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A double prodrug system for colon targeting of benzenesulfonamide COX-2 inhibitors

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Abstract

The design, synthesis and delivery potential of a new type of benzenesulfonamide cyclo-oxygenase-2 (COX-2) inhibitor prodrug is investigated using celecoxib. The approach involves a double prodrug that is activated first by azoreductases and then by cyclization triggering drug release.

We studied the intramolecular aminolysis of the acylsulfonamide. The cyclization was surprisingly rapid at physiological pH and very fast at pH 5. The prodrug is activated specifically under conditions found in the colon but highly stable in the presence of human and rodent intestinal extracts. Finally, the prototype with celecoxib was transported much more slowly in the Caco-2 transepithelial model than the parent. The design therefore shows significant promise for the site specific delivery of benzenesulfonamide COX-2 inhibitors to the colon.

Colorectal cancer is the third leading cause of cancer-related death in the western world with incidence in the range 25–35 per 100,000.[1] Most colonic cancers are preceded by the development of small benign colonic polyps which may take several years to accumulate the mutations required for frank malignant change. The disease is incurable in its later stages and there is an urgent need for new therapies and chemopreventative agents.

Colorectal cancer rates are reduced in patient groups chronically using aspirin or other cyclo-oxygenase (COX) inhibitory drugs.[2] The role of COX-2 in the aetiology and progression of the disease is well characterized and the enzyme is an important pharmacological target.[3-7] The COX-2 inhibitor celecoxib (Celebrex) was approved by the FDA (1999) as add-on therapy for patients with familial adenomatous polypsosis (FAP, 400 mg BID). Subsequent randomized clinical trials affirmed the validity of celecoxib in controlling polyp progression. In the Adenoma Prevention with Celecoxib Trial (APC), which recruited very high risk patients, high dose celecoxib (800 mg) was associated with a 45% reduction in adenoma recurrence. The APC trial is however usually remembered for the discovery of an increase in risk of adverse thrombotic events in the celecoxib treatment group, which had significance far beyond the trial. Clinical evidence supporting a class effect soon followed and the most selective COX-2 inhibitors were withdrawn from the market. Unopposed systemic COX-2 inhibition is now widely accepted to increase the risk of a cardiovascular event.[8] Accordingly, COX-2 inhibitors are indicated in only the very highest risk FAP patients.

Colorectal targeting of COX-2 inhibitors is considered a promising strategem for reducing systemic exposure while focusing the beneficial COX-2 inhibitory effects on the relevant tissue.[9-13] Approaches so far have relied on formulations for local drug release.

In this context it is notable that there are a number of clinically used azo-prodrugs of 5-amino salicylic acid (5-ASA) for treating inflammatory bowel disease (IBD) that act by locally releasing the drug, reducing systemic exposure and intestinal metabolism. Azoprodrugs are activated by azoreductases secreted by the colonic micoflora.[14] The approach is most suitable and directly applicable to those drugs, such as 5-ASA, that bear a primary aromatic amine.

We recently reported the design of a prodrug type that can site specifically deliver prednisolone to the colon using azoreductase as a vector (Figure 1a).[15] The ester prodrug first undergoes reduction by azoreductase liberating an amino ester, followed by spontaneous cyclization. The concept could in principle be applied to benzenesulfonamide COX-2 inhibitors provided that: (i) the required acylation of the sulfonamido group could be accomplished synthetically, and; (ii) intramolecular aminolysis of acylsulfonamide intermediate could happen at realistic rates following azoreductase activation (Figure 1b).

This paper concerns proof of principle studies along these lines along with stability and transport characteristics. In the design, the 5-ASA pendant group is intended to suppress systemic absorption by increasing mass and polarity, but it could in theory impart other interesting characteristics. There is mechanistic evidence for an antiproliferative effect of 5-ASA[16-19] that is consistent with clinical analysis suggesting it has a colorectal cancer chemopreventative effect.[20, 21]

The synthesis of **2** was carried out by attachment of **5** which was obtained as reported previously. The condensation between the carboxylic acid of **5** and the sulfonamide of **1** was accomplished using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), with

an excess of 4-dimethylaminopyridine (DMAP), required because of the low reactivity of the sulfonamide (Scheme 1). We used polymer supported EDC[22] in order to facilitate workup of the reaction by filtration of the polymer. To eliminate the DMAP we used polymer supported sulfonic acid. Finally **6** was treated with TFA at room temperature for 4 h to remove the *tert*butyl ester group to yield **2**.[23]

An important parameter determining the potential capacity of **2** to deliver **1** into the colon is its stability under the conditions in the small intestine and stomach. The stability of **2** was monitored by HPLC following incubation at 20 µM in freshly aspirated duodenal or gastric juices from IBD patients (37 °C) (n=4 in duplicate). Compound **2** could be recovered unchanged from the intestinal preparations after 24 h, with no evidence of formation of **1**. Compound **2** was also stable in human and rat plasma (< 5% hydrolysis at 24 h in 50% plasma buffered at pH 7.4, 37°C). Unsurprisingly, **2** was also stable (<1% hydrolysis) in borate buffer solution at pH 5 and 7.4 over 24 h (HPLC). The stability characteristics of acylsulfonamides have been studied as potential prodrugs in the generic sense and specifically as prodrugs of celecoxib and valdecoxib. Parecoxib sodium, developed originally for IV administration, is a more water soluble propionamide of valdecoxib.[23] Parecoxib is converted to valdecoxib in man but its acetyl analogue is reported to be incompletely converted *in vivo* to the parent.[24] The acyl, propionyl and butyryl amides of celecoxib were reported to be stable in rat plasma over 24 h.[25] The resistance of **2** towards hydrolysis is not surprising in this context and indeed it is a useful feature provided that activation at the target tissue occurs with appropriate kinetics.

The transport characteristics of **1** and **2** were assessed using the transepithelial Caco-2 model in order to predict the potential for passive diffusion and extraction from the gut

(Figure 2). In the apical to basolateral direction (AP>BL, the permeability coefficient (P_{app}) for **1** was $1.88e^{-04}$ cm/sec whereas for **2** it was $2.94e^{-06}$ cm/sec. Mass balance suggests that 98% of celecoxib (**1**) was transported in the AP>BL direction whilst only 18% of **2** was transported in the AP>BL study.

Secretory transport was more similar for **1** and **2** although celecoxib permeability was still higher (P_{app} $1.24e^{-05}$ versus $4.33e^{-06}$ cm/sec). Thus mass balance showed 46% of celecoxib transported versus the 19% of **2** transported in BL>AP. The data suggests that **2** is not likely to undergo passive diffusion to the same extent as **1** following oral administration.

BOC-**3** (Scheme 2) was required in order to study the intramolecular lactamization of **3**. The condensation between **1** and BOC-aminophenylpropionic acid was performed using similar chemistry to before.[26] Deprotection of BOC-**3** was carried out in dichlormethane at 0°C with trifluoroacetic acid for several min, the volatiles removed under a stream of nitrogen and the residue dissolved immediately in the appropriate buffer solution before starting kinetic studies. Lactamization of **3** was studied in BBS at the lower intestinal pH range 5–8 (37°C) and at I= 0.12, using HPLC to measure the disappearance of the parent and the appearance of **1** and dihydroquinolone (DHQ) **4**. The method was validated for precision and accuracy (recovery) at 1.1–16.6 μM for each analyte. Lactamization was unexpectedly rapid given the hydrolytic stability of **2** and it was strikingly pH dependent (Figure 3–4). Apparent first-order rate constants were obtained at each pH by non-linear regression which allowed an estimation of cyclization half-lives. These decreased from around 10 h at pH 7.4 to around 10 min at pH 5.5. The kinetics of aminolysis of acylsulfonamides by amines has not been studied previously. Acylsulfonamides are weak

organic acids with pK_a values in the range of carboxylic acids, for which they are sometimes employed as mimics (parecoxib (Figure 1.) has a reported pK_a of 4.9). The pH-rate behavior of cyclization is likely to be influenced by the ionization state of the acylsulfonamide and anilide amino groups (nucleophile). In its anionic form, the sulfonamide amide group is resistant to nucleophilic attack. Cyclization rate at pH 5 is rapid because of the presence of the neutral anilide (pK_a~4.3) and sulfonamide.

In order to examine both of the necessary mechanistic components of drug release i.e. azo reduction and cyclization, **2** was incubated under anaerobic conditions in a brain-heart-infusion (BHI) suspension that had been inoculated with *C. perfringens*, an azoreductase secreting anaerobe from the human intestine. Incubation and analysis was carried out as recently described.[27] Processing of **2** was measured using HPLC along with the appearance of celecoxib (**1**) and DHQ (**4**). Consumption of **2** was complete in 6 h (85% in 4 h) (Figure 5). The amino intermediate (**3**) was also evident and it too disappeared over time leading to formation of celecoxib (**1**) and DHQ **4**. The experiment was conducted at pH 7.4 at which the HPLC and extraction methods were validated but evidently this is not the optimum pH for the cyclization of **3**. The pH in the gastrointestinal tract (radiotelemetric capsule) varies from 6.4 in proximal small intestine to 7.3 in the distal ileum, falling sharply to 5.7 in the caecum and rising through the colon to 6.6 in the rectum.[28] This pH range is optimal for reduction and cyclization from **2** suggesting that **2** has suitable characteristics for colon targeting of celecoxib.

A double prodrug design involving azoreductase and cyclization can be applied to benzenesulfonamides because of the surprising rate of intramolecular aminolysis of acylsulfonamides. The phenylpropionic acid acyl adduct of celecoxib is highly stable in

intestinal fluid except when azoreductase secreting organisms are present under anaerobic conditions. Then, rapid drug disappearance occurs, generating anilide **3**, drug release from which is rapid especially in the pH range encountered in the diseased colon. The design can be optimised with respect to physicochemical and transport characteristics but in its simplest form, with the propionic acid linker, transepithelial diffusion is effectively suppressed in the Caco-2 model. The design may find most use applied to COX-2 inhibitory sulfonamides that possess highest COX-2 selectivity and potency.

Acknowledgements

References and notes

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23.   Spectroscopic data for **2**: IR$_{vmax}$(KBr): 3434.84, 2923.82, 1642.30, 1095.99 cm^{-1}. ^{1}H-NMR (DMSO-d6) δ: 9.55 (s, 1H, NH), 8.32 (s, 1H), 7.97 (s, 1H), 7.84 (d, 1H, *J* 5.8 Hz), 7.56 (d, 1H *J* 5.24 Hz), 7.44 (d, 2H, *J* 5.04 Hz), 7.36 (m, 2H), 6.80 (d, 1H, *J* 6.04 Hz), 6.62 (d, 1H, *J* 5.8 Hz), 6.11 (s, 1H), 2.64 (t, 2H *J* 5.24 Hz). ^{13}C-NMR (DMSO) δ: 169.90 (C, C=O, C-16), 150.18 (C, C-5), 140.01 (C, C-4), 130.61 (C, C-28), 130.11 (CH, C-9), 127.19, 125.97, 124.84, 122.48, 119.56, 118.10, 115.78, 115.78, 115.77 (CH, C-14), 115.37 (CH, C-6), 107.50 (CH, C-26), 38.37 (CH$_2$, C-2), 27.17 (CH$_2$, C-3), 21.06 (CH$_3$, C-34). HRMS: Expected (M-H^{+}) = 678.16287, Found (M-H^{+}) = 678.16284.

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26.   Spectroscopic data for BOC-**3.** IR$_{vmax}$(KBr): 3437.21, 1564.30, 1415.85, 1261.62 cm^{-1}. ^{1}H-NMR (MeOH-d4) δ: 9.48 (s, 1H, NH), 7.89 (d, 2H, *J* 8.52 Hz), 7.45 (d, 2H *J* 9.04 Hz), 7.37 (d, 1H, *J* 7.56 Hz), 7.21 (d, 3H *J* 8.04 Hz), 7.14 (d, 2H, *J* 8.04 Hz), 6.96 (t, 1H, *J* 7.52 Hz), 6.92 (d, 1H, *J* 6.52 Hz), 6.5 (s, 1H, NH), 2.84 (t, 2H, *J* 7 Hz), 2.48 (t, 2H *J* 7.04 Hz), 2.40 (s, 3H), 1.53 (s, 9H). ^{13}C-NMR (MeOD) δ: 178.64 (C, C=O, C-1), 155.13 (C=O, C-10), 145.48 (C, C-21), 143.87 (C, C-19), 141.05 (C, C-16), 139.44 (C, C-25),

135.59 (C, C-13), 134.96 (C, C-5), 132.19 (C, C-4), 131.01 (C, C-22), 129.22 (CH, C-26, C-24), 129.19 (CH, C-23, C-27), 128.62 (CH, C-14, C-18), 127.80 (CH, C-7, C-9), 125.98 (CH, C-8), 124.89 (C, C-29), 124.82 (CH, C-6), 124.65 (CH,C-17, C-15), 105.29 (CH, C-20), 79.46 (C, C-11), 39.44 (CH$_2$, C-3), 27.34 (CH$_3$, C-12), 26.60 (CH$_2$, C-3), 22.34 (CH$_3$, C-28). HRMS: Expected (M-Na$^+$) = 651.1875, Found (M-Na$^+$) = 651.11865.

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List of Figures, Schemes, Tables

Figure 1. Prednisolone prodrug, celecoxib (**1**), celecoxib prodrug design (**2**).
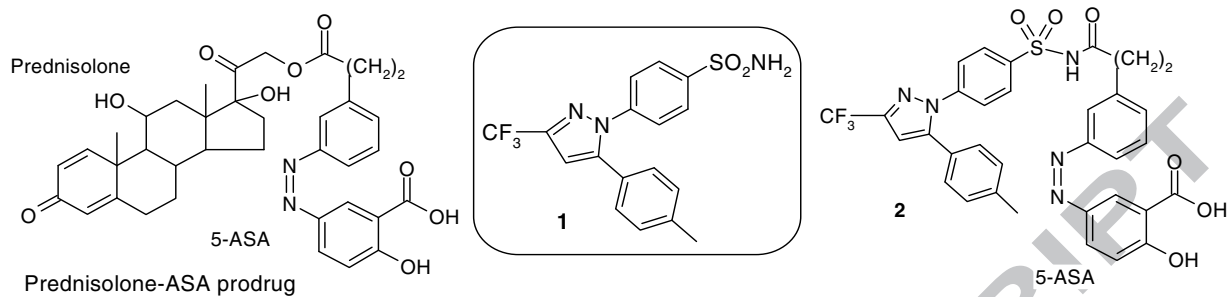
Figure 1b. Mechanism of drug release by spontaneous intramolecular lactamization of **3** following azoreductase activation of **2**.

Figure 2. A: Absorptive transport of **1** (□), **2** (▲): B: Secretory transport of **1** (□), **2** (▲)

Figure 3. Lactamization of **3** (○) with formation of celecoxib, **1** (□), DHQ, **4** (♦) at A: pH 6.4 and B, 5.5 both at 37°C and I=0.05.

Figure 4. Profile of k_{obs} for the lactamization of **3** (●) versus pH at 37°C, I=0.05

Figure 5. Progress curve following incubation of **2** (□) in a suspension of *C. perfringens* under anaerobic conditions at pH 7.4 and 37 ^{o}C and the evolution of celecoxib (●), DHQ (Δ), **3** (○). Also shown is **2** in BHI (♦).

Table 1. Rate data for the cyclization of the **3** in aqueous conditions (I=0.05) in the pH range 5.5-7.4 at 37°C.

Scheme 1. Synthetic route to **2**. Conditions: a: celecoxib (**1**), P-EDC, DMAP in ClCH$_2$CH$_2$Cl: t-butanol (1: 1), A-15; b: TFA, DCM

Scheme 2. Synthetic route to BOC-**3**. Conditions: a: celecoxib (**1**), P-EDC, DMAP in ClCH$_2$CH$_2$Cl: t-butanol (1: 1), A-15.

Figure 1. Prednisolone prodrug, celecoxib (**1**), celecoxib prodrug design (**2**)

Figure 1b. Mechanism of drug release by spontaneous intramolecular lactamization of **3**

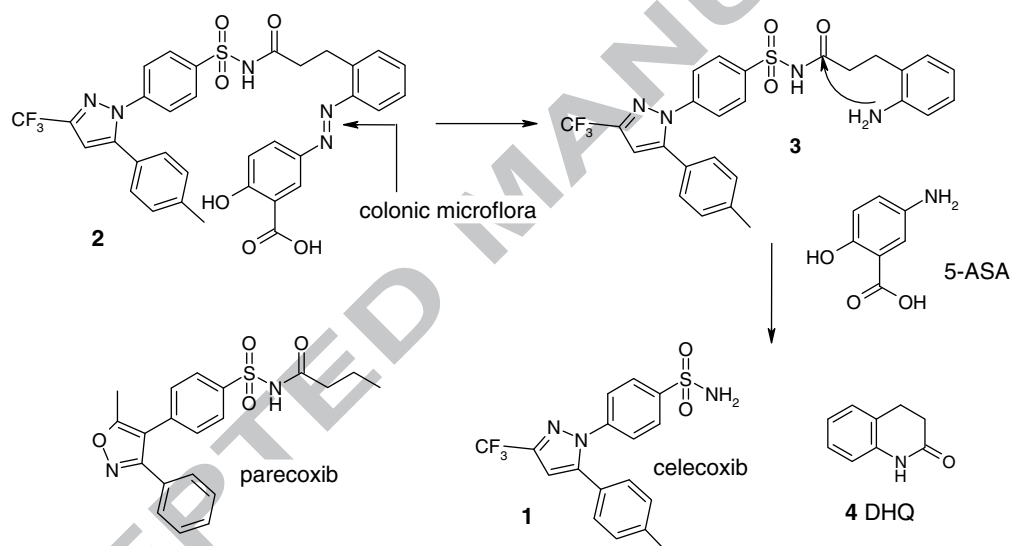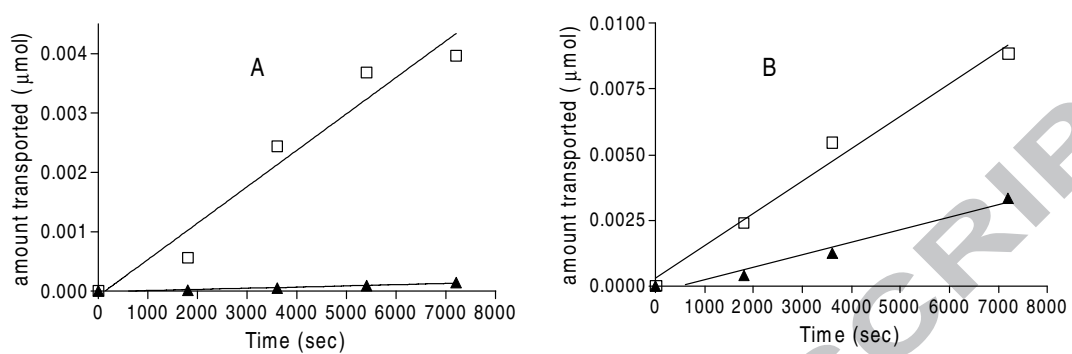following azoreductase activation of **2**

Figure 2. A: Absorptive transport of **1** (□), **2** (▲): B: Secretory transport of **1** (□), **2** (▲)

Figure 3. Representative lactamization progress curves for **3** (○) and liberation of

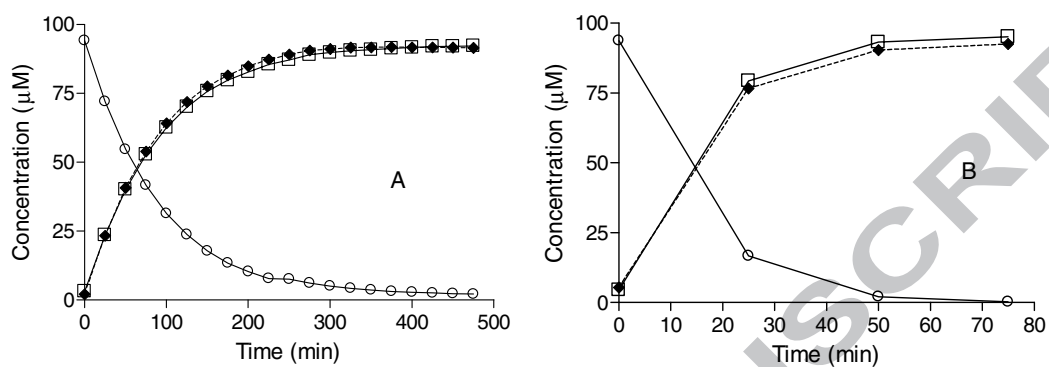celecoxib, **2** (□), DHQ, **4** (♦) at A: pH 6.4 and B, 5.5 both at 37°C and *I*=0.05.

Figure 4. Profile of k_{obs} (min^{-1}) for lactamization of **3** (●) versus pH at 37°C, I=0.05 (each point represents the mean of three determinations: Rate data are in Table 1)
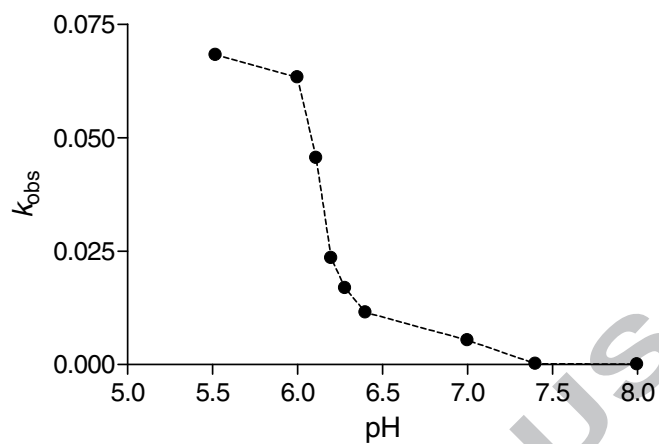
Figure 5. Representative progress curve following incubation of **2** (□) in suspension of *C. perfringens* under anaerobic conditions at pH 7.4 and 37 ºC and the evolution of **1** (●), DHQ (Δ), anilide intermediate **3** (○). Also shown, **2** incubated in BHI (♦) under similar conditions (n=3).
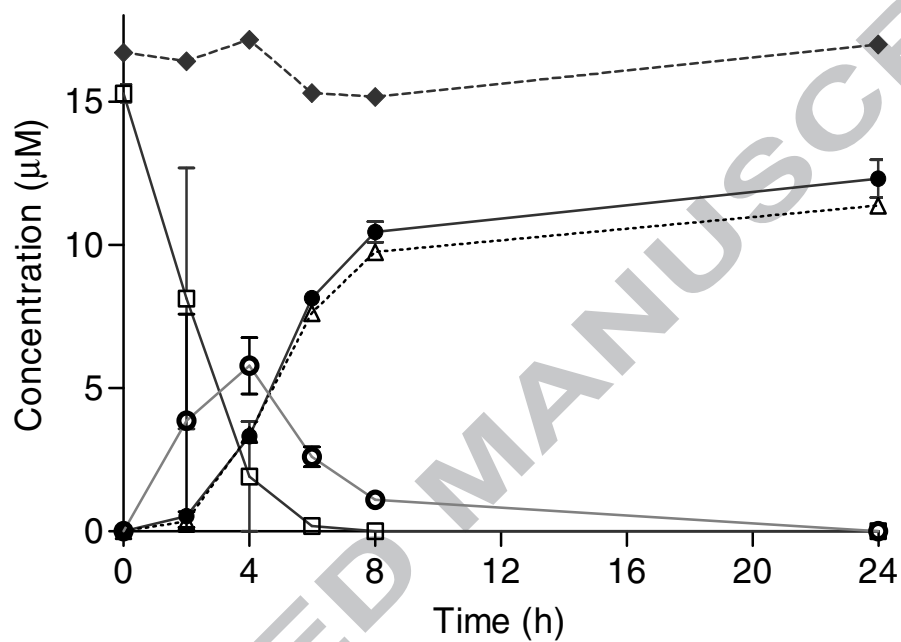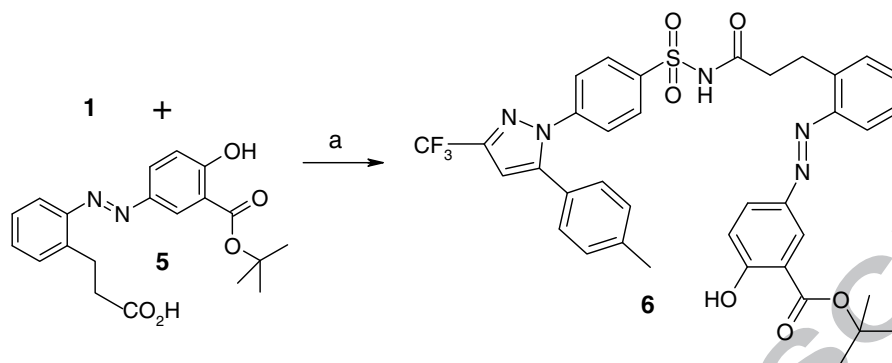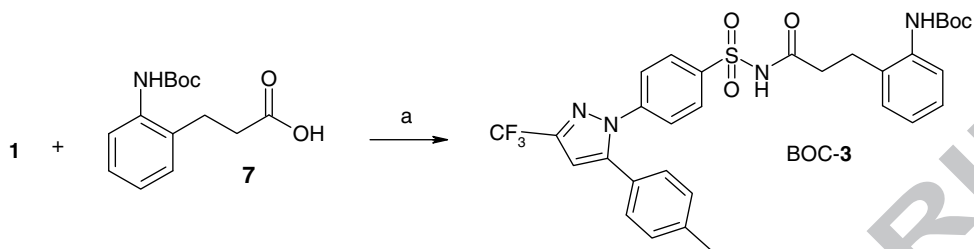
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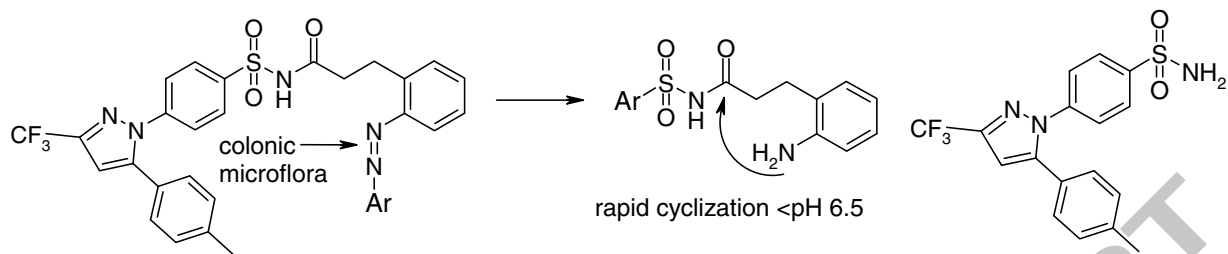
pH	k_{obs} ±SEM (min^{-1})	$t_{1/2}$ (min)
7.4	0.00109±0.00007	631.8
7	0.0053±0.0004	130.4
6.4	0.0115±0.0002	60.3
6.28	0.0168±0.0002	41.2
6.2	0.0234±0.0003	29.5
6.11	0.045±0.004	15.2
6.0	0.063±0.016	10.9
5.52	0.068±0.022	10.2

Scheme 1. Synthetic route to celecoxib prodrug **2**. Conditions: a: Celecoxib (**1**), P-EDC,

DMAP in ClCH$_2$CH$_2$Cl: *t*-butanol (1: 1), A-15; b: TFA, DCM

Scheme 2. Synthetic route to BOC-**3**, conditions: a: celecoxib (**1**), P-EDC, DMAP in

ClCH$_2$CH$_2$Cl: *t*-butanol (1: 1), A-15.

colonic microflora

rapid cyclization <pH 6.5