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Diaryl-dithiolanes and -isothiazoles: COX-1/COX-2 and 5-LOX-inhibitory, ·OH scavenging and anti-adhesive activities

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ABSTRACT

Three series of non-steroidal anti-inflammatory drugs (NSAIDs) inhibiting the cyclooxygenase/5-lipoxygenase (COX/5-LOX) pathways as such as formation of hydroxyl radicals and adhesion were prepared: 4,5-diaryl isothiazoles, 4,5-diaryl 3*H*-1,2-dithiole-3-thiones and 4,5-diaryl 3*H*-1,2-dithiole-3-ones. The aim of the present study was to develop substances which can intervene into the inflammatory processes via different mechanisms of action as multiple target non-steroidal anti-inflammatory drugs (MTNSAIDs) with increased anti-inflammatory potential. The current lead **11a** was evaluated in COX-1/2, 5-LOX and ·OH scavenging in vitro assays and in a static adhesion assay where it proved to inhibit adhesion. Moreover, **11a** treatment attenuated expression of macrophage adhesion molecule-1 (Mac-1) on extravasated polymorphonuclear leukocytes (PMNs) which indicates that the activation was reduced. The assays used are predictive for the in vivo efficacy of test compounds as shown for **11a** in a peritonitis model of acute inflammation in mice. Thus, the novel 5-LOX/COX and ·OH inhibitor **11a** possesses anti-inflammatory activity that, in addition to COX/5-LOX inhibition, implicates effects on leukocyte–endothelial interactions.

1. Introduction

NSAIDS are basically used for the treatment of pain, inflammation and fever. The main mechanism of action of these drugs is the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2),[1,2] which are catalyzing the biotransformation of arachidonic acid to the prostaglandins.

Gastrointestinal haemorrhage, ulceration and kidney failure are the major side effects associated with all currently available NSAIDS (e.g., celecoxib and diclofenac in Scheme 1).[3] These unwanted side activities are caused by the interference of the physiological balance of prostaglandins. To overcome this several approaches were undertaken. One attempt haunts the strategy of selective inhibition of COX-2. Nevertheless, clinical studies have suggested that selective COX-2 inhibitors could cause typical COX-mediated side effects such as gastrointestinal injury, increased systemic blood pressure and hypersensitivity.[4] Due to safety concerns of an increased risk of cardiovascular events (including heart attack and stroke) in patients on Vioxx™ a voluntary withdrawal of Vioxx™ (rofecoxib) was announced on the worldwide market since 1999. Consequently the American Gastroenterological Association (AGA) differentiated the therapy for patients with higher gastrointestinal (GI) risks or higher cardiovascular (CV) risk in three cases.[5]

Recently the target switched to a combined 5-LOX/COX-inhibition, interfering both pathways, the production of prostaglandins and the biosynthesis of leukotrienes (LTs).[4,6]

This strategy i.g. led to tepoxalin which is a new non-steroidal anti-inflammatory drug and up to now approved for veterinary use only in the United States and the European Union under the brand name Zubrin® for the relief of pain associated with musculoskeletal disorders. Tepoxalin inhibits in a dual mode of action both the cyclooxygenase and 5-lipoxigenase pathway of the arachidonic acid metabolism. In addition, Tepoxalin inhibits migration of neutrophilic granulocytes in the inflammatory tissue.[7]

Another example represents licofelone (formerly known as ML3000), is an anti-inflammatory drug that inhibits the COX and 5-lipoxygenase pathway and is currently undergoing phase III trials for osteoarthritis.[8,9] The effectiveness of licofelone has been demonstrated in animal models as well as in studies on humans, and is attributed mainly to the efficient suppression of PGE_2 formation.[9]

Interestingly, in contrast to NSAIDs and selective COX-2 inhibitors, licofelone shows an improved gastrointestinal and potentially

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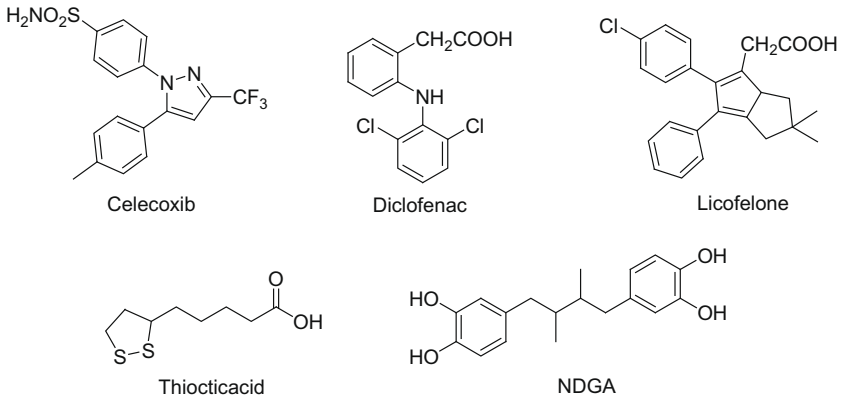


Scheme 1. Chemical structures of some established NSAIDS and used standard compounds.

cardiovascular safety.[10–12] This effect of licofelone might be attributable to the accompanied suppression of leukotrienes,[8] which significantly contribute to gastric epithelial injury as well as to atherogenesis.[13,14]

Reactive oxygen species (ROS) especially the hydroxyl radicals are very important in the inflammatory pathways. ROS like hydrogen peroxide, superoxide anion and HOCl can be easily converted into the most reactive hydroxyl radical.[15,16] These hydroxyl radicals can cause a loss of the membrane lipid functions and induce apoptosis. Another important inflammatory mechanism is the inactivation of IkappaB (IκB) by hydroxyl radicals which leads to a degradation of the IκB and nuclear factor-kappaB (NF-κB) complex and to a nuclear translocation of NF-κB. By virtue of its capability to modulate gene expression NF-κB can activate as transcription factor the synthesis of cytokines like IL-1, IL-6, Il-8 and tumor necrosis factor-alpha (TNF-α, the synthesis of inducible nitric oxide synthase (iNOS) and adhesion molecules like E-selectin and vascular cell adhesion molecule-1 (VCAM-1).[15–20]

Polymorphonuclear leukocyte recruitment is fully accomplished through further homotypic and heterotypic cell–cell adhesive interactions, sustained by adhesion receptors, including the β_2-integrin Mac-1 (CD11b/CD18). This molecule is in part constitutively expressed on the surface of polymorphonuclear leukocytes and is also stored in secretory granules. Upon activation by inflammatory stimuli, rapid translocation of Mac-1 from intracellular pools to the cell surface occurs (upregulation) and, in parallel, conformational changes take place within the molecule to allow competent binding to its counter receptors on polymorphonuclear leukocytes (homotypic cell adhesion), as well as on platelets and on the vascular cell surface (heterotypic cell adhesion).

The rationale for the synthesis of diaryl-dithiolanes and -isothiazoles is that the iselenazoles previously showed high efficacies in inhibiting COX-1, COX-2 and 5-LOX.[21] Consequently, the aim of this study was to develop compounds which maintain these activities and additionally possess significant hydroxyl radical scavenging potencies. Therefore the isoselenazol heterocycle was exchanged by an isothiazole moiety unfortunately with no improvement with regard to hydroxyl radical scavenging activities (Scheme 2).

From there a new approach was needed. It is supposed that compounds like anetholtrithion[22] or oltipraz[23,24] (Scheme 2) exhibit potent hydroxyl radical scavenging activities. The dithiol thione heterocycle of these compounds is a key structure for the radical scavenging efficacy. This led us to the idea to introduce this heterocycle system in our compound with potent COX-1, COX-2 and 5-LOX inhibitory activity. As a consequence **11a** was identified as COX/5-LOX-inhibitor which additionally has the potential to scavenge hydroxyl radicals. The next step was the modification of the dithiol thione core to achieve more potent leads.

In summary, we describe 4,5-diaryl isothiazoles, 4,5-diaryl dithiol-3-thiones and 4,5-diaryl dithiol-3-ones as COX/5-LOX-inhibitors which possess anti-adhesive activities.

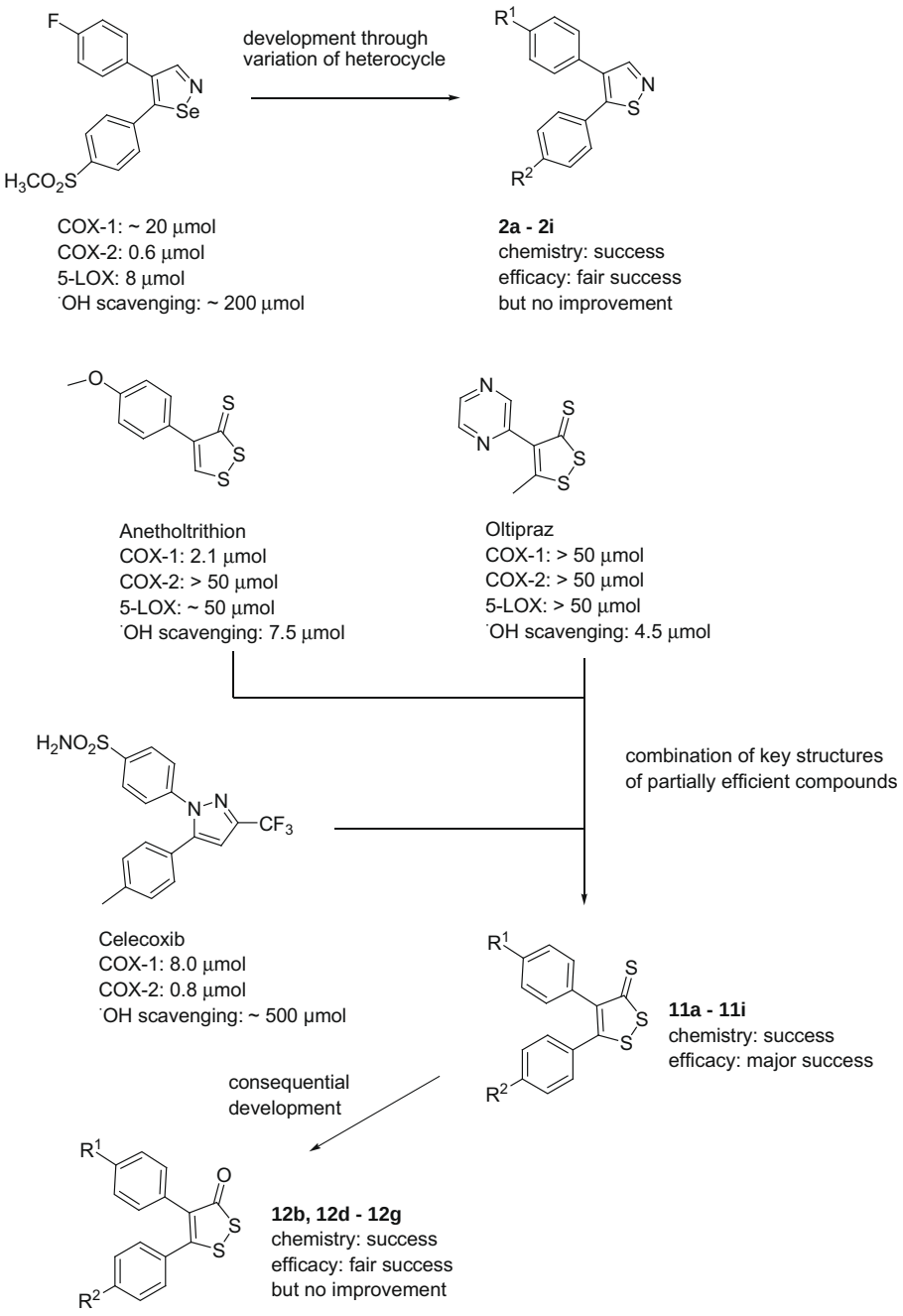
Additionally, those compounds have the potential to reduce the level of reactive oxygen species such as hydroxyl radicals which are well known for supporting inflammation processes in several diseases and therefore could offer new opportunities for the treatment of Parkinson's disease,[25] Alzheimer's disease[26] and rheumatoid arthritis.[27]

2. Chemistry

We focused our synthesis on three different diaryl heterocyclic ring systems. To receive the isothiazoles **2a**–**h** (Scheme 3) different α-chloro formyl stilbene derivatives **1a**–**h** were converted with ammonium thiocyanate[21,22] to the corresponding ring heterocycles. The synthesis of the α-chloro formyl stilbenes was described previously.[21,28,29] The isothiazole derivatives **2a**–**h** were obtained in yields between 30% and 80%. In an ether cleaving reaction **2a** reacted with borontribromide to obtain **2i**.[30]

For the dithiol-thiones different methods were applied. Procedure 1 (Scheme 4) started with a Friedel Crafts reaction of the acid chlorides **3a** or **3b** and thioanisole **4a** to give the ketones **5a** or **5b**.[31] The acid chloride **3a** was prepared by heating the analogue carboxylic acid in thionylchloride.[32] The ketone **5c** was synthesized by heating a mixture of the carboxylic acid **3c,** polyphosphoric acid and thioanisole **4a**. In the next step the ketones **5a**–**c** were methylated with methyl iodide to afford the α-methylketones **6a**–**c** (Scheme 4). It was important to oxidize the thioether derivatives quantitatively with Oxon to get the methylsulfonyl derivatives **6d**–**f**. For the ring closure reaction (Scheme 4) **6d**–**f** reacted with sulfur and Lawesson's reagent to furnish the dithiol-thiones **11a**–**c**.[33]

A Grignard reaction[34] (Scheme 5) of the chloromethylbenzenes **7a**–**c** and the ketones **8a**–**c** provided the tertiary alcohols **9a**–**d** in good yields. The ketone **8a** was generated by a Friedel Crafts reaction of thioanisole **4a** and acetyl chloride. The dehydration of the tertiary alcohols **9a**–**d** with phosphoric acid gave rise to the methylstilbenes **10a**–**d**. The methylstilbene **10e** was synthesized using anisole **4b** and chloroacetone in concentrated sulfuric acid. The thioether intermediates **10c** and **10d** were oxidized with Oxon to the methylsulfonyl derivatives **10f** and **10g**. The ring closure (Scheme 5) of **10d**–**g** using sulfur on a graphite bath (220 °C) led to dithiole-thiones **11d**–**h**.[35] Compound **11i** was obtained by cleavage of **11e** with boron tribromide. The dithiol-thiones **11b** or **11d**–**g**



Scheme 2. Rationale for the synthesis of **2a–i**, **11a–i, 12b** and **12d–g**.

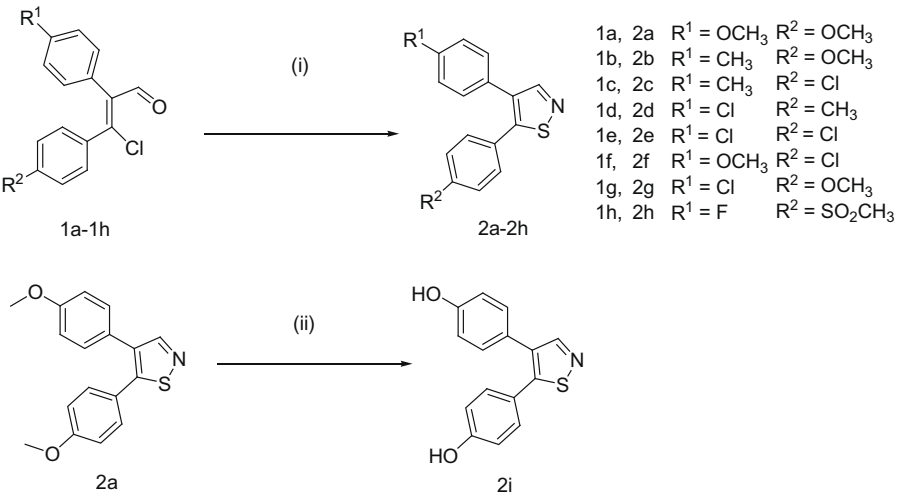
were synthesized using potassium permanganate to yield the dithiole-ones **12b** and **12d–g** (Scheme 6).

3. Results and discussion

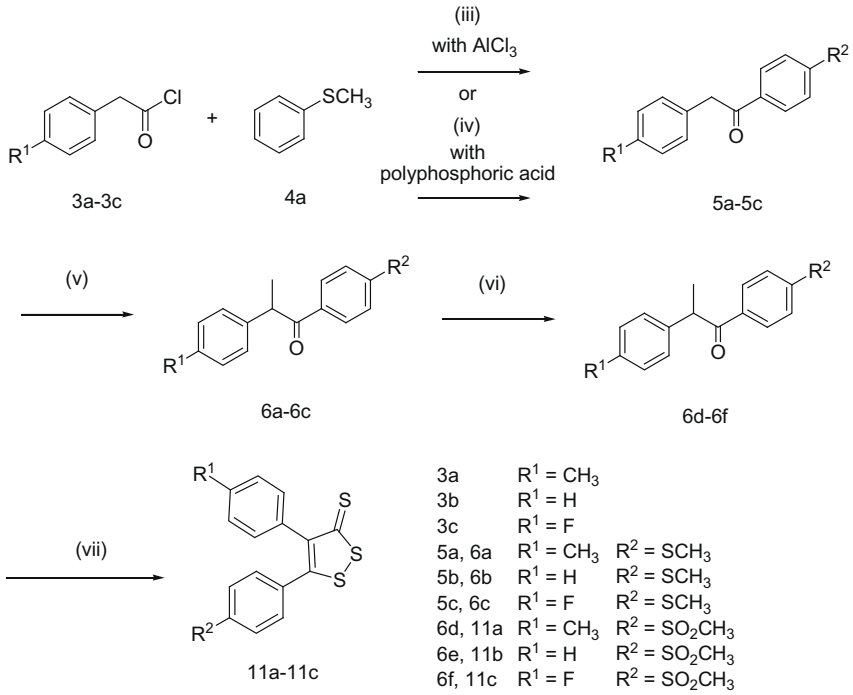
The 4,5-diaryl isothiazoles (**2a–i**), the 4,5-diaryl 3*H*-1,2-dithiole-3-thiones (**11a–i**) and the 4,5-diaryl 3*H*-1,2-dithiole-3-ones (**12b–g**) were initially evaluated in vitro to determine their COX-1/2 and 5-LOX inhibitory activities. Standard compounds were evaluated in parallel. The results are summarized in Table 1. The acquired structure activity relationship (SAR) data show a broad range of inhibitory activities in all test systems (IC_{50} COX-1: 0.0003 μM (**12f**) to ≫10 μM range, IC_{50} COX-2: 0.8 μM (**2a**) to ≫10 μM range, IC_{50} 5-LOX: 3 μM (**11b**) to ≫10 μM range, ·OH scavenging: IC_{50}: 5 μM (**11g**) to ≫100 μM range). Some compounds were also tested for their ability to inhibit leukocyte endothelium interactions (Table 2). One compound was selected for in vivo experiments in a mouse peritonitis model.

In general many substances inhibited the COX-1 enzyme independently from the heterocyclic core systems. This phenomenon was observed for the symmetrically substituted dimethoxyphenyl (R^1 and R^2) isothiazole **2a** and the dithiol-3-thione derivative **11f** (IC_{50} COX-1: 0.003 vs IC_{50} COX-1: 0.03 μM), and analogously for **12f** (IC_{50}: 0.0003 μM). Substitution of the 4-position of the aryl moiety with $R^1 = CH_3$ and $R^2 = Cl$ provided also potent inhibitors of COX-1: **2c** (IC_{50} COX-1: 0.04 μM), **11d** (IC_{50} COX-1: 2 μM) and **12d** (IC_{50} COX-1: 0.2 μM).

Interestingly, in the series of the asymmetrically substituted compounds such as **2c** ($R^1 = CH_3$, $R^2 = Cl$; IC_{50} COX-1: 0.04 μM), **11e** ($R^1 = Cl$, $R^2 = CH_3$; IC_{50} COX-1: 0.006 μM) and **12e** ($R^1 = Cl$,



Scheme 3. Synthesis of the isothiazoles: method i: **1** and ammonium thiocyanate in acetone reflux, 4 h, method ii: **2a** and borontribromide in dichloromethane, −50 °C→rt.



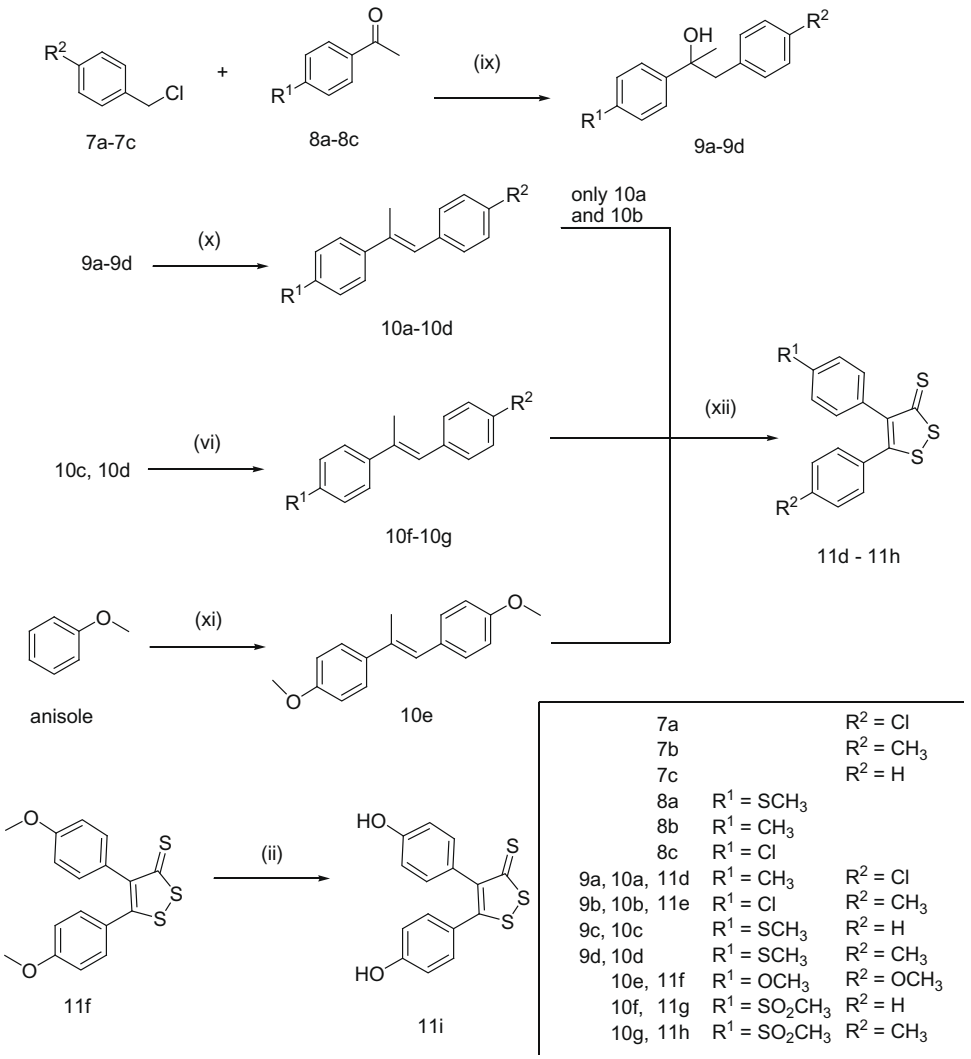
Scheme 4. Synthesis of the 3*H*-1,2-dithiole-3-thiones (Part 1): method iii: **3a** or **3b**, with **4a**, dichloromethane, $AlCl_3$, 0 °C; method iv: **3c**, **4a** (thioanisole), polyphophoric acid, 90–120 °C, 5 min; method v: **5a**, **5b** or **5c** NaH in DMF, methyl iodide, nitrogen atmosphere, rt; method vi: **6a–c** in methanol/THF 1:1, Oxon, 0 °C→rt, over night, method vii: **6d–f,** sulfur, Lawesson's reagent in biphenyl, 210–220 °C.

$R^2 = CH_3$; IC_{50} COX-1: 0.02 μM) a significant difference in potency was observed comparing the regioisomers **2d** ($R^1 = Cl$, $R^2 = CH_3$; IC_{50} COX-1: 0.2 μM), **11d** ($R^1 = CH_3$, $R^2 = Cl$; IC_{50} COX-1: 2 μM) and **12d** ($R^1 = CH_3$, $R^2 = Cl$; IC_{50} COX-1: 0.2 μM).

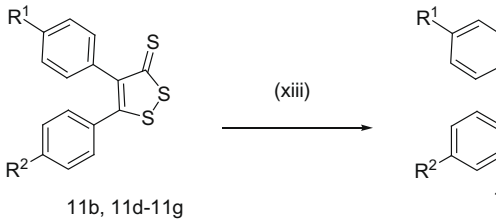
With regard to the COX-1 inhibitory activities ($R^1 = CH_3$) and ($R^2 = OCH_3$), ($R^1 = OCH_3$) and ($R^2 = Cl$), and dichloro ($R^1 = R^2$) substitution worked well for example, **2b** (IC_{50} COX-1: 0.01 μM), **2f** (IC_{50} COX-1: 0.07 μM), **2g** (IC_{50} COX-1: 0.02 μM) and, **2e** (IC_{50} COX-1: 0.1 μM), respectively.

Taken together the highest COX-1 inhibiting potency was achieved by compounds bearing two methoxy groups ($R^1 = R^2$ **2a** (IC_{50} COX-1: 0.003) and (**12f**) (IC_{50} COX-1: 0.0003 μM). The data discussed above demonstrate the weak impact of the heterocyclic system towards COX-1 activity contrary to the influence of the substituentes R^1 and R^2 at the phenyl rings. These findings are in line with previous findings of Janusz et al. who investigated different 5-substituted Dihydrodimethylbenzofurans as combined COX/5-LOX inhibitors.[36]

No clear decisions can be drawn with regard to explain the COX-2 inhibition activities. The introduction of a methylsulfonyl group at both positions R^1 or R^2 resulted in a loss of COX-1 activity (Table 1). This is in line with our previous findings.[21,37] Three compounds exhibited COX-2 inhibitory activities as potent as celecoxib (IC_{50} COX-2: 1 μM): **2a** (IC_{50} COX-2: 0.8 μM); **12b** (IC_{50} COX-2: 1.1 μM) and **12f** (IC_{50} COX-2: 1 μM). However, only **12b** is a methylsulfonyl (R^2) derivative. But it is interesting to notice that the combination of methylsulfonyl (R^2) with fluorine (R^1, **2h**) or methyl (R^1, **11a**) yielded further compounds with moderate COX-



Scheme 5. Synthesis of the 3*H*-1,2-dithiole-3-thiones (Part 2): method ii: **11f**, borontribromide in dichloromethane, −50 °C→rt; method vi: **10c–d** in methanol/THF 1:1, Oxon, 0 °C→rt, over night; method ix: grignard: **7** and Mg in ether, addition of **8** stirred for 10 min, heated for 2 h; method x: **9a–d**, H_3PO_4, reflux, 1 h; method xi: **4b** (anisole), acetone, H_2SO_4, 4–5 h rt; method xii: **10a–b** or **10e–g**, sulfur, 220 °C until the formation of hydrosulfite ended.

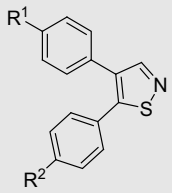


Scheme 6. Synthesis of the 3*H*-1,2-dithiole-3-ones: method xiii: **11b, 11d–g**, acetone, potassium permanganate, 12 h, rt.

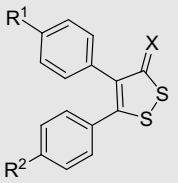
2 activities (both IC_{50} COX-2: 9.0 μM). Moreover, the dichloro derivative **2e** (IC_{50} COX-2: 4.0 μM) provided also moderate COX-2 activity. As already seen for the COX-1 activity the dimethoxy substituents at R^1 and R^2 (**2a** and **12f**) (IC_{50} COX-2: 0.8 μM vs IC_{50} COX-2: 1.0 μM, respectively) proofed to be superior with regard to the COX-2 inhibitory effect compared to a combination of methylsulfonyl (R^2) and fluorine (R^1) (**2h**) (IC_{50} COX-2: 9.0 μM) or methyl (R^1) (**11a**) (IC_{50} COX-2: 9.0 μM). However**,** it is difficult to draw conclusions about the impact of the isothiazole-, dithiolthione- or dithiolone-heterocyclic ring system on COX-2 activity to generalize the results**:** In each series a candidate with a very promising COX-2 inhibiting profile is present (**2a**, IC_{50} COX-2: 0.8 μM; **11a**, IC_{50} COX-2: 9.0 μM; **12b**, IC_{50} COX-2: 1.1 μM**).**

The 5-LOX inhibition values show a very interesting SAR. Despite it was not possible to meet the IC_{50}-value of licofelone[38] (IC_{50} 5-LOX: 0.18 μM) or NDGA (IC_{50} 5-LOX: 1.0 μM), we characterized three substances with a remarkable 5-LOX inhibiton: (**11a,** IC_{50} 5-LOX: 9 μM; **11b,** IC_{50} 5-LOX: 3 μM; **11i**, IC_{50} 5-LOX: 6 μM). The substituents R^1 and R^2 at the isothiazole-, dithiolthione- or dithiolone- systems showed no clear tendency to influence the 5-LOX activity. The 5-LOX in vitro data for the different heterocyclic systems illustrate that compounds containing a dithiolthione- or an isothiazole-ring system seemed to be more active compared to the heterocyclic dithiolone ring system. The replacement of the dithiolthione sulfur of **11b** (R^1 = H, R^2 = methylsulfonyl; IC_{50} 5-LOX: 3.0 μM) by oxygen of the dithiolone **12b** (IC_{50} 5-LOX: > 50 μM (13% inhibition at a concentration of 10 μM)) resulted almost in a loss of 5-LOX potency.

Table 1
COX1/2, 5-LOX and ·OH scavenging activities of the 4,5-diaryl isothiazoles, 4,5-diaryl-3*H*-1,2-dithiole-3-thiones and 4,5-diaryl-3*H*-1,2-dithiole-3-ones



2a-2i,

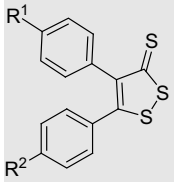


11a-11i, 12b,12d-12g

Cpd	R^1	R^2	X	IC_{50}[a,*] (μM)	IC_{50}[a,*] (μM)	Ratio	IC_{50}[a,*] (μM)	IC_{50}[b,**] (μM)
				COX-1	COX-2	COX-1/COX-2	5-LOX	·OH
2a[29]	OCH_3	OCH_3		0.003	0.8	0.004	~20	>500
2b	CH_3	OCH_3		0.01	>50	—	8.0	>500
2c	CH_3	Cl		0.04	>50	—	~50	>500
2d	Cl	CH_3		0.2		—	>50	>500
2e	Cl	Cl		0.1	4.0	0.03		>500
2f	OCH_3	Cl		0.07		—	~50	500
2g	Cl	OCH_3		0.02	>50	—	~20	>500
2h[49]	F	SO_2CH_3		~50	9.0	—	~10	>500
2i	OH	OH		1.0	>50	—	>50	500
11a	CH_3	SO_2CH_3	S	7.0	9.0	0.8	9.0	9.0
11b	H	SO_2CH_3	S		>50	—	3.0	7.0
11c	F	SO_2CH_3	S	>50	>50	—	~20	11.0
11d	CH_3	Cl	S	2.0	>50	—	~20	~100
11e	Cl	CH_3	S	0.006	~50	—	>50	~100
11f	OCH_3	OCH_3	S	0.03	~20	0.0015	~10	~200
11g	SO_2CH_3	H	S	~20	>50	—	>50	5.0
11h	SO_2CH_3	CH_3	S	0.6	>50	—	>50	9.0
11i	OH	OH	S	~20	>50	—	6.0	11.0
12b	H	SO_2CH_3	O	8.0	1.1	7.3	>50	>500
12d	CH_3	Cl	O	0.2	>50	—	>50	>500
12e	Cl	CH_3	O	0.02	>50	—	>50	>500
12f	OCH_3	OCH_3	O	0.0003	1.0	0.0003	>50	>500
12g	SO_2CH_3	H	O	8.0	>50	—	>50	~500
Celecoxib				8.0	0.8	10	—	~500
Diclofenac				0.003	0.01	0.3	>50	—
Licofelone				0.2[9]	—	—	0.18[9]	—
Thioctic acid				—	—	—	—	11.0
NDGA				~50	>50	—	1.0	29

[a] Values are means of at least two determinations.
[b] Values are means of at least three determinations.
[*] For clarity and better comparison the % inhibition values are represented by ranges of IC_{50}-values (μmol) as follows: 45–55% inhibition = ~10 μmol, 35–44.9% inhibition = ~20 μmol, 25–34.9% inhibition = ~50 μmol, <24.9% inhibition = >50 μmol.
[**] For clarity and better comparison the % inhibition values are represented by ranges of IC_{50}-values (μmol) as follows: 45–55% inhibition = ~100 μmol, 35–44.9% inhibition = ~200 μmol, 25–34.9% inhibition = ~500 μmol, <24.9% inhibition = >500 μmol.

Table 2
In vitro anti-adhesive activities the of 4,5-diaryl-3*H*-1,2-dithiole-3-thiones



11a-11i,

Cpd	R^1	R^2	Static assay[a,c] %inhibition at 100 μM	Mouse peritonitis model
11a	CH_3	SO_2CH_3	71 ± 13%	49%[*]
11b	H	SO_2CH_3	9 ± 7%	—
11c	F	SO_2CH_3	85 ± 7%	—
11h	SO_2CH_3	CH_3	26 ± 14%	—
11g	SO_2CH_3	H	38 ± 16%	
11i	OH	OH	54 ± 26%	—
Celecoxib	—	—	85 ± 13%[b]	—
Licofelone	—	—	56% at 30 μM[10]	55%[**]

[a] Values are the mean of ± SEM of at least six determinations.
[b] Inhibition at a concentration of 40 μM.
[c] In this assay compounds at a concentration of 100 μM were evaluated for inhibition of adhesion of MM6 cells to BAEC and sLeX was chosen as reference. To compare the results for compounds which have been tested on different plates, the number of adherent MM6 cells on TNF-α-stimulated endothelial cells is set to 100%.[43]
[*] Inhibition at a dose of 50 mg/kg.
[**] Inhibition at a dose of 100 mg/kg, n.a.: not active (in adhesion assay, n.a. is defined as less than 10% inhibition at 100 μM).

However, the ·OH inhibiting in vitro data acquired for the different heterocyclic ring systems revealed a clear trend in the potency of scavenging hydroxyl radicals. The dithiolthione heterocycles (**11a–i**) exhibited very potent ·OH scavenging activities (**11a,** IC_{50}: 9.0 μM, **11b** IC_{50}: 7.0 μM, **11c** IC_{50}: 11.0 μM, **11g** IC_{50}: 5.0 μM, **11h** IC_{50}: 9.0 μM, **11i** IC_{50}: 11.0 μM). This is not very sur-

prising since their structures are close related to thioctic acid (IC_{50}: 11.0 μM), a very potent and well known hydroxyl radical scavenger in a variety of hydroxyl radical test assays.[39] The dithiolthiones derivatives bearing a methylsulfonyl moiety (**11a–c**, **11g–h**) displayed ~10-fold higher ·OH scavenging activities compared to compounds missing this structural feature (**11d–f**) (·OH scavenging $IC_{50} \sim 10.0$ μM vs 100 μM) (Table 1).

In contrast the corresponding dithiolones (**12b**, **12d–g**) showed no **12d–f** (0% inhibition at a concentration of 100 μM) or less hydroxyl radical scavenging effects (Table 1), for example, **12b** ($R^1 = H$, $R^2 = SO_2CH_3$; ·OH scavenging $IC_{50} > 500$ μM (22% inhibition at a concentration of 100 μM) and **12g** ($R^1 = SO_2CH_3$, $R^2 = H$; (·OH scavenging $IC_{50} \sim 500$ μM (26% inhibition at a concentration of 100 μM). The methylsulfonyl moiety seemed to be beneficial to support hydroxyl radical scavenging activities. Celecoxib (·OH scavenging $IC_{50} \sim 500$ μM (·OH inhibition at a concentration of 100 μM: 27%)) behaved as weak ·OH scavenging substance.

Among the isothiazoles only **2f** exhibited a weak ·OH inhibiting activity (10% inhibition at a concentration of 100 μM). It is interesting to notice that **2h** (0% inhibition at a concentration of 100 μM) did not show ·OH inhibiting activity at all even it bears a methylsulfonyl group (R^2).

Reactive oxygen species (ROS) produced by cytokines, growth factors, and vasoactive agents contribute to the intracellular signalling cascades associated with inflammatory responses.[40,41] ROS induce NF-κB activation by modifying the activity of one or more of the kinase enzymes in the NF-κB activation cascades.[42] Since NF-κB is a major transcription factor involved in the transcriptional regulation of cell adhesion molecules we studied the effect of some potent ·OH scavenging compounds (**11a–c** and **11h–i**) on adhesion of leukocytes on inflamed endothelium. In this assay compounds at a concentration of 100 μM were evaluated for inhibition of adhesion of MM6 cells to BAEC.[43] To compare the results for compounds which have been tested on different plates, the number of adherent MM6 cells on TNF-α-stimulated endothelial cells was set to 100% (Table 2).

Interestingly, we found that the dithiolthiones **11a** ($R^1 = CH_3$, $R^2 = SO_2CH_3$) and **11c** ($R^1 = F$, $R^2 = SO_2CH_3$) gave equivalent good inhibitory activities in the cell based static assay (71% vs 85%) (Table 2). It is worth to mention that the unsymmetrical substituted regioisomer **11h** ($R^1 = SO_2CH_3$, $R^2 = CH_3$) was less active (26%). The introduction of a hydroxyl group at the position R^1 and R^2 in case of **11i** resulted in an increase of inhibition of adhesion compared to **11h** (26% vs 54%). This is surprising since **11h** and **11i** behaved both as very potent hydroxyl radical scavenger (IC_{50}: 9 vs 11 μM) in our hydroxyl radical test assays. In addition it is noteworthy that introduction of hydrogen (**11b**) instead of a methyl (**11a**) at position R^1 led almost to a complete loss of ·OH scavenging activities (9% vs 71%).

Taken together we succeeded to prepare novel compounds able to inhibit 4 targets. The overall best hit is the dithiolthione **11a** (COX-1 IC_{50}: 7 μM, COX-2 IC_{50}: 9 μM, 5-LOX $IC_{50} \sim 10$ μM, IC_{50} ·OH: 9 μM) with a balanced inhibition of all 4 targets. Furthermore **11a** inhibits the adhesion of leukocytes in vitro.

4. In vivo evaluations

Based on its overall biological profile, **11a** was chosen for further investigations. The effect of **11a** on leukocyte recruitment in vivo was tested in a mouse peritonitis model. Leukocyte extravasation was induced by i.p. compound 48/80[44] challenge. Treatment of mice with compound 48/80 and the test substance did not lead to any obvious signs of discomfort. The white blood count and the relative amount of peripheral PMN and mononuclear cells were not different in both groups (data not shown). Compound 48/80 induces a strong activation of mast cells which involves the release of prostaglandins and leukotrienes which is related to a rapid influx of PMN. As seen in Figure 1, pre-treatment with **11a** (50 mg/kg) reduced the total cell count in the peritoneal cavity, the majority of which were PMNs (≈49%) ($n = 8$, $P < 0.02$). Earlier mouse peritonitis model studies revealed that treatment with the COX/5-LOX inhibitor licofelone (100 mg/kg) reduced the total cell count in the peritoneum, the majority of which were PMNs, from 24×10^6 cells in cytokine-treated mice to 13×10^6 cells (~55%) ($n = 4$, $P < 0.05$).[14] Only few monocytes and lymphocytes extravasated in response to compound 48/80 or cytokine stimulation. This indicates that our in vitro assays predicted anti-inflammatory efficacy in vivo of the test compound. In order to investigate if the anti-adhesive effect of **11a** seen in vitro was mediated by changes in expression of adhesion molecules we assessed the effect of **11a** on cell adnesion molecule (CAM) expression on protein level. Peritoneal PMNs from the compound 48/80-treated group responded with strong expression of CD 11b (Mac-1) ($n = 8$, $P < 0.008$) (Fig. 2), which was attenuated by **11a** (50 mg/kg) by 40%, but not other NSAIDs (data not shown), respectively. This indicates that the activation of extravasated PMN was reduced. This effect may

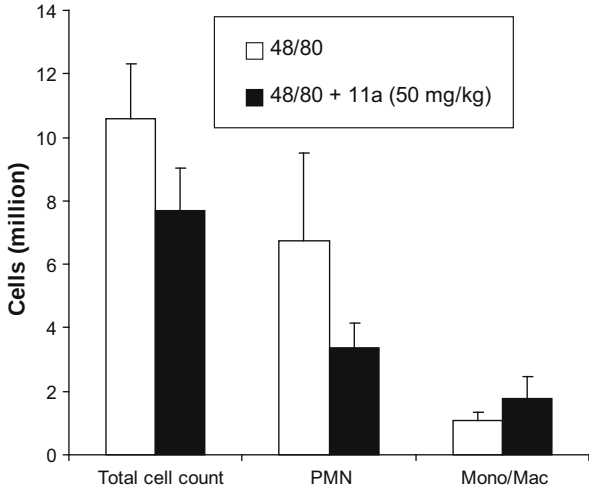


Figure 1. Effect of **11a** on leukocyte recruitment in a mouse peritonitis model. Leukocyte extravasation into the peritoneal cavity was analyzed in mice after intraperitoneal challenge with compound 48/80 for 4 h (25 μg/mouse, white bars). One group of animals received **11a** (50 mg/kg) intraperitoneally 1 h before injection of compound 48/80 (black bars). Leukocytes harvested from the peritoneal cavity were differentiated by FACS analysis. Values are mean ± SD of eight independent experiments (*$P < 0.05$ vs control).

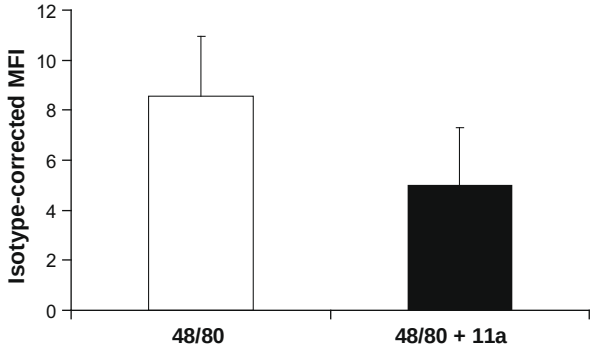


Figure 2. Effect of **11a** on expression of the membrane receptor CD11b (Mac-1) on neutrophils recruited to the peritoneum in mice exposed to compound 48/80. Peritonitis was induced as described for Figure 1. Expression of CD11b was measured by FACS analysis. Data are expressed as isotype-corrected MFI. Values are mean ± SD of eight independent experiments (*$P < 0.05$ vs control).

contribute to the markedly reduced leukocyte accumulation found after **11a** treatment in the mouse peritonitis model in vivo.

To exclude the possibility that inhibition of CD 11b expression seen with **11a** might have resulted from a cytotoxic effect of this compound on leukocytes we investigated viability by trypan blue exclusion. After 6 h, trypan blue exclusion was 96 ± 4% and 94 ± 2%, indicating that loss in cell viability does not account for changes in CAM expression on EC.

5. Conclusion

Three series of different substituted diaryl heterocycles were synthesized. The 4,5-diaryl-dithiol-3-thione derivative **11a** reported here represents a candidate which is able to inhibit different pathways involved for example, inflammation: (i) **11a** inhibits three arachidonic acid utilizing pathways: COX-1, COX-2 and 5-LOX; (ii) **11a** reduces ROS via hydroxyl radical scavenging activities; (iii) **11a** inhibits the expression of Mac-1 and adhesion and infiltration of leukocytes in vitro and in vivo.

Recapitulatory, we showed that this moderate overall in vitro potency was translated in an additive in vivo activity. Up to date we do not have an explanation for this phenomenon. One speculation is that the intervention in the inflammatory cascade at different signal transduction pathways might be beneficial for the good in vivo activity. However, the precise mechanism of action remains to be elucidated. Especially the effect of **11a** on the proinflammatory transcription factor nuclear factor-κB (NF-κB) is interesting in this regard because NF-κB activation represents an essential pathway for the induction of numerous proinflammatory genes in the vascular wall, including cell CAMs and cytokines.

Further improvements on the potency of this compound could lead to novel and safe anti-inflammatory agents with potential therapeutic utilities. These drugs represent a novel class of multiple target non-steroidal anti-inflammatory drugs (MTNSAIDs).

6. Experimental

The docking experiments (see SI-file),[21] COX-1, 5-LOX,[45] COX-2,[46] Hydroxyl radical scavenging, inhibition assays[21] and the adhesion assay[42,47] were performed as described previously. For clarity a brief summary of the procedures are given below.

6.1. Enzyme assays

COX-1 inhibition was determined by using platelets isolated from bovine blood, incubated with the test substance, and stimulated by the Ca ionophor A23187. The inhibition of COX-1 was assessed by quantitative HPLC determination of the formation of 12-hydroxyheptadecatrienoic acid.[45]

5-LOX inhibition was investigated by using bovine polymorphonuclear leucocytes, incubated with the test substance, and stimulated by the Ca ionophor A23187. The inhibition of 5-LOX was assessed by quantitative HPLC determination of the formation of leukotriene B_4.[45]

COX-2 inhibition was determined by using aliquots of human blood samples, containing sodium heparin. The samples were incubated in absence and presence of LPS and the test substances as described.[48]

6.2. EPR spin trapping

Test assay for hydroxyl radical scavenging: DEPMPO was purchased from Calbiochem (San Diego, USA). The test solution was prepared by adding 2 μL of a solution of the test substance in acetonitrile (concentration 10, 1 or 0.1 mM/l) or acetonitrile, as zero value, to 192 μL demineralized water (Millipore) in a 1.5 mL micro-centrifuge tube. 2 μL DEPMPO solution (20 mM/l), 2 μL $FeSO_4$ solution (5 mM/l) and 2 μL hydrogen peroxide solution (2.5 mM/l) were put on different points on the wall of the tube. The reaction was started by vortexing the tube. After 10 s a sample was gathered with a 100 μL ringcap from HIRSCHMANN® Laborgeräte and closed with wax. After exact 90 s the EPR experiment was started. Instrumental parameters were as follows: Receiver Gain 3.99×10^5, Modulation Amplitude 3.0 G, Time Constant 0.64 ms, Conversion Time 81.92 ms, Attenuation 4 ms. The spectra were analyzed with the WinEPR® (Version 1.0) software. The raw data were integrated, the background noise was subtracted and the heights of the second peaks were analyzed. Each concentration was tested 3 times. Thioctic acid in concentrations of 10, 1 and 0.1 mM/l was used as standard substance in every test series. For an amount of 30 spectra totalling 6 zero values as control were analyzed. The EPR Spectra were acquired on a Bruker EMX EPR spectrometer.

Melting points were determined with a Büchi apparatus according to Dr. Tottoli and are uncorrected. ^{1}H NMR spectra (300 MHz) were recorded on a Bruker AC 300 spectrometer. Combustion analyses were performed with a Carlo Erba Strumentiazione 1106 analyzer.

Column chromatography was performed with Merck Silica Gel 60 (0.063–0.200 mm). The progress of the reactions was monitored by thinlayer chromatography (TLC) performed with Merck Silica Gel 60 F-245 plates.

All reagents and solvents were obtained from commercial sources and used as received. Reagents were purchased from Sigma-Aldrich Chemie Steinheim, Germany, or Acros, Nidderau, Germany.[21]

6.3. Static assay

The adhesion assay was performed as described previously.[43] Briefly, BAEC monolayer 7 days post seeding; passage 1-3, were grown in 24 well plates. Prior to assay, the BAEC were examined microscopically to confirm the integrity and uniformity of the monolayer. After the culture medium was removed and the endothelial cell monolayer was washed with DPBS (2×1 mL/well at 37 °C) the cells were treated with cell culture medium containing 20 ng/mL recombinant tumor necrosis factor alpha (TNF-α) for 4 or 16 h and the respective test substance dissolved in DMSO or vehicle for 20 min (in other settings for 4 or 16 h) at 37 °C/5% CO_2. The BAEC were washed with DPBS (2×1 mL/well at 37 °C) before 900 μL of culture medium and 100 μL of PMN suspension (1×10^6 cells/mL) per well were added and incubated for 30 min at 37 °C/5% CO_2. Non-adherent PMNs were removed by washing with DPBS (3×1 mL/well at 37 °C). Culture medium (1 mL/well at 37 °C) was added prior to microscopic examination. (In inhibition experiments, various concentrations of drugs were used (1×10^{-4}–1×10^{-7} mol/L)). After incubating with cytokines or drugs the cell viability of BAEC was confirmed by trypan blue exclusion and additional microscopic examination. IC_{50} values were calculated using the program GRAFIT, Erithacus Software Ltd., UK.[42,47]

6.4. Compound 48/80-induced Mouse peritonitis

Female Balb/c mice were obtained from B&K, Stockholm, Sweden. Animals of 20–25 g were fed normal chow and water ad libitum. Peritonitis was induced by a single injection of compound 48/80 (Sigma) at 25 μg/mL in 1 mL sterile PBS. The test compound was injected intraperitoneally at a concentration of 50 mg/kg 1 h before challenge with compound 48/80, control animals were injected with vehicle only. Four hours after injec-

tion of compound 48/80, peritoneal cells were harvested by injecting 4 mL cold HBSS containing 5 mM EDTA. Cells were spun down, counted after staining with turck solution and the proportion of PMN and monocytes/macrophages was assessed by FACS analysis using cell specific antibodies. PMN were stained with rat anti-Gr1 PE/Cy5 (Biolegend), monocytes/macrophages were stained with rat anti-F4/80 FITC (Serotec). After 30 min of incubation, cells were washed twice, resuspended in PBS and analyzed in FACSort (Becton Dickinson). Gating for leukocytes was performed in forward and side scatter, and the relative amount of PMN and monocytes/macrophages was estimated by the number of $Gr1^+/F4/80^-$ (PMN) or $F4/80^+/Gr1^-$ and $F4/80^+/Gr1^{lo}$ (both monocytes). The activation of emigrated PMN was assessed by staining with rat anti-mouse CD11b PE antibody (Serotec). All experiments were approved by the local ethical committee for animal experimentation (N149/06).

6.5. Chemistry

Compounds were analyzed for C, H, N, S and elemental analyses were within 0.4% for elements unless indicated otherwise.

Methods iii–vi and methods viii–xi are described in the Supplementary data.

6.5.1. Method i

A mixture of the 3-chloro-acrylaldehyde **1a**–**h** (0.1 mol) and ammonium thiocyanate (22.8 g, 0.3 mol) in acetone (200 mL) was refluxed for 4 h (CAUTION: HCN formation!). After cooling to rt the mixture was poured in a saturated solution of aqueous sodium hydrogen carbonate (300 mL) and extracted with diethyl ether (3 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum and the residue purified by SiO_2, ethyl acetate/petroleum ether 3:7 to yield the isothiazoles **2a**–**h** (30–80% yield) as colourless solids.

6.5.2. Method ii

The anisol derivative **2a** or **11e** (4.8 mmol) was dissolved in 50 mL anhydrous dichloromethane. The reaction mixture was cooled to −50 °C and stirred vigorously in a nitrogen atmosphere. A solution of borontribromide (9.6 mmol) in 20 mL dichloromethane was added using a septum and the mixture was stirred over night slowly warming up to rt. The mixture was poured into ice water; The organic layer was separated and extracted with a diluted solution of NaOH (3 × 50 mL). The aqueous layers were acidified by dropwise addition of aqueous hydrochloric acid (1.0 M) and extracted with diethyl ether (3 × 100 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the phenol derivative **2i** or **11i** (15–25% yield).

6.5.3. Method vii

To a mixture of ketone **6d**, **6e** or **6f** (13.87 mmol), sulfur (4.44 g, 138.75 mmol) and Lawesson's reagent (5.6 g, 13.87 mmol) biphenyl were added and heated on a graphite bath (210–220 °C). After completion of hydrosulfite formation, the mixture was cooled to 50 °C and ethyl acetate (100 mL) was added. The reaction mixture was refluxed for 1 h, stirred at rt over night, filtered over Celite® and concentrated under reduced pressure. By column chromatography purification (SiO_2, ethyl acetate/petroleum ether 5:95) and recrystallization with methanol the 5-(4-methylsulfonyl)phenyl)-4-phenyl-3H-1,2-dithiole-3-thiones **11a**, **11b** or **11c** (5–9% yield) were obtained as dark red crystals.

6.5.4. Method xii

A mixture of (*E*)-1,2-diphenylprop-1-ene **10a**–**b** or **10e**–**g** (1 mmol) and sulfur (4 mmol) was heated on a graphit bath to 220 °C until the formation of hydrosulfite ended. After addition of ethyl acetate (25 mL) the mixture was refluxed for 30 min, filtered over Cellite®, washed and concentrated under reduced pressure. By column chromatography purification (SiO_2, ethyl acetate/petroleum ether 1:9) the dithiole-3-thiones **11d**–**h** (9–19%) were obtained as red solids.

6.5.5. Method xiii

To a solution of the dithiole-3-thione **11b** or **11d**–**g** (1 mmol) in acetone a solution of potassium permanganate (5 mmol) in acetone was added slowly. The violet mixture was stirred for 12 h at rt until it was decolourized. The mixture was filtered, dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and recrystallized with ethyl acetate to obtain of the dithiole-3-ones **12b** or **12d**–**g** (29–83%).

6.6. Compounds

Compounds **1a**–**h** and **2a** were described previously.[21,28,29] Compounds **5a**–**c**, **6a**–**f**, **8a**, **9a**–**d** and **10a**–**g** are described in the Supplementary data.

6.6.1. 4,5-Bis(4-methoxyphenyl)isothiazole (2a)[29]

Method i; Reactant: **1a**; yield: 60%; mp: 81 °C; ^{1}H NMR (DMSO) 8.61 (s, 1H), 7.245 (d, 8.69 Hz, 4H, A″A‴ and B″B‴), 6.95 (m, 4H, AA′ and BB′), 3.76 (s, 3H), 3.75 (s, 3H). FD: *m*/*z* (rel. Int.) = 297.6 (100%). Anal. Calcd for $C_{17}H_{15}NO_2S$: C, 68.66; H, 5.08; N, 4.71; S, 10.78. Found: C, 69.01; H, 5.00; N, 4.64; S, 10.62.

6.6.2. 5-(4-Methoxyphenyl)-4-*p*-tolylisothiazole (2b)

Method i; Reactant **1b**; yield: 40%; mp: 94 °C; ^{1}H NMR (DMSO 8.62 (s, 1H), 7.24 (d, 8.65 Hz, 2H, BB′), 7.18 (m, 4H), 6.96 (d, 8.73 Hz, 2H, AA′), 3.76 (s, 3H), 2.30 (s, 3H); FD: *m*/*z* (rel. Int.) = 281.2 (100%). Anal. Calcd for $C_{17}H_{15}NOS$: C, 72.57; H, 5.37; N, 4.98; S, 11.40. Found: C, 72.65; H, 5.32; N, 4.77; S, 10.97.

6.6.3. 5-(4-Chlorophenyl)-4-*p*-tolylisothiazole (2c)

Method i; Reactant: **1c**; yield: 76%; mp: 79 °C; ^{1}H NMR (DMSO) 8.69 (s, 1H), 7.49 (d, 8.51 Hz, 2H, BB′), 7.33 (d, 8.51 Hz, 2H, AA′), 7.19 (m, 4H), 2.3 (s, 3H); FD: *m*/*z* (rel. Int.) = 285.4 (100%).

6.6.4. 4-(4-Chlorophenyl)-5-*p*-tolylisothiazole (2d)

Method i; Reactant: **1d**; yield: 59%; mp: 108 °C; ^{1}H NMR (DMSO) 8.7 (s, 1H), 7.45 (d, 8.42 Hz, 2H, B″B‴), 7.34 (d, 8.29 Hz, 2H, BB′), 7.24 (m, 4H, AA′and A″A‴), 2.31(s, 3H); FD: *m*/*z* (rel. Int.) = 285.4 (100%).

6.6.5. 4,5-Bis(4-chlorophenyl)isothiazole (2e)

Method i; Reactant: **1e**; yield: 57%; mp: 94 °C; ^{1}H NMR (DMSO) 8.73 (s, 1H), 7.5 (d, 8.51 Hz, 2H, B″B‴), 7.46 (d, 8.54 Hz, 2H, A″A‴), 7.34 (d, 8.44 Hz, 4H, BB′ and AA′); FD: *m*/*z* (rel. Int.) = 305.4 (100%).

6.6.6. 5-(4-Chlorophenyl)-4-(4-methoxyphenyl)isothiazole (2f)

Method i; Reactant: **1f**; yield: 66%; ^{1}H NMR (DMSO) 8.67 (s, 1H), 7.49 (d, 8.54 Hz, 2H, B″B‴), 7.33 (d, 8.54 Hz, 2H, A″A‴), 7.24 (d, 8.77 Hz, 2H, BB′), 6.94 (d, 8.78 Hz, 2H, AA′); FD: *m*/*z* (rel. Int.) = 301.5 (100%). Anal. Calcd for $C_{16}H_{12}ClNOS$: C, 63.68; H, 4.01; N, 4.64; S, 10.62. Found: C, 63.73; H, 4.08; N, 4.44; S, 10.41.

6.6.7. 4-(4-Chlorophenyl)-5-(4-methoxyphenyl)isothiazole (2g)

Method i; Reactant: **1g**; yield: 70%; mp: 99 °C; ^{1}H NMR (DMSO) 8.67 (s, 1H), 7.44 (d, 8.46 Hz, 2H, B″B‴), 7.34 (d, 8.51 Hz, 2H, A″A‴), 7.24 (d, 8.68 Hz, 2H, BB′), 6.98 (d, 8.71 Hz, 2H, AA′), 3.76 (s, 3H); FD: *m*/*z* (rel. Int.) = 301.5 (100%). Anal. Calcd for $C_{16}H_{12}ClNOS$: C, 63.68; H, 4.01; N, 4.64; S, 10.62. Found: C, 63.68; H, 4.11; N, 4.55; S, 10.58.

6.6.8. 4-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)isothiazole (2h)[49]

Method i; reactant **1h**; yield: 48%; mp: 104 °C; ^{1}H NMR (DMSO 8.77 (s, 1H), 7.96 (d, 8.23 Hz, 2H, BB′), 7.58 (d, 8.26 Hz, 2H, AA′), 7.39 (m, 2H), 7.25 (m, 2H), 3.26 (s, 3H); FD: *m*/*z* = 333.1 (M^+).

6.6.9. 4-(5-(4-Hydroxyphenyl)isothiazol-4-yl)phenol (2i)

Method ii; Reactant: **2a**; yield: 25%; mp: 227 °C; ^{1}H NMR (DMSO) 9.86 (s, 1H), 9.59 (s, 1H), 7.13 (d, 7.94 Hz, 4H, BB′ and AA′), 6.76 (m, 4H), 8.55 (s, 1H); FD: *m*/*z* (rel. Int.) = 269.1 (100%).

6.6.10. 5-(4-(Methylsulfonyl)phenyl)-4-*p*-tolyl-3*H*-1,2-dithiole-3-thione (11a)

Method vii; Reactant: **6d**; yield: 5%; mp: 243 °C; ^{1}H NMR (DMSO) 7.91 (d, 8.4 Hz, 2H, B″B‴), 7.69 (d, 8.6 Hz, 2H, A″A‴), 7.13 (d, 7.9 Hz, 2H, BB′), 7.01 (d, 7.9 Hz, 2H, AA′), 3.23 (s, 3H), 2.26 (s, 3H); ^{13}C NMR: (DMSO) 21.5 (Ar-CH_3); 44.3 (SO_2CH_3); 128.0 (2CH); 129.7 (2CH); 130.0 (2CH); 130.2 (Cq); 130.6 (2CH); 138.7 (Cq); 139.0 (Cq); 142.1 (Cq); 146.8 (Cq); 166.4 (Cq); 215.1 (C═S); EI: *m*/*z* = 378.7 (M^+). Anal. Calcd for $C_{17}H_{14}O_2S_4$: C, 53.94; H, 3.73; N, 0; S, 33.88. Found: C, 53.62; H, 3.61; N, 0; S, 33.58.

6.6.11. 5-(4-(Methylsulfonyl)phenyl)-4-phenyl-3*H*-1,2-dithiole-3-thione (11b)

Method vii; Reactant: **6e**; yield 6%; mp: 193 °C; ^{1}H NMR (DMSO) 7.9 (d, 8.4 Hz, 2H), 7.58 (d, 8.4 Hz, 2H), 7.39–7.24 (m, 3H), 7.18–7.07 (m, 2H), 3.23 (s, 3H); ^{13}C NMR: (DMSO) 43.3 (SO_2CH_3); 127.7 (2CH); 128.66 (2CH); 128.72 (CH); 130.3 (2CH); 131.1 (2CH); 134.0 (Cq); 137.8 (Cq); 142.6 (Cq); 146.8 (Cq); 169.3 (Cq); 215.0 (C═S); EI: *m*/*z* = 364.5 (M^+). Anal. Calcd for $C_{16}H_{12}O_2S_4$: C, 52.72; H, 3.32; N, 0; S, 35.18. Found: C, 52.60; H, 3.24; N, 0; S, 34.93.

6.6.12. 4-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-3*H*-1,2-dithiole-3-thione (11c)

Method vii; Reactant: **6f**; yield: 5%; mp: 201 °C; ^{1}H NMR ($CDCl_3$) 8.01–7.92 (m, 2H), 7.7–7.62 (m, 2H), 7.25–7.17 (m, 2H), 7.04–6.94 (m, 2H), 3.24 (s, 3H); EI: *m*/*z* = 382.5 (M^+). Anal. Calcd for $C_{16}H_{11}FO_2S_4$: C, 50.24; H, 2.90; N, 0; S, 33.53. Found: C, 50.22; H, 3.06; N, 0; S, 33.46.

6.6.13. 5-(4-Chlorophenyl)-4-*p*-tolyl-3*H*-1,2-dithiole-3-thione (11d)

Method xii; Reactant: **10a**; yield: 16%; mp: 175 °C; ^{1}H NMR (DMSO) 7.45 (d, 8.5 Hz, 2H, BB′), 7.32 (d, 8.5 Hz, 2H, AA′), 7.12 (d, 8.0 Hz, 2H, B″B‴), 6.97 (d, 8.0 Hz, 2H, A″A‴), 2.265 (s, 3H); EI: *m*/*z* = 334.9 (M^+). Anal. Calcd for $C_{16}H_{11}ClS_3$: C, 57.38; H, 3.31; N, 0; S, 28.72. Found: C, 57.36; H, 3.34; N, 0; S, 28.81.

6.6.14. 4-(4-Chlorophenyl)-5-*p*-tolyl-3*H*-1,2-dithiole-3-thione (11e)

Method xii; Reactant: **10b**; yield: 19%; mp: 156 °C; ^{1}H NMR (DMSO) 7.39 (d, 8.35 Hz, 2H, BB′), 7.19 (s, 4H), 7.12 (d, 8.35 Hz, 2H, AA′), 2.27 (s, 3H); FD: *m*/*z* (rel. Int.) = 334.6 (100%).

6.6.15. 4,5-Bis(4-methoxyphenyl)-3*H*-1,2-dithiole-3-thione (11f)

Method xii; Reactant: **10e**; yield: 15%; mp: 179 °C; ^{1}H NMR ($CDCl_3$) 7.19 (d, 8.8 Hz, 2H, B″B‴), 7.07 (d, 8.8 Hz, 2H, A″A‴), 6.89 (d, 8.8 Hz, 2H, BB′), 6.81 (d, 9.1 Hz, 2H, AA′), 3.79 (s, 6H); EI: *m*/*z* (rel. Int.) = 346.5. Anal. Calcd for $C_{17}H_{14}O_2S_3$: C, 58.93; H, 4.07; N, 0; S, 27.76. Found: C, 58.79; H, 4.07; N, 0; S, 27.73.

6.6.16. 4-(4-(Methylsulfonyl)phenyl)-5-phenyl-3*H*-1,2-dithiole-3-thione (11g)

Method xii; Reactant: **10f**; yield: 18%; mp: 191 °C; ^{1}H NMR (DMSO) 7.86 (d, 8.1 Hz, 2H, AA′), 7.5–7.24 (m, 7H), 3.22 (s, 3H); EI: *m*/*z* (rel. Int.) = 364.5. Anal. Calcd for $C_{16}H_{12}O_2S_4$: C, 52.72; H, 3.32; N, 0; S, 35.18. Found: C, 52.65; H, 3.48; N, 0; S, 34.92.

6.6.17. 4-(4-(Methylsulfonyl)phenyl)-5-*p*-tolyl-3*H*-1,2-dithiole-3-thione (11h)

Method xii; Reactant: **10g**; yield: 9%; ^{1}H NMR (DMSO) 7.88 (d, 8.1 Hz, 2H, BB′), 7.4 (d, 8.4 Hz, 2H, AA′), 7.19 (s, 4H), 3.23 (s, 3H), 2.27 (s, 3H);); ^{13}C NMR: (DMSO) 21.2 (Ar-CH_3); 43.6 (SO_2CH_3); 127.1 (2CH); 129.1 (2CH); 129.7 (Cq); 130.1 (2CH); 132.3 (2CH); 140.0 (Cq); 140.6 (Cq); 141.6 (Cq); 144.0 (Cq); 173.0 (Cq); 214.3 (C═S); EI: *m*/*z* (rel. Int.) = 378.5. Anal. Calcd for $C_{17}H_{14}O_2S_4$: C, 53.94; H, 3.73; N, 0; S, 33.88. Found: C, 53.71; H, 3.60; N, 0; S, 33.68.

6.6.18. 4,5-Bis(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (11i)

Method ii; Reactant: **11f**; yield: 24%; mp: 249 °C; ^{1}H NMR (DMSO) 10.16 (s, 1H), 9.56 (s, 1H), 7.11(d, 8.6 Hz, 2H, AA′), 6.97–6.62 (m, 6H); EI: *m*/*z* (rel. Int.) = 318.4.

6.6.19. 5-(4-(Methylsulfonyl)phenyl)-4-phenyl-3*H*-1,2-dithiol-3-one (12b)

Method xiii; Reactant: **11b**; yield: 28%; mp: 183 °C; ^{1}H NMR (DMSO) 7.93 (d, 7.9 Hz, 2H, BB′), 7.58 (d, 7.6 Hz, 2H, AA′), 7.31 (s, 3H), 7.14 (s, 2H), 3.24 (s, 3H); ^{13}C NMR: (DMSO) 43.4 (SO_2CH_3); 127.9 (2CH); 128.7 (CH); 128.8 (2CH); 129.9 (2CH); 130.5 (2CH); 132.2 (Cq); 132.3 (Cq); 138.6 (Cq); 142.7 (Cq); 164.4 (Cq); 193.2 (C═O) EI: *m*/*z* (rel. Int.) = 348.4. Anal. Calcd for $C_{16}H_{12}O_3S_3$: C, 55.15; H, 3.47; N, 0; S, 27.60. Found: C, 54.98; H, 3.17; N, 0; S, 27.63.

6.6.20. 5-(4-Chlorophenyl)-4-*p*-tolyl-3*H*-1,2-dithiol-3-one (12d)

Method xiii, Reactant: **11d**; yield: 83%; mp: 130 °C; ^{1}H NMR (DMSO) 7.47 (d, 8.53 Hz, 2H, BB′), 7.32 (d, 8.52 Hz, 2H, AA′), 7.12 (d, 8.02 Hz, 2H, B″B‴), 6.99 (d, 7.96 Hz, 2H, A″A‴), 2.26 (s, 2H); FD: *m*/*z* (rel. Int.) = 318,5 (100%).

6.6.21. 4-(4-Chlorophenyl)-5-*p*-tolyl-3*H*-1,2-dithiol-3-one (12e)

Method xiii; Reactant: **11e**; yield: 59%; mp: 110 °C; ^{1}H NMR (DMSO) 7.38 (d, 8.19 Hz, 2H, BB′), 7.2 (s, 4H), 7.13 (d, 8.26 Hz, 2H, AA′), 2.29 (s, 3H); FD: *m*/*z* (rel. Int.) = 318.5 (100%). Anal. Calcd for $C_{16}H_{11}ClOS_2$: C, 60.27; H, 3.48; N, 0; S, 20.11. Found: C, 60.27; H, 3.52; N, 0; S, 19.77.

6.6.22. 4,5-Bis(4-methoxyphenyl)-3*H*-1,2-dithiol-3-one (12f)

Method xiii; Reactant: **11f**; ^{1}H NMR ($CDCl_3$) 7.21 (d, 8.87 Hz, 2H, BB′), 7.08 (d, 8.80 Hz, 2H, AA′), 6.87–6.77 (m, 4H), 3.80 (s, 3H), 3.79 (s, 3H).

6.6.23. 4-(4-(Methylsulfonyl)phenyl)-5-phenyl-3*H*-1,2-dithiol-3-one (12g)

Method xiii; Reactant: **11g**; yield: 69%; mp: 176 °C; ^{1}H NMR ($CDCl_3$) 7.86 (d, 8.6 Hz, 2H, AA′), 7.49–7.31 (m, 5H), 7.29–7.19 (m, 2H), 3.03 (s, 3H); EI: *m*/*z* (rel. Int.) = 348.4 (100%). Anal. Calcd for $C_{16}H_{12}O_3S_3$: C, 55.15; H, 3.47; N, 0; S, 27.60. Found: C, 55.27; H, 3.62; N, 0; S, 27.61.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.11.074.

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