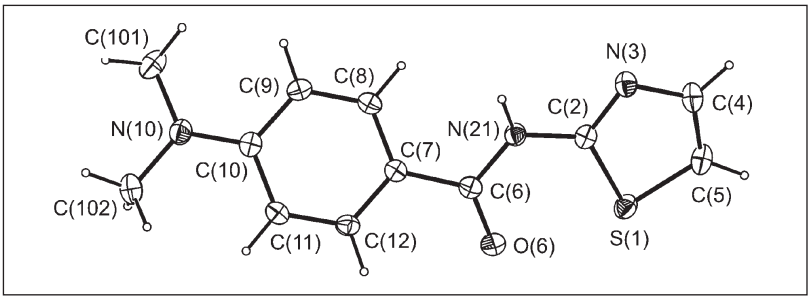
Synthesis and Activity of Four (*N,N*-dimethylamino)benzamide Non-steroidal Anti-inflammatory Drugs Based on Thiazole and Thiazoline.

Daniel E. Lynch, Ravinder Hayer, Samantha Beddows, Joy Howdle and C. Douglas Thake

School of Science and the Environment, Coventry University, Coventry CV1 5FB, UK.

Received April 22, 2005



Four compounds derived from 2-aminothiazole and 2-amino-2-thiazoline were prepared by coupling the respective bases with the acid chlorides of either 3- or 4-(*N,N*-dimethylamino)benzoic acid. Products were identified using infrared spectroscopy, ^{1}H NMR spectroscopy and electrospray mass spectroscopy and in two cases by single-crystal X-ray diffraction. Of the four, *N*-(thiazol-2-yl)-3-(*N,N*-dimethylamino)-benzamide (1), *N*-(thiazolin-2-yl)-4-(*N,N*-dimethylamino)benzamide (2), *N*-(thiazolin-2-yl)-3-(*N,N*-dimethylamino)benzamide (3) and *N*-(thiazolin-2-yl)-4-(*N,N*-dimethylamino)benzamide (4), the hydrochloride salts of compounds 3 and 4 showed anti-inflammatory activity across a concentration range of 10^{-2} – 5 x 10^{-4} *M* while 3 (at a concentration of 10^{-5} *M*) was found to have no adverse effect on myocardial function. The X-ray crystal structure of 2 and the 1:1 adduct structure of 3 with 3-(*N,N*-dimethylamino)benzoic acid are reported.

J. Heterocyclic Chem., **43**, 191 (2006).

Introduction.

Non-steroidal anti-inflammatory drugs, or NSAIDs, are one of the most widely used drug classifications in modern society and include a variety of different chemical agents with differing chemical classes. The word non-steroidal is used in the context that the drugs do not resemble corticosteroids which themselves are anti-inflammatory compounds. NSAIDs are involved in a variety of clinical situations such as in the treatment of analgesia, for anti-inflammatory purposes (mainly for rheumatoid arthritis and osteoarthritis), antipyretic and uricosuric effects [1]. NSAIDs have also been associated with a reduction in risk for developing Alzheimer's desease [2,3] and in the prevention of ocular inflammation related to cataract surgery [4]. Common NSAIDs work by inhibiting cyclooxygenase (COX) activity in prostaglandin H_2 synthase, a key enzyme in the biosynthesis of prostoglandins [5], although other modes of action are known. Other actions that are related to the treatment of inflammation, such as the inhibition of lipoxygenases [6], can be used to distinguish between individual NSAIDs. NSAIDs are also being investigated for application in combination therapy for the stimulation of hair growth [7] and photo-protection compositions [8] (*i.e*. sunscreens). NSAIDs consist of several subcategories, with the main ones being the salicylates, propionic acids (profens), the aryl and heteroaryl acetic acids, the anthranilates (fenamates), the anilides, the phenylpyrazolones and oxicams (enolic acids) [9], with oxicams being characterised by the 4-hydroxybenzothiazine heterocycle. However, an important characteristic of an oxicam molecule is the presence of the carboxyamide substituent at the 3-position of the benzothiazine ring, which contributes towards the acidity of the molecule by stabilising any negative charge during ionisation. Both Sudoxicam and Meloxicam [10] are examples of an oxicam with a carboxamino-1,3-thiazole attachment, and their anti-inflammatory and analgesic properties as well as toxicity, have been reported [11-13]. Sudoxicam is no longer marketed due to severe side effects but Meloxicam is used medically for the treatment of arthritic conditions in several countries [14].

Both aminothiazoles [15-17] and carboxamino-1,3-thiazoles [18,19] have been investigated for anti-inflammatory action, while the crystal structures of several carboxamino-1,3-thiazoles have also been published [20-24]. In addition to these studies we have prepared four carboxamino-1,3-thiazole derivatives containing *N,N*-dimethylaminophenyl moieties with the intention of investigating their anti-inflammatory activity. Anti-inflammatory activity can be easily appraised by using the bioassay, neutrophil oxidative activity qualitative nitroblue tetrazolium test (or NBT reduction test). The use of nitroblue tetrazolium (NBT), 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)bis-2-(*p*-nitrophenyl)-5-phenyl-2*H*-tetrazolium [25], was introduced in 1968 by B.H. Park *et al.* for recognising bacterial infections [26]. Park and co-workers reported that the NBT test might be useful in differentiating patients with bacterial infection

from individuals with non-bacterial disease. The NBT test theory is based on the hypothesis that a metabolic change and an increased reduction of NBT dye would take place when leukocytes are involved in phagocytosis *in-vivo* during a natural infection [27]. NBT is a clear yellow dye that is absorbed by the neutrophils during phagocytosis. As a result of the superoxide produced by the activated neutrophil [in this study induced by *N*-formylmethionylleucylphenylalanine (fMLP)], NBT accepts H^+ and is reduced to formazan but is observed as deep purple precipitates inside the phagocytes when stained. Thus the presence of these coloured zones within each neutrophil is a visual indication of inflammatory activation. However, the percentage of activated neutrophils is suppressed if the sample has been treated with an anti-inflammatory compound.

As a prequel to their anti-inflammatory experiments, Andreani *et al*. tested for and reported that none of their compounds were chemotaxins for human neutrophils, secretagogue agents or were able to trigger superoxide anion production [19]. In their anti-inflammatory tests, Andreani *et al*. reported that all compounds investigated showed effects, at concentrations 10^{-5} – 10^{-9} *M*, but not all were statistically significant. For this reason we chose to examine higher concentrations (10^{-2} – 5 x 10^{-4} *M*) to be sure of any effect. In addition to our own compounds we also tested the commercial anti-inflammatory drug, Diclofenac, so that some comparative strengths of effect could be evaluated. For this purpose Andreani *et al*. used Indomethacin and although Meloxicam may have been a more structurally similar drug for us to use (Diclofenac being a fenamate), Meloxicam's mode of action is preferentially COX-2 inhibition whereas Diclofenac is known to act through both COX inhibition and the down-regulation of L-selectin adhesion molecules thus making it a more effective anti-inflammatory agent [28,29]. The testing of compounds for their own potential toxic affect is important and the results of Andreani *et al*. indicate that our compounds will be passive to neutrophil activity. However, as an addition to the results of Andreani *et al*. we decided to investigate the effects, if any, that our compounds would have on myocardial function. Reported here is the synthesis and chemical characterisation of *N*-(thiazol-2-yl)-3-(*N*,*N*-dimethylamino)benzamide (**1**), *N*-(thiazol-2-yl)-4-(*N*,*N*-dimethylamino)benzamide (**2**), *N*-(thiazolin-2-yl)-3-(*N*,*N*-dimethylamino)benzamide (**3**) and *N*-(thiazolin-2-yl)-4-(*N*,*N*-dimethylamino)benzamide (**4**) (Figure 1). All compounds were characterised using infrared spectroscopy, ^{1}H NMR and electrospray mass spectrometry. The single crystal X-ray structures of compound **2** and the 1:1 adduct of **3** with (*N,N*-dimethylamino)benzoic acid **3a** are also reported. NBT tests were performed on the hydrochloride salts of **3** and **4**, while **3** was investigated for effect on myocardial function.

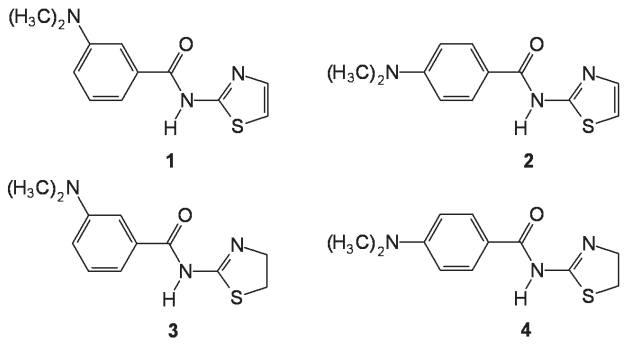


Figure 1. Chemical diagrams for the compounds prepared in this study.

Results and Discussion

Carboxamide linkages are commonly produced by the coupling of an acid chloride with an amine group with the loss of hydrochloric acid, thus an equimolar amount of triethylamine should be present to form the hydrochloride salt which can be easily removed by aqueous extraction in the work-up. Although an inert atmosphere is not required dry conditions are still necessary else some of the acid chloride hydrolyses back to the carboxylic acid and then both react to form the anhydride [30]. In our procedure, completion of the addition of the acid chloride to the amine was followed by removal of the solvent under reduced pressure to yield an expected mixture of compounds including the water soluble salt and, assuming that some acid chloride had been hydrolysed, the benzoic acid derivative. The removal of both of these side-products was easily achieved by washing the resultant mass with dilute aqueous base. However in the case of **3** removal of the reaction solvent yielded crystals that redissolved in an organic solvent, indicating that they were organic and not triethylammonium chloride. Subsequent analysis of crystals of this unknown material yielded the structure of **3a**, proving the presence of some benzoic acid derivative in the final reaction mixture. Thus, washing with dilute aqueous base instead of just water was an essential part of the work-up process. Dissolution in minimal ethyl acetate and then precipitation in light petroleum was found to be an effective procedure for the removal of small amounts of the aminoheterocycle and any anhydride that had formed. As a final process, and a precursor to preparing the compounds for pharmacological testing, the products were dissolved in acid, filtered, and then re-precipitated with excess base. This last step ensured that the entire final product was capable of being made water-soluble by production of the hydrochloride salt. Many drugs containing base units are stored and administered as protonated salts. These salts are of course more water-soluble and are mainly chlorides, sulfates and hydrogen sulfates, although very insoluble compounds are complexed with citrates or tartrates [31]. The preparation of the hydrochloride salts of **1** – **4** consisted of a measured amount of each compound being dissolved in an aqueous solution containing a stoichiometric equivalent of hydrochloric acid, filtering and evaporation to dryness. Although

initially soluble, aqueous solutions of **1** and **2** over time encountered problems with precipitation of the compound salt. The additional use of both citric and tartaric acids did not improve the long-term solubility of **1** and **2** at the concentrations needed to match **3** and **4** so **1** and **2** were not tested for anti-inflammatory activity.

Two characteristic bands are commonly observed in the infrared spectra of amides, or carboxamides, these are the carbonyl stretching vibration between 1690 – 1640 cm^{-1} and the N-H stretching absorption around 3200 – 3300 cm^{-1}. The frequency of the carbonyl vibration is lower for amide C=O than for esters or ketones (≥ 1700 cm^{-1}) and can be reduced by as much as 20 – 60 cm^{-1} [32,33]. In the case of **4** the carbonyl stretch is lowered to 1600 cm^{-1}, becoming essentially a shoulder on the side of the strong aromatic peak typically observed at that wavenumber. Similarly, the carbonyl stretch for **3** is also lower than expected for a secondary amide. In support of these observations, Andreani *et al.* also reported that the carbonyl stretching frequencies for their thiazoline derivatives were lower than those of the thiazoles [19]. In the ^{1}H NMR spectra of **1** – **4**, none displayed distinct N-H peaks, observed around δ = 12.5 ppm in previous reports [19]. Interestingly, the aromatic peaks in the ^{1}H NMR of **2** show the possible existence of a second conformation in solution with minor peaks neighbouring the main aromatic proton doublets between δ = 6.6 – 8.1 ppm (Figure 2). Integration across both minor and major doublets in both cases gives the whole proton value, thus the existence of the minor peaks is potentially due to a second conformation that is most likely to be one where the phenyl ring is inclined to the approximate plane of the rest of the molecule. This second conformation is not observed in the solid-state crystal structure. The electrospray mass spectrometry spectra show a variety of results in addition to MH^+ with compounds **2** – **4** also assembling with Na as either MNa^+ or $2MNa^+$.

The single crystal structure of **2** comprises a slightly twisted molecule with a dihedral angle of 30.00(6)° between

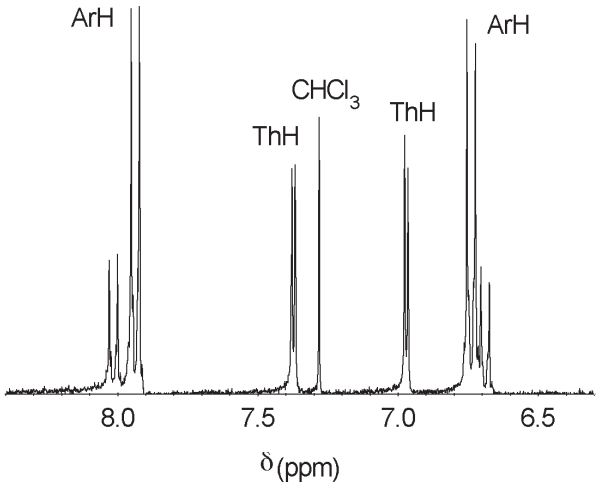


Figure 2. The aromatic region of the ^{1}H nmr for **2**.

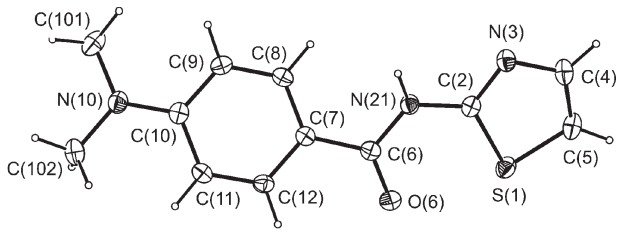


Figure 3. Molecular configuration and atom numbering scheme for **2**, showing 50% probability ellipsoids.

the phenyl and thiazole rings (Figure 3). One hydrogen-bonding association exists between the carboxamide NH and the thiazole nitrogen atom; listed in Table 1 as well as selected torsion angles for the carboxamide

Table 1

Hydrogen bonding geometries and selected torsion angles for compounds **2** and **3a**.

	D--H···A	D–H(Å)	H…A(Å)	D…A(Å)	D–H…A(°)
2	N(21)--H···N(3)	0.85(2)	2.16(2)	3.000(2)	170(2)
3a	N(21A)--H···O(10B)	0.89(2)	1.94(2)	2.830(2)	173(3)
	O(11B)--H···N(3A)	1.26(2)	1.28(2)	2.540(1)	173(3)

Torsion angles

2		**3a**	
S(1)-C(2)-N(21)-C(6)	0.5(2)	S(1A)-C(2A)-N(21A)-C(6A)	-4.7(2)
C(2)-N(21)-C(6)-C(7)	165.4(1)	C(2A)-N(21A)-C(6A)-C(7A)	-163.4(1)
N(21)-C(6)-C(7)-C(12)	163.4(1)	N(21A)-C(6A)-C(7A)-C(12A)	-173.1(1)
C(2)-N(21)-C(6)-O(6)	-13.6(2)	C(2A)-N(21A)-C(6A)-O(6A)	15.9(2)
O(6)-C(6)-C(7)-C(8)	157.6(1)	O(6A)-C(6A)-C(7A)-C(8A)	-167.7(1)

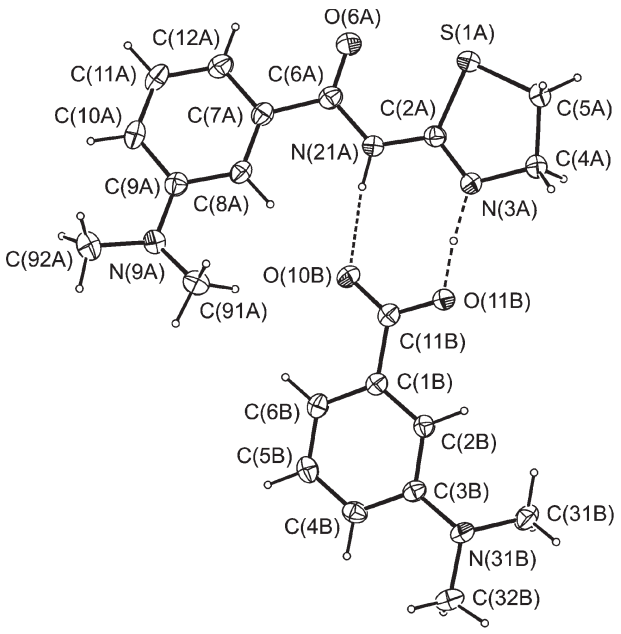


Figure 4. Molecular configuration and atom numbering scheme for **3a**, showing 50% probability ellipsoids. Hydrogen-bonding associations are shown as dotted lines.

Table 2

Percentage inhibition of neutrophil activity for each compound, concentration and run (normalized against saline solution).

Compound	**Conc. (mM)**	**Run 1**	**Run 2**	**Run 3**
3	0.5	8	33	11
	1.0	50	54	41
	5.0	38	35	15
	10.0	32	39	27
4	0.5	28	29	24
	1.0	31	37	29
	5.0	36	33	32
	10.0	50	50	50
Diclofenac	0.5	43	33	34
	1.0	47	49	37
	5.0	74	73	71
	10.0	71	73	74

linkage. The single crystal structure of the 1:1 adduct of *N*-(thiazolin-2-yl)-3-(*N*,*N*-dimethylamino)benzamide with (*N*,*N*-dimethylamino)benzoic acid **3a** comprises the two constituent molecules associated *via* a hydrogen-bonded $R^2_2(8)$ graph set motif involving the carboxylic acid group across the N(21A) / N(3A) site of the aminoheterocyclic moiety (Figure 4). Hydrogen-bonding geometries of this association and selected torsion angles are also listed in Table 1. The dihedral angle in molecule A between the phenyl ring and the calculated median plane of the thiazoline ring is 26.65(7)°.

Andreani *et al*. reported their anti-inflammatory results as percentage inhibition of human neutrophils [19] but Viallet *et al*. undertook *in vivo* experiments on rats and reported their data as dose responses (*i.e*. LD_{50} and ED_{50}) [18]. For this study, results are given in percentage inhibition compared to the number of activated neutrophils in physiological saline (baseline). Results for the hydrochloride salts of **3** and **4**, and for Diclofenac are listed in Table 2 with the results for each run being the average of three slides (twice counted) prepared from the same mixture. For **3**, the 0.5 m*M* concentration exhibited an average range of inhibition of neutrophil activity of 8 – 33% with the higher value arising due to the second of the three runs with a difference of >20%. The 1 m*M* concentration gave higher, and more consistent, results with an average of 41 – 54% inhibition of neutrophil activity. The 5 m*M* and 10 m*M* had 15 – 38% and 27 – 39% inhibition of neutrophil activity with the third run at 5 m*M* being *c*. 20% lower than the other two runs. The results for **4** are more consistent and inhibition slowly increases with concentration. The 0.5 m*M* concentration of **4** had an average range of neutrophil inhibition of 24 – 28%. The 1 m*M* and 5 m*M* concentration gave slightly higher results with an average of 29 – 37% and 32 – 36% inhibition of neutrophil activity while the 10 m*M* concentration had 50% inhibition of neutrophil activity for all three runs. Results for Diclofenac were expected to be much higher and for the higher concentrations (5 and 10 m*M*) a range of 71 – 74% inhibition of neutrophil activity is observed but the lower concentrations (0.5 and 1 m*M*) give results that are not too different from **3** and **4** with percentages ranging from 33 – 43% inhibition and 37 – 49% inhibition, respectively.

Unfortunately, Andreani *et al*. did not examine the anti-inflammatory activity of their pre-cursor compounds that **1** – **4** are different analogues of. Instead the compounds they did examine gave similar anti-inflammatory responses (35 – 55% inhibition) compared to **3** and **4** but at much weaker concentrations [10^{-5} – 10^{-9} *M* as opposed to 10^{-2} – 5 x 10^{-4} *M* for **3** and **4**]. At their most effective concentrations, compounds **3** and **4** both show approximately 50% inhibition of neutrophil activation, which directly relates to a 20% reduction in the best anti-inflammatory activity of Diclofenac, across the same concentration range although at a concentration of 1 m*M*, **3** shows slightly more inhibition activity than Diclofenac. Testing the effects of **3** on myocardial function added to the studies of Andreani *et al*. that reported that this series of compounds were not chemotaxins for human neutrophils. Compound **3**, at a lower concentration of 0.01 m*M* or 10^{-5} *M*, was introduced after an ischemic insult was induced in the heart (*i.e.* a heart attack) but before reperfusion began. The haemodynamic properties of the heart (*i.e.* left ventricular developed pressure, coronary flow and heart rate) remained unaffected with the inclusion of **3** and continuing throughout the reperfusion process. In addition to this, the percent necrosis (infarction), calculated by evaluating slices of the stained heart and determining the percentage of stained or healthy tissue against non-stained or dead tissue, was found to be 36.7%,

Table 3

Crystallographic details for **2** and **3a**.

	Compound **2**	Compound **3a**
CCDC reference	211677	211678
Formula	$C_{12}H_{13}N_3OS$	$C_{21}H_{26}N_4O_3S$
M_r	247.31	414.52
Crystal class	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$
a (Å)	7.34590(10)	15.1552(12)
b (Å)	11.3488(2)	8.3453(7)
c (Å)	14.3981(3)	16.7265(13)
β (°)	103.8536(8)	108.0190(10)
V(Å^3)	1165.41(4)	2011.7(3)
D_c (g cm^{-3})	1.410	1.369
Z	4	4
μ(Mo-K$_\alpha$) (mm^{-1})	0.264	0.192
T_{min}, T_{max}	0.897, 0.920	0.945, 0.987
Colour	colourless	colourless
Crystal size (mm)	0.42 x 0.34 x 0.32	0.30 x 0.27 x 0.07
Total data	5188	11179
Unique data	2668	4115
R_{int}	0.014	0.018
N [I>2.0 (I)]	2334	3389
R1	0.040	0.035
wR2	0.090	0.095
S	1.05	1.04
A, B [a]	0.0506, 0.4053	0.0627, 0.0000

[a] $w = [\sigma^2(F_o^2) + (AP)^2 + BP]^{-1}$ [where $P = (F_o^2 + 2F_c^2)/3$]

which is a minimal variation from the control value of 35% [34]. Thus, compounds **3** and **4** display anti-inflammatory activity and compound **3** has no adverse effect on myocardial function which is a good basis for further studies on phenylcarboxamides based on derivatives of 1,3-thiazoles.

EXPERIMENTAL

All chemicals were purchased from Sigma-Aldrich. Triethylamine was distilled before use. Pyridine was distilled, dried and stored over potassium hydroxide. Dichloromethane was dried using molecular sieves. Infrared spectra were recorded as pressed KBr discs on a Nicolet 205 FT-IR spectrometer. ^{1}H NMR data were recorded on a Bruker AC250 NMR spectrometer for **1** and **3** and a Bruker Spectrospin 400 NMR spectrometer for **2** and **4**. Electrospray mass spectra were recorded in positive ion mode on a Micromass Platform mass spectrometer (Southampton University).

3-(*N*,*N*-Dimethylamino)benzoic Chloride.

Oxalyl chloride (3.10 g, 24 mmol) was drop-wise added to a stirred solution of 3-(*N*,*N*-dimethylamino)benzoic acid (1.00 g, 6 mmol) in dry dichloromethane (20 mL) with 3 drops of dry pyridine. After further stirring for 30 minutes the solvent was removed under reduced pressure to yield an off-white powder, 1.05 g (95%), mp 138 – 140º (lit. 138 – 140º) [35].

4-(N,N-Dimethylamino)benzoic Chloride.

Oxalyl chloride (3.10 g, 24 mmol) was drop-wise added to a stirred solution of 4-(*N*,*N*-dimethylamino)benzoic acid (1.00 g, 6 mmol) in dry dichloromethane (20 mL) with 3 drops of dry pyridine. After further stirring for 30 minutes the solvent was removed under reduced pressure to yield an off-white powder, 1.06 g (96%), mp 135 – 136º (lit. 135 – 136º) [35].

N-(Thiazol-2-yl)-3-(*N,N*-dimethylamino)benzamide (**1**).

3-(*N*,*N*-Dimethylamino)benzoic chloride (1.00 g, 5.5 mmol) was slowly added to a stirred solution of 2-aminothiazole (0.55 g, 5.5 mmol) and triethylamine (0.56 g, 5.5 mmol) in dry dichloromethane (20 mL). After further stirring for 30 minutes, the solvent was removed under reduced pressure to yield an off-white gelatinous material. The crude product was then washed with dilute sodium hydroxide solution, filtered, dissolved in minimal ethyl acetate, filtered and then precipitated by adding to 50 mL of light petroleum (40 - 60 °C). The solid was redissolved in dilute hydrochloric acid solution and reprecipitated by again adding to dilute sodium hydroxide solution in excess. The product was collected *in vacuo* to afford a white powder, 0.71 g (52%), mp 134 – 136°; ir (KBr): 3140m (NH), 1680 (CO); ^{1}H nmr (250 MHz; $CDCl_3$; Me_4Si): δ 2.99 (s, 6 H, NCH_3), 6.92 (d, 1 H, J = 3.5, ThH), 6.95 (d, 1 H, J = 8.3, ArH), 7.06 (d, 1 H, J = 3.5, ThH), 7.28 – 7.38 (m, 3 H, ArH); es-ms: m/z 248 (100) (MH^+), 495 (8) ($2MH^+$).

Anal. Calcd. for $C_{12}H_{13}N_3OS$: C, 58.3; H, 5.3; N, 17.0. Found: C, 58.0; H, 5.1; N, 17.1.

N-(Thiazolin-2-yl)-4-(*N,N*-dimethylamino)benzamide (**2**).

4-(*N*,*N*-Dimethylamino)benzoic chloride (1.00 g, 5.5 mmol) was slowly added to a stirred solution of 2-aminothiazole (0.55 g, 5.5 mmol) and triethylamine (0.56 g, 5.5 mmol) in dry dichloromethane (20 mL). After further stirring for 30 minutes, the solvent was removed under reduced pressure to yield an off-white powder. The crude product was then washed with dilute sodium hydroxide solution, filtered, dissolved in minimal ethyl acetate, filtered and then precipitated by adding to 50 mL of light petroleum (40 - 60 °C). The solid was redissolved in dilute hydrochloric acid solution and reprecipitated by again adding to dilute sodium hydroxide solution in excess. The product was collected *in vacuo* to afford a white powder, 0.68 g (50%), mp 138-140°; ir (KBr): 3165w (NH), 1654m (CO); ^{1}H nmr (400 MHz; $CDCl_3$; Me_4Si): δ 3.10 (s, 6 H, CH_3), 6.69 (25%) & 6.74 (75%) (d, 2 H, J = 9, ArH), 6.97 (d, 1 H, J = 3, ThH), 7.37 (d, 1 H, J = 4, ThH), 7.94 (75%) & 8.02 (25%) (d, 2 H, J = 9, ArH); es-ms: m/z 248 (55) (MH^+), 470 (100) (MNa^+).

Anal. Calcd. for $C_{12}H_{13}N_3OS$: C, 58.3; H, 5.3; N, 17.0. Found: C, 58.2; H, 5.2; N, 17.3.

N-(Thiazolin-2-yl)-3-(*N,N*-dimethylamino)benzamide (**3**).

3-(*N*,*N*-Dimethylamino)benzoic chloride (1.00 g, 5.5 mmol) was slowly added to a stirred solution of 2-amino-2-thiazoline (0.56 g, 5.5 mmol) and triethylamine (0.56 g, 5.5 mmol) in dry dichloromethane (20 mL). After further stirring for 30 minutes, the solvent was removed under reduced pressure to yield an off-white crystalline material. The crude product was redissolved in minimal ethyl acetate, filtered and then precipitated by adding to 50 mL of light petroleum (40 - 60 °C). A small amount of the product was then crystallized from ethyl acetate and analysed using single crystal X-ray diffraction techniques to give the 1:1 adduct structure of *N*-(thiazolin-2-yl)-3-(*N*,*N*-dimethylamino)benzamide with 3-(*N*,*N*-dimethylamino)benzoic acid (**3a**), mp 110 – 112°.

Anal. Calcd. for $C_{21}H_{26}N_4O_3S$: C, 60.85; H, 6.3; N, 13.5. Found: C, 61.1; H, 6.5; N, 13.3.

The remaining solid product was washed with dilute sodium hydroxide solution, filtered, redissolved in dilute hydrochloric acid solution and reprecipitated by again adding to dilute sodium hydroxide solution in excess. The product was collected *in vacuo* to afford a white powder, 0.44 g (32%), mp 123 – 125°, ir (KBr): 3154w (NH), 1623s (CO); ^{1}H nmr (250 MHz; $CDCl_3$; Me_4Si) δ 2.98 (s, 6 H, NCH_3), 3.20 (t, 2 H, J = 8.0, CH_2), 3.59 (t, 2 H, J = 8.0, CH_2), 6.66 (d, 1 H, J = 8.0, ArH), 7.20 – 7.47 (m, 3 H, ArH); es-ms: m/z 250 (100) (MH^+), 499 (12) ($2MH^+$), 521 (15) ($2MNa^+$).

Anal. Calcd. for $C_{12}H_{15}N_3OS$: C, 57.8; H, 6.1; N, 16.9. Found: C, 58.0; H, 6.2; N, 16.7.

N-(Thiazolin-2-yl)-4-(*N,N*-dimethylamino)benzamide (**4**).

4-(*N,N*-Dimethylamino)benzoic chloride (1.00 g, 5.5 mmol) was slowly added to a stirred solution of 2-amino-2-thiazoline (0.56 g, 5.4 mmol) and triethylamine (0.56 g, 5.5 mmol) in dry dichloromethane (20 mL). After further stirring for 30 minutes, the solvent was removed under reduced pressure to yield an off-white powder. The crude product was then washed with dilute sodium hydroxide solution, filtered, dissolved in minimal ethyl acetate with a few drops of added chloroform (to enhance dissolution), filtered and then precipitated by adding to 50 mL of light petroleum (40 - 60 °C). The solid was redissolved in dilute hydrochloric acid solution and reprecipitated by again adding to dilute sodium hydroxide solution in excess. The product was collected *in vacuo* to afford a white powder, 0.75 g (55%); mp 128-131°; ir (KBr): 3216m (NH), 1600s (CO); ^{1}H nmr (400 MHz; $CDCl_3$; Me_4Si) δ 3.07 (s, 6 H, CH_3), 3.31 (t, 2 H, J = 8, CH_2), 3.89 (t, 2 H, J = 8, CH_2), 6.69 (d, 2 H, J = 9, ArH), 8.04 (d, 2 H, J = 9, ArH); es-ms: m/z 250 (100) (MH^+), 272 (65) (MNa^+).

Anal. Calcd. for $C_{12}H_{15}N_3OS$: C, 57.8; H, 6.1; N, 16.9. Found: C, 57.9; H, 6.0; N, 17.0.

X-ray Structure Analysis.

General crystallographic details for compounds **2** and **3a** are listed in Table 3. Crystals were grown from ethyl acetate solutions. Crystallographic data was collected on a Bruker Nonius Kappa CCD area diffractometer using monochromatised Mo-Kα x-ray radiation (λ = 0.71073Å) equipped with an Oxford Cryosystems low temperature device (Southampton University). Lattice parameters were calculated using 7564 **2** and 8432 **3a** reflections with 2.91º < θ < 27.48º. Intensity data were collected at a temperature of 120 K **2** and 150 K **3a** using φ-ω scans to a maximum 2θ value of 50° **2** and 53º **3a**. Multi-scan absorption corrections were applied to all data sets using the program SORTAV [36]. Structures were solved by direct methods and refined using the SHELX-97 package [37]. All hydrogen atoms not involved in the strong hydrogen-bonding associations were included in the refinement at calculated positions as riding models with C-H set to 0.95 Å (Ar-H) and 0.98 Å (CH_3). The NH and OH hydrogen atoms were located by difference methods and both positional and thermal parameters were refined.

Nitroblue Tetrazolium (NBT) Reduction Test.

All tests were performed using venous blood from resting healthy male subjects aged 20 – 30 years. The hydrochlorides of **3** and **4**, and diclofenac acid sodium salt were prepared in 10.0, 5.0, 1.0 and 0.5 m*M* aqueous solutions. 50 μL of each concentration for each of the three compounds or 50 μL of a fresh saline solution (0.9% w/v NaCl) were in turn added to separate mixtures of 100 μL of 0.1% nitroblue tetrozolium (NBT) solution, made with 0.9% phosphate-buffered saline (PBS), and 5 μL of 10^{-3} *M* fMLP in 1:9 DMSO:0.9% PBS, followed by the respective addition of 50 μL of blood to each solution. The samples were gently mixed and placed in a water bath and incubated at 37 ºC for 10 minutes. Following this time they were removed and allowed to cool to room temperature for another 10 minutes completed with further gentle agitation. 3 x 40 μL of each mixture were then gravity spread respectively over 3 separate glass slides. Each smear was air dried and then stained using 1 mL of ACCUSTAIN Wright Stain, allowing it to flood the sample for 90 seconds. To the flooded smears 1 mL of deionised water was added and the slides were again allowed to stand for another 90 seconds. The stain was then rinsed off with deionised water and the slides left to air dry. Each slide was examined twice on separate days by counting 100 neutrophils and recording the number that were activated. All slides were blind counted (*i.e*. labels covered) and randomly ordered. In total, for every concentration of every compound there were three runs performed and each run consisted of treating and twice measuring three slides whose results were averaged to obtain a result for each run. The background activation percentage was obtained by averaging three slides (twice counted) treated with the saline solution.

Myocardial Ischaemia/Reperfusion [34].

A male Sprague-Dawley rat heart, in a classic Langendorff heart set up, was perfused retrogradely with modified Krebs-Hensleit bicarbonate buffer that contained (in m*M*) NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.7, and glucose 12.0. The buffer was gassed with a mixture consisting of 95% O_2 and 5% CO_2. The heart was allowed to stabilise for 15 minutes prior to being subjected to 30 minutes of regional ischemia followed by 100 minutes of reperfusion. The heart temperature was maintained between 36.5 and 37.5 ºC. Regional ischemia was induced by tightening a suture around the left main coronary artery and reperfusion initiated by releasing the ends of the suture. An aqueous solution containing the hydrochloride of **3** was administered to the Krebs buffer, resulting in an overall concentration of 0.01 m*M* of **3**, 10 minutes before reperfusion commenced. Throughout the duration of the experiment recordings were taken every 5 minutes of left ventricle developed pressure, coronary flow and heart rate. Left ventricle developed pressure was monitored by inserting and inflating a latex balloon in the left ventricle resulting in an end-diastolic pressure of 8 – 10 mmHg. The heart was then stained with triphenyltetrazolium and frozen for 24 hours after which it was sliced and placed into formaldehyde for a further 24 hours. From the slices the percentage of necrosis was determined.

Acknowledgements.

The authors thank the School of Science and the Environment (Coventry) for financial assistance, the EPSRC National Crystallography Service (Southampton) and Dr G. J. Langley (Southampton) for the collection of es-ms data. Dr H. Maddock and Mr B. Harb (Coventry) are thanked for performing the myocardial experiment.

REFERENCES AND NOTES

[1] H. P. Rang, M. M. Dale and J. M. Ritter, Pharmacology, Churchill Livingstone, London, 1998, pp 246-248.

[2] F. Landi, M. Cesari, G. Onder, A. Russo, S. Torre and R.

Bernabei, *Am. Journ. Geriatric Psych.*, **11**, 179 (2003).

[3] T. Morihava, T. Chu, O. Ubeda, W. Beech and G. M. Cole, *J. Neurochem.*, **83**, 1009 (2002).

[4] R. Schalnus, *Ophthalmologica*, **2**, 89 (2003).

[5] D. G. Munroe and C. Y. Lau, *Chemistry and Biology*, **2**, 343 (1995).

[6] D. R. Laurence, P. N. Bennet and M. J. Brown, Clinical Pharmacology, 8th Edn, Churchill Livingstone, London, 1998, p 250.

[7] J. F. Grollier and G. Rosenbaum, United States Patent No. 5053410, October 1991; *Chem. Abstr.*, **Vol 113**, P11923f (1990).

[8] D. L. Bissett and R. Chatterjee, United States Patent No. 4946671, August 1990; *Chem. Abstr.*, **Vol 112**, P223133c (1990).

[9] A. Galbraith, S. Bullock, E. Manias, B. Hunt and A. Richards, Fundamentals of Pharmacology, Addison Wesley Longman Ltd, Harlow, 1999, pp 338-341.

[10] P. Luger, K. Daneck, W. Engel, G. Trummlitz and K. Wagner, *Eur. J. Pharm. Sci*., **4**, 175 (1996).

[11] D. Binder, O. Hromatka, F. Geissler, K. Schmied, K. Noe and R. Christian, *J. Med. Chem*., **30**, 678 (1987).

[12] K. T. Olkkola, A. V. Brunetto and M. J. Mattila, *Clin. Pharmacokinet*., **26**, 107 (1994).

[13] E. Albengres, S. Urien, J. Barre, P. Nguyen, F. Bree, P. Jolliet, J. P. Tillement, R. S. Tsai, P. A. Carrupt and B. Tiesta, *Int. J. Tissue React*., **15**, 125 (1993).

[14] S. Noble and J. A. Balfour, *Drugs*, **51**, 424 (1996).

[15] K. Satsangi, V. S. Misra, *Pharmazie*, **38**, 341 (1983).

[16] A. A. Geronikaki, J. D. Hadjipavlou-Litina, *Pharmazie*, **48**, 948 (1993).

[17] J. D. Hadjipavlou-Litina, A. A. Geronikaki, E. D. Sotiropoulou, *Res. Commun. Chem. Pathol. Pharmacol.*, **79**, 355 (1993).

[18] M. P. Viallet, A. Boucherle, C. Cohen-Addad, *Eur. J. Med. Chem. – Chim. Ther.*, **14**, 553 (1979).

[19] A. Andreani, L. Alberto, A. Locatelli, R. Morigi, M. Rambaldi, J. C. Gehret, S. Traniello, A. Cariani and S. Spisani, *Collect. Czech. Chem. Commun.*, **64**, 299 (1999).

[20] C. Cohen-Addad, M. P. Viallet and A. Boucherle, *Acta Cryst*., **B35**, 2109 (1979).

[21] C. Cohen-Addad, *Acta Cryst*., **B38**, 1753 (1982).

[22] N. H. Dung, B. Viossat, J. -C. Lancelot and M. Robba, *Chem. Pharm. Bull*., **34**, 951 (1986).

[23] B. Viossat, N. H. Dung, J. -C. Lancelot, F. Robert and M. Robba, *Chem. Pharm. Bull*., **35**, 2419 (1987).

[24] J. K Maurin, Z. Czamocki and B. Paluchowska, *Pol. J. Chem*., **73**, 377 (1999).

[25] R. K. Bouhafs and C. Jarstrand, *Medline*, **36**, 727 (2002).

[26] B. H. Park, S. M. Fikrig and E. M. Smithwick, *Lancet*, **2**, 532 (1968).

[27] M. Grzegorowski, J. Antyborzec and J. Szyd_owski, *New Medicine*, **3,** 2 (1999).

[28] M. V. Gómez-Gaviro, I. González-Alvaro, C. Domínguez-Jiménez, J. Peschon, R. A. Black, F. Sánchez-Madrid and F. Díaz-González, *J. Biol. Chem*., **277**, 38212 (2002).

[29] M. V. Gómez-Gaviro**,** C. Domínguez-Jiménez**,** J. M. Carretero**,** P. Sabando**,** I. González-Alvaro**,** F. Sánchez-Madrid and F. Díaz-González, *Blood*, **96**, 3592 (2000).

[30] D. E. Lynch, R. Hayer, S. Bagga and S. Parsons, *Aust. J. Chem.*, **53**, 593 (2000).

[31] G. Thomas, Chemistry for Pharmacy and the Life Sciences Including Pharmacology and Biomedical Science, Prentice Hall, London, 1996, p 406.

[32] W.O. George and P. S. McIntyre, Infrared Spectroscopy, John Wiley & Sons, New York, 1987, p 296.

[33] J. R. Dyer, Applications of Absorption Spectroscopy of Organic Compounds, Prentice-Hall, Englewood Cliffs, 1965, p 82.

[34] H. L. Maddock, M. M. Mocanu and D. M. Yellon, *Amer. J. Physiol-Heart*, **283**, H1307. (2002).

[35] J. C. Howard and F. E. Youngblood, *J. Org. Chem*., **31**, 959 (1966).

[36] R. H. Blessing, *Acta Cryst.*, A**51**, 33 (1995); R. H. Blessing, *J. Appl. Cryst*., **30**, 421 (1997).

[37] G. M. Sheldrick, SHELX-97, University of Göttingen, Germany, 1997.