chaperone for SOD (CCS), inserting Cu into the appropriate protein receptor [305,306]. Whilst little is known about the structure and function of the specific chaperone proteins, the crystal structure of yCCS derived from yeast shows two SOD-like domains forming a dimer [307]. It is reported that CCS is necessary for the expression in vivo of SOD, despite the high affinity of SOD for Cu ($K_D = 6$ fM) and high intracellular concentrations of both SOD (10 μM in yeast) and copper (70 μM in yeast) [280]. CCS reportedly directly inserts Cu into SOD (via Cu(I)–yCCS protein in yeast) and so protects the metal ion from binding to other intracellular ligands [279]. This insertion is postulated to involve the cooperation of three distinct regions of yCCS, whereby domain I recruits cellular Cu; domain II

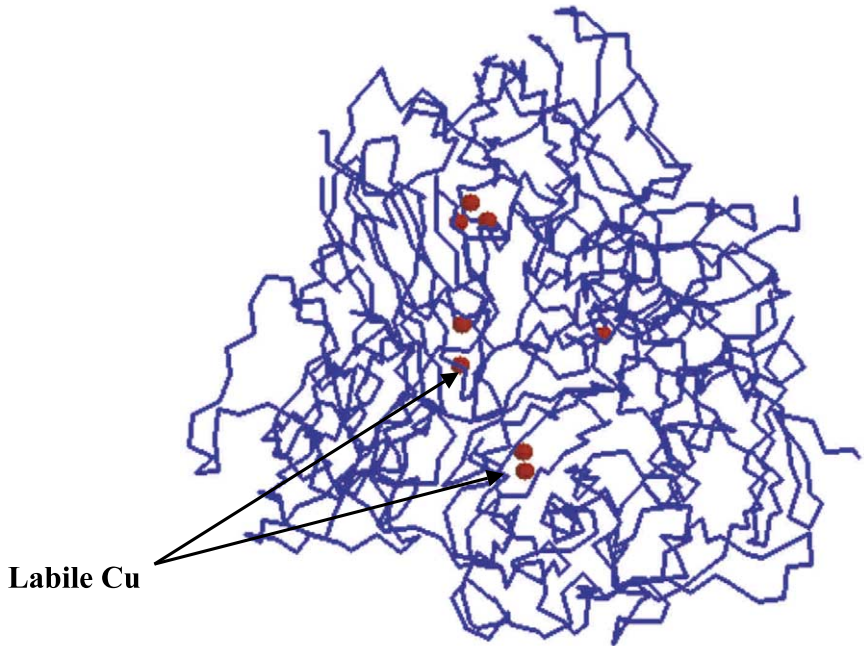


Fig. 6. Carbon-chain representation of CP generated by the RASMOL molecular visualization program [199] showing the two labile Cu-sites. Data taken from ref. [302].

facilitates target recognition and domain III (in concert with domain I) mediates Cu insertion into apo-SOD [279]. Yeast CCS exhibits no SOD activity [308].

3.3. The anti-inflammatory role of Cu

A potential scientific basis for the anti-inflammatory Cu bracelet remedy emerged when it was shown that metallic Cu can dissolve in sweat and be absorbed through skin [309]. Copper is believed to possess anti-inflammatory activity [10,14]. Patients with RA and oesteoarthritis exhibit changes in the Cu distribution in the blood [310,311]. For instance, there is an observed increase in total serum Cu in arthritis sufferers compared with controls. This is observed as an increase in CP-bound Cu and a decrease in albumin-bound and low-molecular-weight Cu effectively resulting in lower levels of bioavailable Cu, in the blood [310,311]. Altered Cu concentrations have also been observed in the synovial fluid of RA and OA patients [10]. There is some debate as to the reasons for these observations; some researchers have suggested that the alterations in Cu are a cause of the disease while others believe that it is a physiological response to the disease and that Cu plays a pertinent role in its control [311].

Whilst the roles of Cu in inflammation are still a matter of debate, a change in its metabolism is observed in acute and chronic inflammatory conditions. In acute inflammation, there are significant increases in both total Cu(II) and CP concentrations in serum, without notable changes in the Cu concentration in the liver [254]. Likewise, in chronic inflammation, Cu serum concentrations are increased during the active phase, with appreciably higher than normal CP levels found in the synovial fluid of patients with RA [253] and a net accumulation of Cu in inflamed areas [312–314]. It is proposed that there is increased demand for Cu during inflammatory conditions, which is compensated for by enhanced intestinal absorption and/or decreased intestinal excretion of Cu [314]. Moreover, a Cu deficiency is reported to have a pro-inflammatory effect [314]. It has long been suggested that the mode of action of salicylates and other such anti-inflammatory drugs may involve the chelation of bioactive metal ions such as Cu(II), so facilitating the transfer of the metal to and from a site of inflammation or pain [20,315,316].

The beneficial role of Cu in minimizing inflammation has been attributed to its redox activity—in particular, the ability of Cu in such enzymes as SOD to remove the highly reactive pro-inflammatory superoxide radical anion $O_2^{-\bullet}$ [317,318]. Indeed, SOD has been used clinically in the treatment of inflammation in animals [319,320]. The Cu(II)–Cu(I) redox cycle can be summarized as follows [255]:

$$Cu(I) + O_2 \rightleftharpoons CuO_2^+ \quad (1)$$

$$CuO_2^+ + H^+ \rightleftharpoons Cu(II) + HO_2 \quad (2)$$

$$Cu(I) + HO_2 \rightleftharpoons Cu(II) + HO_2^- \quad (3)$$

$$H^+ + HO_2^- \rightleftharpoons H_2O_2 \quad (4)$$

with the reduction of O_2 to water ($O_2 + 4e^- + 4H^+ \rightleftharpoons 2H_2O$) and an accompanying transfer of oxygen to a substrate [255]. Hence, many Cu enzymes are either hydroxylases or oxidases [321]. The dismutation of $O_2^{-\bullet}$ by Cu in SOD is summarized as follows [317,318]:

$$Cu(II) + O_2^{-\bullet} \rightarrow Cu(I) + O_2 \quad (5)$$

$$Cu(I) + O_2^{-\bullet} + 2H^+ \rightarrow Cu(II) + H_2O_2 \quad (6)$$

$$\text{Net}: \quad 2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (7)$$

Superoxide, generated in biological systems by the one-electron reduction of O_2, is capable of tissue damage by virtue of its ability to act as a weak base (undergoing protonation ($pK_a = 4.8$)) to $HO_2^{\bullet}$ and as a one-electron reductant of oxidized metal enzymes, e.g. ferricytochrome *c* [317]. The damaging effects of H_2O_2 in cells are minimized by its conversion to water by such enzymes as catalase (CAT) and glutathione-peroxidase (GSSH) [322]. CAT dismutates two H_2O_2 molecules to O_2 and two H_2O molecules [319]:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (8)$$

and GSSH reduces H_2O_2 to two H_2O molecules and disulfide-bridged oxidized glutathione (GSSG):

$$2GSSH + H_2O_2 \rightarrow 2H_2O + GSSG \quad (9)$$

H_2O_2 is not a radical, but it can cross cell membranes (unlike its precursor $O_2^{-\bullet}$) and is postulated to produce more potent oxidants, such as $OH^{\bullet}$, via the metal-catalyzed Haber–Weiss (or Fenton) reaction or, alternatively, highly oxidizing Cu(III) or Fe(IV) species [323,324]. The transition metals implicated in this catalysis are Cu(II)/Cu(I) and Fe(III)/Fe(II) [317,318,320] e.g.:

$$O_2^{-\bullet} + Fe^{3+}\text{chelate} \rightarrow O_2 + Fe^{2+}\text{chelate} \quad (10)$$

$$Fe^{2+}\text{chelate} + H_2O_2 \rightarrow Fe^{3+}\text{chelate} + OH^- + OH^{\bullet} \quad (11)$$

$$\text{Net}: \quad O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet} \quad (12)$$

where the transition metal is reduced and subsequently re-oxidized by H_2O_2, yielding deleterious entities such as $OH^{\bullet}$ or $FeH_2O_2^{2+}$, Fe(IV) and FeO^{2+}, or the equivalent Cu compounds ($CuH_2O_2^{+}$, Cu(III) or CuO^{2+}) [323,324]. NO dramatically inhibits this $O_2^{-\bullet}$-driven Fenton reaction in vitro [325,326]. While such equations are often used to imply the presence of $OH^{\bullet}$ radicals in such cycles, it is not clear whether $OH^{\bullet}$ exists as an oxidizing intermediate or whether high metal oxidation states are involved since these metal ions produce the same products with spin traps as $OH^{\bullet}$ [327,328]. It has been suggested that NSAIDs and other selected ligands inactivate the $OH^{\bullet}$ radical and contribute to an anti-inflammatory action [329–331], but they could equally react with high oxidation states of metal ions.

It is contradictory that Cu(II) and Cu(II)-complexes exert anti-inflammatory activity yet, at the same time, are capable of contributing to the production of pro-inflammatory species $OH^{\bullet}$ or Cu(III) via the Fenton reaction [320,329]. Furthermore, treatment of collagen with Cu(II)–H_2O_2 gives rise to degradation and fragmentation of collagen [332,333]. However, under physiological conditions, there may be insufficient free Cu(II) to catalyze the pro-oxidant pathways, due to its avid binding to such biological proteins as CP, albumin and l.m.w. proteins [334]. It is postulated that the nature of the ligand bound to the active Cu ion may also determine the nature of the biological effect observed, with the ligand either enhancing or affording protection against Cu-induced oxygen free radical damage [329]. Despite apparently conflicting roles for Cu in inflammation, the toxicity and inflammatory response to oxidants in the cell wall is due to damage of the lipid membrane of the cell by the production of lipid superoxide radicals (lipid-O_2) and peroxides (lipid-O_2H) [317,334–336]. Lipid peroxides may also augment the inflammatory process by promoting chemotaxis of immune cells [337].

Copper(II)-complexes, including Cu-NSAIDs, exhibit significant anti-inflammatory activities as well as SOD-mimetic activity [134,338,339]. Some researchers propose that NSAIDs, in addition to their inhibitory effects on the synthesis of PGs [3,170,184], may also inhibit the production, or act as scavengers of free radicals in vivo [147]. Whatever the mechanism of anti-inflammatory action of Cu(II)-complexes in vivo, their beneficial function has long been recognized and their clinical effect has been investigated extensively over the last 50 years [15,148,253,340–342]. Whilst the effect of Cu(II) on PG synthesis in tissues has been reported [241,264–267], there are conflicting reports on the effects of Cu(II)-complexes, e.g. of amino acids and NSAIDs, on PG production [129,343,344]. A review of the proposed modes of action of Cu-NSAIDs will be presented later in this paper.

4. Copper complexes of NSAIDs

4.1. Copper NSAIDs as anti-inflammatory agents

Some of the earliest trials of the efficacy of Cu-complexes for the treatment of arthritis included the use of intravenous Cupralene Na[Cu^{I}(3-(allythiouredo-1-benzoate)] [345] (19% Cu content) [10] and intravenous Dicuprene (a mixture of diethylamine:bis(dihydrogen 8-hydroxy-5,7-quinolinedisulfonato)copper(II) (4:1) containing 6.5% Cu content) [10,346,347] in the 1940s. This research followed on from the hypothesis that arthritis was bacterial in nature and may respond to treatment with heavy metals, e.g. Cu and Au [10,348]. Promising evidence of the benefit of Permalon, (an intravenous solution of 12.5 mM sodium salicylate and 3.9 mM $CuCl_2$) [10] was reported in 1952 [340] and 1977 [17]. There was a decline in experimental work on Cu anti-inflammatory agents from the 1950s, which is attributed to the appearance of the corticosteroids and later the NSAIDs [10,348].

Interest in the possible beneficial effects of Cu-complexes was renewed by Sorenson's report in 1976 that the active forms of anti-inflammatory drugs may well be the Cu(II) complexes of such drugs in vivo [20]. Sorenson reported that Cu-complexes of these anti-inflammatory drugs were more active in animal models than either their parent inorganic Cu(II) salt or the parent NSAID [20]. The pharmacological activity was proposed to be due to the inherent physico-chemical properties of the complex itself rather than just that of its constituents, since the amount of Cu in such complexes does not correlate with anti-inflammatory activity [14,338]. Sorenson reported that a salicylate complex of Cu(II) was ~30 times more effective than aspirin as an anti-inflammatory agent [218]. In addition, Cu(II) complexes of many non-anti-inflammatory agents exhibited anti-inflammatory action [10]. Sorenson reported extensively on the anti-epileptic, anti-cancer, anti-diabetic, radiation injury protective, anti-bacterial [10,14], and, most significantly for NSAIDs, the gastric sparing activities of Cu(II) complexes [10,41,218]. The enhanced potency of Cu-NSAID complexes, compared with their individual components, was corroborated in 1989, when a Cu-salicylate complex was reported to be significantly more active than $Cu(NO_3)_2$ mixed with Na-salicylate [19].

The findings of Sorenson on the potential of metal chelates in medicine follow others from the 1950s and 1960s [349–353]. It was suggested as early as 1966 that salicylates may deliver Cu to target cells in the body [349]. The commitment of early researchers to the beneficial use of Cu–salicylate for the treatment of arthritis [347] is evidenced by the comments of Hangarter on his comparison of Permalon therapy with NSAIDs, corticosteroids and gold [17]: 'In general, Permalon therapy was superior to all of these'.

In two previous reviews, Sorenson reported over 140 Cu(II) complexes with anti-inflammatory activity [10,15]. These included $[Cu_2(CH_3COO)_4(OH_2)_2]$ and Cu(II) complexes of amino acids, aromatic carboxylic acids, salicylates, corticoids, tetrazoles, histamines, and penicillamines, and were described as effective 'anti-ulcer agents' in animals [10]. A recent overview (1995) included Cu complexes of the newer enolic acid NSAIDs (piroxicam and tenoxicam), amino acid and the anti-ulcer histamine antagonists ranitidine (*N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine) and cimetidine (*N*-cyano-*N*′-methyl-*N*″-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]-guanidine) [15]. However, limited information is available on the nature of the Cu complexes of NSAIDs in biological matrices and in pharmaceutical formulations. SOD activity, redox behavior, lipophilicity and stability constants may be useful parameters in evaluating the biological activity of these Cu compounds [16].

4.2. Structures of Cu-NSAIDs

The structure of the Cu-NSAID is likely to be an important factor for its biological activity. For example, the anti-tumor activity of the monomeric Cu(II) complex of aspirin ($[Cu(Asp)_2(Py)_2]$) is reportedly more effective than the dimeric $[Cu_2(Asp)_4]$ complex [354]. The magnetic and electronic properties of Cu allow, therefore, the application of a range of analytical techniques to assist in the characterization of the coordination around the Cu(II) center of Cu-NSAIDs. UV–Vis and IR spectroscopy are used to assign the d–d transition of the Cu(II) ion (~700 nm) and the symmetric (ν_{sym}) and antisymmetric (ν_{asym}) stretches of the carboxylato ligand, respectively [285,355–359]. X-band [130,285,355–360] and zero-field (ZF) [285] EPR spectroscopy, electrochemistry [355,360], magnetic susceptibility [285,355,356,361] and the analysis of NMR isotropic shifts of the coordinated ligand in solution [140] are also used. The latter is used to assist in the investigation of the contribution of solvent adducts to the super-exchange mechanism of the dimeric Cu-NSAIDs. Recently, X-ray absorption fine structure (XAFS) analysis and X-band EPR spectroscopy have been used to both determine the coordination geometry around the Cu(II) ion of $[Cu_2(Indo)_4L_2]$ (L = DMF, DMA, NMP, OH_2) and to quantify the Cu(II) monomer/dimer content of veterinary formulations, respectively [362]. X-ray absorption spectroscopy (XAS) may become an important additional technique for investigating the nature of Cu-NSAIDs in biological and pharmaceutical samples.

In the solid-state, Cu-NSAIDs (arylalkanoic NSAIDs) are typically monomeric or dimeric (Table 3), with bonding to the Cu(II) ion via the carboxylato group. The structure of the Cu(II) complexes of the arylalkanoate NSAIDs is dependent upon the electronic properties of the carboxylate groups and the coordinating and steric properties of the added base [363]. A review of the synthesis and crystal structures of a number of anti-inflammatory compounds in metal complexes has recently been reported [364].

4.2.1. Dimeric Cu-NSAIDs

The coordination of the Cu center in the Cu(II) dimers of the NSAIDs of the general formula ($[Cu_2(RCOO)_4L_2]$ (R = aryl/phenyl alkanoic acid), containing either CuO_4O' or CuO_4N' coordination environments (L = O′ or N′ axial adduct), is characteristically octahedral and is typified by the Cu(II) acetate structure ($[Cu_2(CH_3COO)_4(OH_2)_2]$) [365]. The carboxylato ions act as bridging ligands (exhibiting a center of symmetry midway between the Cu atoms) and the solvent used in the synthesis binds in the position *trans* to the Cu···Cu axis. To date, there are ~147 CuO_4N' and ~188

Table 3
Some copper-NSAID complexes

Compound	Structure
$[Cu_2(Asp)_4]$ [a]	Dimer [358,374]
$[Cu(Asp)_2L_2]$ [a] L = benzimidazole, 2-methylbenzimidazole, metronidazole [b] 2-methylimidazole, 1,2-dimethylimidazole, pyridine, 3-picoline, 4-picoline, imidazole, 1-methylimidazole, diethylamine, nicotinamide, *N*,*N*-dimethylsulfoxide	Monomer [130,358,373,374,377]
$[Cu_2(Sup)_4(CH_3CN)_2]$ [c]	Dimer [355]
$[Cu_2(Sup)_4(OH_2)_2]$	Dimer [361]
$[Cu(Tol)_2(pyridine)_2]$ [d]	Monomer [356,360]
$[Cu_2(Tol)_4(DMSO)_2]$	Dimer [356,360]
$[Cu(Nap)_2(pyridine)_2]$ [e]	Monomer [356,360]
$[Cu_2(Nap)_4(DMSO)_2]$	Dimer [356,360]
$[Cu(Ibu)_2(pyridine)_2]$ [f]	Monomer[356,360]
$[Cu_2(Ibu)_4(DMSO)_4]$ [l]	Dimer [356,360]
$[Cu(Ibu)_2(imidazole)_2]$	Monomer [357]
$[Cu(Ibu)_2(2\text{-methylimidazole})_2]$	Monomer [357]
$[Cu_2(Ibu)_4(caffeine)_2]$	Dimer [357]
$[Cu_2(Ibu)_4(metronidazole)_2]$	Dimer [357]
$[Cu_2(Flufen)_4L_2]$ [g] L = caffeine, papaverine	Dimer [359]
$[Cu(Flufen)_2L_2]$ L = nicotine, nicotinamide, *N*,*N*-diethylnicotinamide	Monomer [359]
$[Cu(Nif)_2L]$ L_2] [h] L = 3-pyridylmethanol, water, *N*,*N*-dimethylformamide	Monomer [132,379]
$[Cu_2(Nif)_4(DMSO)_2]$ [l]	Dimer [132]
$[Cu_2(Indo)_4L_2]$ [i] L = water, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, *N*-methyl-2-pyrrolidone, dimethylsulfoxide	Dimer [285,339,369]
$[Cu(Pirx)_2(DMF)]$ [j,m]	Monomer [380]
$[Cu_2(DicH)_4L_2]$ [k] L = water, *N*,*N*-dimethylformamide, ethanol, dimethylsulfoxide, methanol	Dimer [418,419]

[a] Aspirin = 2-acetylsalacylic acid (AsaH).
[b] Metronidazole = 2-methyl-5-nitrobenzimidazole.
[c] Suprofen = (+)-α-methyl-4-(2-thienyl-carbonyl)phenylacetic acid (SupH).
[d] Tolmentin = 1-methyl-5-(*p*-toluoyl)-1*H*-pyrrole-2-acetic acid (TolH).
[e] Naproxen = 6-methoxy-α-methyl-2-naphthaleneacetic acid (NapH).
[f] Ibuprofen = (+)-α-methyl-4-(isopropylmethyl)benzeneacetic acid (IbuH).
[g] Flufenamic acid = *N*-((trifluoromethylphenyl)anthranilic acid) (FlufenH).
[h] Niflumic acid = 2-((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid (NifH).
[i] Indomethacin = 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid (IndoH).
[j] Piroxicam (Pirx) = 4-hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide.
[k] Diclofenac = 2-[(2,6-dichlorophenyl)amino]phenylacetic acid (DicH).
[l] DMSO = dimethylsulfoxide.
[m] DMF = *N*,*N*-dimethylformamide.

CuO_4O' acetate-type structures deposited in the Cambridge Crystallographic Database [366].

This 'paddle-wheel' dimeric Cu(II) structure typically has a Cu···Cu distance of ~2.64 Å; with an octahedral stereochemistry tetragonally elongated along the Cu–Cu–O_L axis due to the Jahn–Teller effect [367,368]. The structure of the Cu(II) dimer of Indo ($[Cu_2(Indo)_4(DMF)_2]$) [285,369] is shown in Fig. 7. The rigid geometry of the acetate-type Cu(II) dimers is due to the restricted bite of the bidentate carboxylate bridge and allows little variation (~0.3 Å) [255] in the Cu–Cu distance of these carboxylato-bridged dimers. The Cu–Cu distances in the dimeric Cu(II)-NSAIDs are only slightly longer than that in Cu metal (2.56 Å) [370]; consistent with a significant Cu–Cu bonding interaction [285,355,357]. Hydrogen-bonding between the bridging carboxylate oxygens of neighboring dimers may, however, distort the inverted center of symmetry, making the four carboxylate oxygens coordinated to the Cu(II) ions non-equivalent [132,285,355,356].

4.2.2. Monomeric Cu-NSAIDs

The electronic properties and stereochemistry of mononuclear Cu(II) complexes of carboxylato-complexes were reviewed as early as the 1970s [371], with a number of subsequent studies that describe the factors that influence monomer over dimer formation in Cu(II) carboxylate complexes [358,372–376]. Increasing the acid strength of the carboxylate groups, e.g. halogenation of the alkyl group, and increased basicity, e.g. pyridine and imidazole, of the other coordinating ligands, favors the formation of the Cu(II) monomer over the Cu(II) dimer [358,372–378], with steric effects reportedly playing a less important role [373,374]. Cu(II) carboxylate monomers can exist as *cis* or *trans* adducts. The crystal structures of the monomeric Cu(II) arylalk-

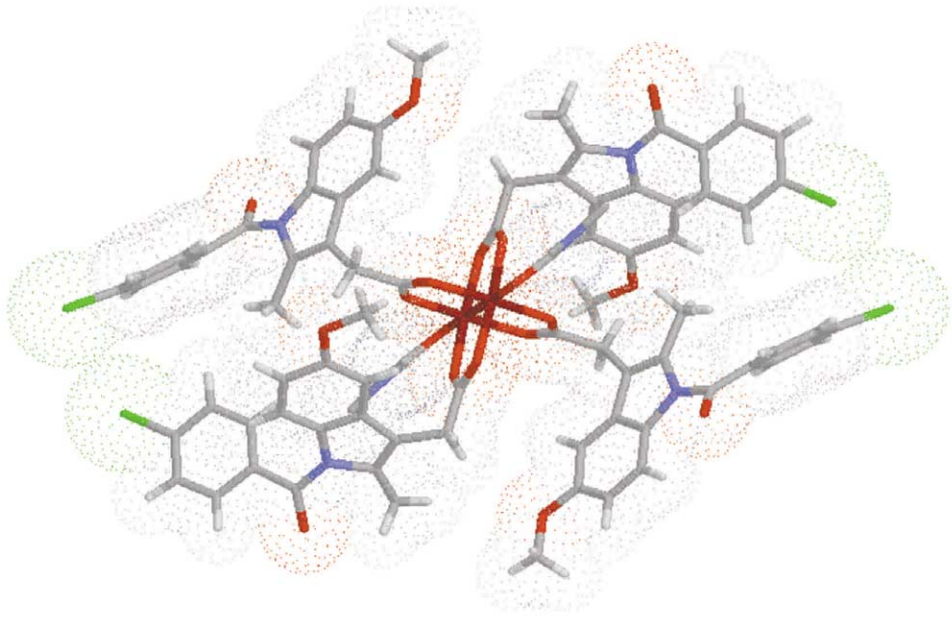


Fig. 7. The crystal structure of $[Cu_2(Indo)_4(DMF)_2]$ [285]. Generated by the molecular visualization program RASMOL [199].

anoic-type NSAIDs typically display a *trans* [359,360,379] unidentate bis (carboxylato) binding. Likewise, a structure with a *trans* bidentate bis(ligand) binding [380] has been reported for the enolic-NSAID piroxicam [380]. The coordination number around the Cu(II) center in the unidentate bis(carboxylato) Cu-NSAID ranges from four to six (Fig. 8).

Both monomer and dimer Cu-NSAID complexes may occur for a given NSAID, depending upon the electronic properties of the added solvent. Both monomer [130,358,373,377] and dimer [358] complexes of aspirin have been reported (Table 3) and this may explain their different anti-tumor effects [354]. The nature [358,374] and structure [381] of dimeric Cu–Asp are typical of Cu(II) acetate monohydrate ($[Cu_2(CH_2COO)_4(OH_2)_2]$) [367] with, a somewhat longer $Cu–O_L = 2.24(2)$ Å coordination to a neighboring acetyl residue, compared with $[Cu_2(CH_2COO)_4(OH_2)_2]$ ($Cu–O_L = 2.156(4)$ Å) [365]. The crystal structure of the Cu(II) monomer of aspirin ($[Cu(Asp)_2(Py)_2]$) displays a CuN_2O_2 core in a *trans* square-planar arrangement with the N atoms of the two pyridine molecules ($Cu–N = 2.003(4)$ Å) and a carboxylate oxygen from each of the two Asp moieties ($Cu–O = 1.949(3)$ Å) (Fig. 8) [373]. Recently, a number of Cu(II) monomer and dimer [285,382,383] complexes of Indo have been prepared, including Cu(II) dimer complexes of the metabolites of IndoH, i.e. desmethyl- and desbenzoyl-IndoH [384]. An investigation of the structure–activity relationship of these Cu(II) monomer and dimer complexes of Indo is yet to be undertaken.

4.3. Cu-NSAIDs and proposed modes of action

In a 1995 review, Sorenson discussed modes of anti-inflammatory action of the Cu complexes, including gastric sparing activities due to down-regulation (inhibition) of SOD activity [15]. Other proposed modes of action of Cu-NSAIDs include down-regulation (and stabilization) of polymorphonuclear leukocytes (PMNL), which are part of the immune system and exert phagocyte activity, (including inhibition of $O_2^{-\bullet}$ synthesized by PMNLs), down-regulation of phospholipase A_2 (which activates/releases membrane-bound arachidonic acid prior to its conversion by COXs and lipoxygenases to, e.g. PGs and leukotrienes, respectively), inhibition of lipid peroxidation and microsomal NADPH oxidation [10,15], and modulation of nitric oxide synthetase (NOS) activity [385].

The amelioration of inflammation by Cu complexes has been attributed to the role of free radicals in disease [320]. Scavenging of free radicals [219,320] and the activation of lysyl oxidases (collagen cross-linking enzymes) [10,15] are proposed modes of action. Inhibition of the release of tissue necrosis factor-alpha (TNF-α), IL-1 and IL-2 from macrophages by Cu-carboxylates (down-regulating the synthesis of pro-inflammatory PGs and leukotrienes) is also reported [179].

Whatever the actual mechanisms of action of Cu-NSAIDs, they do exhibit a marked SOD-mimetic activity and this is commonly proposed to account for their anti-inflammatory activities [15,134,339,386,387].

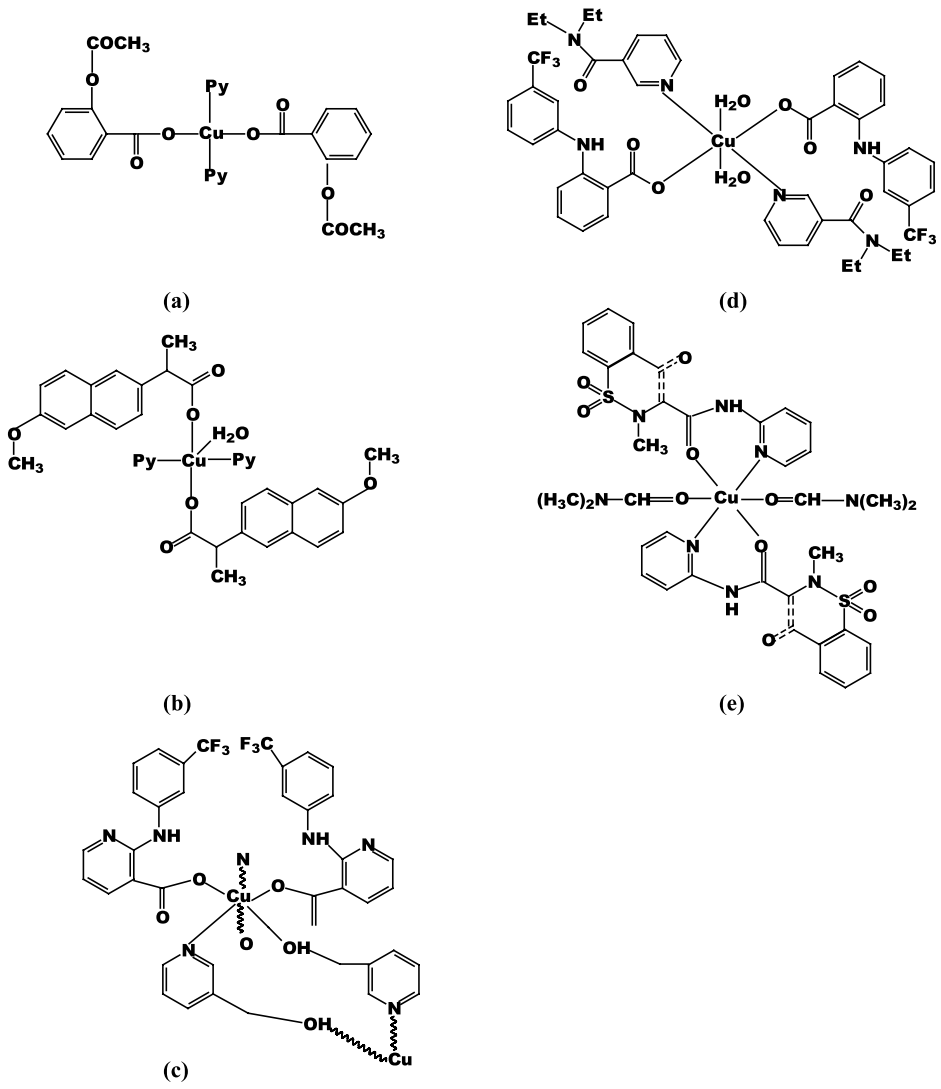


Fig. 8. The unidentate Cu(II) monomer structures of (a) aspirin ($[Cu(Asp)_2(Py)_2]$ Py = pyridine),[373] (b) naproxen (Nap),[360] (c) niflumic acid (Nif),[379] (d) flufenamate (Fen)[359] and (e) piroxicam (Pirx) [380].

The SOD activity of specific Cu-NSAIDs will be dealt with separately in this review.

NO may also be important in the mode of action of Cu-NSAIDs. NO has a variety of pharmacological and pathophysiological actions in the body [388–391]. However, studies of NO release are hampered by the short half-life of the molecule [392], with NO disappearing in blood within seconds because of avid binding with hemoglobin [392]. Reported values of the in vivo half-life (in humans) of free NO range from 'very short', 'only a few seconds' [393–396] to 5 s [397], with an estimated half-life of 1.8 ms in whole blood [398]. The half-life of NO in vivo is also difficult to specify due to its dependence upon both location and tissue vascularity [398], and target, i.e. direct or indirect effects [318]. In addition, the half-life of free NO depends upon its concentration, because of second-order reactions [391]. Direct effects, e.g. NO interactions with metal-containing proteins or organic free radicals, reputedly occur at lower concentrations or fluxes of NO, whereas higher NO fluxes result in indirect effects, e.g. effects mediated by reactive nitrogen oxide species derived from the interactions of NO with O_2 or $O_2^{-\bullet}$. The rapid and spontaneous interaction between $O_2^{-\bullet}$ and NO forms the potent oxidants peroxynitrous acid (ONOOH) and peroxynitrite ($ONOO^-$) (Equation 13) [325,396].

$$NO + O_2^{-\bullet} \rightarrow ONOO^- \quad (13)$$

This is suggested to represent an important pathway by which tissue may be injured during inflammation [326,391,399]. Peroxynitrite is an inorganic toxin of biological importance [400], implicated in the pathology of several neuro-degenerative disorders [401] and programmed cell death [402].

SOD competes with NO for $O_2^{-\bullet}$ scavenging, and hence promotes the vasodilatory action of NO [396]. NO is now recognized as a critical mediator of GI

mucosal defenses [403,404], and may even account for the GI sparing effect of Cu-NSAIDs. In addition, small amounts of NO stimulate the production of PGs [405], with $O_2^{-\bullet}$ reputedly inhibiting COX, and possibly other enzymes involved in PG synthesis, by reducing the heme iron in COX to its inactive Fe(II) state [318]. It is postulated that the presence of small amounts of NO may prevent the reduction of heme by scavenging $O_2^{-\bullet}$, so maintaining the COX-associated heme iron in the Fe(III) state and resulting in the formation of GI protective PGs [318]. NO-releasing derivatives of NSAIDs, e.g. NO-releasing naproxen (NapH = 6-methoxy-α-methyl-2-naphthaleneacetic acid), are reported to produce significantly less GI toxicity than the parent NSAID [235].

Oxidation of NOS [385], as well as disproportionation of $O_2^{-\bullet}$ by the anti-inflammatory Cu(II) dimer of diisopropylsalicylate (DIPS) ($[Cu_2(DIPS)_4]$) is reported [10]. This observation by Sorenson adds weight to the proposal that the anti-inflammatory, anti-convulsant, anti-diabetic and other pharmacological activities of Cu-NSAIDs may be due to NO-mediated events [385]. However, whilst NO is recognized as a mediator of mucosal blood flow, mucus release, repair of mucosal injury, and an inhibitor of neutrophil activation [219,404], no significant difference is reported between the NOS activities stimulated by IndoH (~ 0.06 mM kg^{-1}) or $[Cu_2(Indo)_4(DMF)_2]$ (~ 0.01 mM kg^{-1}) [219].

4.4. Stability of Cu-NSAIDs in biological media

Doubts over the stability of Cu-NSAIDs in biological media arose from early computer simulations and in vitro studies for salicylic acid that predicted that Cu-NSAID could not exist in plasma [406]. Likewise, simulations in 1997 on Cu(II)–anthranilic acid (anthranilic acid is not anti-inflammatory, whilst its Cu(II)-complex is) showed an insignificant fraction of Cu(II)–anthranilic acid in plasma [330]. A synergistic absorption of Cu(II) and anthranilic acid from the alkaline lower intestine (the major site of drug absorption) was proposed, with re-formation of the Cu(II)-anthranilate complex in the acidic conditions of inflamed synovial fluid [330]. However, there was no therapeutic difference between the administration of the pre-formed complex dissolved in solution, and a mixture of its individual components at equivalent concentrations. The pre-formed complex administered as suspensions was postulated to exhibit a greatly enhanced bioavailability compared with the mixture of the individual reagents [330]. The observations on the potency and efficacy of Cu-complexes, along with the therapeutic use of the Cu(II) dimer of IndoH ($[Cu_2(Indo)_4(DMF)_2]$) in dogs [42] (where free IndoH can be fatal) [407–409], indicate some intact Cu-NSAID complex may survive in the stomach and perhaps be absorbed into the plasma. Consequently, the in vitro studies and computer simulations may not mirror the fate of Cu-NSAIDs in vivo.

Absorption of Cu(II) complexes of Indo from the small intestine of rats has been reported [410]. Notwithstanding poor aqueous solubility of Cu-Indo compared with IndoH, comparable blood levels and rapid penetration of Cu-Indo across the intestinal cell membrane were reported [410]. Unfortunately, the analytical method used to measure Cu-Indo in the blood was not presented in detail [410]. This suggests that the Cu-complex may have a distinctly different absorption profile compared with IndoH with dissolution of the complex not being a major controlling factor for absorption, but rather a more important factor being its excellent partitioning capability across the intestinal cell membrane [410].

Whilst the percentage of a Cu-Indo dose absorbed across the gut lumen is currently uncertain, even less is known of its systemic disposition. A ternary human serum albumin (HSA)-Cu(II) monomer or Cu(II) dimer complex of DIPS is proposed as the vehicle for distribution of this anti-inflammatory drug throughout the body [386,411]. A ternary complex is recognized as the mode of Cu binding to albumin [290,296]. This hypothesis was based upon changes in the observed UV–Vis absorbance of HSA before and after the removal of $[Cu_2(DIPS)_4]$ from a HSA sample by dialysis [386]. Such direct interactions were not confirmed in subsequent studies by EPR, NMR and UV–Vis spectroscopies, where 95% of both Cu and DIPS are reported to be bound separately at different sites of the albumin [411]. This dissociation of the Cu-complex on binding to HSA, whilst retaining its potent therapeutic action, led to the proposal that the action of the complex may be due to only small ($< 5\%$) amounts of either the mononuclear or dinuclear complex being bound intact to HSA [411]. It was concluded that either HSA delivers Cu(II) and DIPS to the same cellular site, or that the administration of the Cu(II) complex of DIPS is important only in facilitating intestinal absorption. Moreover, it was proposed that chelates of Cu(II) formed with plasma/cellular proteins, e.g. L-histidine, following ligand-exchange reactions, and that it was these plasma/cellular Cu(II) chelates that accounted for the drug's pharmacological action, rather than $[Cu_2(DIPS)_4]$ [411]. Whereas little is known of the binding of Cu-NSAIDs to HSA, the major NSAID binding locations of HSA have been reported [412,413]. The crystal structure of HSA complexed with either aspirin or ibuprofen identifies hydrophobic cavities in HSA as the principal regions for NSAID binding [413].

A radio-labeled biodistribution study in inflamed rats following the administration of the ^{64}Cu(II) complex of aspirin reported sequestering of ^{64}Cu to the site of inflammation [283]. The double-labeled ^{67}Cu(II) dimer of DIPS (carboxy-^{14}C) is reported by Sorenson to

rapidly distribute into the blood, liver, intestine, femur, spleen, thymus, kidney, lung and brain within 0.5 h of administration with persistence of the ^{67}Cu and DIPS (carboxy-^{14}C) in the tissues over the 5-day study period [284]. Sorenson proposed that the Cu(II) dimer complex of DIPS is a bioavailable form of Cu and that ligand-exchange reactions occur in the body [284].

Retention of the dinuclear $[Cu_2(DIPS)_4]$ in non-polar solvents is postulated, but predominantly mononuclear $[Cu(DIPS)_2]$ is formed in polar solvents [411]. After 3 days, a DMF solution of $[Cu_2(Indo)_4(DMF)_2]$ (1 mg ml^{-1}) contains $<2\%$ Cu(II) monomer contaminant and exhibits a distinctive Cu(II) dimer EPR signal [414]. Some new veterinary formulations of $[Cu_2(Indo)_4(DMF)_2]$ retain the dimeric structure of the Cu(II) complex (with $<10\%$ Cu(II) monomer by-products) over a 2–3 year period [414,415], compared with $\sim 80\%$ Cu(II) monomer byproducts in carboxypolymethylene pastes [414,415].

4.5. Commercial applications of Cu-NSAIDs

Topical applications of the Cu(II) alcohol adduct of salicylic acid, Alcusal® [315], and the dimethylsulfoxide (DMSO) adduct, Dermusal® [416], were developed and used in Australia in the late 1970s and early 1980s as human pharmaceuticals. Apart from the initial reports of their anti-inflammatory activities, further characterization of these products has not been reported, nor have oral preparations been marketed. Parenterally administered Cu(II) salicylate has been reported [347], i.e. Permalon®, but is not available commercially. Alcusal® is still available commercially. The nature of Cu(II) salicylate in aqueous solutions reportedly depends upon the pH value of the solution with both neutral complexes, such as the pale blue crystals of bis(salicylato)copper(II) tetrahydrate and anionic complexes, such as the olive-green sodium salicylatocuprate(II) having been reported [40]. Furthermore, it is proposed that the presence of water may result in the formation of Cu salicylate polymers [40]. More detailed analysis of aqueous samples of Cu-NSAIDs is required.

The quest for more effective, yet less toxic NSAIDs, has led to several studies in the last 10 years on the preparation, characterization, and veterinary and medical use of divalent metal salts of IndoH, one of the most potent of the NSAIDs [22], and a number of other NSAIDs [39,130,334,339,356,357,360,417]. Table 3 lists some of the monomeric and dimeric Cu-complexes of arylalkanoic and enolic acids NSAIDs reported in the literature.

Whilst a number of researchers have reported the preparation and characterization of Cu(II)-complexes of Indo [339,369,417,420], there were no clinical applications of these complexes to animals or humans prior to patents issued in 1994 [39] and 1995 [18,38] for bis(*N*,*N*′-dimethylformamide)tetrakis-μ-(*O*,*O*′-Indo)dicopper(II) ($[Cu_2(Indo)_4(DMF)_2]$). The full advantage of the gastric sparing activity of $[Cu_2(Indo)_4(DMF)_2]$ is illustrated by its safe veterinary use [42]. In as much as IndoH cannot be used in dogs because of fatal GI hemorrhaging [407–409], $[Cu_2(Indo)_4(DMF)_2]$ (Cu-Algesic®) can be used without side-effects [42]. Approximately 12 million doses of Cu-Algesic® have been safely administered to dogs in Australia, South Africa and South East Asia [43], although some gastro duodenal irritation has been reported in dogs at doses higher than therapeutic levels [421].

5. The pharmacology of some Cu-NSAIDs

Whatever the proposed mode of action of the Cu-NSAIDs, they display a superior anti-inflammatory and anti-ulcerogenic effect compared with the parent drug [316,338]. A comparison of the SOD, anti-inflammatory and ulcerogenic effects of some Cu-NSAIDs will now be reviewed.

5.1. SOD mimetics

The use of SOD as a pharmaceutical has been proposed for treatment of a number of diseases including, hyperoxia, reperfusion injury, auto-immune deficiency disease (AIDS), ulcerative colitis, bronchopulmonary dysplasia in premature neonates, as well as inflammation and inflammation-associated diseases, such as RA and OA [123].

McCord implicated $O_2^{-\bullet}$ in the promotion of arthritis due to its ability to degrade hyaluronic acid (HA) [422]. HA is an important component of synovial fluid that maintains internal joint homeostasis by acting as a lubricant. It assists with the nutrition of articular cartilage and provides direct anti-inflammatory action [319,423]. When $O_2^{-\bullet}$ is added to synovial fluid, the viscosity decreases, indicating depolymerisation of HA [422]. Introduction of SOD provides complete protection against this degradation [422] by removing potentially damaging $O_2^{-\bullet}$ from cells (Equations 5–7) [424].

An increase in SOD activity has been found in the synovial fluid of RA and OA patients compared with controls, with increased SOD activity occurring with the progression of the disease [10]. While it has been suggested that injection of SOD into arthritis patients may have anti-inflammatory effects, there have been conflicting results [422].

Unsurprisingly, as the catalytic role of SOD is based on the cyclic Cu(II)–Cu(I) redox reactions (Equations 5–7), it has been shown that many Cu-containing complexes also exhibit SOD activity (Table 4). For instance, Cu(II) amino acid complexes, $[Cu(tyr)_2]$ and $[Cu(lys)_2]$, produce a 50% inhibition of the reduction of

Table 4
SOD activity of Cu complexes [428,429]

Copper complex	IC50 (μM)
$[Cu(tyr)_2]$ [a]	45 [425]
$[Cu(lys)_2]$ [b]	86 [425]
$CuSO_4$	30 [427] 2 [426]
$[Cu_2(valp)_4]$ [c]	10.4 [430]
$[Cu(valp)_2(2,2'\text{-bpy})] \cdot H_2O$ [d]	4.2 [430]
$[Cu(valp)_2(phen)]$ [e]	4.5 [430]
$[Cu(valp)_2(dmph)]$ [f]	6.3 [430]
$[Cu(valp)_2(\mu\text{-}4,4'\text{-bpy})]$	18.3 [430]
$[Cu_2(valp)_4(\mu\text{-}4,4'\text{-bpy})]$	5.0 [430]
$[Cu(glygly)(2,2'\text{-bpy})] \cdot 3H_2O$ [g]	25 [430]
$[Cu(glygly)(phen)] \cdot 3H_2O$	32 [430]
$[Cu(cimetidine)_2(ClO_4)_2]$ [h]	4.0 [430]
$[Cu_2(bpzbiap)Cl_3]$ [i]	0.255 [431]
$[Cu(stz)(py)_3Cl]$ [j,k]	1.31 [432]
$[Cu(hstz)_2(MeOH)Cl_2]$ [l]	2.510 [432]
$[Cu(hstz)_2(EtOH)Cl_2]$	5.170 [432]
$[Cu(TAAB)]^{2+}$ [m]	0.144 [427]
$[Cu(im)Zn(mbc)]^{3+}$ [n,o]	0.5 [433]
$[Cu(im)Cu(mbc)]^{3+}$	0.5 [433]
Derivatives of Cu(sal) [p]	0.97–1.17 [427]
Derivatives of Cu(1,10-phen)	1.7–28.8 [427]
SOD	0.04 [339]

[a] tyr = tyrosine.
[b] lys = lysine.
[c] valpH = valproic acid.
[d] bpy = 2,2′-bipyridine.
[e] phen = 1,10-phenanthroline.
[f] dmph = 2,9-dimethyl-1,10-phenanthroline.
[g] glygly = glycylglycine.
[h] cimetidine = *N*-cyano-*N*′-methyl-*N*″-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine.
[i] Hbpzbiap = 1,5-bis(1-pyrazoyl)3-[bis(2-imidazoyl)methyl]azapentane.
[j] stz = deprotonated form of sulfathiazole = 4-amino-*N*-2-thiazoyl-bencenosulfonamide.
[k] py = pyridine.
[l] Hstz = protonated form of sulfathiazole.
[m] TAAB = tetraanhydroaminobenzaldehyde.
[n] im = imidazole.
[o] mbc = macrobicyclic ligand = 1,4,12,15,18,26,31,39-octaazapentacyclo[13.13.13.1.1.1]tetratetracontane-6,8,10,22,24,33,35,37-nonaene.
[p] sal = salicylic acid.

nitroblue tetrazolium (NBT) by the oxygen radical at concentrations of 45 and 86 μM, respectively [425]. Similarly, $CuSO_4$ exhibits SOD activity with IC_{50} values reported between 2 and 30 μM [426,427]. These values compare to an IC_{50} of 0.04 μM for SOD [425].

A number of Cu-containing SOD mimics have been developed to try to improve the efficiency of the natural enzyme. For example, the compounds can be modified to: (i) possess the required chemical stability in vivo; (ii) provide access to intracellular space; and (iii) facilitate oral administration. Furthermore, some drugs are more advantageous than SOD itself, because they do not pose potential immunogenetic complications and, depending on the drug, may be financially competitive compared to SOD [123].

The SOD activity of the potential mimics can be tested by a variety of methods using direct assays, e.g. pulse radiolysis detection of $O_2^{-\bullet}$, EPR spectroscopy, stopped-flow spectrometry, flash photolysis and polarography; or indirect assays, e.g. electronic absorption spectroscopic monitoring of the reduction of NBT to blue formazan, inhibition of cytochrome *c* reduction and EPR analysis of spin traps [339,434,435]. Up to 20 assays for determining SOD activity have been listed and referenced in a review by Gartner and Weser [434].

The indirect determination of SOD activity by monitoring the reduction of NBT by $O_2^{-\bullet}$ involves the generation of $O_2^{-\bullet}$ in vitro. This can be afforded by a variety of methods, including enzymatic production using xanthine–xanthine oxidase, or simply using the inorganic source, KO_2 [339]. A gelatin solution is added to ensure that the blue formazan does not precipitate and catalase is also added to remove H_2O_2 from the system [434,436]. As the reaction proceeds, there is a color change from yellow to blue/mauve, which is associated with an increase in the absorption spectrum at 540 nm [339]. When a chemical inhibitor is added, the reduction reaction proceeds slowly or is totally inhibited in which instance the solution remains yellow. The rate of absorption change is determined and the concentration required to produce 50% inhibition (IC_{50}) may be obtained by graphing the rate of NBT reduction versus the concentration of the test solution.

Inhibition of cytochrome *c* reduction employs xanthine–xanthine oxidase as the $O_2^{-\bullet}$ source and quantification is monitored by electronic absorption spectroscopy at 550 nm. The increase in absorbance per minute is determined and the rate obtained from the test solutions is compared with that of the control to yield the IC_{50} value [429].

5.2. SOD activities of Cu-NSAIDs

Table 5 shows the IC_{50} results of the SOD studies performed for a number of Cu-NSAID complexes using the xanthine–xanthine-oxidase-generated $O_2^{-\bullet}$ in the NBT reduction assay [339,429,437,438].

A number of papers have been published on the $O_2^{-\bullet}$-scavenging activity of Cu(II) complexes of aspirin and salicylate compounds [429,438,441]. An EPR spectroscopic study was performed for reactions of $O_2^{-\bullet}$ with Cu(II) salicylate with increasing $O_2^{-\bullet}$:Cu ratios [441]. A reduction in the Cu(II) signal (coincident with the production of Cu(I)) was observed as the $O_2^{-\bullet}$ concentration was increased [441]. Furthermore, there was a significant difference in the reactions of anhydrous $[Cu(Sal)_2]$ (SalH = salicylic acid) and $[Cu(Sal)_2(OH_2)_2]$ in dry DMSO. In the presence of a fourfold excess of $O_2^{-\bullet}$, the reaction of $O_2^{-\bullet}$ with $[Cu(Sal)_2]$ produced

Table 5
SOD activity of Cu-NSAID complexes

Complex	IC_{50} (μM)
Cuprein	0.04 [429]
Cuprein	0.06 [429]
Cuprein	0.5–18 (solvent dependence study) [339]
[Cu(salicylate)$_2$]	16 [429]
[Cu(salicylate)$_2$]	1.3 [429]
[Cu(acetylsalicylate)$_2$]	23 [429]
[Cu(acetylsalicylate)$_2$]	2.15 [429]
[Cu(acetylsalicylate)$_2$]	11–60 (solvent dependence study) [339]
[Cu(*p*-aminosalicylate)$_2$]	28 [429]
[Cu(*p*-aminosalicylate)$_2$]	3.0 [429]
[Cu(DIPS)$_2$] [a]	73 (extrapolated value) [429]
[Cu(DIPS)$_2$] [a]	2.85 [429]
[Cu(DIPS)$_2$] [a]	8.9 [437]
[Cu$_2$(lonazolac)$_4$] [b]	1.5 [436]
[Cu(ϕMeTIM)] [c] monomer	16 [437]
[Cu(TIM)] [d] monomer	210 [437]
[Cu$_2$(Indo)$_4$(DMF)$_2$] [e,f]	2–25 (solvent dependence study) [339]
[Cu$_2$(Indo)$_4$(DMF)$_2$]	0.23 [439]
Cu(II)–penicillamine [g,h]	32–140 (solvent dependence study) [339,440]
Cu(II)–penicillamine [h]	4.3 [438,440]

[a] DIPS = diisopropylsalicylate.
[b] lonazolac = 3-(*p*-chorophenyl)-1-phenylpyrazole-4-acetate.
[c] ϕMeTIM = 2,9-dimethyl-3,10-diphenyl-1,4,8,11-tetraacylotetradeca-1,3,8,10-tetraene.
[d] TIM = 2,3,9,10-tetramethyl-1,4,8,11-tetraazacylcotetradeca-1,3,8,10-tetraene.
[e] Indo = indomethacin.
[f] DMF = *N*,*N*′-dimethylformamide.
[g] penicillamine = 3-mercapto-D-valine.
[h] There is controversy regarding the structure of the tested complex. The complex is believed to be $[Cu^{II}_6Cu^{I}_8(\text{D-penicillamine})_{12}Cl]^{5-}$ but a subsequent article suggests that there is also a Cu(II)–penicillamine disulfide contamination [440].

only 15% divalent Cu(II) salicylate. The analogous reaction of [Cu(Sal)$_2$(OH$_2$)$_2$] resulted in an increase of Cu(II) to 65%, which was attributed to the reoxidation of Cu(I) to Cu(II). This result was attributed to the presence of the water ligand in [Cu(Sal)$_2$(OH$_2$)$_2$] as was necessary for the cycling of Cu(II) to Cu(I) to Cu(II) (Equations 1–3) [441]. In aprotic solution, $O_2^{-\bullet}$ behaved as a base for O_2^{-}:Cu ratios of 1:1 and 2:1 resulting in the deprotonation of the phenol group of the salicylate to produce two new Cu salicylate complexes [441]. No Cu-superoxide complexes were observed [441].

The SOD activity of the Cu(II) salicylate decreased in the order of: [Cu(salicylate)$_2$] > [Cu(acetylsalicylate)$_2$] > [Cu(*p*-aminosalicylate)$_2$] > [Cu(DIPS)$_2$] [429]. As shown in Table 5, all four complexes exhibited SOD activity with IC_{50} values ranging from ca. 1 to 28 μM [429]. In the reported study [429], a number of different assays were performed, including the NBT reduction assay using KO_2 as a source of $O_2^{-\bullet}$ and the inhibition of cytochrome *c* reduction using xanthine–xanthine oxidase as the source of $O_2^{-\bullet}$. All complexes exhibited SOD activity in these assays but, interestingly, the order of activity of the complexes was not preserved across the different assays [429].

The SOD activity of [Cu$_2$(Indo)$_4$(DMSO)$_2$] was first reported by Weser et al. using pulse radiolysis and the NBT reduction assay using KO_2 as the superoxide source [339]. Pulse radiolysis experiments were performed in acetonitrile (to reduce the generation of other radical species by side-reactions). Second-order rate constants of 6×10^9 and 1.1×10^9 M^{-1} s^{-1} were obtained in aqueous and aqueous/aprotic solutions, respectively, with the latter system being studied as a 'lipid-mimicking system' [339]. These values compared with 1.8×10^5 M^{-1} s^{-1} for spontaneous superoxide dismutation, 2.6×10^6 M^{-1} s^{-1} for IndoH and 2.6×10^9 M^{-1} s^{-1} for SOD, indicating that [Cu$_2$(Indo)$_4$(DMSO)$_2$] did, indeed, exhibit high SOD activity. The IC_{50} values of [Cu$_2$(Indo)$_4$(DMSO)$_2$] ranged from 25 μM in acetonitrile to 2 μM in DMSO solutions [339]. SOD activity of [Cu$_2$(Indo)$_4$(DMSO)$_2$] was also observed in the NBT assay employing xanthine–xanthine oxidase as the $O_2^{-\bullet}$ source, whereby 3 μM inhibited NBT reduction by 60% [436]. This was consistent with the IC_{50} value of 0.23 μM obtained using the NBT assay with xanthine–xanthine oxidase. It was noted that the IC_{50} value for [Cu$_2$(Indo)$_4$(DMF)$_2$] lies at the high activity region of the spectrum exhibited by these Cu-NSAID complexes [439].

SOD activity was also observed for other Cu-NSAIDS although IC_{50} values were not determined. Underhill et al. [361] showed that (±) suprofen (SupH = ±α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid) exhibited an absorbance increase of 27% at 560 nm due to the reduction of NBT, whereas the monomeric Cu(II) complex of Sup exhibited an increase of only 18% in the absorbance at this wavelength compared with the control [361]. Bury et al. studied the SOD activity of a number of monomeric metal tenoxicam complexes and showed that the monomeric Cu(II) complex exhibited the greatest activity (2% absorbance increase at 540 nm), followed by Mn(II) (19%), Co(II) (57%), Ni(II) (74%), Fe(III) (78%) and finally the free ligand (79%) [442]. Interestingly, Frechilla et al. showed that the bis(2,4,6-trimethylpyridinium), bis(2,4-dimethylpyridinium), and bis(pyridinium) salts of tetrachlorocuprate(II) inhibited $O_2^{-\bullet}$ production in macrophages at sub-millimolar concentrations [443].

SOD studies have also been performed in the presence of EDTA and bovine serum. These were chosen as competing chelating agents and were examined to determine the degree of stability of the Cu(II) complexes in vivo [323,435]. Roberts and Robinson [435] determined the SOD activity of a number of Cu(II) complexes in the presence of 20 μM EDTA. Compounds that exhibited SOD activity below 20 μM complex were deemed active while those that only possessed activity at

or above a concentration of 20 μM were defined as inactive. Cu(II) complexes of thiol-containing ligands, including D-penicillamine, thiola (thiopronin, 2-(mercaptopropionyl)glycine) and captopril (1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline) were active in the SOD assay while those of salicylate, Indo, DIPS and ±carprofen (CarpH = ±6-chloro-α-methyl-9H-carbazole-2-acetic acid) were inactive and behaved in a similar manner to $[Cu(gly)_2]$. In SOD studies with albumin, the SOD mimetic activity of $[Cu_2(DIPS)_4]$–$[Cu(DIPS)_2]$ decreased in the presence of increasing concentrations of albumin [386], as had also been found for $[Cu_2(Sal)_4]$, $[Cu_2(Indo)_4]$ and $[Cu_2(lonazolac)_4]$ (lonazolac = 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-acetic acid) [436]. If the Cu(II) complexes are regenerated in vivo at the site of inflammation, these "stability" SOD studies may not be less relevant.

5.3. Anti-inflammatory activity

The anti-inflammatory properties of inorganic Cu salts, while not as great as those of Cu-NSAID complexes, have been observed. For example, Lewis [135] showed kaolin paw edema inhibition ($<23\%$, $<27\%$) in the rat and guinea pig, respectively, by Cu(II) salts (Cu_2O, $CuCl_2$ and $[Cu_2(CH_3COO)_4(OH_2)_2]$). It has also been reported that Cu complexes of NSAIDs exhibit greater anti-inflammatory activity than the parent drugs [10,19]. For instance, Cu(II) complexes of salicylic acid, aspirin, 1-phenyl-5-aminotetrazole, 2-[3-trifluoromethylphenyl]aminonicotinic acid, penicillamine (3-mercapto-D-valine) and several corticoids are more active than their parent drugs [10].

In contrast, a number of researchers have found that some complexes show similar but not significantly greater anti-inflammatory activity. For instance, Boyle et al. [129] reported that the anti-inflammatory activities of Cu(II) complexes of (±)-ketoprofen (KetH = (±)-3-benzoyl-α-methyl-benzeneacetic acid), Indo, (+)-naproxen (NapH = (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid), niflumic acid (NifH = 2-[[3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid), clopirac (1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrole-3-acetic acid) and aspirin were similar but not greater than those of the parent drug following assessment using the carrageenan-induced edema assay combined with oral dosing. Unfortunately, no information is provided for the characterization of the complexes except that the structures were consistent with a 2:1 molar ratio of organic carboxylate to Cu(II). Nevertheless, there appears to be no doubt that Cu-NSAIDs exhibit anti-inflammatory activity, which is at least equivalent to if not more effective than the parent NSAID.

It is evident from the following studies that the animal species, the route of administration, the stereochemical configuration, the doses administered, and the assays employed, all need to be considered when reporting the anti-inflammatory activities of these compounds. The anti-inflammatory activities of the Cu(II) complex of aspirin (characterized as $[Cu_2(Asp)_4]$) and free aspirin were similar in the paw edema assay following oral administration of 50, 100 and 200 mg kg^{-1} [444]. Using this assay, Blahová et al. tested the anti-inflammatory activity of a number of Cu(II) complexes of the structure $[Cu(ROCH_2COO)_2(H_2O)_n] \cdot mH_2O$ and found that the anti-inflammatory activity decreased in the following order: R = phenyl, 76%; R = 2-naphthyl, 71%; Cu-salicylate, 58%; R = 4-chlorophenyl, 52%; R = 4-chloro-2-methylphenyl, 40% (where the % represents change in paw volume compared with rats treated with carrageenan only) [445]. When the Cu(II) complexes were compared with the free acids, the aryloxyacetato compounds containing the aryl groups, phenyl, 4-chlorophenyl and 2-naphthyl, exhibited greater anti-inflammatory activity compared with the Cu(II) derivatives, while those of 2-naphthyl and salicylate exhibited similar activities when complexed with Cu(II) [445].

Lewis [135] performed studies of the anti-inflammatory effects of the Cu(II) complex of aspirin in the rat and the guinea pig. While anti-inflammatory activity was observed for the Cu(II) complex of aspirin, [135] no difference was observed in the anti-inflammatory activity of the chelated and free drug when administered orally to rats. In contrast, the Cu(II) complex of aspirin exhibited greater anti-inflammatory activity than aspirin in the rat following subcutaneous injection of the drugs [135].

Mohan and Nagar [446] assessed the anti-inflammatory activity of monomeric Cu(II), Ni(II), Co(II), Zn(II), and Mn(II) complexes of *N*-pyridinobenzamide-2-carboxylic acid (PBCA) using the carrageenan-induced paw edema test, the granuloma pouch assay, and the adjuvant arthritis assay. Doses of metal chelates (200 mg kg^{-1}) were administered subcutaneously in a carboxymethylcellulose (CMC) suspension. In the cotton pellet granuloma test, the Cu(II)–PBCA complex (26.5%) showed a greater decrease in inflammation compared with free PBCA (20.2%). Interestingly, in the adjuvant arthritis assay Zn(II)–PBCA (28.9 ± 5.6%) showed greater anti-inflammatory activity than Cu(II)–PBCA (20.6 ± 2.1%) and PBCA (18.1 ± 1.6%) and, in the carrageenan-induced assay, Co(II)–PBCA (32.8 ± 1.1%) showed greater anti-inflammatory activity than Cu(II)–PBCA (22.6 ± 2.6%) and PBCA (28.6 ± 3.2%) [446].

Recently, Andrade et al. showed that the dimeric Cu(II) complex of (±)-Ibu (IbuH = (±)-α-methyl-4-(2-methylpropyl)benzeneacetic acid) (15.6 mg kg^{-1}) inhibited inflammation in the carrageenan-induced paw edema assay [447]. The increase in paw volume following carrageenan injection was similar for equivalent Ibu doses of $[Cu_2(Ibu)_4]$, 44.9 ± 3.3%; a dimeric Ru(II,III) complex $[Ru_2Cl(Ibu)_4]$, 46.0 ± 2.7%; and Ibu, 45.2 ±

1.9%. All compounds reduced inflammation compared to the control (untreated group—carrageenan alone), 65.3±1.4% [447].

Bertrand et al. [219] reported that both Cu(II)-Indo liposomes (15 mg kg^{-1}) and IndoH (15 mg kg^{-1}) significantly reduced paw edema induced by carrageenan in male Sprague–Dawley rats. Interestingly, Cu(II)-Indo liposomes exhibited similar anti-inflammatory activity to that of IndoH [219]. This result was confirmed by Dillon et al. [439], whereby similar anti-inflammatory activities were observed for $[Cu_2(Indo)_4(DMF)_2]$ (11.7 mg kg^{-1}) and IndoH (10 mg kg^{-1}) in 2% CMC solutions (equivalent molar IndoH doses) in male Sprague–Dawley rats ($n=6$ per group).

5.4. Ulcerogenic activity

Species specificity of the ulcerogenic properties of the Cu-NSAID was shown by Lewis [135], whereby lower gastric irritancy was observed in male Wister rats treated with the Cu(II) complex of Asp compared with AspH, while significantly greater irritancy of the Cu(II) complex was evident in the guinea pig. Not surprisingly, it was also shown that the aspirin compounds induced less gastric irritancy when administered subcutaneously compared with orally [135]. Williams et al. [444] noted no erosions of the stomach for rats treated with the dimeric Cu(II) complex of aspirin $[Cu_2(Asp)_4]$ (100–1200 mg kg^{-1}), compared with an average number of erosions of 0.4–4.1 for aspirin-treated rats (50–300 mg kg^{-1}). The comparatively lower number of ulcerations observed for the dimeric Cu(II) aspirin complex versus aspirin were consistent with findings of other groups [129,135]. However, the total number of erosions for both groups of animals was quite low considering the high doses used. This is consistent with the long (18 h) period between dosing and examination of the stomach, as epithelial restitution and healing would be evident [448–450].

Boyle et al. [129] showed increased ulcerations in female Wistar rats treated with (±)-KetH (6 mg kg^{-1}), IndoH (16 mg kg^{-1}), NapH (24 mg kg^{-1}), NifH (60 mg kg^{-1}), clopirac (150 mg kg^{-1}) and aspirin (300 mg kg^{-1}). Significant decreases in the ulcerations were observed only for the Cu(II) complexes of NifH, clopirac and aspirin (2:1 molar ratio of organic carboxylate to Cu(II)). Importantly, ulcerations were not assessed as a degree of damage, i.e. a summation of the ulcerated area, but were positively evaluated based on evidence of one area of erosion.

Bertrand et al. [219] examined the ulcerogenic damage in the small intestine of male Sprague–Dawley rats following treatment with Cu(II)-Indo (20 mg kg^{-1}) or IndoH (20 mg kg^{-1}) at 8 and 24 h after administration. While the extent of damage was lower for Cu(II)-Indo-treated rats in both instances, only the 24 h result was significantly lower [219]. Bertrand et al. [219] also showed that the intestinal damage was highly dependent on the form of administration of the Cu(II)-Indo. For instance, rats ($n=9$–10) receiving 15 mg kg^{-1} Cu(II)-Indo by oral dosing exhibited the following % ulcerations: DMF solution/RPMI 1640 medium, 20.2±5.3%; liposomes 2.9±0.9%; nanocapsules, 11.5±4.2%. The increase in ulcerations caused by Cu(II)-Indo in DMF/RPMI 1640 solution was likely to be associated with a high amount of monomeric Cu(II) and free IndoH. Low temperature EPR studies indicated a rapid decay of $[Cu_2(Indo)_4(DMF)_2]$ when the complex was added to the growth media from organic solvents [439].

Gastric and intestinal damage was assessed in male Sprague–Dawley rats following oral gavage of 2% CMC suspensions of equivalent Indo doses and $[Cu_2(Indo)_4(DMF)_2]$ (11.7 mg kg^{-1}) induced less macroscopic damage than IndoH (10 mg kg^{-1}). The degree of damage was significantly different in the stomach as noted by the summed area of ulcerations: IndoH = 40.5±11.24 mm^2, $[Cu_2(Indo)_4(DMF)_2]=14\pm6.95$ mm^2 ($P=0.0012$). The degree of damage was extremely significant in the small intestine at 24 h where the ulcerations induced by IndoH were 138±13 mm^2 and those by $[Cu_2(Indo)_4(DMF)_2]$ were 4.3±1.5 mm^2 ($P<0.0001$) [439]. Consistently, the hemoglobin content of the caecums from rats treated with Cu-Indo (1.38 mg ml^{-1}) was significantly lower than that from rats treated with IndoH (5.40 mg ml^{-1}, $P<0.0001$) and was only slightly greater than the control (0.94 mg ml^{-1}, $P=0.045$) [439].

Comparative studies of ulcerogenic damage in the rat stomach were performed for a number of metal complexes of PBCA, namely those of Cu(II), Ni(II), Co(II), Zn(II) and Mn(II), and it was found that the Cu(II) complex exhibited the lowest ulcerogenic effects [446]. This was also observed for a number of monomeric and dimeric complexes of Indo, namely $[Zn(Indo)_2(EtOH)_2]$, $[Zn(Indo)_2(OH_2)_2]$, $[Zn_2(Indo)_4(DMA)_2]$, $[Co(Indo)_2(EtOH)_2]$, $[Ni(Indo)_2(OH_2)_2]$ and $[Ni_2(Indo)_4(EtOH)_2]$, whereby $[Cu_2(Indo)_4(DMF)_2]$ exhibited the lowest ulcerogenic damage in both the stomach and small intestine of all the complexes studied [439]. Similar protective effects were observed for $[Cu_2(Ibu)_4]$ in the rats stomach. For instance, rats treated with IbuH exhibited a lesion index of 597±43, while markedly lower lesion indices were observed for rats treated with the Ru(II,III) complex, $[Ru_2Cl(Ibu)_4]$ (17.3 mg kg^{-1}), 325±14, and $[Cu_2(Ibu)_4]$ (15.6 mg kg^{-1}), 290±31 [447].

Administration of Cu-Algesic® (containing $[Cu_2(Indo)_4(DMF)_2]$) to dogs resulted in only mild GI lesions when administered at anti-inflammatory doses while equivalent doses of IndoH were toxic [421]. Hemorrhages and erosions were also less severe than those produced by low dose aspirin [421]. Furthermore, doses of $[Cu_2(Indo)_4L_2]$ five times the therapeutic levels were

non-toxic in the GI tract and central nervous system of horses while a similar supra therapeutic dose of IndoH was toxic to the central nervous system [18].

The gastro-duodenal mucosa was examined endoscopically following treatment of dogs ($n=6$ per group) with (±) ketoprofen (1 mg kg^{-1} every 24 h), $[Cu_2(Indo)_4(DMF)_2]$ (0.2 mg kg^{-1} every 12 h), prednisolone (1 mg) and cinchophen (2-phenyl-4-quinolinecarboxylic acid) (200 mg per 20 kg every 12 h), aspirin (15 mg kg^{-1} every 12 h) and gelatin (one capsule every 12 h) [421]. These were reportedly administered as anti-inflammatory doses recommended by the manufacturer, except in the case of aspirin, which was administered at a 'low-tolerated dose' and $[Cu_2(Indo)_4(DMF)_2]$, which was administered at double the manufacturer's recommended dose. No ulcerations were observed in the esophagus of any of the dogs examined. Aspirin induced a greater severity of ulcerations overall and the highest prevalence of ulcers was observed in the stomach antrum, followed by equal ulcerations in the stomach cardia/fundus and stomach body. Similarly, the highest number of ulcerations was observed in the stomach antrum for all other drugs tested including $[Cu_2(Indo)_4(DMF)_2]$-treated dogs. It was concluded from the study that ketoprofen, $[Cu_2(Indo)_4(DMF)_2]$ and prednisolone-cinchophen produced mild GI lesions in comparison to aspirin.

Finally, local topical ulcerogenic effects have been examined in human clinical trials following application of a Cu(II)–salicylate gel (1.5 g every 12 h; 4.3 mg g^{-1} Cu) to the inner forearm of patients with OA [451]. Importantly, no ulcerogenic effects were observed, although significantly more dermatological reactions occurred in 17% of the Cu(II)–salicylate-treated group, compared with 1.7% in the placebo-treated group. This was accompanied by insignificant decreases in pain scores experienced by the group compared with the placebo-treated group.

6. Conclusions

If the clinical opportunities presented by the Cu-NSAIDs and other metallic NSAIDs are to be completely realized, much more information is required about the pharmacometrics of the complexes in vivo as well as in vitro. Much is known about the dimeric and monomeric solid-state character of the Cu(II) complexes of the (primarily) carboxylato-type NSAIDs. However, there appears to be limited reported testing and comparison of their pharmacological activity and pharmacokinetic disposition, or information of any structure–activity relationship for such complexes. In addition, the sample sizes used in reported pharmacological testing have either been small and/or the exact nature of the chemical and dosage form of the Cu-NSAIDs administered have not been characterized. Whether or not the mode of action of the Cu-NSAIDs is similar to that of the traditional NSAIDs, i.e. inhibition of PG synthesis via the COX isoenzyme system, or is related to the modulation of SOD, NOS, or other elements of the immune system in addition to COX inhibition, remains unclear. What is irrefutable is the reduced GI toxicity of such complexes, as evidenced by the successful long term veterinary applications of the Cu(II) complex of the NSAID indomethacin [42]. The stability of Cu-NSAID complexes has been shown to be a critical determinant of their activity and toxicity. Investigations of the Cu-NSAIDs in biological matrices are, therefore, essential, if the opportunities of the Cu(II)-complexes are not be to missed and their application extended to the human market.

Acknowledgements

We acknowledge the support of the Australian Research Council for a SPIRT grant and RIEFP grants for EPR, vibrational spectroscopy, XRD, and a 10-element Ge detector. Biochemical Veterinary Research (B.V.R) is gratefully acknowledged for funding, including a postgraduate scholarship for JEW and for helpful discussions from Mr. Barry Warwick on the Cu(II) pharmaceuticals. We also gratefully acknowledge the support of the Australian Synchrotron Research Program (ASRP) at the Australian National Beamline Facility (ANBF) in Tsukuba (Japan), which is funded by the Commonwealth of Australia under the Major National Research Facilities Program; the Stanford Synchrotron Research Laboratory (SSRL), which is operated by the United States of America Department of Energy, Offices of Basic Energy Sciences; the SSRL Biotechnology Program, which is supported by the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program and by the Department of Energy, Office of Biological and Environmental Research; and the Australian facilities at the Advanced Photon Source (APS) at the Argonne National Laboratory in Chicago which is supported by the ASRP and funded by the Commonwealth of Australia under the Major National Research Facilities Program, and operated by the United States of America Department of Energy. We also thank co-workers Ms. Ying R Morgan, Dr. Qingdi Zhou and Dr. Antonio Bonin; including Dr. Gary Foran, Dr. Anne Rich, Dr. James Hester, Dr. Paul Ellis and Dr. Peter Turner (X-ray absorption spectroscopy and analysis), and Dr. Dugald MacLachlan, Dr. Richard Bramley, Dr. Chris Delfs, Dr. Keith S. Murray and Dr. B. Moubaraki (EPR, variable temperature magnetic susceptibility). We also thank Mr. Greg Halder and Ms. Jade Aitken for assistance with the Cambridge Structural Database and

the RASMOL molecular visualization program, respectively.

References

[1] G.P. Rodnan, T.G. Benedek, Arthritis Rheum. 13 (1970) 145.
[2] G.P. Rodnan, T.G. Benedek, Arthritis Rheum. 6 (1963) 317.
[3] J.R. Vane, R.J. Flower, R.M. Botting, Stroke 21 (1990) 12.
[4] H. Haas, Am. J. Med. 14 (1983) 1.
[5] H. Levesque, O. Lafont, Rev. Med. Int. 21 (2000) S8.
[6] M. Judge, New Scient. 2058 (1996) 30.
[7] R.F. Borne, in: W.O. Foye, T.L. Lemke, D.A. Williams (Eds.), Principles of Medicinal Chemistry, Williams & Wilkins, Baltimore, 1995, p. 535.
[8] L.S. Goodman, A. Gilman (Eds.), The Pharmacological Basis of Therapeutics, fifth ed., Macmillan, New York, 1975, p. 1704.
[9] The name aspirin comes from a contraction of the letter a from acetyl and spirin; a name given to spirea plants.
[10] J.R.J. Sorenson, Prog. Med. Chem. 26 (1989) 437.
[11] G. Ebers, Papyrus Ebers: die Arzneimittel der alten Aegypter in hieratischer Schrift, W. Engelmann, Leipzig, 1875.
[12] B. Ebbel, The Papyrus Ebers, Oxford University Press, London, 1937.
[13] W.R. Walker, S.J. Beveridge, M. Whitehouse, Agents Action Suppl. 8 (1981) 359.
[14] J.R.J. Sorenson, Chem. Br. 20 (1984) 1110.
[15] J.R.J. Sorenson, Handbook of Metal–Ligand Interactions in Biological fluids, vol. 2, 1st ed., Marcel Decker, New York, 1995, p. 1318.
[16] L.-O. Klotz, U. Weser, Copper and Zinc in Inflammatory and Degenerative Diseases, Kluwer Academic Publishers, Dordrecht, 1998, p. 19.
[17] W. Hangarter, Copper–Salicylate in Rheumatoid Arthritis and Similar Degnerative Diseases, first ed., Humana Press, New Jersey, 1982, p. 439.
[18] H.L. Regtop, J.R. Biffin, US Patent, Divalent Metal Complexes of Indomethacin, Compositions and Medical Methods of Use Thereof, p. 9 cont.-in-part of US 5310936 1995.
[19] Z. Korolkiewicz, E. Hac, I. Gagalo, P. Gorcyca, A. Lodzinska, Agents Action 26 (1989) 355.
[20] J.R.J. Sorenson, J. Med. Chem. 19 (1976) 135.
[21] J.E.F. Reynolds (Ed.), Martindale. The Extra Pharmacopoeia, 31st ed., The Pharmaceutical Press, London, 1996.
[22] J. Thomas (Ed.), Australian Presciption Products Guide, vol. 1, 26th ed., Australian Pharmaceutical Publishing Co Ltd., Hawthorn, Victoria, 1997, p. 1327.
[23] C.S. Boynton, C.F. Dick, G.H. Mayor, J. Clin. Pharmacol. 28 (1988) 512.
[24] US Department of Health and Human Services, In: FDA Approves Celebrex for Arthritis; 1998, http://www.verity.fda.gov/search97...%26ResultCount%3D10&hlnavigate+ALL.
[25] US Department of Health and Human Services, In Food and Drug Administration: FDA Approves VIOXX for Osteoarthritis, Pain and Menstrual Pain; 1999, http://www.fda.gov/bbs/topics/ANSWERS/ANS00956.html.
[26] J. van Ryn, M. Pairet, Inflamm. Res. 48 (1999) 247.
[27] D.O. Stichtenoth, H. Zeidler, J.C. Frohlich, Medizin. Klinik 93 (1998) 407.
[28] J.L. Wallace, Gastroenterolgy 112 (1997) 1000.
[29] M.T. Donnelly, C.J. Hawkey, Aliment. Pharmacol. Ther. 11 (1997) 227.
[30] W.O. Foye (Ed.), Principles of Medicinal Chemistry, 1st ed., Lea & Febier, Philadelphia, 1974.
[31] V. Wright, Br. J. Rheumatol. 34 (1995) 2.
[32] E.C. Filipponi, T. Violetta, B. Oriana, D. Bonelli, A. Fravolini, J. Comput.-Aided Mol. Des. 14 (2000) 277.
[33] D. Hadjipavlou-Litina, Curr. Med. Chem. 7 (2000) 375.
[34] R.P.C. Pouplana, J. Sanchez, J.J. Lozano, P. Puig-Parellada, J. Comput.-Aided Mol. Des. 13 (1999) 297.
[35] C.K. Lau, W.C. Black, M. Belley, C. Chan, S. Charleson, D. Denis, J.Y. Gauthier, R. Gordon, D. Guay, P. Hamel, S. Kargman, Y. Leblanc, J. Mancini, M. Ouellet, D. Percival, P. Prasit, P. Roy, K. Skorey, P. Tagari, P. Vickers, E. Wong, Adv. Exp. Med. Biol. 407 (1997) 73.
[36] CHEMWINDOW; v 5.0.1, SoftShell International Ltd., BioRad Laboratories, Grand Junction, 1995–1997.
[37] G.L. Maurer US Patent: 82-447424 19821206, Methods and Composition for Treating Inflammation or Arthritis, 1986.
[38] H.L. Regtop, J.R. Biffin, Patent WO 9014337 A1 901129, WO 90-AU209 900521, AU 89-4328 890522: Divalent Metal Salts of Indomethacin as Antiinflammatory and Analgesic Agents, 1990, p. 18.
[39] H.L. Regtop, J.R. Biffin, Patent US 5466824 A 951114, US 94-217520 940324, AU 89-4328 890522 US 92-773601 920115: Preparation of Divalent Metal Salts of Indomethacin p. 9 Cont.-in-part of US 5310936, 1994.
[40] B. Boettcher, W.R. Walker, M.W. Whitehouse, Patent EP Priority: AU 77-2584 19771128, CAN 92:58446, AN 1980:58446: Complexes of Bivalent Copper and Compositions Containing NSAID Complexes, 1981.
[41] J.R.J. Sorenson, Patent Application: US 75-563778 19750331, CAN 94:25225: Anti-inflammatory and Anti-ulcer Compounds and Processes, 1980.
[42] IVS Annual; MIMS Publishing, Crows Nest, NSW, Sydney, 1997, p. 145 & 276.
[43] J.R. Biffin, Safe Administration of Copper Indomethacin to Dogs, Personal Communication (2000).
[44] E. Alexopoulos, Renal Failure 20 (1998) 809.
[45] F. Rodriquez, M.T. Llinas, J.D. Gonzalez, J. Rivera, J. Salazar, Hypertension 36 (2000) 276.
[46] R. Birck, S. Krzossok, T. Knoll, C. Braun, F.J. van der Woude, P. Rohmeiss, Exp. Nephrol. 8 (2000) 173.
[47] M. Komhoff, J.L. Wang, H.F. Cheng, R. Langenbacj, J.A. McKanna, R.C. Harris, M.D. Breyer, Kidney Int. 57 (2000) 414.
[48] J.J. Ares, P.E. Outt, Curr. Pharm. Des. 4 (1998) 17.
[49] E. Rahme, L. Joseph, S.X. Kong, D.J. Watson, J. LeLorier, Arthritis Rheum. 43 (2000) 917.
[50] S. Hernandez-Diaz, L.A.G. Rodriguez, Arch. Int. Med. 160 (2000) 2093.
[51] P. Schoenfeld, M.B. Kimmey, J. Scheiman, D. Bjorkman, L. Laine, Aliment. Pharmacol. Ther. 13 (1999) 1273.
[52] J. Holvoet, L. Terriere, W. Van Hee, L. Verbist, E. Fierens, M.L. Hautekeete, Gut 32 (1991) 730.
[53] S.E. Gabriel, E.L. Matteson, Pharmacoeconomics 8 (1995) 479.
[54] D.J. Bjorkman, M.B. Kimmey, Digest. Dis. 13 (1995) 119.
[55] J. Page, D. Henry, Arch. Int. Med. 160 (2000) 777.
[56] R.N. Dubois, Aliment. Pharmacol. Ther. 14 (2000) 64.
[57] R.W. Stockbrugger, Eur. J. Cancer Prev. 8 (1999) S21.
[58] L. Rosenberg, C. Louik, S. Shapiro, Cancer 82 (1998) 2326.
[59] G.D. Luk, Schweizerische Medizinische Wochenschrift 126 (1996) 801.
[60] R. Chinery, R.D. Beauchamp, Y. Shyr, S.C. Kirkland, R.J. Coffey, J.D. Morrow, Cancer Res. 58 (1998) 2323.
[61] A.J.M. Watson, Histol. Histopathol. 13 (1998) 591.
[62] H.A. Weiss, D. Forman, Scand. J. Gastoent. 31 (1996) 137.
[63] T. Hida, J. Leyton, A.N. Makheja, P. Benav, T. Hla, A. Martinez, J. Mulshine, S. Malkani, P. Chung, T.W. Moody, Anticancer Res. 18 (1998) 775.
[64] P.L. McGeer, M. Schulzer, E.G. McGeer, Neurology 47 (1996) 425.
[65] I.R.A. MacKenzie, D.G. Munoz, Neurology 50 (1998) 986.

[66] K. Sugaya, T. Uz, H. Manev, Jpn. J. Pharmacol. 82 (2000) 85.
[67] E.E. Netland, J.L. Newton, R.E. Majocha, B.A. Tate, Neurobiol. Aging 19 (1998) 201.
[68] L.A.G. Rodriguez, Sem. Arthritis Rheum. 26 (1997) 16.
[69] L.A.G. Rodriguez, H. Jick, Lancet 343 (1994) 769.
[70] D.B. Hogan, N.R.C. Campbell, R. Crutcher, P. Jennett, N. MacLeod, Can. Med. Assoc. J. 151 (1994) 315.
[71] M.R. Griffin, J.M. Piper, J.R. Daugherty, M. Snowden, W.A. Ray, Ann. Int. Med. 114 (1991) 257.
[72] M.M. Elseviers, M.E. Debroe, Drugs Aging 12 (1998) 391.
[73] M.R. Griffin, A. Yared, W.A. Ray, Am. J. Epidemiol. 151 (2000) 488.
[74] T.S. Field, J.H. Gurwitz, R.L. Glynn, M.E. Salive, J.M. Gaziano, J.O. Taylor, C.H. Hennekens, J. Am. Geriatr. Soc. 47 (1999) 507.
[75] S.K. Swan, D.W. Rudy, K.C. Lasseter, C.F. Ryan, K.L. Buechel, L.J. Lambrecht, M.B. Pinto, S.C. Dilzer, O. Obrda, K.L. Sundblad, C.P. Gumbs, D.L. Ebel, H. Quan, P.J. Larson, J.I. Schwartz, T.A. Musliner, B.J. Gertz, D.C. Brater, S.L. Yao, Ann. Int. Med. 133 (2000) 1.
[76] M.C. Hochberg, J. Rheumatol. 19 (1992) 63.
[77] B. Bannwarth, Drugs 59 (2000) 17.
[78] W.E. Smalley, M.R. Griffin, R.L. Fought, L. Sullivan, W.A. Ray, New Engl. J. Med. 332 (1995) 1612.
[79] D.T. Felson, R.C. Lawrence, M.C. Hochberg, T. McAlindon, P.A. Dieppe, M.A. Minor, S.N. Blair, B.M. Berman, J.F. Fries, M. Weinberger, K.R. Lorig, J.J. Jacobs, V. Goldberg, Ann. Int. Med. 133 (2000) 726.
[80] W.D. Blackburn, Am. J. Med. 100 (1996) 24.
[81] C.R.W. Edwards, I.A.D. Bouchier, C. Haslett, E.R. Chilvers (Eds.), Davidson's Principles and Practice of Medicine, 17th ed., Churchill Livingstone, Edinburgh, 1995.
[82] N. Moore, X. Verschuren, C. Montout, J. Callens, S.X. Kong, B. Begaud, Therapie 55 (2000) 133.
[83] K.D. Muirden, Med. J. Aust. 166 (1997) 344.
[84] E. Yelin, L.F. Callahan, F. Arnett, D. Dennis, R. Deyo, D. Felson, W. Felts, E. Giannini, C. Helmick, S. Heyse, R. Hirsch, M. Hochberg, G. Hunder, R. Lawrence, M. Liang, S. Pillemer, L. Shulman, V. Steen, F. Wolfe, Arthritis Rheum. 38 (1995) 1351.
[85] G. de Pouvourville, Br. J. Rheum. 34 (1995) 19.
[86] D.R. Lappin, Arthritis: What We Know Today; National Institute of Arthritis and Muscularskeletal and Skin Diseases, 1997, http://www.elitehealthshop.com/Articles/ArthritisFacts-Myths.htm.
[87] E. Gordon, In: House of Commons Hansard (UK), 1999, http://www.parliament.the-stationery-office.co.uk/cgi-bin/empower.
[88] M.D. McGoldrick, G.R. Bailie, Ann. Pharmacother. 31 (1997) 221.
[89] C. Ryall, In: Facts, Figures and Estimated Costs of Arthritis; British Society for Rheumatology, 1998, http://www.rheumatology.org.uk/noframes/general/facts.htm.
[90] Canadian Arthritis Network, 1999, http://can.klickit.com/content.cfm?page = info_exchange_disease_arthritis.
[91] M.J. Elders, J. Rheumatol. 27 (2000) 6.
[92] E. Ernst, S. Chrubasik, Rheumatol. Dis. Clin. North Am. 26 (2000) 13.
[93] T.E. McAlindon, M.P. La Valley, J.P. Gulin, D.T. Felson, J. Am. Med. Assoc. 283 (2000) 1467.
[94] L.S. Simon, Int. J. Clin. Prac. 54 (2000) 243.
[95] T.J. Standiford, Curr. Pharm. Des. 6 (2000) 633.
[96] A. Geronikaki, D.J. Hadjipavlou-Litina, M. Tzaki, Arzneimittel-Forschung (Drug Res.) 50 (2000) 266.
[97] D.H. Ko, A.S. Heiman, M.Q. Chen, H.J. Lee, Steroids 65 (2000) 210.
[98] O. Bruno, S. Schenone, A. Ranise, E. Barocelli, M. Cjiavarini, V. Ballabeni, S. Bertoni, Arzneimittel-Forschung 50 (2000) 149.
[99] C. Nicolas, M. Verny, I. Giraud, M. Ollier, M. Rapp, J. Maurizis, J. Madelmont, J. Med. Chem. 42 (1999) 5235.
[100] Y. Boutsen, W. Esselinckx, Acta Gastroenterol. Belgica 62 (1999) 421.
[101] S. Radhofer-Welte, X. Rabasseda, Drugs Today 36 (2000) 55.
[102] L.S. Simon, Curr. Opin. Rheumatol. 12 (2000) 163.
[103] E. Filipponi, V. Cecchetti, O. Tabarrini, D. Bonelli, A. Fravolini, J. Comput.-Aided Mol. Des. 14 (2000) 277.
[104] C. Puig, M.I. Crespo, N. Godessart, J. Feixas, J. Ibarzo, J.M. Jimenez, L. Soca, I. Cardelus, A. Heredia, M. Miralpeix, J. Puig, J.M. Huerta, M. Lopez, V. Segerra, H. Ryder, J.M. Palacios, J. Med. Chem. 43 (2000) 214.
[105] N.M. Davies, J.Y. Saleh, N.M. Skjodt, J. Pharm. Pharmacol. Sci. 3 (2000) 137.
[106] D.W. Gilroy, P.R. Colville-Nash, J. Mol. Med. (Berlin) 78 (2000) 121.
[107] L.J. Marnett, A.S. Kalgutkar, Trends Pharmacol. Sci. 20 (1999) 465.
[108] C.K. Lau, W.C. Black, M. Belley, C. Chan, S. Charleson, D. Denis, J.Y. Gauthier, R. Gordon, D. Guay, P. Hamel, S. Kargman, Y. Leblanc, J. Mancini, M. Ouellet, D. Percival, P. Prasit, P. Roy, K. Skorey, P. Tagari, P. Vickers, E. Wong, Adv. Exp. Med. Biol. 407 (1997) 73.
[109] R.S. Spangler, Sem. Arthritis Rheum. 26 (1996) 435.
[110] S. Fiorucci, L. Santucci, B. Federici, E. Antonelli, E. Distrutti, O. Morelli, G. Di Renzo, G. Coata, G. Cirino, P. Del Soldato, A. Morelli, Aliment. Pharmacol. Ther. 13 (1999) 421.
[111] S. Fiorucci, E. Antonelli, L. Santucci, O. Morelli, M. Miglietti, B. Federici, R. Mannucci, P. Del Soldato, A. Morelli, Gastroenterology 116 (1999) 1089.
[112] M.N. Muscara, W. McKnight, P. Delsoldato, J.L. Wallace, Life Sci. 62 (1998) PL235.
[113] J.L. Wallace, Can. J. Physiol. Pharmacol. 72 (1994) 1493.
[114] B.K. Reuter, G. Cirino, J.L. Wallace, Life Sci. 55 (1994) PL1.
[115] J. Panes, M. Molla, M. Casadevall, A. Salas, M. Sans, C. Conill, D.C. Anderson, J. Rosello-Catafau, D.N. Granger, J.M. Pique, Aliment. Pharmacol. Ther. 14 (2000) 841.
[116] C. Raguenes-Nicol, F. Russo-Marie, G. Domage, N. Diab, E. Solito, F. Dray, J.L.G. Mace, G. Streichenberger, Biochem. Pharmacol. 57 (1999) 433.
[117] T. Kirchner, B. Aparicio, D.C. Argentieri, C.Y. Lau, D.M. Ritchie, Prostagl. Leukotri. Ess. Fatty Acids 56 (1997) 417.
[118] H.J. Showell, M.J. Conklyn, R. Alpert, G.P. Hingorani, K.F. Wright, M.A. Smith, E. Stam, E.D. Salter, D.N. Scampoli, S. Meltzer, L.A. Reiter, K. Koch, A.D. Piscopio, S.R. Cortina, A. Lopezanaya, E.R. Pettipher, A.J. Milici, R.J. Griffiths, J. Pharmacol. Exp. Ther. 285 (1998) 946.
[119] W.R. Henderson, Ann. Int. Med. 121 (1994) 684.
[120] S.M. Weisman, M.J. Doyle, K.R. Wehmeyer, B.A. Hynd, T.H. Eichhold, R.M. Clear, C.W. Coggeshell, D.L. Kuhlenbeck, Agents Action 41 (1994) 156.
[121] C.J. Malemud, V.M. Goldberg, Front. Biosci. 4 (1999) 762.
[122] D.P. Riley, Chem. Rev. 99 (1999) 2573.
[123] S.L. Henke, Expert Opin. Ther. Pat. 9 (1999) 169.
[124] J. Aaseth, M. Haugen, O. Forre, Analyst 123 (1998) 3.
[125] C.J. Hawkey, Gastroenterology 119 (2000) 521.
[126] L.M. Lichtenberger, Z.M. Wang, J.J. Romero, C. Ulloa, J.C. Perez, M.N. Giraud, J.C. Barreto, Nature Med. 1 (1995) 154.
[127] C. Dendrinou-Samara, G. Tsotsou, L.V. Ekateriniadou, A.H. Kortsaris, C.P. Raptopoulou, A. Terzis, D.A. Kyriakidis, D.P. Kessissoglou, J. Inorg. Biochem. 71 (1998) 171.
[128] G.E. Jackson, L. Mkhonta-Gama, A. Voye, M. Kelly, J. Inorg. Biochem. 79 (2000) 147.
[129] E. Boyle, P.C. Freeman, A.C. Goudie, F.R. Mangan, M. Thomson, J. Pharm. Pharmacol. 28 (1976) 865.
[130] A.L. Abuhijleh, C. Woods, E. Bogas, G. Le Guenniou, Inorg. Chim. Acta 195 (1992) 67.

[131] V. Brumas, B. Brumas, G. Berthon, J. Inorg. Biochem. 57 (1995) 191.
[132] F.T. Greenaway, E. Rivere, J.J. Girerd, X. Labouze, G. Morgant, B. Viossat, J.C. Daran, M. Roch Arveiller, N.-H. Dung, J. Inorg. Biochem. 76 (1999) 19.
[133] F.T. Greenaway, L.J. Norris, R.J. Sorenson, Inorg. Chim. Acta 145 (1988) 279.
[134] E. Lengfelder, K.H. Sellinger, U. Weser, in: U. Weser (Ed.), Metalloproteins, Thieme Verlag, Stuttgart, NY, 1979, p. 136.
[135] A.J. Lewis, Agents Action 8 (1978) 244.
[136] N.A. Roberts, P.A. Robinson, in: G. Rotilio (Ed.), Superoxide and Superoxide Dismutase in Chemistry and Medicine, Elsevier, Amsterdam, 1986, p. 538.
[137] W.R. Walker, S.J. Beveridge, Inorg. Perspect. Biol. Med. 2 (1979) 93.
[138] P.M. Fraser, R. Doll, M.J.S. Langman, J.J. Misiewicz, H.H. Shawdon, Gut 13 (1972) 459.
[139] D.J. Frommer, Med. J. Aust. 2 (1975) 793.
[140] Q. Zhou, T.W. Hambley, B.J. Kennedy, P.A. Lay, P. Turner, B. Warwick, J.R. Biffin, H.L. Regtop, Inorg. Chem. 39 (2000) 3742.
[141] N.M. Davies, J. Pharm. Pharmacol. Sci. 2 (1999) 5.
[142] F. McKenna, J. Rheumatol. 25 (1998) 21.
[143] A. Figueras, D. Capella, J.M. Castel, J.R. Laporte, Eur. J. Clin. Pharmacol. 47 (1994) 297.
[144] V. Wright, J. Orthop. Rheumatol. 6 (1993) 129.
[145] J. Macleod (Ed.), Davidson's Principes and Practice of Medicine: a Textbook for Students and Doctors, 12th ed., Churchill Livingstone, Edinburgh, 1977.
[146] J.A. Hunter, Treaste in the Blood, Inflammation, and Gun-shot Wounds, Niclos, George, London, 1894.
[147] E. Arrigoni-Martelli, Int. J. Tissue React. 7 (1985) 513.
[148] K.D. Rainsford, K. Brune, M.W. Whitehouse, Trace Elements in the Pathogenesis and Treatment of Inflammation, Berkhauser Verlag, Basel, Boston, Stuttgart, 1981.
[149] I.L. Bonta, J. Thompson, K. Brune (eds.) ,Inflammation: Mechanisms and their Impact on Therapy, Birkhauser Verlag, Basel, 1977.
[150] J.R. Vane, S.H. Ferreira (Eds.), Inflammation, vol. 1 & 2, Springer-Verlag, Berlin, 1978.
[151] Y. Dummy, in: C. Haslett (Ed.), Davidson's Principles and Practice of Medicine, 18th ed., Churchill Livingstone, Edinburgh, 1999.
[152] G.G. Vaday, O. Lider, J. Leuk. Biol. 67 (2000) 149.
[153] M. Maiotti, G. Monteleone, U. Tarantino, G.F. Fasciglione, S. Marini, M. Coletta, Arthroscopy 16 (2000) 522.
[154] H. Yamanaka, Y. Matsuda, M. Tanaka, W. Sendo, H. Nakajima, A. Taniguchi, N. Kamatani, Arthritis Rheum. 43 (2000) 852.
[155] D.R. Yager, B.C. Nwomeh, Wound Repair Regeneration 7 (1999) 433.
[156] H. Birkedalhansen, J. Periodont. Res. 28 (1993) 500.
[157] J.V. Hurley, Acute Inflammation, Churchill Livingstone, Edinburgh, 1972.
[158] B.W. Zweifach, L. Grant, G. Lester, R. McCluskey, The Inflammation Process, Academic Press, New York, 1974.
[159] P.C. Trackman, R.J. Graham, H.K. Bittner, D.L. Carnes, J.A. Gilles, D.T. Graves, Histochem. Cell Biol. 110 (1998) 9.
[160] A.C. Issekutz, Inflamm. Res. 47 (1998) 123.
[161] X.Y. Song, L. Zeng, W.W. Jin, J. Thompson, D.E. Mizel, K.J. Lei, R.C. Billinghurst, A.R. Poole, S.M. Wahl, J. Exp. Med. 190 (1999) 535.
[162] V. Everts, E. Vanderzee, L. Creemers, W. Beertsen, Histochem. J. 28 (1996) 229.
[163] M.R.E. Silva, J.G. Leme, Chemical Mediators of the Acute Inflammatory Reaction, 1st ed., Pergamon Press, Oxford, 1972.
[164] R.L. Smith, Front. Biosci. 4 (1999) 704.
[165] Z. Xing, J. Wang, Curr. Pharm. Des. 6 (2000) 599.
[166] J. Lunec, H.R. Griffiths, Free Radic. Inflamm. (2000).
[167] I.M. Roitt, Roitt's Essential Immunology, Blackwell Science, Oxford, 1997.
[168] J.R. Vane, Nature (London) 231 (1971) 232.
[169] J.C. Frohlich, Trends Pharmacol. Sci. 18 (1997) 30.
[170] J.R. Vane, R.M. Botting, Semin. Arthritis Rheum. 26 (1997) 2.
[171] R. Polisson, Am. J. Med. 100 (1996) S31.
[172] U.S. von Euler, J. Physiol. 88 (1936) 213.
[173] S.R. Bergstrom, R. Samuelsson, B.J. Sjovall, Acta Chem. Scand. 16 (1962) 501.
[174] P.D. Brown, Angiogenesis 1 (1997) 142.
[175] D.E. Levy, A.M. Ezrin, Emerging Drugs 2 (1997) 205.
[176] L. Garcia-Marcos, A. Schuster, Pediat. Allergy Immunol. 10 (1999) 77.
[177] J. Pfeilschifter, H. Muhl, Eur. J. Pharmacol. 375 (1999) 237.
[178] S. Moncada, J.R. Vane, Adv. Int. Med. 24 (1979) 1.
[179] I.H. Hall, K.G. Rajendran, S.Y. Chey, V.N. Norwood, K.W. Morse, A. Sood, B.F. Spielvogel, Appl. Organomet. Chem. 8 (1994) 473.
[180] J.A. Salmon, G.A. Higgs, Br. Med. Bull. 43 (1987) 285.
[181] M. Ouellet, M.D. Percival, Biochem. J. 306 (1995) 247.
[182] C. Luong, A. Miller, J. Barnett, J. Chow, C. Ramasha, M.F. Browner, Nat. Struct. Biol. 3 (1996) 927.
[183] B. Cryer, M. Feldman, Am. J. Med. 104 (1998) 413.
[184] J. Vane, J. Clin. Rheumatol. 4 (1998) S3.
[185] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stalling, Nature (London) 384 (1996) 644.
[186] S.K. Kulkarni, N.K. Jain, A. Singh, Methods Find. Exp. Clin. Pharmacol. 22 (2000) 291.
[187] C. Brideau, S. Kargman, S. Liu, A.L. Dallob, E.W. Ehrich, I.W. Rodger, C.C. Chan, Inflamm. Res. 45 (1996) 68.
[188] J.S. Carter, Expert Opin. Ther. Pat. 10 (2000) 1011.
[189] H. Fenner, Semin. Arthritis Rheum. 26 (1997) 28.
[190] M. Katori, M. Majima, Inflamm. Res. 49 (2000) 367.
[191] M. Suneel, D.W. Croom, New Engl. J. Med. 340 (1999) 2005.
[192] P.M. Brooks, R.O. Day, Med. J. Aust. 173 (2000) 433.
[193] P. Harding, D.H. Sigmon, M.E. Alfie, P.L. Huang, M.C. Fishman, W.H. Beirwlates, O.A. Carretero, Hypertension 29 (1997) 297.
[194] D. Picot, P.J. Loll, R.M. Garavito, Nature (London) 367 (1994) 243.
[195] C.J. Hawkey, Lancet 353 (1999) 307.
[196] I. Siegle, T. Klein, J.T. Backman, J.G. Saal, R.M. Nusing, P. Fritz, Arthritis Rheum. 41 (1998) 122.
[197] M.A. Iniguez, J.L. Pablos, P.E. Carreira, F. Cabre, J.J. Gomezreino, Br. J. Rheumatol. 37 (1998) 773.
[198] A.S. Kalgutkar, B.C. Crews, S.W. Rowlinson, C. Garner, K. Seibert, L.J. Marnett, Science (Washington, DC) 280 (1998) 1268.
[199] R. Sayle Rasmol, Glaxo Wellcome Research and Development, Stevenage, 1996.
[200] J.K. Gierse, J. McDonald, S. Hauser, S. Rangwala, K. Seibert, J. Biol. Chem. 271 (1996) 15810.
[201] A.J. de Brum Fernandes, J. Rheumatol. 24 (1997) 246.
[202] J.L. Wallace, Best Pract. Res. Clin. Gastroent. 14 (2000) 147.
[203] Products in Development; Searle Pty Ltd., 2000, http://www.searlehealthnet.com/searle/proddevl.html.
[204] 'Research and Development: In the Pipeline'; Merck and Co, Inc, Whitehouse Station, NJ, USA, 2001, http://www.merck.-com/newsroom/research_development.html.
[205] S.L. Noble, D.S. King, M.D. Olutade, Am. Family Phys. 61 (2000) 3669.
[206] R. Rowland, in: Cable News Network (CNN), 1999, http://www.cnn.com?HEALTH/9904/20/celebrex.deaths.03.
[207] Editorial, Drug Ther. Perspec. 17 (2001) 6–10.

[208] M. Ostensen, P.M. Villiger, Lupus 9 (2000) 566.
[209] J.A. Mitchell, T.D. Warner, Br. J. Pharmacol. 128 (1999) 1121.
[210] B. Kaplan-Machlis, B.S. Klostermeyer, Ann. Pharmacother. 33 (1999) 979.
[211] H.B. Fung, H.L. Kirschenbaum, Clin. Ther. 21 (1999) 1131.
[212] A.S. Kalgutgar, L.J.Marnet, Patent WO 99-US30219 19991216: Converting COX-inhibiting compounds to derivatives that are selective COX-2 inhibitors as non-steroidal anti-inflammatory drugs, 2000.
[213] A.S. Kalgutkar, A.B. Marnett, B.C. Crews, R.P. Remmel, L.J. Marnett, J. Med. Chem. 43 (2000) 2860.
[214] A.S. Kalgutkar, B.C. Crews, S.W. Rowlinson, A.B. Marnett, K.R. Kozak, R.P. Remmel, L.J. Marnett, A.B. Hancock, Jr., Proc. Natl. Acad. Sci. USA 97 (2000) 925.
[215] C.A. Lanzo, J. Sutin, S. Rowlinson, J. Talley, L.J. Marnett, Biochemistry 39 (2000) 6228.
[216] A.S. Kalgutkar, Expert Opin. Ther. Pat. 9 (1999) 831.
[217] A.S. Kalgutkar, B.C. Crews, S.W. Rowlinson, A.B. Marnett, K.R. Kozak, R.P. Remmel, L.J. Marnett, A.B. Hancock, Jr., Proc. Natl. Acad. Sci. USA 97 (2000) 925.
[218] J.R.J. Sorenson, in: R. Milanino, K.D. Rainsford, G.P. Velo (Eds.), Copper and Zinc in Inflammation, Kluwer Academic Publishers, Dordrecht, 1989, p. 69.
[219] V. Bertrand, F. Guessous, A. Le Roy, B. Viossat, H. Fessi, A. El Abbouyi, J. Giroud, M. Roch-Arveiller, Digest. Dis. Sci. 44 (1999) 991.
[220] S. Kawano, S. Tsuji, J. Gastroenterol. Hepatol. 15 (2000) D1.
[221] M.K. Jones, E. Sasaki, F. Halter, R. Pai, T. Nakamura, T. Arakawa, T. Kuroki, A.S. Tarnawski, FASEB J. 13 (1999) 2186.
[222] D. Vogiagis, E.M. Glare, A. Misajon, W. Brown, P.E. O'Brien, Am. J. Physiol. 278 (2000) G820.
[223] S. Takahashi, N. Kobayashi, S. Okabe, J. Pharmacol. Exp. Ther. 291 (1999) 634.
[224] S. Kato, A. Tanaka, T. Kunikata, H. Mizoguchi, K. Takeuchi, Aliment. Pharmacol. Ther. 14 (2000) 18.
[225] W.H. Sun, M. Tsuji, E.S. Gunawan, H. Sawaoka, N. Kawai, H. Iijima, A. Kimura, Y. Kakiuchi, M. Yasumaru, Y. Sasaki, S. Kawano, H. Hori, J. Gastroenterol. Hepatol. 15 (2000) 752.
[226] M.A. Hull, J.L. Thomson, C.J. Hawkey, Gut 45 (1999) 529.
[227] D.W. Gilroy, P.R. Colville-Nash, D. Willis, J. Chivers, M.J. Paul-Clark, D.A. Willoughby, Nat. Med. 5 (1999) 698.
[228] I. Tegeder, W. Neupert, H. Guhring, G. Geisslinger, J. Pharmacol. Exp. Ther. 292 (2000) 1161.
[229] B.K. Reuter, S. Asfaha, A. Buret, K.A. Sharkey, J.L. Wallace, J. Clin. Invest. 98 (1996) 2076.
[230] H. Mizuno, C. Sakamoto, K. Matsuda, K. Wada, T. Uchida, H. Noguchi, T. Akamatsu, M. Kasuga, Gastroenterology 112 (1997) 387.
[231] A. Schmassman, B.M. Peskar, C. Stettler, P. Netzer, T. Stroff, B. Flogerzi, F. Halter, Br. J. Pharmacol. 123 (1998) 795.
[232] C. Jiang, A.T. Ting, B. Seed, Nature (London) 391 (1998) 82.
[233] M. Ricote, A.C. Li, T.M. Willson, C.J. Kelly, C.K. Glass, Nature (London) 391 (1998) 79.
[234] J.M. Lehmann, J.M. Lenhard, B.B. Oliver, G.M. Ringold, S.A. Kliewar, J. Biol. Chem. 272 (1997) 3406.
[235] N.M. Davies, A.G. Roseth, C.G. Appleyard, W. McKnight, P. Delsoldato, A. Calignano, G. Cirino, Aliment. Pharmacol. Ther. 11 (1997) 69.
[236] K. Takeuchi, K. Suzuki, H. Yamamoto, H. Araki, H. Mizoguchi, H. Ukawa, J. Physiol. Pharmacol. 49 (1998) 501.
[237] S. Fiorucci, L. Santucci, G. Cirino, A. Mencarelli, P. Del Soldato, A. Morelli, J. Immunol. 165 (2000) 5245.
[238] S. Fiorucci, L. Santucci, B. Federici, E. Antolli, E. Distrutti, O. Morelli, G. DiRenzo, G. Coata, G. Cirino, P. Del Soldato, A. Morelli, Aliment. Pharmacol. Ther. 13 (1999) 421.
[239] U. Ravnskov, Br. J. Clin. Pharmacol. 47 (1999) 203.
[240] N.M. Davies, unpublished results (2000).
[241] T. Fujita, N. Ohtani, M. Aihara, K. Nishioka, Y. Fujimoto, J. Pharm. Pharmacol. 39 (1987) 230.
[242] C. van den Branden, B. Ceyssens, D. de Craemer, P. de Bleser, K. Hellemans, A. Geerts, D. Verbleelen, Exp. Nephrol. 8 (2000) 91.
[243] E. Morpurgo, R. Cadrobbi, M. Morpurgo, P. Rigotti, F. Schiavon, O. Schiavon, P. Caliceti, E. Ancona, F.M. Veronese, Transplantation 62 (1996) 1221.
[244] A. Bayati, O. Hellberg, B. Odlind, M. Wolgast, Acta Physiol. Scand. 130 (1987) 367.
[245] M.S. Paller, J.R. Hoidal, T.F. Ferris, J. Clin. Invest. 74 (1984) 1156.
[246] I. Duque, C. Garcia-Escribano, M. Rodriquez-Puyol, M.L. Diez-Marques, J.M. Lopez-Novoa, I. Arribas, L. Hernando, D. Rodriquez-Puyol, Am. J. Physiol. 263 (1992) F466.
[247] M. Leach, S. Frank, A. Olbrich, J. Pfeilschifter, C. Thiemermann, Br. J. Pharmacol. 125 (1998) 817.
[248] M. Noris, G. Remuzzi, Proc. Assoc. Am. Physicians 111 (1999) 602.
[249] E. Titos, J.L. Masferrer, R. Altuna, J.L. Poo, W. Jimenez, V. Arroya, F. Rivera, J. Rodes, Gastroenterology 116 (1999) 1167.
[250] C.P. Vio, C. Cespedes, P. Gallardo, J.L. Masferrer, Hypertension 30 (1997) 687.
[251] E.B. Hart, H. Steenbock, J. Waddell, C.A. Elvehjem, J. Biol. Chem. 77 (1928) 777.
[252] C.M. Elvehjem, Physiol. Rev. 15 (1935) 471.
[253] J.M.C. Howell, J.M. Gawthorne, Copper in Animals and Man, vol. 2, 1st ed., CRC Press, Boca Raton, Florida, 1987.
[254] J.M.C. Howell, J.M. Gawthorne, Copper in Animals and Man, vol. 1, 1st ed., CRC Press, Boca Raton, Florida, 1987.
[255] B.J. Hathaway, Comprehensive Coordination Chemistry. The Synthesis, Reaction, Properties & Application of Coordination Compounds, vol. 5, Pergamon Press, Oxford, 1987, p. 634.
[256] D.W. Cox, Br. Med. Bull. 55 (1999) 544.
[257] R. Uauy, M. Olivares, M. Gonzales, Am. J. Clin. Nutr. 67 (1998) S952.
[258] R.B. Rucker, T. Kosonen, M.S. Clegg, A.E. Mitchel, B.R. Rucjer, J.Y. Uriu-hare, C.L. Keen, Am. J. Clin. Nutrit. 67 (1998) S996.
[259] M.C. Linder, The Biochemistry of Copper, Plenum Press, New York, 1991.
[260] S. Cerone, A. Sansinanea, S. Streitenberger, C. Garcia, N. Auza, Gen. Physiol. Biophys. 19 (2000) 49.
[261] S.S. Percival, Am. J. Clin. Nutr. 67 (1998) S1064.
[262] B. Odell, Nutr. Rev. 51 (1993) 307.
[263] E.D. Rosenstein, J.R. Caldwell, Rheum. Dis. Clin. North Am. 25 (1999) 929.
[264] J.V.H. Forder, Lancet 2 (1974) 1009 (incl.).
[265] Y. Fujimoto, H. Nishida, K. Uchida, Y. Tsuji, S. Sakuma, T. Fujita, J. Clin. Biochem. Nutr. 14 (1993) 45.
[266] I.S. Maddox, Biochim. Biophys. Acta 306 (1973) 74.
[267] P.V. Peplow, P.R. Hurst, Prostaglandins Leukotrienes Med. 21 (1986) 15.
[268] J.R. Turnlund, Am. J. Clin. Nutr. 67 (1998) S960.
[269] J.R. Turnlund, W.R. Keyes, G.L. Peiffer, K.C. Scott, Am. J. Clin. Nutr. 67 (1998) 1219.
[270] R. Wapnir, Am. J. Clin. Nutr. 67 (1998) S1054.
[271] M.C. Linder, L. Wooten, P. Cerveza, S. Cotton, R. Shulze, N. Lomeli, Am. J. Clin. Nutr. 67 (1998) S965.
[272] M.C. Linder, M. Hazegh-Azam, Am. J. Clin. Nutr. 63 (1996) S797.
[273] J.R. Turnlund, W.R. Keyes, H.L. Anderson, L.L. Acord, Am. J. Clin. Nutr. 1989 (1989) 870.
[274] S.C. Luza, H. Speisky, Am. J. Clin. Nutr. 63 (1996) S812.
[275] B. Lonnerdal, Am. J. Clin. Nutr. 63 (1996) S821.

[276] C. Harford, B. Sarkar, in: G. Berthon (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, Marcel Decker, New York, 1995, p. 62.
[277] C.W. Levenson, Am. J. Clin. Nutr. 67 (1998) S978.
[278] E.D. Harris, Y. Qian, E. Tiffany-Castiglioni, A.R. Lacy, M. Reddy, C.M., Am. J. Clin. Nutr. 67 (1998) S988.
[279] P.J. Schmidt, T.D. Rae, R.A. Pufahl, T. Hamma, J. Strain, T.V. O'Halloran, V.C. Culotta, J. Biol. Chem. 274 (1999) 2371.
[280] T.D. Rae, P.J. Schmidt, R.A. Pufahl, V.C. Culotta, T.V. O'Hallaron, Science (Washington, DC) 284 (1999) 805.
[281] B. Sarkar, Chem. Rev. 99 (1999) 2535.
[282] H. Hartmann, K. Felix, W. Nagel, U. Weser, BioMetals 6 (1993) 115.
[283] S.J. Beveridge, I.R. Garrett, M.W. Whitehouse, B. Vernon-Roberts, P. Brooks, Agents Action 17 (1985) 104.
[284] J.R.J. Sorenson, in: K.D. Rainsford, R. Milanino, J.R.J. Sorenson, G.P. Velo (Eds.), Copper and Zinc in Inflammatory Degenerative Diseases, Kluwer Academic Publishers, Dordrecht, 1998, p. 120.
[285] J.E. Weder, T.W. Hambley, B.J. Kennedy, P.A. Lay, D. MacLachlan, R. Bramley, C.D. Delfs, K.S. Murray, B. Moubaraki, B. Warwick, J.R. Biffin, H.L. Regtop, Inorg. Chem. 38 (1999) 1736.
[286] A.T. Florence, D. Attwood, Physiochemical Principles of Pharmacy, Macmillan, London, 1998.
[287] J. Laussac, B. Sarkar, J. Biol. Chem. 255 (1980) 7563.
[288] D.W. Appleton, B. Sarkar, J. Biol. Chem. 246 (1971) 5040.
[289] J. Masuoka, P. Saltman, J. Biol. Chem. 269 (1994) 25557.
[290] P. Predki, C. Harford, P. Brar, B. Sarkar, Biochem. J. 287 (1992) 211.
[291] M. Valko, H. Morris, M. Mazur, J. Telser, E.L.J. McInnes, F.E. Mabbs, J. Phys. Chem. B 103 (1999) 5591.
[292] S. Panwen, Z. Yingqia, W. Sheyi, C. Yunxia, Inorg. Chim. Acta 169 (1990) 161.
[293] T. Peters, Adv. Prot. Chem. 37 (1985) 161.
[294] H. Liang, B. Xin, X. Wang, Y. Yuan, Y. Zhou, P. Shen, Chin. Sci. Bull. 43 (1998) 404.
[295] M. Valko, M. Mazur, H. Morris, J. Telser, E.J.L. McInnes, F.E. Mabbs, Monogr. Ser. Int. Conf. Coord. Chem. 4 (1999) 339.
[296] S.J. Lau, B. Sarkar, J. Biol. Chem. 246 (1971) 5938.
[297] S. Lau, B. Sarkar, Can. J. Chem. 53 (1975) 710.
[298] B. Sarkar, Y. Wigfield, Can. J. Biochem. 46 (1968) 601.
[299] I. Zaitseva, V. Zaitsev, G. Card, K. Moshkov, B. Bax, A. Ralph, P. Lindley, J. Biol. Inorg. Chem. 1 (1996) 15.
[300] P.F. Lindley, G. Card, I. Zaitseva, V. Zaitsev, B. Reinhammer, E. Selin-Lingren, K. Yoshida, J. Biol. Inorg. Chem. 2 (1997) 454.
[301] P.F. Lindley, I. Zaitseva, V. Zaitseva, G. Card, K. M.oshkov, B. Bax, Multi-Copper Oxidases 4 (1997) 81.
[302] I. Zaitseva, V. Zaitsev, G.L. Card, K. Moshkov, B. Bax, A. Ralph, P.F. Lindley, in: X-ray Crystal Structure Ceruloplasmin at 3.0 Angstroms; Protein Data Bank Brookhaven National Laboratory, 1996, http://www.pdb.bnl.gov-bn/pdbibs.
[303] E.D. Harris, Proc. Soc. Exp. Biol. Med. 162 (1991) 130.
[304] E.L. Saenko, A.I. Yaropolov, E.D. Harris, J. Trac. Elem. Exp. Med. 7 (1994) 69.
[305] S.J. Lippard, Science (Washington, DC) 284 (1999) 748.
[306] L.T. Hall, R.J. Sanchez, S.P. Holloway, H. Zhu, J.E. Stine, T.J. Lyons, B. Demeler, V. Schirf, J.C. Hansen, A.M. Nersissian, J.S. Valentine, P.J. Hart, Biochemistry 39 (2000) 3611.
[307] A.L. Lamb, A.K. Wernimont, R.A. Pufahl, V.C. Culotta, T.V. O'Halloran, A.C. Rosenzweig, Nat. Struct. Biol. 6 (1999) 724.
[308] P.J. Schmidt, M. Ramos-Gomez, V.C. Culotta, J. Biol. Chem. 274 (1999) 36952.
[309] W.R. Walker, D.M. Keats, Agents Action 6/4 (1976) 454.
[310] P.C. Freeman, P.O. O'Callaghan, Br. J. Pharmacol. 90 (1987) 49P.
[311] I.L. Bonta, M.J. Parnham, J.E. Vincent, P.C. Bragt, in: G.P. Ellis, G.B. West (Eds.), Progress in Medicinal Chemistry, North Holland Biomedical Press, North Holland, 1980, p. 185.
[312] R. Milanino, K.D. Rainsford, G.P. Velo (Eds.), Copper and Zinc in Inflammation, Kluwer Academic Publishers, Dordrecht, 1989.
[313] R. Milanino, A. Frigo, L.M. Bambara, M. Marrella, U. Moretti, M. Pasquialiccio, D. Biasi, R. Gasperini, L. Maintenti, G.P. Velo, Clin. Exp. Rheumatol. 11 (1993) 271.
[314] R. Milanino, U. Mauro, M. Marrella, M. Pasqualicchio, R. Gasperini, G. Velo, in: G. Berthon (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, Marcel Decker, New York, 1995, p. 886.
[315] W.R. Walker, S.J. Beveridge, M.W. Whitehouse, Agents Action 10 (1980) 38.
[316] J.R.J. Sorenson, in: J.R.J. Sorenson (Ed.), Biology of Copper Complexes, Humana Press, Clifton, New Jersey, 1987, p. 3.
[317] S. Ahmad, Oxidative Stress and Antioxidant Defenses in Biology, 1st ed., Chapman & Hall, New York, 1995.
[318] M.B. Grisham, D. Jourd'Heuil, D.A. Wink, Am. J. Physiol. 276 (1999) G315.
[319] D.E. Auer, J.C. Ng, A.A. Seawright, Equine Vet. J. 22 (1990) 13.
[320] D. Auer, in: Free Radicals, Inflammation and Copper: Conference Proceedings, 11th Bain-Fallon Memorial Lecture, Australian Equine Veterinary Association, Coffs Harbour, Australia, 1989.
[321] G. Zubay (Ed.), Biochemistry, Addison-Wesley, Reading, 1986.
[322] O.I. Aruoma, J. Am. Oil Chem. Soc. 75 (1998) 199.
[323] G. Czapski, S. Goldstein, Free Radic. Res. Commun. 4 (1988) 225.
[324] G.V. Buxton, Q.G. Mulazzani, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 6, Marcel Dekker, New York, 1999, p. 103.
[325] A.M. Miles, D.S. Bohle, P.A. Glassbrenner, B. Hansert, D.A. Wink, M.B. Grisham, J. Biol. Chem. 271 (1996) 40.
[326] H.R. Rubbo, R. Radi, M. Trujillo, R. Telleri, B. Kalyanaraman, S. Barnes, M. Kirk, A. Freeman, J. Biol. Chem. 269 (1994) 26066.
[327] D.A. Wink, R.W. Nims, J.E. Saavedra, W.E. Utermahlen, P.C. Ford, Proc. Nat. Acad. Sci. USA 91 (1994) 6604.
[328] D.A. Wink, C.B. Wink, R.W. Nims, P.C. Ford, Environ. Health Perspect. 102 (1994) 11.
[329] G. Berthon, Agents Action 39 (1993) 210.
[330] H. Miche, V. Brumas, G. Berthon, J. Inorg. Biochem. 68 (1997) 27.
[331] B. Halliwell, J.R. Hoult, D.R. Blake, Fed. Am. Soc. Exp. Biol. J. 2 (1988) 2867.
[332] K.V. Chace, R. Carubelli, R.E. Nordquist, J.J. Rowsey, Free Radic. Res. Commun. 12-13 (1991) 591.
[333] C.L. Hawkins, M.J. Davies, Biochim. Biophys. Acta 1360 (1997) 84.
[334] D.E. Auer, Ph.D. Thesis, Copper, Inflammation and Copper Containing Anti-inflammatory Drugs in the Horse, University of Queensland, Brisbane, 1987.
[335] O. Sergent, I. Morel, J. Cillard, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 6, Marcel Dekker, New York, 1999, p. 251.
[336] J.W. Heinecke, in: G. Berthon (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, Marcel Decker, New York, 1995, p. 986.
[337] S.R. Turner, J.A. Campbell, W.S. Lynn, J. Exp. Med. 141 (1975) 1437.
[338] J.R.J. Sorenson, V. Kishore, A. Pezeshk, L.W. Oberley, S.W.C. Leuthauser, Inorg. Chim. Acta 91 (1984) 285.
[339] U. Weser, K.H. Sellinger, E. Lengfelder, W. Werner, J. Strahle, Biochim. Biophys. Acta 631 (1980) 232.
[340] W. Hangarter, A. Lubke, Dtsch. Wochenschr. 77 (1952) 870.

[341] I.L. Bonta, Acta Physiol. Pharmacol. Neerl. 15 (1969) 188.
[342] K.D. Karlin, J. Zubieta, in: R.K. Crouch, T.W. Kensler, L.A. Oberley, J.R.J. Sorenson (Eds.), Biological and Inorganic Copper Chemistry, Adenine, New York, 1986, p. 139.
[343] D. Frechilla, B. Lasheras, M. Ucelay, E. Parrondo, G. Craciunescu, E. Cenarruzageitia, Arzneim.-Forsch. 40 (1990) 914.
[344] L. Franco, D. Doria, Pharmacol. Res. 36 (1997) 395.
[345] E. Fenz, Munch. Med. Wochenschschr. 88 (1941) 1101.
[346] J.M. Forestier, A. Certonciny, Presse. Med. 64 (1946) 884.
[347] J.R.J. Sorenson, W. Hangarter, Inflammation (NY) 2 (1977) 217.
[348] F. Fernandez-Madrid, in: R. Milanino, K.D. Rainsford, G.P. Velo (Eds.), Copper and Zinc in Inflammation, Kluwer Academic Publishers, Dordrecht, 1989, p. 49.
[349] J. Schubert, Sci. Am. 214 (1966) 40.
[350] M.B. Chenoweth, Pharmacol. Rev. 8 (1956) 57.
[351] M.W. Whitehouse, Prog. Drug. Res. 8 (1965) 321.
[352] F.P. Dwyer, E.C. Gyarfas, W.P. Rogers, J.H. Koch, Nature (London) 170 (1952) 190.
[353] F.P. Dwyer, E. Mayhew, E.M.F. Roe, A. Shulman, Br. J. Cancer 19 (1965) 195.
[354] L.W. Oberley, G.R. Buettner, Cancer Res. 39 (1979) 1141.
[355] P. Kögerler, P.A.M. Williams, B.S. Parajón-Costa, E.J. Baran, L. Lezama, T. Rojo, A. Müller, Inorg. Chim. Acta 268 (1998) 239.
[356] C. Dendrinou-Samara, D.P. Kessissoglou, G.E. Manoussakis, D. Mentzafos, A. Terzis, J. Chem. Soc. Dalton Trans. (1990) 959.
[357] A.L. Abuhijleh, J. Inorg. Biochem. 55 (1994) 255.
[358] R.G. Bhirud, T.S. Srivastava, Inorg. Chim. Acta 173 (1990) 121.
[359] M. Melnik, I. Potocnak, L. Macaskova, D. Miklos, C.E. Holloway, Polyhedron 15 (1996) 2159.
[360] C. Dendrinou-Samara, P.D. Jannakoudakis, D.P. Kessissoglou, G.E. Manoussakis, D. Mentzafos, A. Terzis, J. Chem. Soc. Dalton Trans. (1992) 3259.
[361] A.E. Underhill, S.A. Bougourd, M.L. Flugge, S.E. Gale, P.S. Gomm, J. Inorg. Biochem. 52 (1993) 139.
[362] J.E. Weder, B.J. Kennedy, P.A. Lay, T.W. Hambley, G.J. Foran, A.M. Rich, Inorg. Chem. 40 (2001) 1295.
[363] J. Mrozinski, E. Heyduk, M. Korabik, Bull. Pol. Acad. Sci. Chem. 42 (1994) 169.
[364] R. Cini, Comments Inorg. Chem. 22 (2000) 151.
[365] P. de Meester, S.R. Fletcher, A.C. Skapski, J. Chem. Soc. Dalton Trans. 23 (1973) 2575.
[366] Cambridge Crystallographic Data Centre; http://www.ccdc.cam.ac.uk/.
[367] J.N. van Niekerk, F.R.L. Schoening, Acta Crystallogr. 6 (1953) 227.
[368] S.F.A. Kettle, Physical Inorganic Chemistry. A Coordination Chemistry Approach, 1st ed., Spektrum Academic Publishers, Oxford, 1996.
[369] F. Guessous, J.C. Daran, B. Viossat, G. Morgant, X. Labouze, A. Leroy, M. Roch-Arveiller, N.-H. Dung, Met.-Based Drugs 5 (1998) 337.
[370] G.H. Aylward, T.J.V. Findlay (Eds.), SI Chemical Data, 2nd ed., Wiley, New York, 1971.
[371] B.J. Hathaway, D.E. Billing, Coord. Chem. Rev. 5 (1970) 143.
[372] M. Melnik, Coord. Chem. Rev. 36 (1981) 1.
[373] F.T. Greenaway, A. Pezeshk, A.W. Cordes, M.C. Noble, J.R.J. Sorenson, Inorg. Chim. Acta 92 (1984) 67.
[374] A.L. Abuhijleh, Polyhedron 8 (1989) 2777.
[375] N.E. Heimer, I.Y. Ahmed, Inorg. Chim. Acta 64 (1982) L65.
[376] I.Y. Ahmed, A.L. Abuhijleh, Inorg. Chim. Acta 61 (1982) 241.
[377] A.L. Abuhijleh, C. Woods, I.Y. Ahmed, Inorg. Chim. Acta 190 (1991) 11.
[378] R.J. Doedens, Prog. Inorg. Chem. 21 (1976) 209.
[379] F. Valach, M. Tokarcik, P. Kubinex, M. Melnik, L. Macaskova, Polyhedron 16 (1997) 1461.
[380] R. Cini, G. Giorgi, A. Cinquantini, C. Rossi, M. Sabat, Inorg. Chem. 29 (1990) 5197.
[381] L. Manojilovic-Muir, J. Chem. Soc. Chem. Commun. 2 (1967) 1057.
[382] Y.R. Morgan, P. Turner, B.J. Kennedy, T.W. Hambley, P.A. Lay, J.R. Biffin, H.L. Regtop, B. Warwick, Inorg. Chim. Acta 324 (2001) 149.
[383] Y. Morgan, Personal Communication (2001).
[384] R.Y. Morgan, B.J. Kennedy, T.W. Hambley, P.A. Lay, J.E. Weder, G.J. Foran, J.R. Biffin, H.L. Regtop, to be submitted (2002).
[385] J.G.L. Baquial, J.R.J. Sorenson, J. Inorg. Biochem. 60 (1995) 133.
[386] S.T. Shuff, P. Chowdhary, M.F. Khan, J.R.J. Sorenson, Biochem. Pharmacol. 43 (1992) 1601.
[387] U. Weser, L.M. Schubotz (Eds.), Catalytic Reaction of Copper Complexes with Superoxide, Birkhauser Verlag, Basel, Boston, Stuttgart, 1981.
[388] S. Moncada, M.W. Radomski, R.M.J. Palmer, Biochem. Pharmacol. 37 (1988) 2495.
[389] S. Moncada, R.M.J. Palmer, E.A. Higgs, Pharmacol. Rev. 43 (1991) 109.
[390] S. Moncada, M.A. Marletta, J.J.B. Hibbs, E.A. Higgs, The Biology of Nitric Oxide; Proceedings of the International Meeting, vol. 1 & 2, Portland Press, London, 1992.
[391] D.A. Wink, J.B. Mitchell, Free Radic. Biol. Med. 25 (1998) 434.
[392] T.S. Hakim, K. Sugimori, E.M. Camporesi, G. Anderson, Physiol. Meas. 17 (1996) 267.
[393] L. Viinika, Scan. J. Clin. Lab. Invest. 56 (1996) 577.
[394] A.M. Dondorp, T. Planche, E.E. Debel, B.J. Angus, K.T. Chotivanich, K. Silamut, J.A. Romijn, R. Ruangveerayuth, F.J. Hoek, P.A. Kager, J. Vreeken, N.J. White, Am. J. Trop. Med. Hyg. 59 (1998) 497.
[395] M. Moenkhoff, B. Schmidt, G. Wohlrab, K. Waldvogel, K. Fanconi, O. Baenziger, Crit. Care Med. 26 (1998) 1887.
[396] K.A. Boggess, T.D. Oury, H.H. Kay, J.D. Crapo, Placenta 19 (1998) 417.
[397] J.J. Lancaster (Ed.), Nitric Oxide Principles and Action, Academic Press, San Diego, CA, 1996.
[398] X. Liu, M.J.S. Miller, M.S. Joshi, H. Sadowska-Krowicka, D.A. Clark, J.R. Lancaster, Jr., J. Biol. Chem. 273 (1998) 18709.
[399] A.M. Miles, D.S. Bohle, P.A. Glassbrenner, B. Hansert, D.A. Wink, M.B. Grisham, J. Biol. Chem. 271 (1996) 40.
[400] W.H. Koppenol, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 6, Marcel Dekker, New York, 1999, p. 597.
[401] T. Koppal, J. Draje, S. Yatin, B. Jordan, S. Varadarajan, L. Bettenhausen, D.A. Butterfield, J. Neurochem. 72 (1999) 310.
[402] L. Virag, D.L. Marmer, C. Szabo, Free Radic. Biol. Med. 25 (1998) 1075.
[403] P. Kubes, J.L. Wallace, Med. Inflam. 4 (1995) 397.
[404] B.J.R. Whittle, Br. J. Pharmacol. 110 (1993) 3.
[405] D. Salvemini, T.P. Misko, K. Masferrer, K. Seibert, M.G. Currie, P. Needleman, Proc. Natl. Acad. Sci. USA 90 (1993) 7240.
[406] G. Arena, G. Kavu, D.R. Williams, J. Inorg. Nucl. Chem. 40 (1978) 1221.
[407] H.R. Adams, Veterinary Pharmacology and Therapeutics, 7th ed., Iowa State University Press, 1995, p. 443.
[408] G.O. Ewing, J. Am. Vet. Med. Assoc. 161 (1972) 1665.
[409] R. Menguy, L. Desbaillets, Am. J. Digest. Dis. 12 (1967) 862.
[410] S.-Y. Lee, A.A. Belmonte, J. Pharm. Sci. 83 (1994) 1107.
[411] F.T. Greenaway, J.J. Hahn, N. Xi, R.J. Sorenson, BioMetals 11 (1998) 21.
[412] X.M. He, D.C. Carter, Nature (London) 358 (1992) 209.

[413] D.C. Carter, X.M. He, Science (Washington, DC) 249 (1990) 302.
[414] J.E. Weder, Ph.D. Thesis, Characterisation of Cu(II) Dimers of the Non-steroidal Anti-inflammatory Drug Indomethacin, University of Sydney, Sydney, 2000.
[415] J.E. Weder, P.A. Lay, T.W. Hambley, B.J. Kennedy, G.J. Foran, J.R. Biffin, H.L. Regtop, to be submitted (2002).
[416] S.J. Beveridge, W.R. Walker, M.W. Whitehouse, J. Pharm. Pharmacol. 32 (1980) 425.
[417] L. David, O. Cosar, V. Chis, A. Negoescu, I. Vlasin, Appl. Mag. Res. 6 (1994) 521.
[418] D. Kovala-Demertzi, A. Theodorou, M.A. Demertzis, C.P. Raptopoulou, A. Terzis, J. Inorg. Biochem. 65 (1997) 151.
[419] D. Kovala-Demertzi, J. Inorg. Biochem. 79 (2000) 153.
[420] J.R.J. Sorenson, L.W. Oberley, R.K. Crouch, T.W. Kensler, V. Kishore, S.W.C. Leuthauser, T.D. Oberley, A. Pezeshk, Biol. Trace Elem. Res. 5 (1983) 257.
[421] S.F. Forsyth, W.G. Guilford, C.R.O. Lawoko, NZ Vet. J. 44 (1996) 179.
[422] J.M. McCord, Science (Washington, DC) 185 (1974) 529.
[423] P. Netter, B. Bannwarth, M.-J. Royer-Morrot, Clin. Pharmacokinet. 17 (1989) 145.
[424] D.E. Auer, J.C. Ng, A.A. Seawright, J. Vet. Pharmacol. Ther. 13 (1990) 59.
[425] M. Younes, U. Weser, FEBS Lett. 61 (1976) 209.
[426] J.M. McCord, I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[427] Z. Durackova, J. Labuda, J. Inorg. Biochem. 58 (1995) 297.
[428] J.M.C. Gutteridge, Med. Biol. 62 (1984) 101.
[429] U. Weser, C. Richter, A. Wendel, M. Younes, Bioinorg. Chem. 8 (1978) 201.
[430] A.L. Abuhijleh, J. Inorg. Biochem. 68 (1997) 167.
[431] G. Tabbi, W.L. Driessen, J. Reedijk, R.P. Bonomo, N. Veldman, A.L. Spek, Inorg. Chem. 36 (1997) 1168.
[432] J. Casanova, G. Alzuet, J. Borras, J. Latorre, M. Sanau, S. Garcia-Granda, J. Inorg. Biochem. 60 (1995) 219.
[433] J.-L. Pierre, P. Chautemps, S. Refaif, C. Beguin, A. El Marzouki, G. Serratrice, E. Saint-Aman, P. Rey, J. Am. Chem. Soc. 117 (1995) 1965.
[434] A. Gartner, U. Weser, Top. Curr. Chem. 132 (1986) 1.
[435] N.A. Roberts, P.A. Robinson, Br. J. Rheumatol. 24 (1985) 128.
[436] U. Deuschle, U. Weser, Inorg. Chim. Acta 91 (1984) 237.
[437] J.R. Harrison, D.P. Rillema, J.H. Ham, IV, J.J. Sando, Cancer Res. 46 (1986) 5571.
[438] M. Younes, U. Weser, Biochem. Biophys. Res. Commun. 78 (1977) 1247.
[439] C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay, Q. Zhou, N.M. Davies, J.R. Biffin, H. Regtop, J. Biol. Inorg. Chem. Chem. Res. Toxicol., submitted.
[440] U. Deuschle, U. Weser, Inorg. Chim. Acta 107 (1985) 275.
[441] C.-L. O'Young, S.J. Lippard, J. Am. Chem. Soc. 102 (1980) 4920.
[442] A. Bury, A.E. Underhill, D.R. Kemp, N.J. Oshea, J.P. Smith, P.S. Gomm, Inorg. Chim. Acta - Bioinorg. Chem. 138 (1988) 85.
[443] D. Frechilla, B. Lasheras, M. Ucelay, E. Parrondo, G. Craciunescu, E. Cenarruzabeitia, Arzneim.-Forsch. 40 (1990) 1008.
[444] D.A. Williams, D.T. Walz, W.O. Foye, J. Pharm. Sci. 65 (1976) 126.
[445] M. Blahova, I. Tumova, J. Sokolik, M. Gallasova, P. Svec, Pharmazie 49 (1994) 373.
[446] G. Mohan, R. Nagar, Ind. J. Pharmacol. 24 (1992) 207.
[447] A. Andrade, S.F. Namora, R.G. Woisky, G. Wiezel, R. Najjar, J.A.A. Sertie, D. de Oliveira Silva, J. Inorg. Biochem. 81 (2000) 23.
[448] K. Takeuchi, S. Ueki, S. Okabe, Digest. Dis. Sci. 31 (1986) 1114.
[449] K. Ohe, H. Yokoya, T. Kitura, T. Kunita, A. Miyoshi, Digest. Dis. Sci. 25 (1980) 849.
[450] R. Melarange, C. Gentry, M. Durie, C. O'Connell, P.R. Blower, Digest. Dis. Sci. 39 (1994) 601.
[451] N.A. Shackel, R.O. Day, B. Kellet, P.M. Brooks, Med. J. Aust. 167 (1997) 134.