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NO-NSAIDs: Gastric-sparing nitric oxide-releasable prodrugs of non-steroidal anti-inflammatory drugs

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ABSTRACT

Recently, a new class of nitric-oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) is being studied as 'Safe NSAIDs' because of their gastric-sparing properties. As an extension of our novel disulfide linker technology, we have designed, synthesized and evaluated novel NO-releasing NSAID prodrugs such as NO-Aspirin (**1b**–**d**) and NO-Diclofenac (**2b**–**c**). Although the amide-containing derivative 1d did not show any bioavailability, the remaining compounds have shown fair to excellent pharmacokinetic, anti-inflammatory and gastric-sparing properties. Among them, however, the NO-Diclofenac (**2b**) has shown the most promising pharmacokinetic, anti-inflammatory and NO-releasing properties and protected rats from NSAID-induced gastric damage which could be attributable to the beneficial effects of NO released from these prodrugs.

Aspirin and diclofenac are among the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of pain, fever, and inflammatory diseases such as arthritis.[1] Because of its anti-thrombotic properties, aspirin is now indicated for patients with stable angina, unstable angina, acute myocardial infarction, transient cerebral ischemia, thrombotic stroke, and peripheral arterial disease. The use of aspirin for the prevention of arterial thrombosis is likely to increase further as public awareness of this wonder drug grows. It has been reported that more than 80 billion aspirin tablets are consumed annually in the USA, and more than 37% of the individuals taking aspirin do so to prevent blood clots.[2] Similarly, diclofenac or its sodium salt (Voltaren®) is the most widely prescribed NSAID for the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.[1] However, chronic use of these NSAIDs can induce severe gastrointestinal (GI) toxicity such as bleeding, ulceration and perforation, and also cardiorenal complications which greatly limit their therapeutic usefulness. According to an investigative report on 'Toxic and Deadly NSAIDs',[3] conservative estimates of NSAID-related GI complications account for more than 107,000 hospitalizations and 16,500 deaths annually among arthritis patients in the United States alone. Therefore, there exists an unmet medical need for 'Safe NSAIDs' that do not cause adverse effects on GI tract and cardiorenal systems. As a potential solution to this problem, a new class of nitric oxide (NO)-releasable prodrugs of non-steroidal anti-inflammatory drugs (NO-NSAIDs), also sometimes known as CINODs (cyclooxygenase inhibitory NO donors), has been developed recently by the addition of NO-releasing moiety to an existing NSAID. The rationale behind this strategy is to harness the beneficial effects of NO that is released from these NO-NSAIDs. NO is now widely recognized as a critical mediator of GI mucosal defense and greatly suppresses NSAID-induced COX-1 inhibition related adverse events such as suppression of prostanoid synthesis, reduction in mucosal blood flow and over-expression of inflammatory mediators such as plasma tumor necrosis factor alfa (TNF-α) and the leukocyte–endothelial cell adherence.[4] As anticipated, NO-NSAIDs such as NO-Aspirin (**NCX 4016**), NO-Diclofenac (**NITROFENAC**),[5,6] and NO-Naproxen (**NAPROXCINOD/HCT 3012/AZD 3582**) (Fig. 1) have been shown either pre-clinically or clinically to cause minimal or insignificant gastric damage with comparable analgesic and anti-inflammatory activity to their respective parent NSAIDs.[7] These studies have provided a 'Proof of concept'[8] that is in agreement with their intended design and application. Interestingly, naproxcinod (**AZD 3582/HCT 3012**), whose Phase III studies were completed recently with a very favorable outcome, has been tipped by the media to be one of the ten potential 'Future Blockbusters'.[9]

With an intent to apply our disulfide-based linker and prodrug technology[10] to this emerging area of NO-NSAIDs, we have designed and synthesized novel NO-releasing prodrugs of

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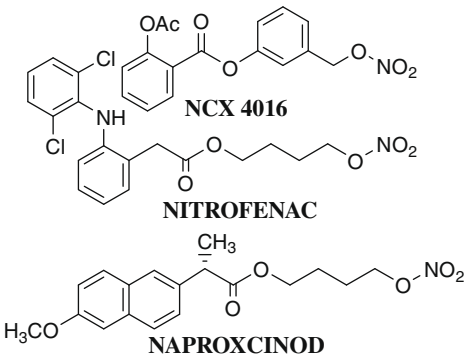


Figure 1. Structures of some known NO-NSAIDs.

anti-inflammatory agents, **1b–c** (NO-Aspirin) and **2b–c** (NO-Diclofenac) by utilizing widely used anti-inflammatory drugs aspirin (**1a**) and diclofenac (**2a**), respectively (Fig. 2), and evaluated their oral bioavailability, anti-inflammatory activity, NO-releasing profile and gastric-sparing properties in rats. We now report these promising preliminary results in this Letter.

The most important feature in our design of these NO-NSAIDs is the presence of an appropriately positioned disulfide linkage that is at β-position to both the carboxyl ester group of NSAID and NO-releasing nitrate ester group (Fig. 2) and their design was partly based on previously proposed sulfhydryl-dependent metabolism of organic nitrates such as glyceryl trinitrate (GTN) to NO.[11,12] Although drug release from ester bearing prodrugs can very likely occur via enzymatic hydrolysis, we have logically proposed a plausible sulfhydryl-assisted cleavage of these novel prodrugs as shown in Scheme 1. We thus suggest that a strategically placed disulfide bond in our NO-NSAID prodrug can get reduced by an intracellular sulfhydryl-containing species such as cysteine or glutathione via the proposed mechanism of cleavage to release free drug and intermediate metabolites, **M1** and **M2**. The presumed toxic episulfide (**M1**) can rapidly get metabolized further to a benign glutathione or cysteine conjugate **M5** as shown in the Scheme. The other presumed metabolite **M2** can undergo further breakdown in the presence of cysteine or glutathione as shown in the scheme which is based on the mechanism proposed earlier for such 'SS-nitrates'.[13] The presumed transient intermediate **M3** can breakdown further under biological conditions in two probable pathways as shown in the Scheme. While pathway 2 can lead to direct generation of NO and a benign glutathione or cysteine conjugate **M6**, pathway 1 may lead to the generation of a benign nitrate group and a reactive episulfide **M1** which can get converted further

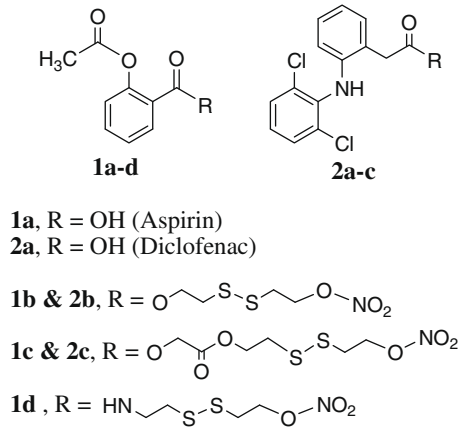
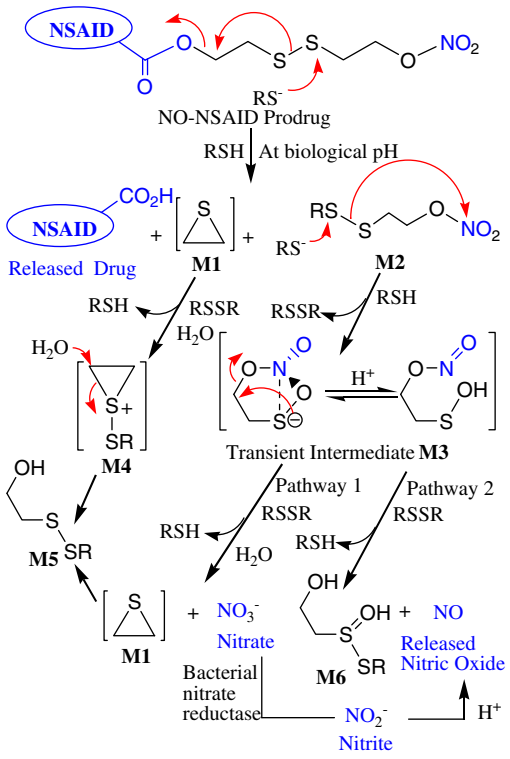


Figure 2. Structures of aspirin (**1a**), diclofenac (**2a**), NO-Aspirin prodrugs (**1b–d**) and NO-Diclofenac prodrugs (**2b–c**).

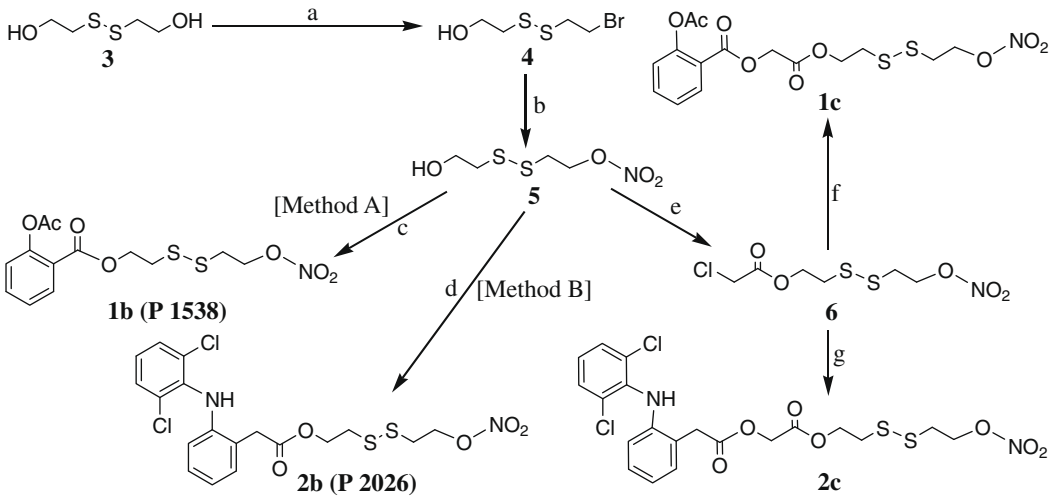


Scheme 1. Plausible mechanism of drug and NO release.

to a benign glutathione or cysteine conjugate **M5** as discussed above. It was also reported that the nitrate ion thus generated can get reduced by oral bacterial nitrate reductase to nitrite ion which in turn can rapidly get reduced to NO via nitrous acid in the acidic environment of the stomach.[14,15]

Synthesis of NO-NSAIDs (**1b–c**) and (**2b–c**) was performed as depicted in Scheme 2.[16] Syntheses of NO-Aspirin (**1b**) and NO-Diclofenac (**2b**) containing ester linkages were carried out by Method A and Method B, respectively. In Method A, selective mono-bromination of 2-hydroxyethyl disulfide (**3**) using carbon tetrabromide, triphenylphosphine and triethylamine afforded 2-((2-bromoethyl)disulfanyl)ethanol (**4**) in 52% yield. The monobromide **4** was treated with silver nitrate in acetonitrile to afford 2-((2-hydroxyethyl)disulfanyl)ethyl nitrate (**5**) in ~59% yield. The nitrate ester **5** was acylated with freshly prepared aspirin acid chloride in the presence of triethylamine to afford 2-((2-(nitrooxy)ethyl)disulfanyl)ethyl 2-acetoxybenzoate (NO-Aspirin, **1b**) in 64% yield. In Method B, the intermediate nitrooxy alcohol **5** was reacted with diclofenac in the presence of DCC and DMAP in 1:4 DMF/DCM to afford 2-((2-(nitrooxy)ethyl)disulfanyl)ethyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate (NO-Diclofenac, **2b**) in 50% yield. For the synthesis of diester-containing NO-NSAIDs (**1c** and **2c**), the intermediate nitrooxy alcohol **5** was first acylated with chloroacetyl chloride in the presence of triethylamine to afford 2-((2-(nitrooxy)ethyl)disulfanyl)ethyl 2-chloroacetate (**6**) which was further reacted with freshly prepared aspirin cesium salt in THF or diclofenac sodium salt in DMF to afford the double ester-containing 2-(2-((2-(nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl 2-acetoxybenzoate (NO-Aspirin, **1c**) or 2-(2-((2-(nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl 2-(2-(2,6-dichlorophenylamino)phenyl)-acetate (NO-Diclofenac, **2c**), respectively.

Similarly, synthesis of amide linkage-containing NO-Aspirin (**1d**) was also performed by using appropriate reagents and reaction conditions.[16]



Scheme 2. Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, 30 min at 0 °C, then 10 h at 25 °C, 52%; (b) Silver nitrate, acetonitrile, 25 °C, 3 h, 59%; (c) aspirin acid chloride, Et_3N, benzene, 25 °C, 5 h (mixed reagents at 0 °C), 64%; (d) diclofenac, DCC, DMAP,1:4 DMF/CH_2Cl_2, 0–5 °C, 3 h, 50%; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2, 2 h, 49%; (f) aspirin cesium, THF, 25 °C, 20 h, 35%; (g) diclofenac sodium, 1:3 DMF/THF, 24 h at 25 °C, then 8 h at 60 °C, 64%.

Biological studies on these novel NO-NSAIDs were carried out in rats for establishing their bioavailability, anti-inflammatory activities, and gastric tolerance relative to their respective parent drugs and the results of these experiments are presented in Table 1. The data presented for aspirin is actually for plasma salicylate levels as aspirin or its NO-Aspirin prodrugs quickly get hydrolyzed enzymatically in vivo to salicylic acid. We have not seen any intact NO-Aspirin derivative in plasma even at initial time points of the study. It is therefore obvious that none of the tested NO-Aspirin compounds behaved like a true prodrug of aspirin, but they may be termed as prodrugs of salicylic acid. Interestingly, only the ester linkage-containing prodrug **1b** showed appreciable bioavailability when compared to that of aspirin. Although the double ester linkage-containing prodrug **1c** showed moderate bioavailability, the amide linkage-containing compound **1d** did not show even traces of salicylate in plasma of the tested rats. It is therefore obvious that the compound **1d** did not act even as a prodrug of salicylic acid.

Interestingly, the bioavailability (AUC value) for the monoester-containing NO-Diclofenac prodrug **2b** is comparable to that of diclofenac. The double ester-containing NO-Diclofenac prodrug (**2c**) has also showed appreciable bioavailability. However, we have not seen any of these intact NO-Diclofenac prodrugs in the plasma at any time point and we therefore measured the plasma concentration of the released diclofenac for calculation of AUC values of NO-Diclofenac prodrugs (Table 1).

Although the NO-Aspirin prodrug **1b** showed appreciable bioavailability, it exhibited only moderate anti-inflammatory activity when compared to that of aspirin at equimolar doses at all the tested time points (Table 1).

As anticipated, the monoester-containing NO-Diclofenac prodrug **2b** showed nearly equal anti-inflammatory activity to that of diclofenac at equimolar doses at all the tested time points (Fig. 3). However, the double ester-containing NO-Diclofenac prodrug **2c** exhibited only moderate anti-inflammatory activity. These results clearly establish that the extent of anti-inflammatory activity is proportional to the plasma concentration of diclofenac.

Based on their extent of bioavailability and/or anti-inflammatory efficacy, we selected the NO-Aspirin prodrug **1b** and NO-Diclofenac prodrug **2b** and studied their gastric tolerance compared to their respective parent drugs in rats and the results of these experiments are presented in Table 1 as well as in Figure 4.

While both the parent drugs caused significant gastric damage, our NO-NSAIDs **1b** and **2b** at equimolar doses did not cause any GI damage. This gastric-sparing effect could be attributable to the beneficial actions of NO released from these promising NO-NSAIDs. However, additional factors such as masking of free acid group as an ester might be partly contributing to the gastric-sparing effect.

In order to experimentally determine the NO-releasing properties of these novel molecules, the most promising NO-Diclofenac prodrug **2b** was selected and studied in rats. The nitrate/nitrite concentration of the plasma of tested rats was determined by using Griess method.[19] As shown in Figure 5, NO-Diclofenac **2b** released significant quantities of NO in a sustained fashion when compared to that of control.

Based on this promising data, we selected the NO-Diclofenac prodrug **2b** and carried out additional in vivo pharmacology and toxicology studies and those results will be described elsewhere. This molecule is currently undergoing IND enabling preclinical toxicology studies.

Interestingly, the non-bioavailability exhibited by the amide derivative **1d** may support the view that an ester bond may be

Table 1
Data for bioavailability, anti-inflammatory activity,[17] and gastric tolerance[18] studies on NO-NSAIDs and their parent drugs

Compd[a]	AUC[d] (μg/ml h)	Activity[c] (% inhibition)[d]		Gastric ulcer area[d] (mm^2)
		4 h	6 h	
Aspirin[b]	4051 ± 312	42 ± 9*	31 ± 8*	**99 ± 32**
1b (**P1538**)	1234 ± 93	20 ± 12	17 ± 7	**0**
1c (**P1911**)	892 ± 140	Inactive	Inactive	ND
1d (**P1537**)	**0**	ND	ND	ND
Diclofenac	**65 ± 2**	**55 ± 9****	**21 ± 12**	**108 ± 36**
2b (**P2026**)	**60 ± 5**	**54 ± 7****	**20 ± 13**	**0**
2c (**P1912**)	49 ± 2	34 ± 10*	Inactive	ND

[a] Aspirin (150 mg/kg) or NO-Aspirin (150 mg/kg equimolar to aspirin), diclofenac (10 mg/kg) or NO-Diclofenac (10 mg/kg equimolar to diclofenac) was administered orally to rats.

[b] For aspirin and its prodrugs, plasma salicylate levels were estimated to calculate their bioavailability.

[c] Activity is expressed as % inhibition of paw volume over control rats. ND = Not determined.

[d] All values are expressed as mean ± S.E.M. (n = 3–4 for pharmacokinetic study; n = 5–8 for anti-inflammatory activity; n = 5 for gastric tolerance study).

* P <0.05.

** P <0.01 versus control.

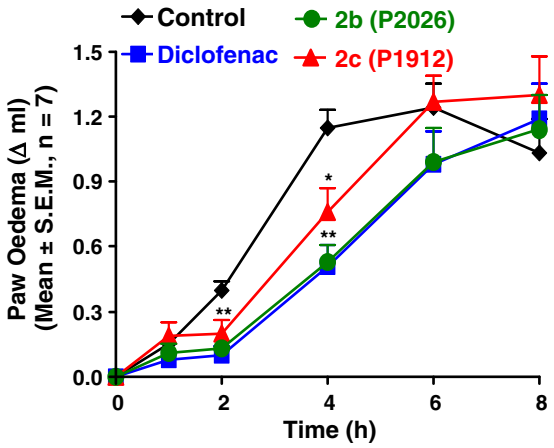


Figure 3. Anti-inflammatory activity of diclofenac (10 mg/kg) or NO-Diclofenac at equimolar dose in carrageenan-induced paw odema in rat. $^{*}P$ <0.05, $^{**}P$ <0.01 versus control.

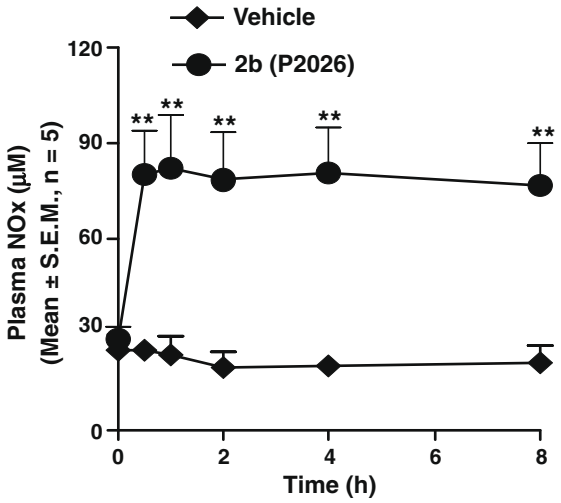


Figure 5. Plasma NOx (nitrite/nitrate) following oral administration of NO-Diclofenac (5 mg/kg equimolar diclofenac) in rat. $^{**}P$ <0.01 versus control.

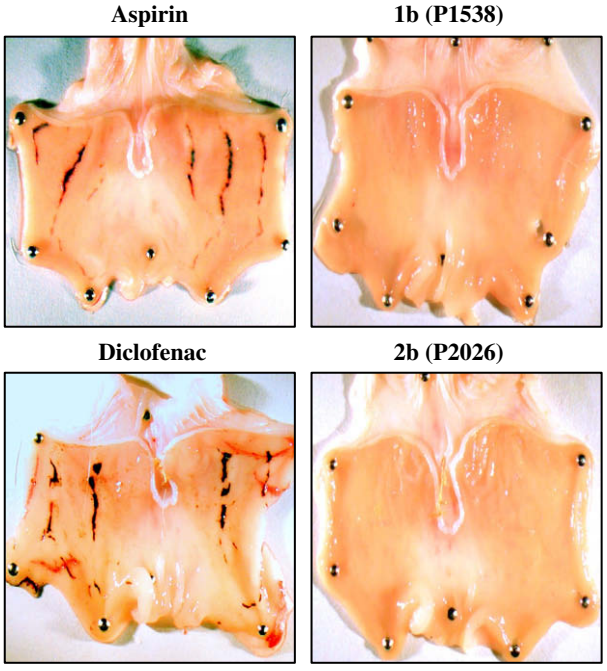


Figure 4. Representative images of rat stomachs showing gastric ulcer induction/sparing following oral administration of NSAID (100 mg/kg) or NO-NSAID (100 mg/kg equimolar to NSAID) in rats.

essential for this class of NO-NSAIDs to act as prodrugs. This structural requirement may also support the view that these ester prodrugs might be releasing free drugs via enzymatic hydrolysis. However, the proposed sulfhydryl-dependent mechanism of NO release from these novel NO-NSAIDs still looks very plausible.

In summary, we have designed, synthesized, and evaluated biological activity of novel NO-NSAID prodrugs such as NO-Aspirin and NO-Diclofenac. They have shown promising pharmacokinetic, anti-inflammatory and NO-releasing properties and also protected rats from NSAID-induced gastric damage which could be most likely due to their NO-releasing capability. Among the evaluated prodrugs, the NO-Diclofenac prodrug **2b** has shown excellent gastro-protective and NO-releasing properties in addition to exhibiting comparable bioavailability and anti-inflammatory activities to those of diclofenac. These preliminary results clearly establish the 'Proof of concept' in that our novel disulfide linker-containing NO-NSAIDs are indeed acting as potentially gastric-sparing 'Safe NSAIDs'. Further application of this promising disulfide linker and prodrug technology to other prominent NSAIDs such as naproxen, ibuprofen, indomethacin, flurbiprofen, and ketoprofen, to identify a few more potentially gastric-sparing 'Safe NSAIDs', is in progress.

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Supplementary data

Supplementary data (experimental details for the synthesis and characterization of all reported compounds; procedures for biological experiments and some additional biological data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.142.

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16. Refer to Supplementary data for complete experimental details and compound characterization data. Analytical and spectral data for final compounds are given below: *Compound* **1b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.97 (t, J = 7.5 Hz, 2H), 3.05 (t, J = 7.5 Hz, 2H), 4.53 (t, J = 7.5 Hz, 2H), 4.69 (t, J = 7.5 Hz, 2H), 7.11 (dd, J = 6 Hz, 1H), 7.33 (dt, J = 6 Hz, 1H), 7.58 (dt, J = 6 Hz, 1H), 8.02 (dd, J = 1.8, 1.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.1, 34.8, 37.1, 62.6, 70.6, 122.7, 123.8, 126.1, 131.7, 134.2, 150.8, 164.0, 169.7. HRMS ESI (m/z): $[M+Na]^+$ calcd for $C_{13}H_{15}NO_7NaS_2$: 384.0188; found: 384.0193 (mass accuracy: 1.3 ppm); *compound* **1c**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.80–3.03 (m, 4H), 4.46 (t, J = 9.5 Hz, 2H), 4.62 (t, J = 7 Hz, 2H), 4.90 (s, 2H), 7.14 (d, J = 8 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 8.11 (d, J = 8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0, 34.8, 36.7, 61.04, 62.7, 70.6, 122.2, 123.9, 126.1, 132.0, 134.1, 150.9, 163.6, 167.3, 169.6. HRMS ESI (m/z): $[M+Na]^+$ calcd for $C_{15}H_{17}N_1Na_1O_9S_2$: 442.0237; found: 442.0233 (mass accuracy: 0.90 ppm); *compound* **1d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.92 (t, J = 6.1 Hz, 2H), 2.98 (t, J = 6.81 Hz, 2H), 3.76 (q, J = 6.05 Hz, 2H), 4.71 (t, J = 6.73 Hz, 2H), 6.70 (br s, 1H), 6.90,7.12 (dd, J = 1.04 Hz, 1H), 7.31(t, J = 3 Hz, 1H), 7.48 (t, J = 3 Hz, 1H), 7.77, 7.79 (dd, J = 1.68 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.3, 34.5, 37.6, 38.2, 70.5, 123.2, 126.3, 127.7, 129.8, 132.0, 148.0, 165.8, 169.1. HRMS ESI (m/z): $[M+Na]^+$ calcd for $C_{13}H_{16}N_2O_6S_2Na$: 383.0347; found: 383.0342 (mass accuracy: 1.5 ppm); *compound* **2b**: ^{1}H NMR ($CDCl_3$): δ 2.93–3.03 (m, 4H), 3.86 (s, 2H), 4.44 (t, J = 6.6 Hz, 2H), 4.67 (t, J = 6.9 Hz, 2H), 6.57 (d, J = 7.8 Hz, 1H), 6.83 (br s, 1H), 6.96–7.04 (m, 2H), 7.13–7.18 (m, 1H), 7.25–7.28 (m, 1H), 7.36–7.38 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.3, 36.5, 37.9, 62.4, 70.0, 117.8, 121.6, 123.6, 127.6, 128.4, 129.0, 130.4, 137.2, 142.1, 171.5. HRMS ESI (m/z): $[M+H]^+$ calcd for $C_{18}H_{19}Cl_2N_2O_5S_2$: 477.0107; found: 476.0114 (mass accuracy: −1.47 ppm); *compound* **2c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 (t, J = 6.6 Hz, 2H), 2.95 (t, J = 6.6 Hz, 2H), 3.96 (s, 2H), 4.41 (t, J = 6.6 Hz, 2H), 4.69 (t, J = 6.6 Hz, 2H), 4.72 (s, 2H), 6.56–6.59 (m, 1H), 6.72 (br s, 1H), 6.97–7.04 (m, 2H), 7.13–7.19 (m, 1H), 7.27–7.30 (m, 1H), 7.36 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.3, 36.1, 37.5, 60.6, 62.4, 70, 117.9, 121.7, 123.3, 123.6, 127.7, 128.4, 129, 130.5, 137.3, 142.2, 166.7, 170.9. HRMS ESI (m/z): $[M+H]^+$ calcd for $C_{20}H_{21}Cl_2N_2O_7S_2$: 535.0162; found: 535.0181 (mass accuracy: −3.55 ppm).
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