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Bioorthogonal release of sulfonamides and mutually orthogonal liberation of two drugs†

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Abstract

Sulfonamide derivatives have been used in pharmaceutics for decades. Here we report a new approach to release sulfonamides efficiently using a bioorthogonal reaction of sulfonyl sydnonimines and dibenzoazacyclooctyne (DIBAC). The second-order rate constant of the cycloaddition reaction can be up to 0.62 M^{-1} s^{-1}, and the reactants are highly stable under physiological conditions. Most significantly, we also discovered the mutual orthogonality between the sydnonimine–DIBAC and benzonorbornadiene–tetrazine cycloaddition pairs, which can be used for selective and simultaneous liberation of sulfonamide and primary amine drugs.

Sulfonamides constitute an important family of drugs that have been extensively applied in human and veterinary medicine due to their general stability, bioavailability, and ease of preparation.[1] The compounds of this class (Fig. 1) have been clinically in use for decades, and some of which showed potent antitumor activity.[2] Over decades, various strategies for controlled release of drugs under biological conditions have been developed,[3] such as multi-function polymeric nanoformulations[4] and photoresponsive biomaterials for targeted drug delivery.[5] However, in the case of sulfonamides, few examples had been reported, such as the use of an epichlorohydrin-crosslinked semi-interpenetrating polymer network to control the release of the sulfonamide drug sulpiride.[6] In biological systems, the physiological triggers, such as pH value,[7] are subtle, more general and reliable triggers that involve rapid bond formation and selective bond cleavage[8] to release drug molecules are highly desirable.

In recent years, with bioorthogonal chemistry as a powerful tool,[9] click-release reactions have been developed and applied to drug delivery, enzyme activation, and so on.[10] In 2013,

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Conflicts of interest

There are no conflicts to declare.

Robillard and coworkers reported the inverse-electrondemand Diels–Alder (IEDDA) reaction of carbamate-modified *trans*-cyclooctene (TCO) with tetrazine that allows release of a primary amine drug doxorubicin attached to the TCO after the cycloaddition step under ambient conditions.[10a] Royzen *et al.* applied this drug release strategy into treating soft tissue sarcoma *in vivo*.[10g] Very recently, Weissleder and coworkers discovered that the TCO–tetrazine-ligation-based drug release is sensitive to pH value, and can only achieve partial release due to an intramolecular side reaction.[11] They further designed and synthesized the tetrazine-acids for rapid cleavage reactions that are less sensitive to pH value. In addition, the Gamble group reported the strain-promoted 1,3-dipolar cycloaddition of TCO and azide, the product of which undergoes degradation, hydrolysis, and elimination to release doxorubicin.[10d]

Sydnone and its derivatives are a relatively new class of bioorthogonal reagents, introduced into bioorthogonal reactions by Taran,[12,13] and some of them have been used in click-release reactions.[14] Chin first described the reaction of *N*-phenyl sydnone with strained alkyne bicyclo-[6.1.0]-nonyne (BCN) in protein labeling.[15] The rate constant in MeOH/H_2O (55 : 45) was measured to be 0.054 M^{-1} s^{-1} at 21 °C. Previously we predicted that dibenzoazacyclooctyne (DIBAC) had better reactivity than BCN with *N*-phenyl sydnone by DFT calculations. Our prediction was confirmed by the Murphy group, and the measured rate constant is 0.90 M^{-1} s^{-1} (Fig. 2a).[16] Inspired by the fact that sydnone cycloaddition is followed by the extrusion of CO_2, which has a very low barrier,[16] we envision that the sulfonyl sydnonimine cycloaddition will lead to the simultaneous generation of sulfonyl isocyanate, which is extremely unstable in aqueous media and quantitatively hydrolyzed to form CO_2 and sulfonamide.[17] Therefore, a new strategy for the controlled release of sulfonamides is proposed: sulfonyl-modified sydnonimines (N_6-sulfonyl-SIN **4**, with aliphatic and aromatic substituents) undergo 1,3-dipolar cycloadditions with DIBAC **5**, resulting in rapid release of sulfonamide **8** *via* sulfonyl isocyanate 7 (Fig. 2b).

Before launching into experiments, we carried out DFT calculations at the M06-2X level of theory[18] on the (3+2) cycloaddition of N_6-methanesulfonyl-SIN **4a** and DIBAC. The computed activation free energy for this reaction is 21.5 kcal mol^{-1} (**TS_4a-DIBAC**, Fig. 3), corresponding to a predicted second-order rate constant of 0.12 M^{-1} s^{-1}, which is reasonably fast for a bioorthogonal reaction. Subsequently, we synthesized sydnonimine hydrochloride **9**.[19] After optimization, we found that lowering the reaction temperature to −10 to 0 °C, the desired products N_6-sulfonyl-SIN **4a–c** can be obtained in good yields from the reaction of **9** and sulfonyl chlorides **10** in dichloromethane with triethylamine (Fig. 4).[20]

To test the feasibility of the click-release strategy, we monitored the cycloaddition reaction of **4a** (c_0 = 2 mM) with DIBAC-COOH 5 (c_0 = 2.2 mM) in 9 : 1 DMSO-d_6/D_2O at 22 °C with ^{1}H NMR spectroscopy (Fig. 5a). The process of the reaction is characterized by the disappearance of the methyl proton signal of **4a** at 2.97 ppm (▪, Fig. 5a). After 2 hours, we observed 40% conversion of **4a** into the cycloaddition product **6** (★, Fig. 5a) and methanesulfonamide (**8a**) (*, δ= 2.91 ppm, Fig. 5a). After 6 hours, 65% of the starting material was consumed. Nearly complete transformation took place within 24 hours, and no side product or intermediate signals were observed during the whole process. This result confirmed our prediction of the effciency of the cycloaddition reaction and the release of

sulfonamide. We also monitored the reaction of pro-drug **4c** (N_6-CXB-SIN) with **5** in 9 : 1 DMSO-d_6/D_2O using the same method (see ESI†). The release of drug **8c** (celecoxib, CXB) is very effcient.

We further studied the kinetics of the reactions of N_6-sulfonyl-SIN **4a–c** with 5. As shown in Fig. 5b, the measured second-order rate constants (k_2) of N_6-methanesulfonyl-SIN **4a** was 0.043 M^{-1} s^{-1} in 9 : 1 DMSO-d_6/D_2O at 22 °C, and N_6-sulfonyl-SIN **4b–c** have better reactivities than **4a** (0.067 M^{-1} s^{-1} and 0.050 M^{-1} s^{-1} for **4b** and **4c**, respectively). To our delight, the second-order rate constants are increased by 5–9 fold in 1 : 1 DMSO-d_6/D_2O (Fig. 5b).[21] Moreover, the stability of pro-drug N_6-CXB-SIN **4c** is tested in phosphate-buffered saline (PBS) and fetal bovine serum (FBS) at 37 °C. No degeneration was observed within a week in PBS/DMSO (1 : 1 v/v), while less than 20% of **4c** decomposed after a week in FBS/PBS (1 : 1 v/v). The high stability of the pro-drug and efficiency of the click-release reaction make **4c** very promising for *in vivo* applications.

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID), a specific COX-2 inhibitor for pain and inflammation without inhibiting COX-1,[22] and also shows potent antitumor activity.[23] Since the bioorthogonal release of CXB has proved to be effcient, we further test the inhibition effect of this method against human COX-2 (Fig. 5c). When pro-drug **4c** was treated with DIBAC 5 (100 mM) in the system, it shows excellent performance with IC_{50} of 0.106 mM, which is very close to that of the direct use of CXB (IC_{50} of 0.065 μM). Decreasing the concentration of DIBAC to 50 μM, the measured IC_{50} slightly dropped to 0.169 μM. The pro-drug **4c** and DIBAC **5** are both nontoxic at the testing concentration with IC_{50} > 25.0 μM. Control experiments indicate that the combination of **4b** and **5** showed no toxicity even at maximal testing concentration, demonstrating that the cycloadduct **6** is nontoxic. These results confirmed the biostability and high cycloaddition-release effciency of **4c**, which can be potentially applied to targeted drug release.

Recently, the Franzini group reported that 7-aza-benzonorbornadiene (ABNBD) derivatives react with tetrazine to liberate a primary amine drug doxorubicin.[10i] Tetrazines react rapidly with ABNBD but are inert to DIBAC.[24] Our calculations predicted that sydnonimines have selectivity opposite to that of tetrazines, and react with DIBAC but not with ABNBD (Fig. 3). The computed activation barrier for the reaction between sydnonimine **4a** and ABNBD is 27.2 kcal mol^{-1}, corresponding to a rate constant of 3×10^{-4} M^{-1} s^{-1}. Based on these facts, we designed click-release pairs that can be used for orthogonal release of two different drugs simultaneously, where sydnonimine–DIBAC and tetrazine–ABNBD cycloaddition pairs are delivery vehicles for celecoxib **8c** and doxorubicin **13**, respectively (Fig. 6a). ^{1}H NMR spectroscopy confirmed that there is no reaction between sydnonimine **4c** and ABNBD **11** within 25 hours (Fig. 6b). We successfully released celecoxib (after 4.5 h) or doxorubicin (after **11** h) by introducing DIBAC **5** or 3,6-di(2-pyridyl)-1,2,4,5-tetrazine (DPTz, **12**) correspondingly to the system where both **4c** and 11 were present (Fig. 6b). When both **5** and **12** were added, the selective and simultaneous liberation of two drugs **8c** and **13** can be complete after 11 hours (Fig. 6b). While a few examples of multi-component bio-labeling

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using mutually orthogonal cycloaddition pairs were reported,[24a,25] this established the first example of using mutually orthogonal click-release pairs to liberate multiple bioactive molecules.

In summary, we describe a bioorthogonal reaction of sulfonyl sydnonimines with DIBAC for effcient release of sulfonamides. The sulfonyl sydnonimine as pro-drug exhibits excellent bio-stability with low toxicity under physiological conditions, which is essential in targeted drug release. With the aid of DFT calculations, we also discovered the first mutually orthogonal click-release pairs—sydnonimine–DIBAC and tetrazine–ABNBD, which realized the liberation of sulfonamide and primary amine drugs selectively and simultaneously in one system. This method is promising for applications in dual-drug delivery and targeted therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. (a) Supuran CT, Nat. Rev. Drug Discovery, 2008, 7, 168; [PubMed: 18167490] (b) Supuran CT, Molecules, 2017, 22, 1642.
2. (a) Monti SM, Supuran CT and Simone GD, Expert Opin. Ther. Pat, 2013, 23, 737; [PubMed: 23672415] (b) Krall N, Pretto F, Decurtins W, Bernardes GJ, Supuran CT and Neri D, Angew. Chem., Int. Ed, 2014, 53, 4231.
3. Bernkop-Schnurch A and Jalil A, J. Controlled Release, 2018, 271, 55.
4. (a) Ding C and Li Z, Mater. Sci. Eng., C, 2017, 76, 1440;(b) Mai BT, Fernandes S, Balakrishnan PB and Pellegrino T, Acc. Chem. Res, 2018, 51, 999. [PubMed: 29733199]
5. (a) Yue X, Zhang Q and Dai Z, Adv. Drug Delivery Rev, 2017, 115, 155;(b) Ruskowitz ER and DeForest CA, Nat. Rev. Mater, 2018, 3, 17087.
6. Hoosain FG, Choonara YE, Kumar P, Tomar LK, Tyagi C, du Toit LC and Pillay V, AAPS PharmSciTech, 2017, 18, 654. [PubMed: 27184677]
7. Wang Z, Deng X, Ding J, Zhou W, Zheng X and Tang G, Int. J. Pharm, 2018, 535, 253. [PubMed: 29113804]
8. Li J and Chen PR, Nat. Chem. Biol, 2016, 12, 129. [PubMed: 26881764]
9. (a) Prescher JA and Bertozzi CR, Nat. Chem. Biol, 2005, 1, 13; [PubMed: 16407987] (b) Jewett JC and Bertozzi CR, Chem. Soc. Rev, 2010, 39, 1272; [PubMed: 20349533] (c) Devaraj NK and Weissleder R, Acc. Chem. Res, 2011, 44, 816; [PubMed: 21627112] (d) Lang K and Chin JW, ACS Chem. Biol, 2014, 9, 16; [PubMed: 24432752] (e) Patterson DM, Nazarova LA and Prescher JA, ACS Chem. Biol, 2014, 9, 592; [PubMed: 24437719] (f) Lang K and Chin JW, Chem. Rev, 2014, 114, 4764; [PubMed: 24655057] (g) Liu F, Liang Y and Houk KN, Acc. Chem. Res, 2017, 50, 2297; [PubMed: 28876890] (h) Row RD and Prescher JA, Acc. Chem. Res, 2018, 51, 1073; [PubMed: 29727171] (i) Wu H and Devaraj NK, Acc. Chem. Res, 2018, 51, 1249. [PubMed: 29638113]

10. (a) Versteegen RM, Rossin R, ten Hoeve W, Janssen HM and Robillard S, Angew. Chem., Int. Ed, 2013, 52, 14112;(b) Li J, Jia S and Chen PR, Nat. Chem. Biol, 2014, 10, 1003; [PubMed: 25362360] (c) Wu H, Cisneros BT, Cole CM and Devaraj NK, J. Am. Chem. Soc, 2014, 136, 17942; [PubMed: 25495860] (d) Matikonda SS, Orsi DL, Staudacher V, Jenkins IA, Fiedler F, Chen J and Gamble AB, Chem. Sci, 2015, 6, 1212; [PubMed: 29560207] (e) Wu H, Alexander SC, Jin S and Devaraj NK, J. Am. Chem. Soc, 2016, 138, 11429; [PubMed: 27510580] (f) Fan X, Ge Y, Lin F, Yang Y, Zhang G, Ngai WS, Lin Z, Zheng S, Wang J, Zhao J, Li J and Chen PR, Angew. Chem., Int. Ed, 2016, 55, 14046;(g) Mejia Oneto JM, Khan I, Seebald L and Royzen M, ACS Cent. Sci, 2016, 2, 476; [PubMed: 27504494] (h) Rossin R, van Duijnhoven SM, ten Hoeve W, Janssen HM, Kleijn LH, Hoeben FJ, Versteegen RM and Robillard MS, Bioconjugate Chem, 2016, 27, 1697;(i) Xu M, Tu J and Franzini RM, Chem. Commun, 2017, 53, 6271;(j) Rossin R, Versteegen RM, Wu J, Khasanov A, Wessels HJ, Steenbergen EJ, ten Hoeve W, Janssen HM, van Onzen A, Hudson PJ and Robillard MS, Nat. Commun, 2018, 9, 1484; [PubMed: 29728559] (k) Tu J, Xu M, Parvez S, Peterson RT and Franzini RM, J. Am. Chem. Soc, 2018, 140, 8410. [PubMed: 29927585]
11. Carlson JCT, Mikula H and Weissleder R, J. Am. Chem. Soc,2018, 140, 3603. [PubMed: 29384666]
12. Kolodych S, Rasolofonjatovo E, Chaumontet M, Nevers MC, Creminon C and Taran F, Angew. Chem., Int. Ed, 2013, 52, 12056.
13. Decuypere E, Plougastel L, Audisio D and Taran F, Chem. Com-mun, 2017, 53, 11515.
14. (a) Bernard S, Audisio D, Riomet M, Bregant S, Sallustrau A, Plougastel L, Decuypere E, Gabillet S, Kumar RA, Elyian J, Trinh N, Koniev O, Wagner A, Kolodych S and Taran F, Angew. Chem., Int. Ed, 2017, 56, 15612;(b) Riomet M, Decuypere E, Porte K, Bernard S, Plougastel L, Kolodych S, Audisio D and Taran F, Chem. – Eur. J, 2018, 24, 8535. [PubMed: 29665096]
15. Wallace S and Chin JW, Chem. Sci, 2014, 5, 1742. [PubMed: 25580211]
16. Narayanam MK, Liang Y, Houk KN and Murphy JM, Chem. Sci, 2016, 7, 1257. [PubMed: 29910881]
17. Field L and Settlage PH, J. Am. Chem. Soc, 1954, 76, 1222.
18. (a) Zhao Y and Truhlar DG, Theor. Chem. Acc, 2008, 120, 215;(b) Zhao Y and Truhlar DG, Acc. Chem. Res, 2008, 41, 157; [PubMed: 18186612] (c) de la Concepci´on JG, A´valos M, Cintas P and Jim´enez JL, Chem. – Eur. J, 2018, 24, 7507; [PubMed: 29534312] (d) Tao H, Liu F, Zeng R, Shao Z, Zou L, Cao Y, Murphy JM, Houk KN and Liang Y, Chem. Commun, 2018, 54, 5082.
19. Beal EN and Tumbull K, Synth. Commun, 1992, 22, 673.
20. Daeniker HU and Druey J, Helv. Chim. Acta, 1962, 45, 2462.
21. Introducing an ester group at the *para* position of the *N*-phenyl group of sydnonimine can enhance the reaction rate by about 3 fold. For details, see ESI†.
22. Penning TD, Talley JJ, Bertenshaw SR, Carter JS,Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, Anderson GD,Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY and Isakson PC, J. Med. Chem, 1997, 40, 1347. [PubMed: 9135032]
23. Lu XY, Wang ZC, Ren SZ, Shen FQ, Man RJ and Zhu HL,Bioorg. Med. Chem. Lett, 2016, 26, 3491. [PubMed: 27349331]
24. (a) Karver MR, Weissleder R and Hilderbrand SA, Angew. Chem., Int. Ed, 2012, 51, 920;(b) Liang Y, Mackey JL, Lopez SA, Liu F and Houk KN, J. Am. Chem. Soc, 2012, 134, 17904. [PubMed: 23061442]
25. (a) Willems LI, Li N, Florea BI, Ruben M, van der Marel GA and Overkleeft HS, Angew. Chem., Int. Ed, 2012, 51, 4431;(b) Patterson DM, Nazarova LA, Xie B, Kamber DN and Prescher JA, J. Am. Chem. Soc, 2012, 134, 18638; [PubMed: 23072583] (c) Cole C, Yang J, ˇSeˇckut˙e J and Devaraj NK, ChemBioChem, 2013, 14, 205; [PubMed: 23292753] (d) Patterson DM, Jones KA and Prescher JA, Mol. BioSyst, 2014, 10, 1693; [PubMed: 24623192] (e) Sachdeva A, Wang K, Elliott T and Chin JW, J. Am. Chem. Soc, 2014, 136, 7785; [PubMed: 24857040] (f) Nikic I, Plass T, Schraidt O, Szymanski J, Briggs JAG, Schultz C and Lemke EA, Angew. Chem., Int. Ed, 2014, 53, 2245.

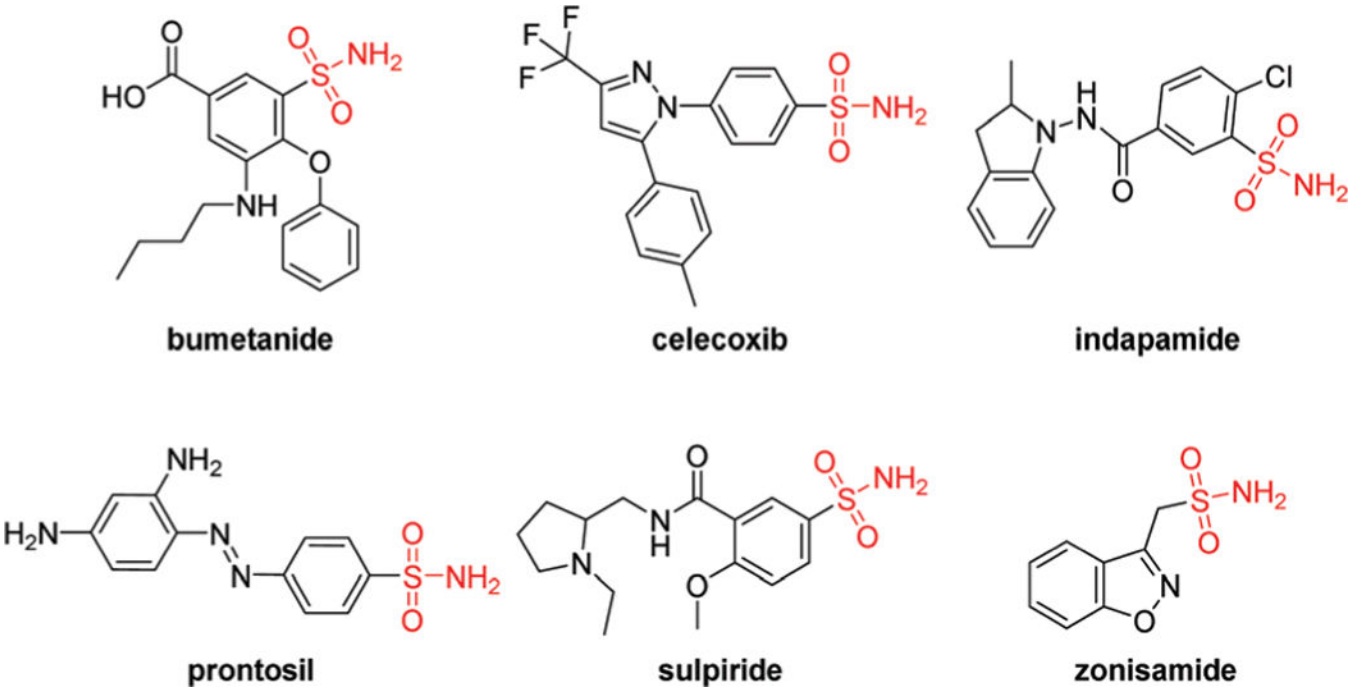


Fig. 1.
Sulfonamide drugs in clinical use.

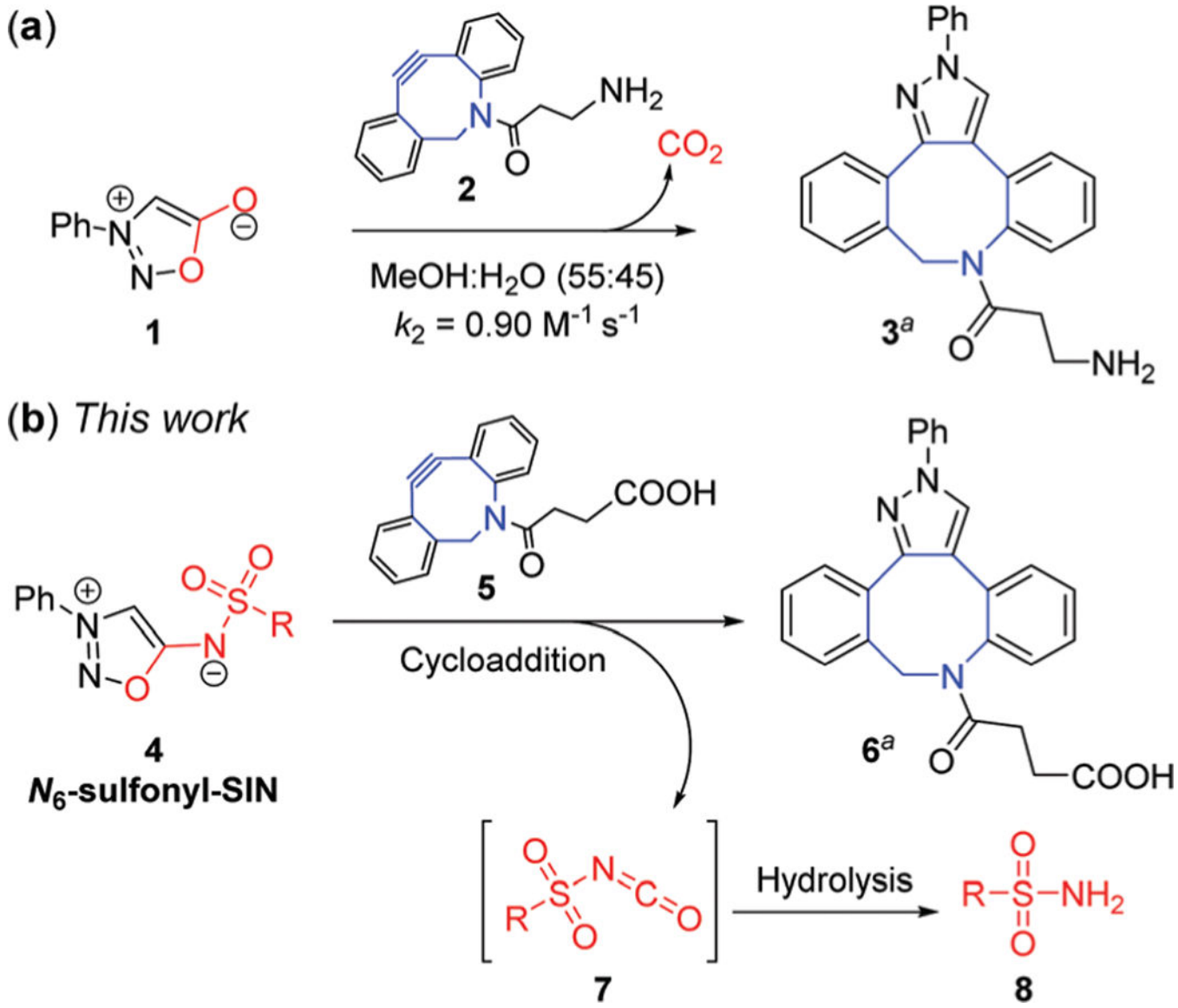


Fig. 2.

(a) The (3+2) cycloaddition reaction of sydnone; (b) release of sulfonamides through the (3+2) reaction of N_6-sulfonyl sydnonimines. [a]Only one regioisomer is depicted.

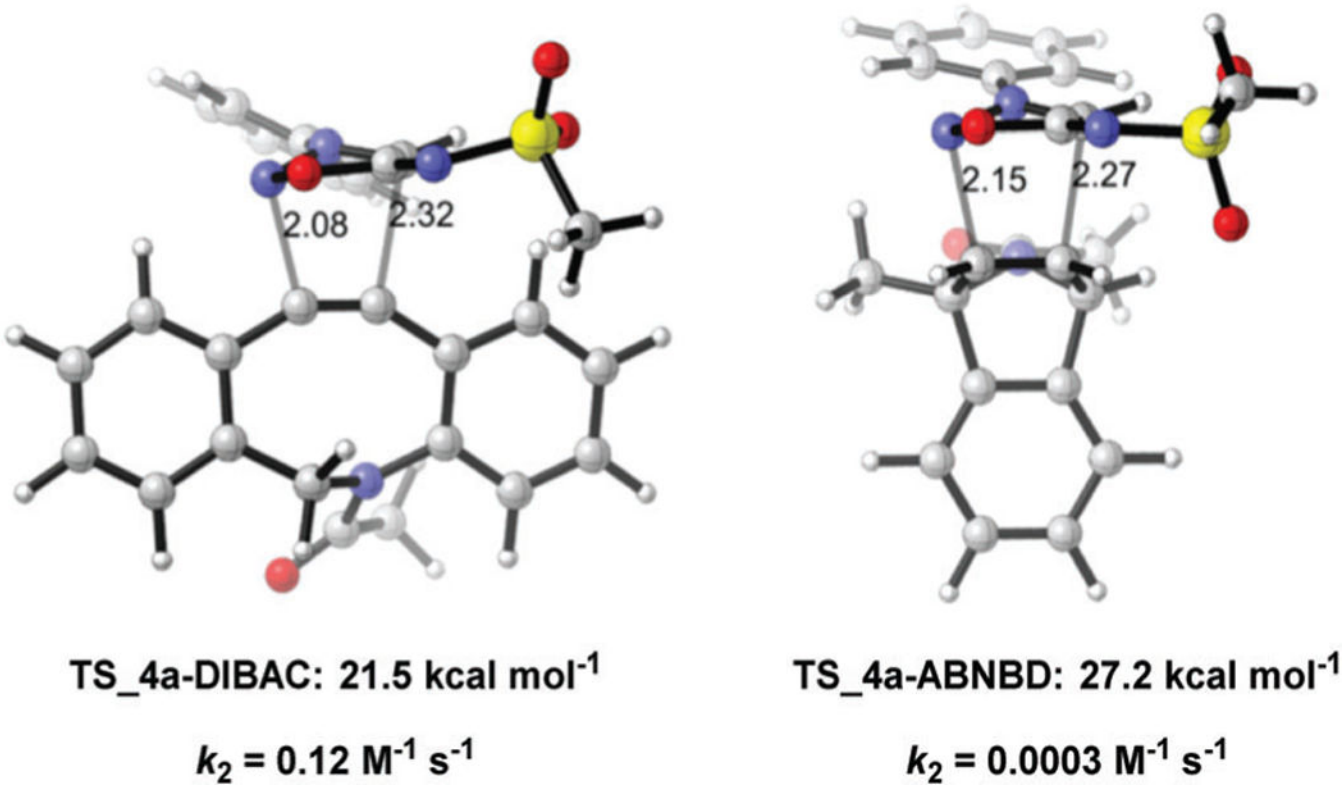


Fig. 3.

Transition-state structures for the (3+2) cycloadditions of **4a** with DIBAC and ABNBD (activation free energies and predicted rate constants are shown below each structure).

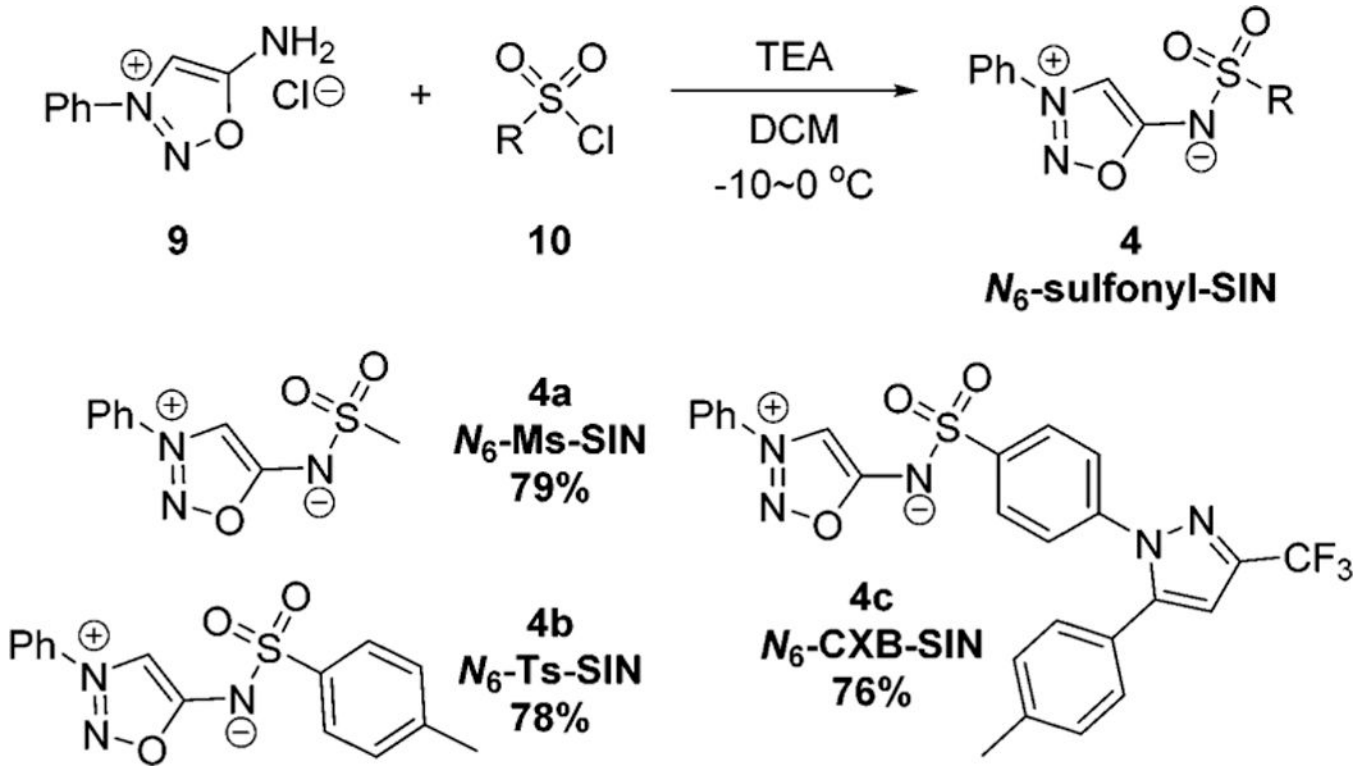
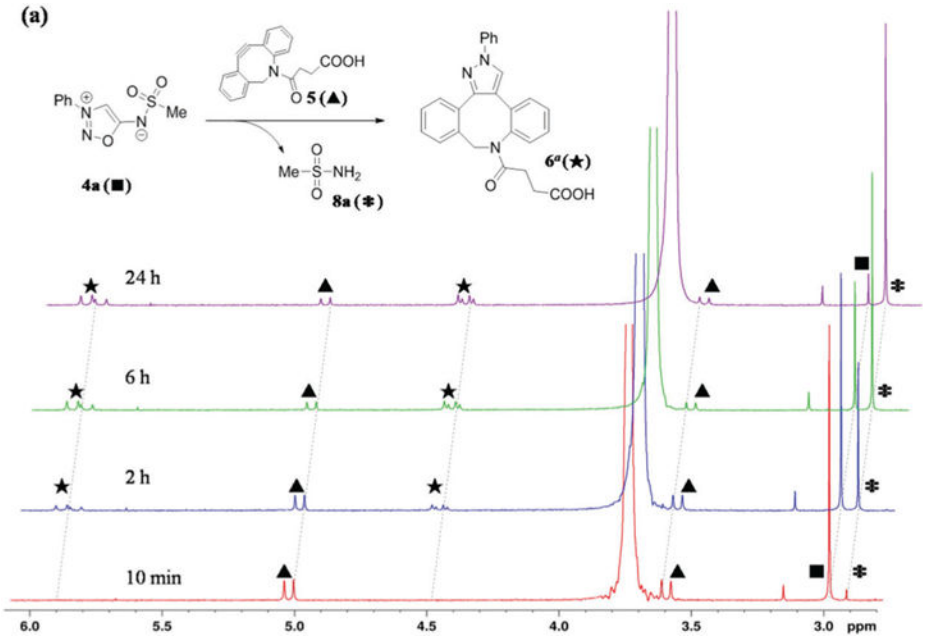


Fig. 4.
Synthesis of N_6-sulfonyl sydnonimines.



(b)

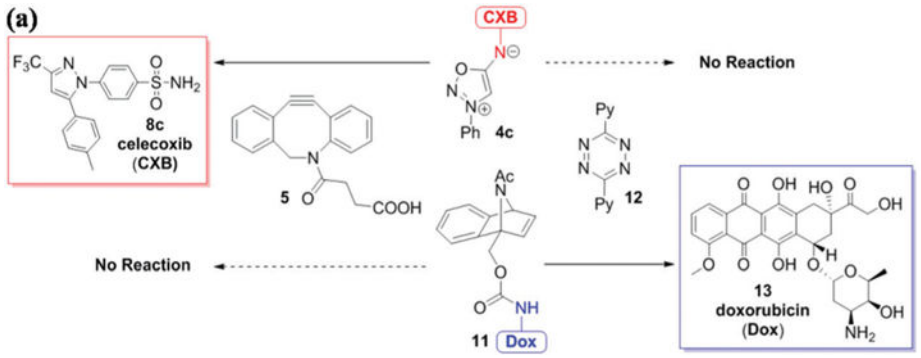
	DIBAC-COOH **5**	
	90 % DMSO-d_6/D_2O	50 % DMSO-d_6/D_2O
4a	0.043 ± 0.004	0.20 ± 0.03
4b	0.067 ± 0.008	0.62 ± 0.08
4c	0.050 ± 0.007	0.45 ± 0.02

(c)

Compounds	IC_{50}, μM[b]
CXB	0.065 (0.045 – 0.094)
4c + **5** (100 μM)	0.106 (0.042 – 0.270)
4c + **5** (50 μM)	0.169 (0.117 – 0.244)
4c	> 25.0
5	> 25.0
4b + **5** (100 μM)	> 25.0

Fig. 5.

(a) ^{1}H NMR analysis of the reaction of N_6-Ms-SIN **4a** (2 mM) and DIBAC-COOH **5** (2.2 mM) in DMSO-d_6/D_2O (9 : 1 v/v). δ = 3.82 ppm is H_2O. [a]Only one regioisomer is depicted. (b) Measured rate constants (k_2, in M^{-1} s^{-1}) for reactions between **4a–c** and **5** at 22 °C. (c) IC_{50} values against human COX-2. [b] 95% confidence interval (n = 2) is given in parentheses.



(b)

Conditions				Drug release	
4c	5	11	12	CXB	Dox
+	−	+	−	×	×
+	+	+	−	√	×
+	−	+	+	×	√
+	+	+	+	√	√

Fig. 6.

Orthogonal drug release of celecoxib and doxorubicin. (a) Mutually orthogonal click-release pairs. (b) ^{1}H NMR analysis of drug release in DMSO-d_6/D_2O (9 : 1 v/v). Attendance reagent (+), or no reagent (−); release drug (√), or no drug (×).