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Brief Article

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Bacterial Reduction as Means for Colonic Drug Delivery: Can Other Chemical Groups Provide an Alternative to the Azo Bond?

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Supporting Information

ABSTRACT: We compared the rate of colonic bacterial reduction of disulfide and nitro bonds to that of an azo counterpart. The disulfide and nitro reduction rates are comparable to that of azo having a similar molecular structure. We further explored QSAR of bacterial reduction of different nitro compounds giving a Hammett correlation with $\rho = 0.553$, $R^2 = 0.97$. We conclude that disulfide and nitro compounds have an unexploited potential for use in prodrugs and drug delivery systems targeted to the colon.

INTRODUCTION

Specific delivery of oral drugs to the colon is important in addressing several types of local medical conditions such as colon cancer and inflammatory bowel disease (IBD)[1] and has potential for systemic applications such as vaccine and peptide delivery.[2−4] There are a number of methods for specific delivery to the colon such as pH, time, or pressure based systems. However, biodegradable delivery systems are considered to be potentially the most accurate.[2,5−8] Colonic bacterial azo reduction has long been employed for the specific delivery of drugs to the colon. The earliest example is that of the prodrug sulfasalazine, which upon reduction releases the active drug 5-aminosalicylic acid (5-ASA), extensively used for the treatment of IBD. Over the past 2 decades, azo reduction has been exploited in the design and preparation of many colonic drug delivery systems in the form of prodrug, polymeric coatings, and matrices.[9] Polymeric colon delivery systems designed for bacterial degradation were based on hydrolysis of polysaccharides[10,11] or azo bond reduction.[12] Despite many publications on this topic, advancement to new products utilizing azo bond reduction is lacking. This may be because of toxicity concerns of the azo group and sluggish reduction rates of azo polymers and matrices.[12] Despite the fact that many xenobiotics with a large variety of chemical groups have been shown to be reduced in the gut, the only chemical bond utilized to date for drug delivery by reduction is the azo bond. Colonic delivery systems using other reducible groups are almost nonexistent. During our work on azo polymers for colon drug delivery,[13] we were interested in expanding our knowledge on other reducible chemical bonds and information about structural requirements necessary for obtaining rapid and efficient reduction. Although the bacterial reduction of many xenobiotics has been evaluated in the past, to the best of our knowledge, comparison of the reduction rate of different chemical groups having similar structures by colonic bacteria has not been published yet.

While it is clear that disulfides have a reduction potential suitable for cleavage in the colon, studies showing directly the reduction of disulfide containing compounds by colonic bacteria are lacking. This is in contrast to the extensive use of disulfide bonds for intracellular drug delivery systems.[14,15] In the area of mucosal absorption, disulfide bonds are being used to improve mucoadhesion of carrier systems to the mucosal membrane in the intestine.[16] There is some mention of the preparation of polymeric colon delivery systems based on disulfide bonds.[17−19] However, to the best of our knowledge, a detailed study describing preparation and in vivo results has not been published.

Many aromatic nitro compounds have been shown to undergo reduction in the colon; however, these reactions were explored in the past, mainly using drugs and other xenobiotics.[20−22] The reduction of nitro groups by nitro reductase have been applied for the design of prodrugs in the fields of antibody-directed enzyme prodrug therapy (ADEPT) and gene (or virus) directed enzyme prodrug therapy (GDEPT or VDEPT).[23] However, to date, nitro groups were not incorporated into colonic prodrugs or drug delivery systems. The reduction of polymeric azo and nitro dyes by suspensions of rat cecal bacteria led to the conclusion that the bacterial reduction may be extracellularly mediated by nonenzymic electron donors.[24] Furthermore, for azo compounds, a relationship was found between structural features and/or redox potentials and rate of reduction, which is consistent with this mechanism.[25,26] Such investigations of nitro compounds are warranted and may improve the ability to predict reduction efficiency of particular nitro xenobiotic compounds in the colon[22] and aid to design novel, nitro based prodrugs or colonic drug delivery systems.

In this work, we compared the reduction of structurally related molecules differing in one chemical bond, azo, nitro, or disulfide, all of which have a potential use in colonic delivery systems. Reduction rates were compared to that of the commercial prodrug sulfasalazine, known to be efficiently reduced in the colon. Subsequently, we focused on the reduction of different nitro compounds and studied the

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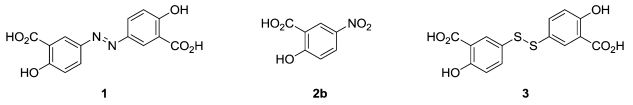
structure−activity relationship (SAR) of this reaction. These studies were aimed to expand the "toolbox" of the medicinal chemist with additional chemical groups to be used in prodrugs or biodegradable matrices and coatings targeted to the colon and to enable a more knowledgeable design of such future systems.

RESULTS AND DISCUSSION

A common structure (5-ASA), an anti-inflammatory drug used for the treatment of IBD and that has been incorporated in several azo prodrugs including sulfasalazine,[25] was used. Since sulfasalazine is used clinically and known to be reduced almost entirely within the gut with only a few percent of parent molecule retrieved from the feces,[26] its rate of reduction is useful as a gold standard in comparing reduction rates for colonic delivery purposes.

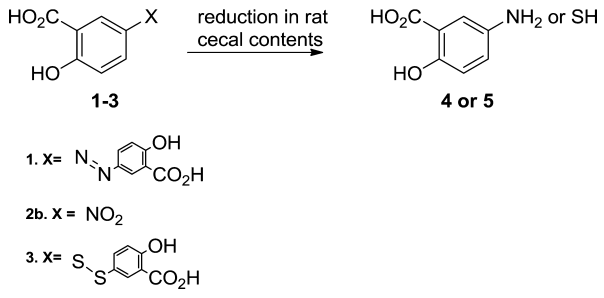
The three compounds tested were (**1**) olsalazine, the symmetrical azo compound formed from two units of 5-ASA, which is also a clinically used prodrug; (**2b**) 5-nitrosalicylic acid, which may also be regarded as a prodrug of 5-ASA (as a prodrug, this compound may be superior to some other azo based prodrugs, as it does not release a residual moiety upon reduction); (**3**) a symmetrical disulfide derivative, having an equivalent structure to that of olsalazine, substituting the azo bond with a disulfide bond (Chart 1).

Chart 1. Azo, Nitro, and Disulfide Compounds Used



Reduction was evaluated ex vivo in a suspension of rat cecal content, which is a common model for the evaluation of colonic reduction and has been found to reduce many molecules to a similar extent as the human colon.[27] Indeed, all three molecules were found to be reduced by the cecal suspension to obtain the expected amine or thiol products (Scheme 1).

Scheme 1. Reduction of the Different Chemical Groups



The reduction was investigated by following the accumulation of the final reduction product (amine or thiol) and not the disappearance of the starting compound. This was to ensure that we were studying the full cleavage of the chemical bond. Following only the starting material disappearance (commonly performed for colored azo compounds) may lead to overestimation of reduction rate, resulting in the design of polymers or prodrugs that are not completely reduced, leading to sluggish or incomplete drug release.[28]

The reduction of sulfasalazine, azo **1**, and nitro **2b** was completed within a few hours (Figure 1). In contrast, the

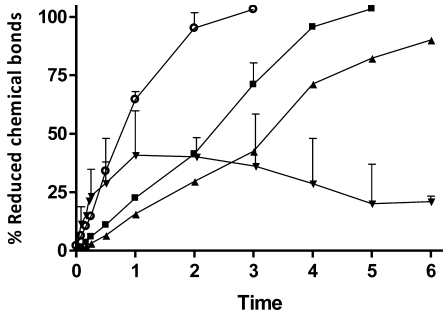


Figure 1. Reduction of different chemical bonds: (○) sulfasalazine, (▲) olsalazine (**1**), (■) nitro (**2b**), (▼) disulfide (**3**).

reduction of disulfide **3** could not be followed to completion. At ∼2 h, the product thiol began to disappear from the suspension. This is due to its instability probably owing to the electron donating groups that facilitate oxidation of the thiol. This phenomenon was also evident when the thiol was directly added to cecal suspensions for calibration purposes. Nevertheless, for practical purposes, in a delivery system based on polymeric matrix or film, drug release following initial reduction and pore formation may still occur irreversibly.

After establishing the reduction process for the three chemical groups, we looked more closely at the initial rates of the different reductions. As can be expected from the single bond of disulfide **3**, it had the fastest initial rate of reduction. The nitro and azo groups were reduced at a similar initial rate (Figure 2). Since our ex vivo reducing system is based on rat

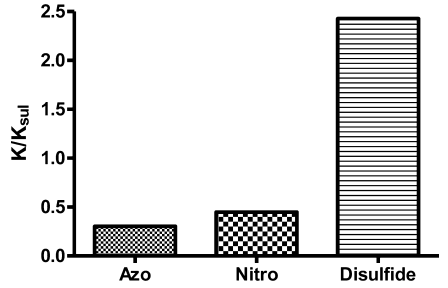


Figure 2. Relative initial rates of reduction of azo (**1**), nitro (**2b**), and disulfide (**3**).

cecal content, different experimental batches may differ in absolute rate. For this reason, the rate of sulfasalazine reduction was remeasured in every experiment as a standard and all other rates were calculated compared to it (K_{sul}).

This result emphasizes the potential of disulfides to be used for colonic delivery. Disulfides could be incorporated into polymers, as has been done previously for azo compounds, and the degradation rate of the disulfide polymers may be expected to proceed more rapidly. Thiol formation at the colonic site may also lead to enhanced mucoadhesion.[29]

Overall, the reduction rates of the disulfide and nitro compounds are of the same magnitude as that of the azo having a similar structure and as that of sulfasalazine with k/k_{sul} = 0.3−2.4 for the three chemical groups.

To study further the parameters necessary for obtaining a higher reduction rate of nitro compounds, we conducted a SAR study using eight nitro compounds (Chart 2 and Table 1). The carboxylic acid group present in all nitro compounds served to gain water solubility, additionally maintaining structural

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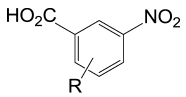
Chart 2. Structure of Nitro Aromatic Compounds Used for Hammett Correlation



2a-h

Table 1. Data on the Different Substituents for Hammett Correlation

compd	R	σ	σ^-	$K = k/k_{sul}$	$\log(K/K_0)$
2a	*p*-H	0	0	$K_0 = 0.759$	0
2b	*p*-OH	−0.38	−0.37	0.450	−0.228
2c	*p*-CH_3	−0.14	−0.17	0.603	−0.100
2d	*p*-$N(CH_3)_2$	−0.63	−0.12	0.724	−0.020
2e	*p*-Cl	0.24	0.19	1.013	0.125
2f	*p*-COOH	0.44	0.31 (charged) 0.77 (neutral)	0.113	−0.827
2g	*m*-COOH	0.35	0.02 (charged) 0.56 (neutral)	0.128	−0.772
2h	*m*-CF_3	0.46	0.41	1.222	0.207

similarity to 5-ASA. It was assumed that the electronic effects of the different substituents would be additive. Indeed, the high correlation obtained has confirmed this assumption.

The σ^- constants were used for the correlation, since the reaction center in the reduction process is expected to have strong resonance interaction with the substituents. k is the pseudo-zero-order rate constant derived from the initial reduction rates.[30] By use of the Hammet equation, a high correlation was observed for the reduction of nitro compounds, with electron-withdrawing groups accelerating the process: $\rho = 0.5526$, $R^2 > 0.97$ (Figure 3)

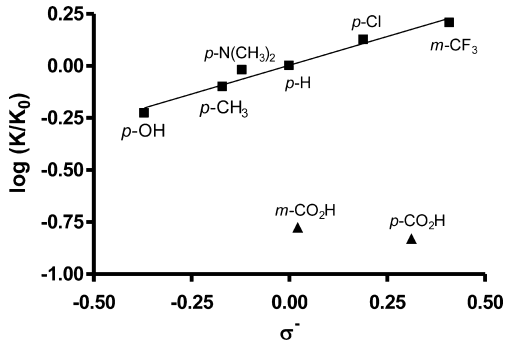


Figure 3. Hammet correlation for the reduction of nitro compounds in cecal contents.

Despite their electron withdrawing nature, two phthalic acids had very slow reduction rates and did not fit this correlation. Carboxylic acids are known to be out of correlation especially when the substituents are negatively charged.[30,31] Under the pH conditions of the current system, these carboxylic acids are the only charged substituents in a series of neutral ones. Slow rates of aromatic nitro reduction in the presence of carboxylic acid substituents have been observed by others. For example, in a chemical nitro reduction performed under similar pH and anaerobic conditions using hydrazine hydrate, nitrobenzoic acids had relatively long reaction times and lower yields and they were not incorporated into the Hammett correlation presented.[32,33] Specifically, under the conditions of the cecal contents, sluggish reaction rates may also result from binding effects of the diacids to different components of the system.

In ref 33 a correlation was obtained using σ and not σ^- values. When examining such a correlation using our data, most substituents have similar σ and σ^-, leading to similar ρ (0.5285) and a similar, even higher R^2 (0.99), provided that the dimethylamino substituent is excluded. For the dimethylamine substituent, σ and σ^- values differ greatly because of the resonance formed by the nitrogen with the aromatic ring which influences the reaction rate. Attempting to correlate all six substituents using σ therefore leads to a very weak correlation with $R^2 = 0.59$.

The high correlation ($R^2 > 0.97$) obtained in the present work indicates that the reduction of nitro compounds is most likely performed extracellularly, mediated by electron donors, as has been suggested before for azo compounds.[24,34,35] Although the R^2 obtained was very high relative to other biological systems, the ρ was relatively low. In the literature similar values for chemical reduction of nitro compounds can be found,[33] although ρ may also reach to ∼2.[31] This indicates that under the conditions of the cecal contents, the role of the substituents is relatively reduced.

Two of the nitro compounds containing the electron withdrawing groups *p*-Cl and *m*-CF_3 were found to undergo reduction at similar or even somewhat faster rates than sulfasalazine. The results emphasize the potential of nitro groups for colon specific drug delivery and the importance of considering SAR for the design of novel systems.

CONCLUSIONS

The human colon serves as an important target for the development of prodrugs and drug delivery systems for local and systemic therapy. The local bacterial population produces a stable reducing environment with the potential to reduce many chemical bonds. Although quite a few delivery systems were designed based on the familiar reduction of azo bonds, other reducible bonds have not been explored. In addition, little thought was given to the structure of the azo molecule in different polymers, leading to slow reduction rates in many of the azo delivery systems. In this work we have shown that other groups, namely, disulfide and nitro, are also potential bonds for the design of novel prodrugs and drug delivery systems to the colon. The SAR of the reduction of nitro aromatic compounds has demonstrated that taking under consideration the electronic effects may be useful for the medicinal chemist in optimizing the design of new prodrugs and drug delivery systems targeted to the colon.

EXPERIMENTAL PROCEDURES

General Methods. See Supporting Information for details. Reduction process was followed and purity of all compounds was determined using HPLC. HPLC was performed on a Dionex Ultimate 3000 system equipped with an autosampler, a photodiode array detector, and Chromeleon software system. The mobile phase consisted of potassium phosphate buffer, pH 6.8, 50 mM (+0.1% tetrabutylammonium hydroxide), and methanol. Analysis was run at a flow rate of 1 mL/min and column temperature of 30 °C. A C18 RP column (Altima, 150 mm × 4.6 mm, 5 μm mean particle size, Alltech) was used. A gradient method was developed to allow the different compounds to be analyzed by the same method. All compounds were determined to be ≥95% pure.

Synthesis. Compounds **1** and **2b** were commercially available. Disulfide **3** was prepared starting from 5-ASA through the preparation of the xantogenate derivative. For reference purposes, the reduction

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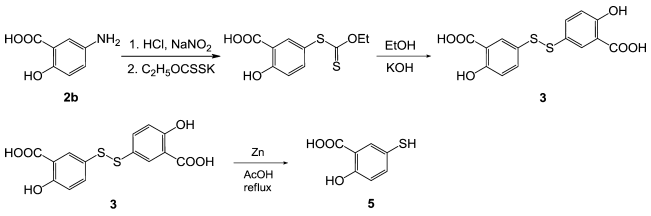
product 5-thiosalicylic acid **5** was also prepared by chemical reduction using Zn in acidic conditions (Scheme 2).

Scheme 2. Synthesis of Disulfide 3 and Its Reduction Product



All nitro compounds used for the Hammett correlation and their aniline derivatives were commercially available except for 5-amino-2-dimethylaminobenzoic acid (**4d**), which was obtained from 2-dimethylamino-5-nitrobenzoic acid (**2d**) by hydrogenation on Pd/C.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental information, syntheses, spectral characterization data, bacterial reduction tests, and HPLC analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

5-ASA, 5-aminosalicylic acid; ADEPT, antibody-directed enzyme prodrug therapy; GDEPT, gene directed enzyme prodrug therapy; VDEPT, virus directed enzyme prodrug therapy

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