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Oxicams, a Class of NSAIDs and beyond

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Abstract

Oxicams are a class of non-steroidal anti-inflammatory drugs (NSAIDs) structurally related to the enolic acid class of 4-hydroxy-1,2-benzothiazine carboxamides. They are used clinically to treat both acute and chronic inflammation by inhibiting the activity of the two cyclooxygenase (COX) isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Oxicams are structurally distinct from all other NSAIDs, exhibiting a novel binding pose in the COX channel. The 4-hydroxyl group on the thiazine ring partners with Ser-530 via hydrogen bonding while two coordinated water molecules mediate a polar interaction between the oxicam and COX. The rotation of Leu-531 in the complex opens a new pocket, which is not utilized for binding other NSAIDs to the enzyme. This structure provides the basis for understanding documented structure-activity relationships (SAR) within the oxicam class. In addition, from the oxicam template, a series of potent microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors represents a new direction for drug development. Here, we review the major route of oxicam synthesis and SAR for COX inhibition, as well as recent advances in oxicam-mediated mPGES-1 inhibition.

Keywords

oxicams; NSAIDs; COX; mPGES-1; anti-inflammatory activity; drug R&D

Introduction

Products of the prostaglandin (PG) biosynthetic pathway (Fig. 1) are chief physiological mediators of both acute and chronic inflammation (1,2). Two COX isoforms, COX-1 and COX-2, are the rate-limiting enzymes in this pathway (1,2). The product of the COXs, prostaglandin H₂ (PGH₂), is rapidly converted into bioactive PGs through the activity of various synthase enzymes. PGE₂, which plays a particularly important role in the inflammatory response, is synthesized by three different enzymes, microsomal PGE₂ synthase 1 (mPGES-1), cytosolic PGE₂ synthase (cPGES) and microsomal PGE₂ synthase 2 (mPGES-2) (Fig. 1). The latter two enzymes are structurally and biologically distinct from mPGES-1, and mPGES-1 is the major enzyme responsible for PGE₂ biosynthesis during the

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inflammatory response (3). During inflammation, COX-2 and mPGES-1 are rapidly induced within hours by pro-inflammatory cytokines in macrophages and fibroblasts (4,5). Therefore, both enzymes are well-recognized targets for the research and development of anti-inflammatory drugs (3).

The importance of PG biosynthesis in inflammation is illuminated by the fact that the non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used to treat inflammation, fever, and pain, act primarily by blocking the COX enzymes (2). Among many NSAIDs marketed for human and veterinary use, the great majority contain a carboxylic acid functional group (Fig. 2), including aspirin, indomethacin, diclofenac, mefenamic acid, and all “profens”, such as ketoprofen, flurbiprofen, naproxen, and ibuprofen. Exceptions to this general rule include phenylbutazone and the oxicams, which do not contain a carboxyl group. It is notable that phenylbutazone served as the scaffold for the discovery of DuP-697, laying the foundation for the development of the COX-2-selective diarylheterocycles, celecoxib, rofecoxib, and valdecoxib, which also lack a carboxyl group (Fig. 2) (6).

The term “oxicam” was chosen by the United States Adopted Names Council to describe NSAIDs belonging to the enolic acid class of 4-hydroxy-1,2-benzothiazine 3-carboxamide derivatives, which share the fewest structural elements with other NSAIDs (Fig. 2). Extensive research by the Pfizer group to discover new, potent, non-carboxylic acid-containing inflammatory agents led to the development of the oxicams (7-10). The first member of this class, piroxicam, was introduced in the United States in 1982 as Feldene (Pfizer) and gained immediate acceptance in the market, becoming one of the top 50 prescribed drugs for several years. After piroxicam, other oxicams, including isoxicam, meloxicam, tenoxicam, and lornoxicam, were introduced. Recently, interest in this class of NSAIDs has increased due to the discovery that some oxicam derivatives are potent inhibitors of mPGES-1.

The primary aim of this review is to cover current knowledge of oxicam chemistry and pharmacology, including chemical synthetic routes, structure-activity relationships (SAR) for COX inhibition, and the recent evidence of oxicam-dependent inhibition of mPGES-1.

Discovery of Oxicams and Chemical Synthesis

Structurally, oxicams belong to a subgroup of the 1,2-benzothiazine class of compounds, which were first synthesized by Braun as early as 1923 (11). Thousands of benzothiazine derivatives have been evaluated and found biologically active as analgesic, antipyretic, hypoglycemic, anti-hypertensive, or anti-inflammatory agents (12). The first oxicams were synthesized in the 1950s with the application of the Gabriel-Colman rearrangement of phthalimides, giving 3-benzoyl-2*H*-1,2-benzothiazin-4(3*H*)-one-1,1-dioxide, but anti-inflammatory activity of this compound was not reported (13).

Carboxylate-containing NSAIDs are susceptible to facile elimination by glucuronide and/or sulfate conjugation, leading to a short plasma half-life, a characteristic that is obviously undesirable for the long-term treatment of diseases such as chronic arthritis (14). Thus, Lombardino and his colleagues at Pfizer initiated a quest for new non-carboxylic acid-

containing anti-inflammatory agents (8). The initial lead was a series of 2-aryl-1,3-indandiones, which demonstrated anti-inflammatory activities with co-existing anticoagulant activity and a short half-life in the dog (Scheme 1) (15). Anti-inflammatory potency in this series further correlated with acidity, with the most active compounds demonstrating pK_a values in the range of 4 to 5.6. Further exploration of the anti-inflammatory activities of structurally related 2-arylbenzo[*b*]thiophene-3(2H)-one 1,1-dioxides (16) and 1,3(2*H*,4*H*)-dioxisoquinoline-4-carboxanilides (17) revealed compounds with similar potency to that of phenylbutazone, with extended plasma half-life in animal models. The related scaffold, 3,4-dihydro-2-alkyl-3-oxo-2*H*-1,2-benzothiazine-4-carboxamide 1,1-dioxide, which contained an isosteric heterocyclic replacement of the dioxisoquinoline nucleus, provided an even longer plasma half-life and potent anti-edema activity (7,18,19). As this series of 4-carboxamides was being developed, compounds based on a 2-alkyl-4-hydroxy-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide scaffold were also synthesized to fully develop the SAR for the 1,2-benzothiazine 1,1-dioxide nucleus (10). Isoelectric replacements of the carboxamide of the first 'oxicam', CP-14304, led to the discovery of piroxicam, and later oxicams, such as isoxicam, lornoxicam, and sulfoxicam (8,20). Even though most oxicams are non-selective COX-1 and COX-2 inhibitors, meloxicam, which was introduced in 2000, is a moderately selective COX-2 inhibitor that is used for the treatment of various arthritic conditions and post-operative inflammation (21).

Lombardino and colleagues (7) established a generalized synthetic method for oxicams, starting with a Gabriel-Colman rearrangement of 3-oxo-1,2-benzisothiazoline-2-acetic acid methyl ester 1,1-dioxide, adapted from the work of Abe and coworkers (13). This reaction of the benzisothiazoline with sodium methoxide in dimethylsulfoxide gave a 3 carboxylic ester of 4-hydroxy-2*H*-1,2-benzothiazine 1,1-dioxide (Scheme 2). N-alkylation at the 2 position of the benzothiazine nucleus followed by replacement of the ester with an amine under forcing conditions provided the desired 3-carboxamides of 4-hydroxy-2-alkyl-2*H*-1,2-benzothiazine 1,1-dioxide. This route proved to be more versatile than the original route, which involved reaction of 4-keto-2-methyl-2*H*-1,2-benzothiazine 1,1-dioxide with an isocyanate to form the 3-carboxamide (7).

Oxicams Bind to the COX Active Site Differently from Other NSAIDs

Both COX-1 and COX-2 are sequence homodimers, of which each monomer comprises an N-terminal epidermal growth factor-like domain, a four helical membrane-binding domain, and a relatively large catalytic domain. The catalytic domain contains two separate but functionally linked active sites, the COX active site and the peroxidase active site. Most NSAIDs, including oxicams, bind to the COX site and inhibit the activity of both COX-1 and COX-2, thereby blocking the synthesis of thromboxane and prostaglandins (2). The COX site is an L-shaped hydrophobic channel that penetrates the center of the protein. At the mouth of the channel is a large opening, often referred to as the lobby, followed by a constriction through which substrates and inhibitors must pass. The substrate AA has been found to exhibit two conformations in the COX site (22-24). The productive conformation in which the carboxylic acid group of AA forms hydrogen-bonding interactions with Arg-120 and Tyr-355 at the constriction (22,23), and the 13-pro-*S*-hydrogen of AA is a short distance away from the critical catalytic residue, Tyr-385, which abstracts this hydrogen atom in the

first step of the reaction. In the non-productive conformation, the carboxylic acid group of AA interacts with Tyr-385 and Ser-530 above the constriction site, and the remainder of the molecule projects toward the lobby, precluding abstraction of any of AA's allylic hydrogen atoms by Tyr-385 (24). Both sets of residues that form hydrogen bonds with AA have also been found to interact with carboxylic acid-containing NSAIDs bound to COX-1 or COX-2 (25-32). In contrast, COX-2-selective diarylheterocycles, such as celecoxib and rofecoxib, exhibit an alternative binding pose with insertion of their sulfur-containing substituent into a larger COX-2 specific side-pocket in addition to interactions with the main active site channel (27,28,32).

The structural basis for the binding of oxicams to COX was unknown until recently (33). Two independent computational models suggested that meloxicam and tenoxicam might bind to the COX site in a mode that places the benzothiazine ring adjacent to the catalytic Tyr-385 and the 3-carboxamide right above the constriction at the entrance of the active site (34,35). However, both predictions failed to explain our early observation that the S530A, R120A, and Y355F COX-2 mutants were not inhibited by piroxicam (29).

Recently, crystal complexes of COX-1:meloxicam, COX-2:meloxicam, and COX-2:isoxicam from our group disclosed that oxicams bind in the COX active site in a strikingly different manner than that of other reported NSAIDs (Fig. 3). (33). In the COX active site, the oxicams display a planar conformation that features an intramolecular hydrogen bond between the nitrogen atom of the carboxamide and the 4-hydroxyl group of the benzothiazine. Oxicams bind to the active site channel through numerous hydrophobic interactions and a single immediate hydrogen bond between the oxycam 4-hydroxyl group and Ser-530 of the enzyme. Interestingly, two highly ordered water molecules in the active site provide additional polar bridges between oxicams and COX residues at the catalytic apex and the constriction site (Fig. 3). The first of these mediates hydrogen bonding between the oxycam benzothiazine ring and the side chains of Tyr-355 and Arg-120 at the constriction site; the second bridges a hydrogen bonding network between two critical residues (Tyr-385 and Ser-530) at the apex of the active site and the heteroatom of the 3-carboxamide substituent. These findings demonstrate a critical role for Ser-530, Tyr-355, and Arg-120 in oxycam binding, thereby explaining why mutation of these residues leads to loss of oxycam inhibitory potency.

Compared to other COX:NSAID complexes, the side chain of Leu-531 in the COX:oxycam complexes displays a different rotamer (Fig. 3 in red) with a CH- π interaction between Leu-531 and the fused phenyl ring from the oxycam benzothiazine nucleus. This rotation opens a new hydrophobic pocket composed of Met-113, Val-116, Leu-117, Ile-345, Val-349, Leu-531, Leu-534, and Met-535, which had not previously been recognized and explored for drug development. Surprisingly, the sulfonyl dioxide of the benzothiazine ring, the hypothesized binding candidate for interaction with Tyr-385 and Ser-530 in prior simulations (34,35), is located approximately 3 Å above the constriction site and at a distance of 3.7 Å to the backbone oxygen of Ala-527, while the other oxygen of the dioxide sterically interferes with the side chain of Val-116.

The complexes of meloxicam bound to COX-1 and COX-2 suggested an overall similar binding mode as was seen with isoxicam in COX-2. However, two conformations of the 3-carboxamide thiazole ring of the inhibitor were suggested. Both conformations form a similar hydrogen-bonding network between a coordinated water molecule and the catalytic apex and are consistent with the principles of bonding interactions (Fig. 3B). As noted above, meloxicam displays an approximately 6-fold selectivity for COX-2 over COX-1. Site-specific mutagenesis studies demonstrated that the inhibitory potency of meloxicam for a V434I mutant of COX-2 was similar to its potency for COX-1. Comparison of the crystal structures of meloxicam complexed to COX-1 and COX-2 revealed that the presence of isoleucine in this position, as is found in COX-1, forces Phe-518 into the active site channel, providing less space for meloxicam to bind than is available when valine is present in this position, as is found in COX-2. Thus, the two crystal structures provide some insight into the semi-selectivity of meloxicam towards COX-2 inhibition (33).

Structural Foundation for the SAR of Oxicam-Dependent COX Inhibition

The SAR of oxicams has been extensively explored for optimization of anti-inflammatory activity, mainly during the first decades when the class of NSAIDs was introduced (7,9,10,18,19,36,37). As most of these experiments were conducted before the discovery of the importance of PGs and COX in inflammation, pharmacological models without *in vitro* experiments were utilized to carry out SAR investigations.

It was recognized in the very early stages of oxicam development that, among over 50 analogs, compounds bearing a methyl substituent at the 2-position of the benzothiazine ring exhibited the best anti-inflammatory activity (7). The recent crystal structures of COX:oxicam complexes confirmed, for the first time, that this methyl group fits, via hydrophobic interactions, into a small pocket comprising Val-349, Tyr-355, and Leu-359. Consistently replacement of this methyl group with a bulkier substituent (ethyl, propyl, benzyl, allyl) results in loss of activity (7), presumably due to a steric clash in the pocket, while the removal of the 2-methyl group also diminishes the activity by eliminating the hydrophobic interactions with the protein residues in this region (7,37). Similar SAR at the 2-position of the benzothiazine ring was found for the more recently discovered 4-hydroxy-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide class of oxicams (36) suggesting that these inhibitors bind to COX in the same mode as that observed in the COX:oxicam complexes.

As indicated in the COX:oxicam crystal structures, the 3-carboxamide substituent is surrounded by Leu-384, Tyr-385, Trp-387, Phe-518, and Met-522. Compounds containing rigid hydrophobic moieties, such as substituted anilides and some heterocyclic ring systems were more potent anti-inflammatory agents than those bearing flexible alkyl substituents at the 3-position (7,9,10), suggesting that an aryl ring is the preferred ligand for this pocket. In the anilide series, *meta*-substituted derivatives were generally more potent than the corresponding *para*-isomers, and maximal activity was observed with *meta*-chloro substitution (7). These findings suggest that *para*-substituents may interfere sterically with the hydrophobic pocket. All marketed oxicams are primary carboxamides. This is likely attributable to the correlation between the acidity of the oxicams and their anti-inflammatory

activity and favorable plasma half-life. This acidity most likely results from stabilization of the enolate anion through intramolecular hydrogen bonding with the hydrogen bound to the carboxamide nitrogen. Such stabilization would not be possible in the case of a secondary carboxamide (10,38).

It is notable that all oxicams currently on the market bear an aromatic heterocyclic substituent at the 3-carboxamide position, eg. 2-pyridyl for piroxicam, 2-thiazolyl for meloxicam, and 3-(5-methyl) isoxazolyl for isoxicam. This heteroaryl substitution is associated with increased anti-inflammatory potency (9,10) which may be attributable to tighter binding affinity resulting from the additional hydrogen bonding between the heteroatom in the carboxamide substituent and one of the two structural water molecules observed in the COX:oxicam crystal structures as described above. Similarly, in a study of 4-hydroxy-2*H*-thieno-[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxide analogs, the 2-pyridyl carboxamide (tenoxicam) exhibited better anti-inflammatory activity than isomeric 3- or 4-pyridyl amides in a kaolin-induced edema model, consistent with an essential role for a hydrogen bond acceptor for ligand binding to the water molecule coordinated to Tyr-385 and Ser-530 (36).

Oxicams Provide a New Scaffold for the Inhibition of mPGES-1

The reduction of PGE₂ production by the inhibition of mPGES-1 has emerged as a therapeutic strategy for the treatment of inflammatory diseases that avoids the typical adverse side effects observed with NSAIDs. These side effects include gastrointestinal, renal, and cardiovascular toxicity (3), all of which have been attributed the broad inhibition of prostanoid biosynthesis. PGE₂, an important mediator of acute and chronic inflammation, is mainly synthesized from PGH₂ by the membrane-associated glutathione- (GSH)-dependent protein, mPGES-1 (Fig. 4A) (5). A series of benzothiopyran *S*-dioxides were identified as lead compounds in a high throughput screen for human mPGES-1 inhibitors. The most potent compound in this series displayed moderate inhibition against human recombinant mPGES-1 with an IC₅₀ of 1.68 μM, and in the IL-1-stimulated fetal fibroblast cell assay, it exhibited an over 26-fold selectivity for the reduction of PGE₂ (IC₅₀ of 3.4 μM) over PGF_{2α} (IC₅₀ > 90 μM) (39).

The structural similarity between benzothiopyran *S*-dioxides and oxicams was quickly realized, leading to the screening of a series of oxicam derivatives for mPGES-1 inhibition and the discovery of PF-9184, containing a biphenyl carboxamide at the 3-position and no substituent at the 2-position (Figure 4) (39,40). SAR studies revealed that the two phenyl rings (C and D) of the biphenyl group of PF-9184 could be separated by a linker, and that the length and the nature of the linker were not as important in determining potency of mPGES-1 inhibition as were the nature and positions of the D ring substituents (Fig 4B). Substituting chloride at positions 3 and 4 on the D ring of PF-9184 resulted in a potent *in vitro* inhibitor of mPGES-1 (IC₅₀ of 16 nM) (40). Based on an examination of the crystal structure of the mPGES-1:bis-phenyl-GSH complex (41), we speculate that the 3-biphenylcarboxamide substituent of PF-9184 is localized in the active site where the bis-phenyl moiety of bis-phenyl-GSH binds, while the benzothiozine ring mimics the GSH moiety, forming several polar interactions with active site residues (Fig 4C). Examination of

the selectivity of the oxicam confirmed that the PF-9184 analogs were indeed mPGES-1 inhibitors with weak or no COX inhibitory activity in both cell-free and cell-based assays (39). However, as has been observed with other mPGES-1 inhibitors, PF-9184 failed to inhibit mPGES-1 in the rat air pouch carrageenan-induced inflammatory model. This observation may be due to primary sequence disparities among different species (3). In addition, PF-9184 and its analogs have poor aqueous solubility, and further efforts to address this problem by modification of the chemical structure led to a great reduction of potency (40).

Other applications of oxicams

Other than COX and mPGE-1 inhibition, new interesting properties of oxicam analogs have been identified. Bearing several heteroatoms in the structure, oxicams are excellent ligands for chelating metals (42-46). An excellent example is the ability of piroxicam and tenoxicam to form iron (III) complexes with different stoichiometries (47). Cu(II)-oxicam coordination complexes were also found to directly bind to DNA, causing distortions of the DNA backbone (48,49). This observation may explain the antiproliferative and chemosuppressive effects exerted by oxicams on various cell lines at both the protein and the transcriptional level (50-52). In addition, oxicam analogs elicited a potent neuroprotective effect against 1-methyl-4-phenyl pyridinium- (MPP^+)-induced toxicity in human dopaminergic SH-SY5Y neuroblastoma cells and in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease model in the mouse. In both cases, the oxicams acted via the phosphatidylinositol 3-kinase/Akt pathway in addition to known COX inhibition (53,54).

Perspective

Recent crystal structures of both COX-1 and COX-2 in complex with oxicams provide the first detailed image of the binding mode of the oxicams via a two-water-mediated network in the main channel of the COX active site (33). The replacement of one or both of the two water molecules by incorporation of appropriate substituents into the oxicam scaffold may provide the foundation for a novel class of new, more potent COX inhibitors. It is noted that, despite numerous interactions throughout the COX channel, oxicams establish few interactions with the COX-2-specific "side pocket", which is an important binding site for most COX-2-selective inhibitors (2). This is consistent with the lack of isoform selectivity of most of the oxicams. Future design focused on addition of substituents that insert into this side pocket may lead to COX-1/COX-2 specific inhibitors in the oxicam series. In addition, the active site subpocket formed by movement of Leu-531 upon oxicam binding offers a new target for drug design. Notably, flexibility of Leu-531 also appears to play a role in the binding of the COX-2-selective endocannabinoid substrates, 2-arachidonoylglycerol and arachidonylethanolamide. Thus, this subpocket may be a site that can be specifically exploited for the design of selective inhibitors of endocannabinoid oxygenation (23,55).

The oxicam-mediated potent inhibition of mPGES-1 in *in vitro* cell-based assays makes them attractive molecules for the development of novel anti-inflammatory drugs targeting the selective biosynthesis of PGE_2 . A recent crystal structure of mPGES-1 in the native state or in complex with GSH or an inhibitor mimic (41,56), have provided details of the active

site of this trimeric target. These details have illuminated the molecular determinants of the observed interspecies differences in the response to mPGES-1 inhibitors (41). However, it is noted that mPGES-1 shares many structural similarities with other membrane-associated proteins involved in eicosanoid and glutathione metabolism (the MAPEG superfamily) (57). Thus, the selectivity of the oxicams and other mPGES-1 inhibitors should be of great concern.

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Abbreviations

AA	arachidonic acid
COX	cyclooxygenase
cPGES	cytosolic prostaglandin E ₂ synthase
GSH	glutathione
SAR	structure-activity relationship
mPGES-1	microsomal prostaglandin E ₂ synthase-1
mPGES-2	microsomal prostaglandin E ₂ synthase 2
NSAID	non-steroid anti-inflammatory drug
PGD₂	prostaglandin D ₂
PGE₂	prostaglandin E ₂
PGF_{2α}	prostaglandin F _{2α}
PGG₂	prostaglandin G ₂
PGH₂	prostaglandin H ₂
PGI₂	prostaglandin I ₂

Fig 1.

The PG synthetic pathway. AA is metabolized into PGG_2 and sequentially to PGH_2 via the cyclooxygenase and peroxidase activities, respectively. PGH_2 , the unstable PG intermediate, is rapidly converted into various prostanoids by specific PG synthases. Those synthases include PGI_2 synthase (PGIS) for PGI_2 , PGD_2 synthase (PGDS) for PGD_2 , $\text{PGF}_{2\alpha}$ synthase (PGFS) for $\text{PGF}_{2\alpha}$, thromboxane A_2 synthase (TXAS) for TXA_2 , and the PGE synthases (mPGES-1, mPGES-2, and cPGE). The chief inflammatory mediator, PGE_2 , is boxed. Oxicams, a class of NSAIDs, were found to be potent inhibitors of both COX-1 and COX-2, and mPGES-1 as well.

Fig 2. Chemical structures of some non-steroidal anti-inflammatory drugs, showing representative carboxylate-containing drugs (top), and drugs that lack a carboxyl group (bottom).

Fig 3. Oxicams bind to the COX active site via a novel mode. (A) Isoxicam bound to the COX active site of COX-2 (PDB id: 4M10). (B) Meloxicam bound to COX active site of COX-1 (PDB id: 4O1Z). One of the two conformations of meloxicam in the COX-1 (in color orange) is virtually the same as seen for isoxicam in panel A. Selected interacting residues, isoxicam, and meloxicam are represented in balls and sticks. The rotated Leu-531 side chains are colored in red while the Phe-518 side chains are in yellow. The coordinated waters are illustrated in magenta spheres. Hydrogen bonds are represented in dashes (A) or omitted (B) for clarity.

Fig 4. The structure of microsomal PGE₂ synthase-1 (mPGES-1) and the oxycam template for mPGES-1 inhibition. A: mPGES-1-GSH complex (PDB id: 4LA0). B: The general template of an oxycam exhibiting potent mPGES-1 inhibition. (C) Stereo diagram of the active site of mPGES-1 in complex with bis-phenyl-GSH and β-octyl glucoside (PDB id: 4LA1). The protein structure is shown in cartoon. The molecule of GSH is in red sticks (A), and bis-phenyl-GSH is in yellow sticks, while β-octyl glucoside is colored in light pink.

Scheme 1.
Early development of the oxicams.

Scheme 2.

The ester synthetic route to 4-hydroxy-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxides.