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Original article

Synthesis, identification and antiplatelet evaluation of 2-morpholino substituted benzoxazines

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Abstract

A number of 2-morpholino substituted benzoxazines have been prepared in order to test their effectiveness against ADP and collagen induced platelet aggregation. The reaction of 2-thio-1,3-benzoxazines with morpholine has been generalised to enable the use of substituted benzoxazines. Two separate methods were used to prepare 7-*O*-2-morpholino substituted benzoxazines from 7-hydroxy-2-morpholino benzoxazines. Antiplatelet testing was carried out on 15 of the title compounds. 7-(2-Chloroethoxy)-8-methyl-2-morpholin-4-yl-4*H*-1,3-benzoxazin-4-one **15d** and 7-[2-(4-methylpiperazin-1-yl)ethoxy]-8-methyl-2-morpholin-4-yl-4*H*-1,3-benzoxazin-4-one **16d** showed potent activity against ADP and collagen induced platelet aggregation. The structures of the newly prepared compounds were confirmed by microanalysis as well as the analysis of IR, ^{1}H and ^{13}C NMR.

Keywords: 2-Morpholino substituted benzoxazines; Antiplatelet

1. Introduction

The use of 1,3-benzoxazines has been important in the development of antimicrobial, antiviral, and antifungal drugs [1–3]. In earlier work we reported a generalised method for the production of 2-thio-(1,3-benzoxazines, 1,3-benzothiazines, and quinazolines) from 2-(hydroxy, thio, or amino) aromatic acids, respectively, using triphenylphosphine thiocyanogen [4]. The presence of the thio group in the C-2 position of the benzoxazines results in the 2-mercapto tautomer. This reacts with benzylamine (primary amines) to open the oxazine ring between positions 1 and 2 to give *N*-benzylthioureas which have been found to possess antiviral, antiproliferation and antibacterial properties [5]. Furthermore the 2-mercapto tautomer provides a good leaving group for the reaction with morpholine (secondary amines) to give 2-morpholino-1,3-benzoxazine antiatherosclerotic compounds [6].

The use of 2-thio-1,3-benzoxazine **1** in the synthesis of 2-morpholino-1,3-benzoxazine **2** has been reported earlier [7] (Scheme 1) and requires the dropwise addition of morpholine to **1** in dioxane followed by heating at reflux for 3 h. 2-Morpholino chromones are structurally similar to 2-morpholino benzoxazines and have been reported to exhibit potent antiplatelet properties [8].

The antiplatelet properties of 2-aminochromones in general have been investigated by many researchers [6,8–11]. 2-Morpholino chromones, however, have been suggested to be the most potent analogue, and work has been done to determine which aromatic ring substituents would enhance the antiplatelet activity [8]. The presence of substituent groups on the C-7 and C-8 positions of the aromatic ring has been found to enhance the activity of the 2-morpholino chromone with the maximum activity found with 7-[2-(amino)ethoxy]-8-methyl derivatives. In particular the 7-[2-(4-methylpiperazin-1-yl) ethoxy]-8-methyl-2-morpholinyl chromone **5** (Scheme 2) was shown to have potent antiplatelet properties against

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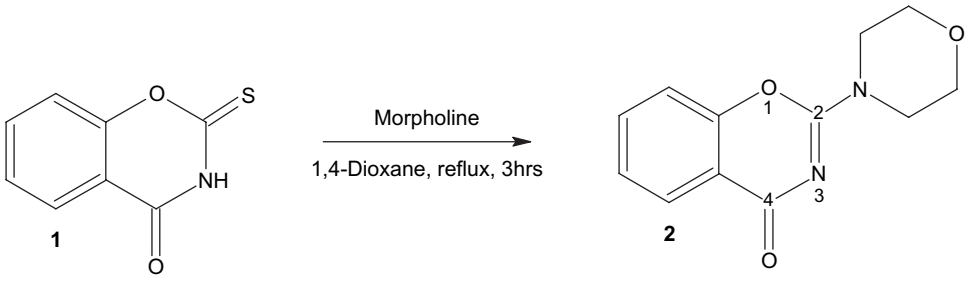


Scheme 1.

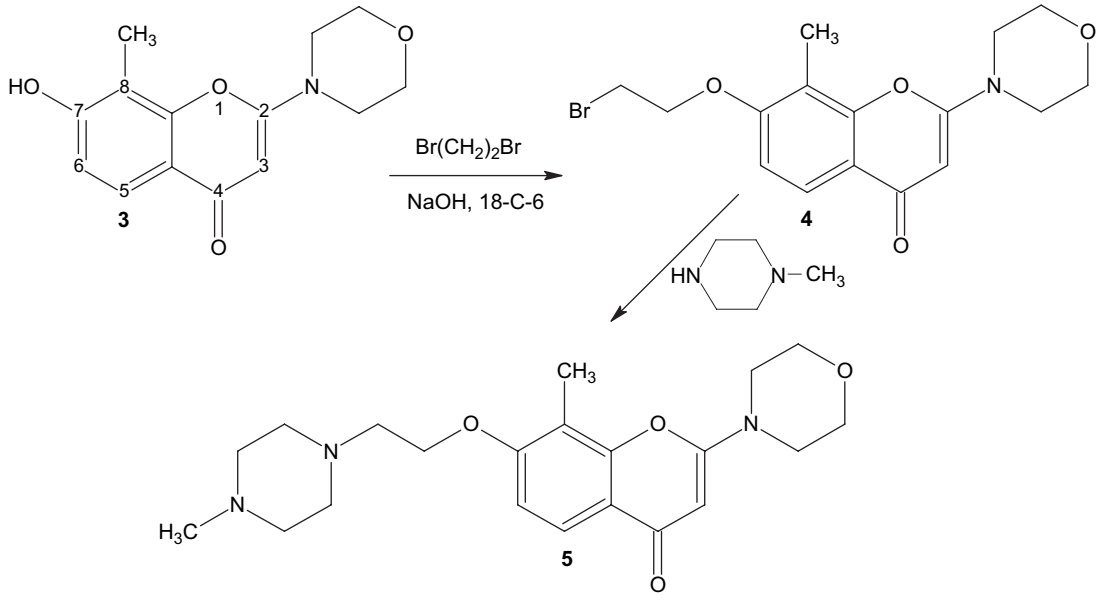
ADP induced aggregation with the IC_{50} reported to be 0.85 ± 0.3 μM [8]. Furthermore ongoing work is being undertaken to determine the activity of 2-morpholino chromones against platelet phosphodiesterases [12].

The synthesis of 2-morpholino chromones has been achieved via the reaction of a 2′-hydroxyacetophenone BF_2 complex with 4-(dichloromethylene)morpholinium chloride [8]. Furthermore the placement of the 7-[2-(4-methylpiperazin-1-yl)ethoxy] substituent group on the aromatic ring of the chromone was achieved via the production of a 7-(2-bromoethoxy) intermediate **4** from the reaction of the 7-hydroxychromone **3** with NaOH and dibromoethane in the presence of a phase transfer catalyst. The intermediate **4** was then allowed to react with *N*-methylpiperazine to give compound **5** [8] (Scheme 2). Alternatively compound **3** was treated with NaH and the alkyl halide 4-methyl-1-(2-chloroethyl)piperazine in DMF to give compound **5** [8]. Other substitutions on C-7 have reportedly been achieved by alkylation of the 7-hydroxychromone using K_2CO_3 in acetonitrile [6,8] or NaH in DMF [8].

In addition 8-phenyl-2-morpholino chromone (LY294002) **6** (Fig. 1) has been reported to inhibit phosphatidylinositol 3-kinase (PI 3-K) [13] and its activity against platelet phosphodiesterases has also been investigated [12]. The PI 3-K inhibitory activity of compound **6** has also led to its use as a lead structure in the development of DNA-dependent protein kinase (DNA-PK) inhibitors with the 2-morpholino substituent found to be important for DNA-PK inhibition [14–16].

2. Chemistry

In this paper we present a modification to the method for the preparation of 2-morpholino benzoxazines, and also report the synthesis and antiplatelet properties of 7-*O*-substituted-2-morpholino benzoxazines. The substituent group at C-7 was varied to produce 2-morpholino-7-substituted benzoxazines including structures **14i**, **14j**, **14k**, **16c** and **16d** (Fig. 2) which were found to give potent antiplatelet activity in the corresponding chromone analogues [8]. In particular **16d** was prepared in order to test its antiplatelet activity in direct comparison to the most potent chromone analogue **5** [8]. The 2-morpholino-8-phenyl benzoxazine **12** (Scheme 4) was also synthesised and tested as a direct comparison to **6** which shows activity against platelet phosphodiesterases [12].



Scheme 2.

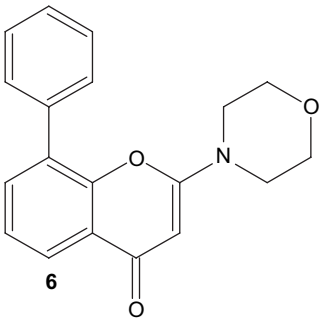


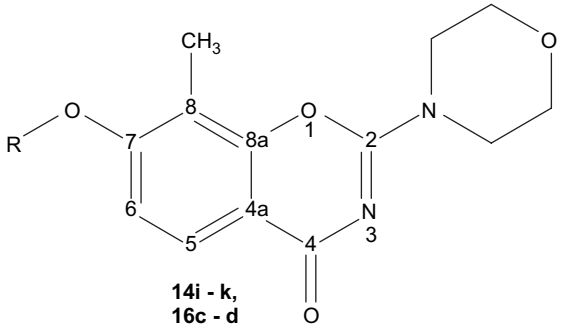
Fig. 1. 8-Phenyl-2-morpholinylchromone (LY294002).

Initially substituted 2-thio-1,3-benzoxazines prepared by earlier methods [4] were allowed to react with morpholine to give the 2-morpholino analogues **7a**, **7b**, **8** and **9**. The previously reported method [7], however, had only been used on unsubstituted 2-thio-1,3-benzoxazine **1** and often resulted in a complicated workup due to incomplete reaction progress. The method was therefore modified and the problem was overcome by the use of an excess of morpholine rather than a one to one ratio (Scheme 3). The presence of the excess amine acted as an additional solvent for the starting material, therefore having the twofold benefit of promoting a more complete reaction by increased interaction and also the total removal of any unreacted 2-thio-1,3-benzoxazine when filtering off the solid product. It was found that the reaction time could also be reduced to 2 h without any decrease in yield. Furthermore it was found that the need for a solvent could be eliminated if an excess of amine was added directly to the 2-thio-benzoxazine and the reaction was left for 2 days at room temperature. A general method for the preparation of 2-morpholino-substituted-1,3-benzoxazines from substituted 2-thio-1,3-benzoxazines is outlined in Section 5.2. Similarly the 8-phenyl-2-thio-benzoxazine **11** was prepared from 3-phenylsalicylic acid **10** as per earlier method [4] and was then allowed to react with morpholine to give **12** (Scheme 4).

Substitution at the C-7 hydroxyl group of compounds **7a** and **7b** is achieved via one of two methods. The first method, referred to as ‘‘Method A’’, involves the reaction of the 2-morpholino-7-hydroxybenzoxazines (**7a**, **b**) with sodium hydride followed by the introduction of an alkyl bromide 2-phenoxy ethyl bromide, 2-(4-bromophenoxy) ethyl bromide or 2-(4-methoxyphenoxy) ethyl bromide (**13a**, **b** and **c**, respectively) to the reaction mixture to give compounds **14a–e**. It was found, however, that the yield was improved if the reaction was continued for a further 5 h after increasing the temperature to 100 °C (Scheme 5, Method A). This can be illustrated in the case of the reaction between compounds **7a** and **13b** to produce compound **14b** where the yield was seen to increase from 33% to 67% when the reaction was heated to 100 °C for a further 5 h.

Method A, however, was only successful when using substituted 2-phenoxy ethyl bromide ($BrCH_2CH_2OPh$) as with **13a–c** and was ineffective in the absence of the 2-phenoxy ethyl bromide structure. ‘‘Method B’’ was devised and the use of K_2CO_3 was explored as a possible reagent for the *o*-alkylation of 7-hydroxy-2-morpholino-1,3-benzoxazines **7a–b** where Method A was unsuccessful. This did prove to be successful, however, the method was also improved slightly by replacing K_2CO_3 with Cs_2CO_3 to give **14f–l** in higher yields (Scheme 5, Method B). Method B was also used in the synthesis of the 7-(2-bromoethoxy) derivatives **15a** and **15b** as well as 7-(2-chloroethoxy) derivatives **15c** and **15d** which act as intermediates in the synthesis of 7-[2-(amino) ethoxy] analogues **16a–d** (Scheme 6). Method B was modified slightly in the preparation of **15a** and **15b** to prevent the formation of an ethane linkage between the two 7-hydroxy benzoxazine molecules and hence the synthesis of a dimer.

It has been reported previously that 2-morpholino-7-hydroxy-chromones were allowed to react with dibromoethane



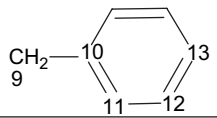
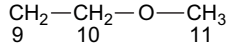
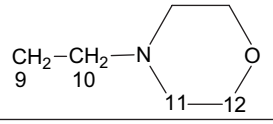
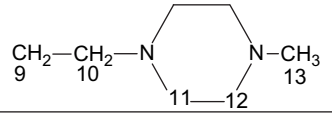
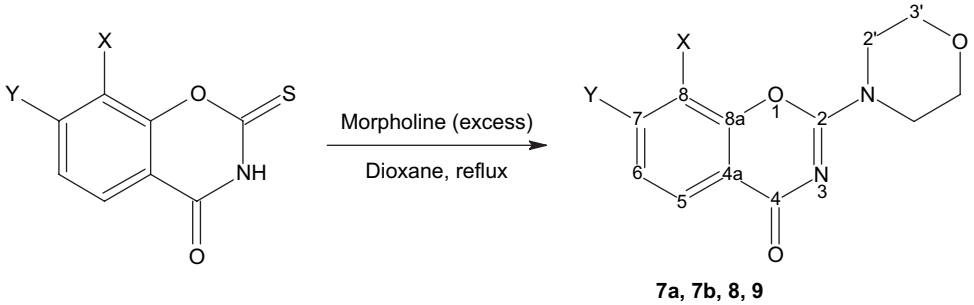
	R
14i	
14j	
14k	
16c	
16d	

Fig. 2. 2-Morpholino-substituted benzoxazines which are structurally similar to antiplatelet chromones.



Scheme 3. Compound **7** (**a** X = H, Y = OH; **b** X = CH_3, Y = OH); compound **8** X = H, Y = OCH_3; compound **9** X = OCH_3, Y = H.

and NaOH in the presence of a phase transfer catalyst to produce compound **4** [8], however, we found that no product could be isolated from the reaction of **7a** and **7b** with NaOH and dibromoethane. It was instead found that, if a low concentration of NaOH was used, no reaction occurred and the starting material (**7a** or **7b**) was recovered. When the concentration of NaOH was increased then the 2-morpholino benzoxazine (**7a**, **7b**) was decomposed to give the corresponding dihydroxy benzoic acid.

Compounds **15a–d** can all act as intermediates for the production of 7-[2-(amino)ethoxy] derivatives **16a–d**. However, higher yields were recorded for the synthesis of **15a** and **b** and their reaction with secondary amines (morpholine or *N*-methylpiperazine) in DMF [17] proceeded more cleanly to give **16a–d** (Scheme 6).

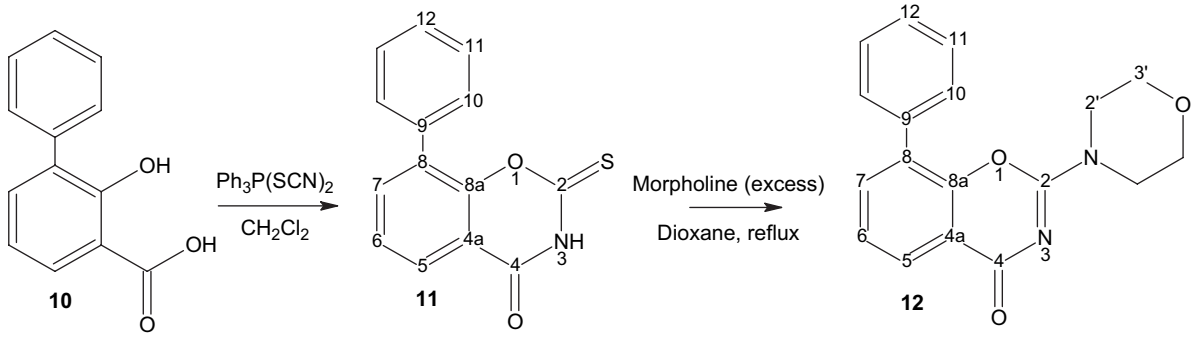
The preparation of the alkyl halide 4-(2-bromoethyl)morpholine from the reaction of morpholine and 1,2-dibromoethane was also achieved and 4-(2-bromoethyl)morpholine was allowed to react with **7b** to give **16c** using Method A. However, a very low yield was obtained (20%) suggesting that the preparation of compounds **16a–d** is more practical via the synthesis of the intermediate 2-bromoethoxy compounds **15a–b**.

The structure of the 2-thio-1,3-benzoxazine **11** was confirmed using IR, ^{1}H NMR, and ^{13}C NMR spectroscopy and microanalysis. The ^{1}H NMR and IR spectra supported the proposed structure and showed strong resemblance to the previously prepared oxazine **1** [18]. Assignment of the carbon-13 chemical shifts was made using calculated values starting from previously reported chemical shifts of **1** [18] and substitution increments of the aromatic ring of 8-C_6H_5 [19]. Previously reported ^{13}C NMR assignment of the parent acid **10** [20] was also used to aid with the assignment of **11**.

All 2-morpholino compound structures were confirmed using IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR and IR spectra supported the proposed structures and showed strong resemblance to the previously prepared oxazine **2** [7]. Assignment of the carbon-13 chemical shifts was made using calculated values starting from previously reported chemical shifts of **2** [18] and substitution increments of the aromatic ring [19]. A comparison with the known carbon-13 chemical shifts of 2-phenoxy ethyl bromide **13a**, 2-(4-bromophenoxy) ethyl bromide **13b**, 2-(4-methoxyphenoxy) ethyl bromide **13c**, benzyl bromide, (2-bromoethyl)benzene, 2-methoxy ethyl bromide, 1,2-dibromoethane,1-bromo-2-chloroethane, morpholine and *N*-methylpiperazine were also used where appropriate [20].

3. Results and discussion

Following the successful synthesis of the 2-morpholino benzoxazines, in vitro testing was carried out on a selection of compounds to determine their inhibitory effect on human platelet aggregation induced by ADP and collagen (Table 1). A total of 15 2-morpholino benzoxazines were tested in order to determine if 2-morpholino benzoxazines could exhibit similar antiplatelet activity to that seen with 2-morpholino chromones. Compounds **12**, **14i**, **14j**, **14k**, **16c** and **16d** were



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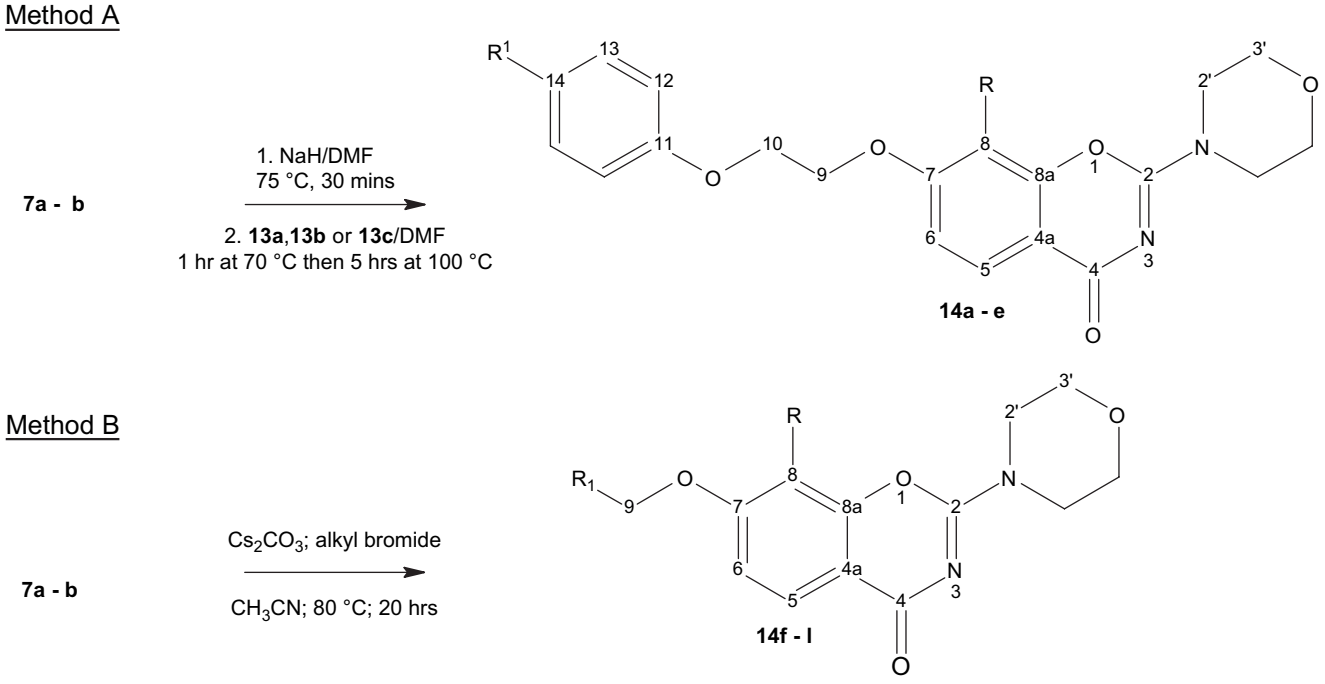
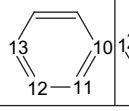
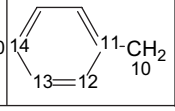
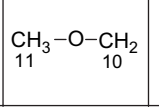
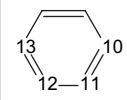
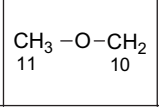
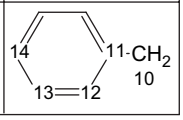
Scheme 4.



	14a	14b	14c	14d	14e	14f	14g	14h	14i	14j	14k	14l
R	H	H	H	CH_3	CH_3	H	H	H	CH_3	CH_3	CH_3	CH_3
R_1	H	Br	OCH_3	H	Br						H	

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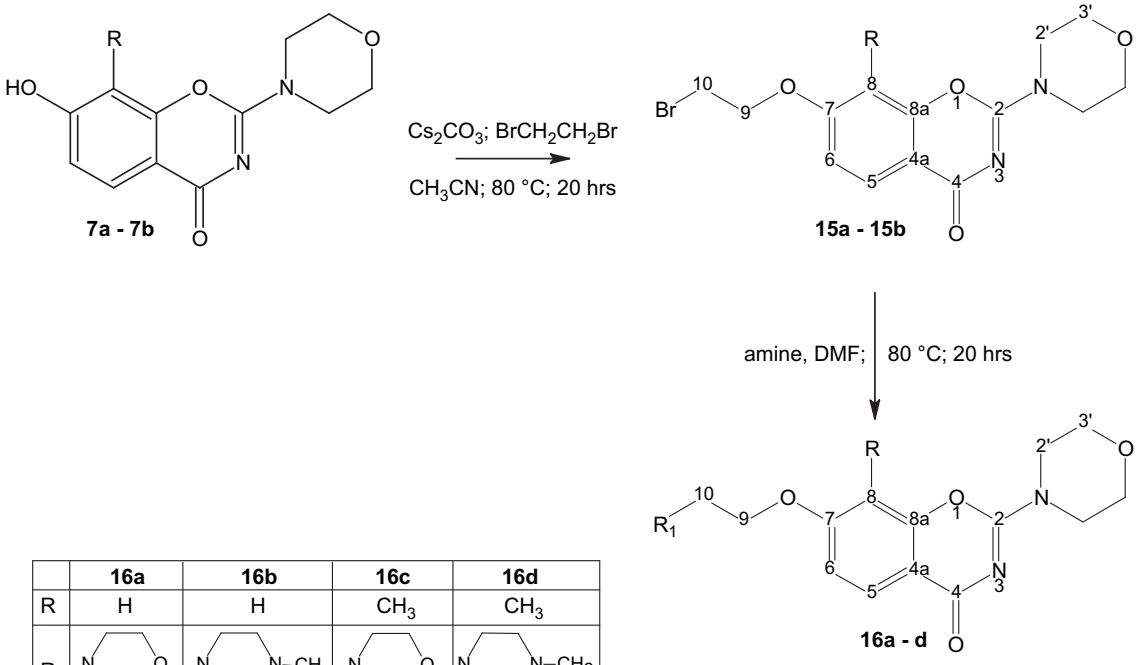
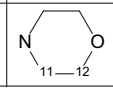
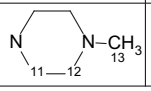
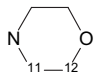
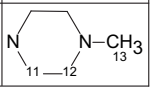
Scheme 5.

tested to give a direct comparison to similar previously reported chromone structures [8,12]. Compounds **14f**, **14h**, **16a** and **16b**, which lack the presence of a methyl group in the C-8 position, were tested to give comparison data on the role of 8-methyl group. Six other analogues **7b**, **14d**, **14l**, **15b** and **15d** were also tested in order to obtain further structure activity relationship data as no reported antiplatelet data could be found for structurally similar 2-morpholino chromones.

From our results it appears that the 2-morpholino 1,3-benzoxazine compound showing the strongest antiplatelet activity is the 7-[2-(4-methylpiperazin-1-yl)ethoxy]-8-methyl analogue **16d**. This agrees with the previously reported data on the structurally similar 7-[2-(4-methylpiperazin-1-yl)ethoxy]-8-methyl-2-morpholinyl chromone **5**, although the activity of **16d** against ADP inducted platelet aggregation ($IC_{50} = 6 \pm 1.4$ μM) is not as potent as that seen for **5** ($IC_{50} = 0.85 \pm 0.3$ μM) [8]. Compound **16d** was also seen to have a similar effect when platelet aggregation was induced by collagen ($IC_{50} = 8 \pm 1.3$ μM). In comparison the 8-methyl-7-(2-morpholin-4-yl-ethoxy) analogue **16c** showed reduced activity against ADP and collagen induced aggregation, $IC_{50} = 32 \pm 6.8$ and 20 ± 4 μM, respectively. The 7-[2-(amino)ethoxy] derivatives **16a** and **16b** which lack the presence of an 8-methyl group were found to require a much higher concentration, $IC_{50} = 78 \pm 12.2$ and >85 μM, respectively, to inhibit ADP induced aggregation and they both required over 85 μM to inhibit collagen induced aggregation. However, another analogue showing similar activity to **16d** in the presence of ADP is the intermediate 7-(2-chloroethoxy)-8-methyl analogue **15d** ($IC_{50} = 6 \pm 1.3$ μM).

In general our results suggest that the presence of a substituent group at C-8 (either methyl or phenyl) is very important for antiplatelet activity. All compounds tested which lacked a substituent at C-8 gave very poor inhibition and in most cases required a concentration in excess of 80 μM. Similarly it was found that the presence of a 2-substituted ethoxy group at C-7 was also important. This was demonstrated by the comparison between **14i** and **14l** which contain a benzyloxy and phenylethoxy group, respectively, at C-7 and shows that the inhibitory effect of the phenylethoxy substituent **14l** is far greater than the benzyloxy substituent **14i**. The importance of the 2-substituted ethoxy group at C-7 was further supported by the activity shown by analogues **14j**, **15b** and **15d**. However, the presence of a 2-phenoxyethoxy group at C-7, as seen with **14d**, resulted in a reduction in the antiplatelet activity against both ADP and collagen induced aggregation.



	16a	16b	16c	16d
R	H	H	CH_3	CH_3
R_1				

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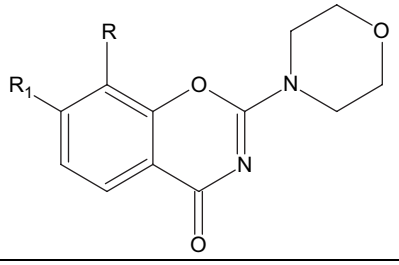
Scheme 6.

Table 1
Inhibitory data for 2-morpholino benzoxazines against ADP and collagen induced human platelet aggregation



Compound	R	R_1	Inhibition	
			IC_{50} (μM)[a]	
			ADP	Collagen
7b	CH_3	OH	58 ± 2.9	>100
12	Ph	H	30 ± 1.4	42 ± 4.6
14d	CH_3	$O(CH_2)_2OPh$	>85	64 ± 6.0
14f	H	OCH_2Ph	>83	>83
14h	H	$O(CH_2)_2OCH_3$	>80	>80
14i	CH_3	OCH_2Ph	>80	>80
14j	CH_3	$O(CH_2)_2OCH_3$	23 ± 3.3	40 ± 2.0
14k	CH_3	OCH_3	51 ± 0.8	>72
14l	CH_3	OCH_2CH_2Ph	44 ± 5.9	23 ± 1.3
15b	CH_3	$O(CH_2)_2Br$	39 ± 1.2	36 ± 4.3
15d	CH_3	$O(CH_2)_2Cl$	6 ± 1.3	36 ± 3.0
16a	H	$O(CH_2)_2$(4-morpholinyl)	78 ± 12.2	>85
16b	H	$O(CH_2)_2$(4-Me-1-piperazinyl)	>85	>85
16c	CH_3	$O(CH_2)_2$(4-morpholinyl)	32 ± 6.8	20 ± 4
16d[b]	CH_3	$O(CH_2)_2$(4-Me-1-piperazinyl)	6 ± 1.4	8 ± 1.3

[a] All values are mean ± SD.
[b] The structurally similar chromone **5** had an IC_{50} (μM) of 0.85 ± 0.3 μM against ADP induced platelet aggregation [8].

4. Conclusion

In conclusion, we have prepared a selection of novel 2-morpholino-substituted-1,3-benzoxazines and have evaluated their activity against ADP and collagen induced platelet aggregation. It appears from our work that, like the 2-morpholino chromones [8], 2-morpholino 1,3-benzoxazines have the potential to give promising antiplatelet activity. Furthermore, as with the 2-morpholino chromone analogues the presence of a methyl or phenyl group in the C-8 position of 2-morpholino benzoxazines was found to be essential to the antiplatelet activity. Similarly substitution at the C-7 position was found to require a 2-substituted ethoxy group with the 7-[2-(4-methylpiperazin-1-yl)ethoxy]-8-methyl analogue **16d** exhibiting the most potent activity against both ADP and collagen induced platelet aggregation. Previous antiplatelet studies carried out on structurally similar 2-morpholino chromones have focused mainly on ADP induced aggregation [8]. Our results, however, show that 2-morpholino benzoxazines can exhibit broad spectrum antiplatelet activity against both ADP and collagen induced aggregation.

5. Experimental protocols

Infrared spectra were obtained using a Perkin Elmer FT-IR spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained using a Bruker AC 200 NMR spectrometer at 200 and 50 MHz, respectively. All ^1H and ^{13}C NMR spectral results are recorded as chemical shifts (δ) and in the case of $CDCl_3$ are relative to the internal TMS and 77.0 ppm, respectively, while chemical shifts recorded in DMSO-d_6 are relative to

the solvent peak of 2.5 and 39.4 ppm, respectively. Microanalysis was performed by Chemical and Microanalytical Services (CMAS), Australia. Melting point determinations were carried out using a Stuart Scientific (SMP3) melting point apparatus and all melting points are uncorrected.

5.1. Starting materials

All 2-thio-benzoxazines (with the exception of **11**) were prepared from the corresponding 2-hydroxy aromatic acid as reported earlier [4]. (2-Bromoethoxy)benzene derivatives **13a**, **b** and **c** were prepared from the reaction of substituted phenols with 1,2-dibromoethane [21]. The IR, ^{1}H NMR and ^{13}C NMR data collected for compounds **13a–c** were found to agree with previously reported data [20–24]. Phenol, 4-bromophenol, 4-methoxyphenol, morpholine, *N*-methylpiperazine, sodium hydride, cesium carbonate, (2-bromoethyl)benzene, 2-bromoethyl methyl ether, benzyl bromide, methyl iodide and 1-bromo-2-chloroethane were purchased from Aldrich Chemical Company and were used as received. 1,2-Dibromoethane was purchased from Asia Pacific Specialty Chemicals Ltd and used as received.

5.1.1. 8-Phenyl-2-thioxo-2,3-dihydro-4H-1,3-benzoxazin-4-one *11*

3-Phenylsalicylic acid **10** was allowed to react with triphenylphosphine thiocyanogen as per previously reported method [4] to give **11** in 53% yield after recrystallisation from absolute ethanol, mp 223–225 °C; ν_{max} (KBr) 3188w and 3083w (N–H), 1703s (4-C=O), 1590w (C=C), 1213s (C=S) cm^{-1}; ^{1}H (DMSO-d_6) δ 13.6 (bs, 1H, NH), 8.0 (dd, 1H, $J_{H5,H7} = 1.73$ Hz, $J_{H5,H6} = 7.70$ Hz, H-5), 7.9 (dd, 1H, $J_{H7,H5} = 1.73$ Hz, $J_{H7,H6} = 7.70$ Hz, H-7), 7.6 (m, 6H, H-6 and Ph at C-8); ^{13}C (DMSO-d_6) δ 181.5 (C-2), 157.6 (C-4), 152.1 (C-8a), 136.8 (C-7), 134.5 (C-9), 129.3 (C-11), 128.8 (C-8), 128.4 (C-10), 128.2 (C-6), 126.0 (C-12), 125.9 (C-5), 116.1 (C-4a) (found C, 65.83; H, 3.63; N, 5.43; $C_{14}H_9NO_2S$ requires C, 65.87; H, 3.55; N, 5.49).

5.2. Synthesis of 2-morpholine benzoxazines

5.2.1. General method

Substituted 2-thioxo-1,3-benzoxazine-4-one (2.5 mmol) is suspended in 10 mL of 1,4-dioxane in a 25 mL round bottom flask. Morpholine (12.5 mmol) was added dropwise with stirring and the reaction mixture was heated to reflux for 2 h. At the completion of the reaction the mixture was evaporated to dryness in vacuo, washed with diethyl ether before being filtered and recrystallised from a suitable solvent.

5.2.1.1. 7-Hydroxy-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one *7a*

7-Hydroxy-2-thioxo-2,3-dihydro-4*H*-1,3-benzoxazin-4-one was allowed to react with morpholine as described in the general method to give **7a**. The solid collected was recrystallised from ethanol, 68% yield; mp 282 °C decomp.; ν_{max} (KBr) 3200–2500broad (O–H), 1635s (4-C=O), 1612s (C=C), 1548s (C=N) cm^{-1}; ^{1}H (DMSO-d_6) δ 10.7 (bs, 1H, 7-OH), 7.7 (d, 1H, $J_{H5,H6} = 8.6$ Hz, H-5), 6.8 (dd, 1H, $J_{H6,H8} = 2.2$ Hz, $J_{H6,H5} = 8.6$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.2$ Hz, H-8), 3.7 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C (DMSO-d_6) δ 165.2 (C-4), 162.5 (C-7), 156.3 (C-2), 154.8 (C-8a), 128.2 (C-5), 114.0 (C-6), 108.8 (C-4a), 101.1 (C-8), 65.4 (C-3′), 44.0 (C-2′) (found C, 57.98; H, 4.90; N, 11.21; $C_{12}H_{12}N_2O_4$ requires C, 58.06; H, 4.87; N, 11.29).

5.2.1.2. 7-Hydroxy-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one *7b*

Similarly, the general method was used for the reaction of 7-hydroxy-8-methyl-2-thioxo-2,3-dihydro-4*H*-1,3-benzoxazin-4-one with morpholine to give **7b**. The solid collected was recrystallised from ethanol, 80% yield; mp 240 °C decomp.; ν_{max} (KBr) 3300–2800broad (O–H), 1629s (4-C=O), 1603s (C=C), 1552s (C=N) cm^{-1}; ^{1}H (DMSO-d_6) δ 10.6 (bs, 1H, 7-OH), 7.6 (d, 1H, $J_{H5,H6} = 8.6$ Hz, H-5), 6.8 (d, 1H, $J_{H6,H5} = 8.6$ Hz, H-6), 3.7 (bm, 8H, $4 \times CH_2$ of morpholine), 2.1 (s, 3H, 8-CH_3); ^{13}C (DMSO-d_6) δ 165.8 (C-4), 160.2 (C-7), 156.5 (C-2), 152.8 (C-8a), 124.8 (C-5), 112.6 (C-6), 109.9 (C-8), 108.8 (C-4a), 65.4 (C-3′), 43.9 (C-2′), 7.8 (8-CH_3) (found C, 59.44; H, 5.31; N, 10.54; $C_{13}H_{14}N_2O_4$ requires C, 59.54; H, 5.38; N, 10.68).

5.2.1.3. 7-Methoxy-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one *8*

7-Methoxy-2-thioxo-2,3-dihydro-4*H*-1,3-benzoxazin-4-one was allowed to react with morpholine as described in the general method to give **8**. The crude solid was recrystallised from ethanol, 75% yield, mp 192–193 °C (lit. mp 197–200 °C [6]); ν_{max} (KBr) 1624s (4-C=O), 1599s (C=C), 1559s (C=N) cm^{-1}; ^{1}H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.8$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H8} = 2.4$ Hz, $J_{H6,H5} = 8.8$ Hz, H-6), 6.6 (d, 1H, $J_{H8,H6} = 2.4$ Hz, H-8); 3.9 (s, 3H, 7-OCH_3), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 164.1 (C-7), 156.6 (C-2), 154.9 (C-8a), 128.9 (C-5), 113.1 (C-6), 110.4 (C-4a), 99.3 (C-8), 66.1 (C-3′), 55.7 (7-OCH_3), 44.4 (C-2′) (found C, 59.51; H, 5.43; N, 10.56; $C_{13}H_{14}N_2O_4$ requires C, 59.54; H, 5.38; N, 10.68).

5.2.1.4. 8-Methoxy-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one *9*

8-Methoxy-2-thioxo-2,3-dihydro-4*H*-1,3-benzoxazin-4-one was allowed to react with morpholine as described in the general method to give **9**. The crude solid was recrystallised from ethanol, 71% yield, mp 198–200 °C; ν_{max} (KBr) 1682s (4-C=O), 1603s (C=C), 1567s (C=N) cm^{-1}; ^{1}H ($CDCl_3$) δ 7.7 (d, 1H, $J_{H5,H6} = 7.9$ Hz, H-5), 7.3 (t, 1H, $J_{H6,H5\&H6,H7} = 7.9$ Hz, H-6), 7.1 (d, 1H, $J_{H7,H6} = 7.9$ Hz, H-7), 3.9 (s, 3H, 8-OCH_3), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C ($CDCl_3$) δ 166.9 (C-4), 162.4 (C-8a), 156.6 (C-2), 146.9 (C-8), 125.0 (C-6), 118.5 (C-7), 118.2 (C-4a), 115.3 (C-5), 66.3 (C-3′), 56.2 (8-OCH_3), 44.5 (C-2′) (found C, 59.52; H, 5.23; N, 10.53; $C_{13}H_{14}N_2O_4$ requires C, 59.54; H, 5.38; N, 10.68).

5.2.1.5. 2-Morpholin-4-yl-8-phenyl-4H-1,3-benzoxazin-4-one *12*

8-Phenyl-2-thioxo-2,3-dihydro-4*H*-1,3-benzoxazin-4-one **11** was allowed to react with morpholine as described in the

general method to give **12**, 70% yield, recrystallised from ethyl acetate mp 209–211 °C; ν_{max} (KBr) 1672m (4-C=O), 1620s (C=C), 1562s (C=N) cm^{-1}; ^1H (DMSO-d_6) δ 7.9 (dd, 1H, $J_{H5,H7} = 1.8$ Hz, $J_{H5,H6} = 7.6$ Hz, H-5), 7.7 (dd, 1H, $J_{H7,H5} = 1.8$ Hz, $J_{H7,H6} = 7.6$ Hz, H-7), 7.6 (dd, 2H, $J_{H10,H12} = 1.6$ Hz, $J_{H10,H11} = 8.0$ Hz, H-10), 7.5 (m, 4H, H-6, H-11 & H-12) 3.7 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C (DMSO-d_6) δ 165.4 (C-4), 156.5 (C-2), 150.1 (C-8a), 134.8 (C-9), 134.5 (C-7), 129.1 (C-11), 128.9 (C-8), 128.5 (C-10), 128.1 (C-12), 125.9 (C-6), 125.3 (C-5), 117.4 (C-4a), 65.2 (C-3′), 44.1 (C-2′) (found C, 70.22; H, 5.25; N, 9.06; $C_{18}H_{16}N_2O_3$ requires C, 70.12; H, 5.23; N, 9.09).

5.3. Synthesis of compounds *14* and *15*

5.3.1. Method A

A suspension of **7a** or **7b** (1 mmol) in 4 mL of dry DMF was treated with sodium hydride (60% in mineral oil, 1.5 mmol) and then stirred at 75 °C for 30 min. The temperature was reduced to 70 °C before the addition of the appropriate alkyl bromide (**13a**, **13b** or **13c**) (3 mmol) and the reaction mixture was stirred at 70 °C for 1 h, then the temperature was increased to 100 °C for 5 h. At the completion of the reaction the mixture was cooled and poured into 8 mL 2 M NaOH and 8 mL of saturated NaCl. The mixture was extracted four times with 10 mL of chloroform and then dried over Mg_2SO_4 before being evaporated in vacuo. The mixture was distilled in vacuo to remove the remaining DMF and then the resulting slurry was diluted with either diethyl ether or ethyl acetate (depending on the solubility of the product) to give the crude solid.

5.3.2. Method B

A suspension of **7a** or **7b** (1 mmol) in 10 mL of dry acetonitrile was treated successively with cesium carbonate (6.2 mmol) and the appropriate alkyl bromide [(2-bromoethyl)benzene, benzyl bromide, 1,2-dibromoethane or 1-bromo-2-chloroethane] (2 mmol) then was stirred for 20 h at 80 °C. At the completion of the reaction the mixture was cooled and the acetonitrile evaporated off in vacuo. The remaining residue was washed with 4×15 mL of $CHCl_3$ and filtered, and then the filtrate was concentrated in vacuo. The resulting residue was triturated with diethyl ether to give the crude solid.

*5.3.2.1. 2-Morpholin-4-yl-7-(2-phenoxyethoxy)-4H-1,3-benzoxazin-4-one **14a***. Compound **7a** was allowed to react with **13a** according to Method A to give **14a** in 38% yield, recrystallised from toluene, mp 181 °C; ν_{max} (KBr) 1670m (4-C=O), 1603s (C=C), 1558s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.8$ Hz, H-5), 7.3 (dd, 2H, $J_{H13,H12} = 7.4$ Hz, $J_{H13,H14} = 7.4$ Hz, H-13), 7.0 (m, 4H, H-6, H-12 & H-14), 6.7 (d, 1H, $J_{H8,H6} = 2.2$ Hz, H-8), 4.4 (bm, 4H, H-9 & H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C ($CDCl_3$) δ 166.7 (C-4), 163.2 (C-7), 158.3 (C-2), 156.7 (C-11), 155.0 (C-8a), 129.6 (C-5), 129.2 (C-13), 121.3 (C-14), 114.6 (C-12), 113.5 (C-6), 110.9 (C-4a), 100.4 (C-8), 67.2/66.0 (C-9/C-10), 66.3 (C-3′), 44.5 (C-2′) (found C, 65.27; H, 5.56; N, 7.63; $C_{20}H_{20}N_2O_5$ requires C, 65.21; H, 5.47; N, 7.60).

*5.3.2.2. 7-[2-(4-Bromophenoxy)ethoxy]-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14b***. Similarly **7a** was allowed to react with **13b** to produce **14b**, 67% yield, recrystallised from acetonitrile, mp 221–223 °C; ν_{max} (KBr) 1670m (C=O), 1600s (C=C), 1554s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.1 (d, 1H, $J_{H5,H6} = 8.9$ Hz, H-5), 7.4 (d, 2H, $J_{H13,H12} = 8.9$ Hz, H-13), 6.9 (dd, 1H, $J_{H6,H8} = 2.2$ Hz, $J_{H6,H5} = 8.9$ Hz, H-6), 6.8 (d, 2H, $J_{H12,H13} = 8.9$ Hz, H-12), 6.7 (d, 1H, $J_{H8,H6} = 2.2$ Hz, H-8), 4.4 (bm, 4H, H-9 & H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 163.0 (C-7), 157.4 (C-11), 156.7 (C-2), 154.9 (C-8a), 132.4 (C-13), 129.3 (C-5), 116.4 (C-12), 113.5 (C-14), 113.3 (C-6), 111.0 (C-4a), 100.3 (C-8), 66.9 (C-3′), 66.3/66.2 (C-9/C-10), 44.6 (C-2′) (found C, 53.85; H, 4.22; N, 6.27; $C_{20}H_{19}BrN_2O_5$ requires C, 53.71; H, 4.28; N, 6.26).

*5.3.2.3. 7-[2-(4-Methoxyphenoxy)ethoxy]-2-morpholin-4-yl-4H-1,3-benzoxazine-4-one **14c***. Compound **7a** was allowed to react with **13c** according to Method A to give **14c** in 95% yield, recrystallised from toluene, mp 158–160 °C; ν_{max} (KBr) 1677m (C=O), 1601s (C=C), 1561s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.8$ Hz, H-5), 7.0 (dd, 1H, $J_{H6,H5} = 8.8$ Hz, $J_{H6,H8} = 2.3$ Hz, H-6), 6.9 (m, 4H, H-12 & H-13), 6.7 (d, 1H, $J_{H8,H6} = 2.3$ Hz, H-8), 4.3 (bm, 4H, H-9 & H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 3.7 (s, 3H, OCH_3); ^{13}C (DMSO-d_6) δ 165.1 (C-4), 162.7 (C-7), 156.4 (C-2), 154.8 (C-8a), 153.5 (C-14), 152.1 (C11), 128.0 (C-5), 115.4/114.6 (C-12/C-13), 113.8 (C-6), 110.2 (C-4a), 100.3 (C-8), 67.2/66.4 (C-9/C-10), 65.4 (C-3′), 55.3 (OCH_3), 44.0 (C-2′) (found C, 63.50; H, 5.86; N, 6.75; $C_{21}H_{22}N_2O_6$ requires C, 63.31; H, 5.57; N, 7.03).

*5.3.2.4. 8-Methyl-2-morpholin-4-yl-7-(2-phenoxyethoxy)-4H-1,3-benzoxazin-4-one **14d***. Compound **7b** was allowed to react with **13a** according to Method A to give **14d**, 48% yield, recrystallised from toluene, mp 177–178 °C; ν_{max} (KBr) 1685m (4-C=O), 1600s (C=C), 1580s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.0$ Hz, H-5), 7.3 (t, 2H, $J_{H13,H12\&H13,H14} = 9.0$ Hz H-13), 7.0 (m, 4H, H-6, H-12 & H-14), 4.4 (bm, 4H, H-9 & H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.2 (C-4), 160.7 (C-7), 158.4 (C-11), 156.9 (C-2), 152.6 (C-8a), 129.5 (C-5), 126.1 (C-13), 121.2 (C-14), 114.6 (C-6), 112.8 (C-8), 110.8 (C-4a), 109.0 (C-12), 67.4 (C-3′), 66.2 (C-9/C-10), 44.4 (C-2′), 8.1 (8-CH_3) (found C, 65.91; H, 5.75; N, 7.37; $C_{21}H_{22}N_2O_5$ requires C, 65.96; H, 5.80; N, 7.33).

*5.3.2.5. 7-[2-(4-Bromophenoxy)ethoxy]-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14e***. Similarly **7b** was allowed to react with **13b** to produce **14e**, 70% yield, recrystallised from ethyl acetate, mp 220–222 °C; ν_{max} (KBr) 1684m (C=O), 1602s (C=C), 1579s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.6$ Hz, H-5), 7.4 (d, 2H, $J_{H13,H12} = 8.9$ Hz, H-13), 6.9 (d, 1H, $J_{H6,H5} = 8.6$ Hz, H-6), 6.8 (d, 2H, $J_{H12,H13} = 8.9$ Hz, H-12), 4.4 (bm, 4H, H-9 & H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 2.2 (s, 3H, 8-CH_3); ^{13}C (DMSO-d_6) δ 167.3 (C-4), 160.6 (C-11), 157.6 (C-7), 156.9 (C-2), 152.6 (C-8a), 132.3 (C-13), 126.2

(C-5), 116.4 (C-12), 113.4/112.8 (C-4a/C-14), 110.9 (C-8), 109.0 (C-6), 67.3 (C-3′), 66.6/66.2 (C-9/C-10), 44.5 (C-2′), 8.2 (8-CH_3) (found C, 54.67; H, 4.55; N, 5.98; $C_{21}H_{21}BrN_2O_6$ requires C, 54.68; H, 4.59; N, 6.07).

*5.3.2.6. 7-(Benzyloxy)-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14f***. Compound **7a** was allowed to react with benzyl bromide according to Method B to give **14f** in 76% yield, recrystallised from toluene, mp 185–186 °C; ν_{max} (KBr) 1670m (C=O), 1599s (C=C), 1560s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.7$ Hz, H-5), 7.4 (m, 5H, H-11, H-12 & H-13), 7.0 (dd, 1H, $J_{H6,H8} = 2.3$ Hz, $J_{H6,H5} = 8.7$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.3$ Hz, H-8), 5.1 (s, 2H, H-9), 3.8 (bm, 8H, 4 × CH_2 of morpholine); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 163.2 (C-7), 156.6 (C-2), 154.9 (C-8a), 135.5 (C-10), 129.1/128.7/128.3 (C-11/C-12/C-13), 127.3 (C-5), 113.8 (C-6), 110.7 (C-4a), 100.3 (C-8), 70.4 (C-9), 66.2 (C-3′), 44.4 (C-2′) (found C, 67.48; H, 5.39; N, 8.21; $C_{19}H_{18}N_2O_4$ requires C, 67.44; H, 5.36; N, 8.28).

*5.3.2.7. 2-Morpholin-4-yl-7-(2-phenylethoxy)-4H-1,3-benzoxazin-4-one **14g***. Similarly Method B was used in the reaction of **7a** and (2-bromoethyl)benzene to give **14g** in 36% yield, recrystallised from ethanol, mp 190–191 °C; ν_{max} (KBr) 1673m (C=O), 1602s (C=C), 1561s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.8$ Hz, H-5), 7.3 (m, 5H, H-12, H-13, & H-14), 6.9 (dd, 1H, $J_{H6,H8} = 2.3$ Hz, $J_{H6,H5} = 8.8$ Hz, H-6), 6.6 (d, 1H, $J_{H8,H6} = 2.3$ Hz, H-8), 4.2 (t, 2H, H-9), 3.8 (bm, 8H, 4 × CH_2 of morpholine), 3.1 (t, 2H, H-10); ^{13}C ($CDCl_3$) δ 166.7 (C-4), 163.4 (C-7), 156.7 (C-2), 155.0 (C-8a), 137.5 (C-11), 129.1/128.9/128.6 (C-12/C-13/C-14), 126.7 (C-5), 113.5 (C-6), 110.6 (C-4a), 100.0 (C-8), 69.3 (C-9), 66.3 (C-3′), 44.5 (C-2′), 35.5 (C-10) (found C, 68.09; H, 5.80; N, 7.84; $C_{20}H_{20}N_2O_4$ requires C, 68.17; H, 5.72; N, 7.95).

*5.3.2.8. 7-(2-Methoxyethoxy)-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14h***. Compound **7a** was allowed to react with 2-methoxy ethyl bromide (10 mmol) and heated to 60 °C for 20 h using Method B to give **14h** in 88% yield, recrystallised from ethyl acetate, mp 163 °C; ν_{max} (KBr) 1666m (C=O), 1601s (C=C), 1558s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H8} = 2.0$ Hz, $J_{H6,H5} = 9.0$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.0$ Hz, H-8), 4.2 (t, 2H, H-9), 3.8 (bm, 10H, 4 × CH_2 of morpholine & H-10), 3.5 (s, 3H, H-11); ^{13}C ($CDCl_3$) δ 166.7 (C-4), 163.4 (C-7), 156.7 (C-2), 154.9 (C-8a), 129.2 (C-5), 113.5 (C-6), 110.8 (C-4a), 100.2 (C-8), 70.6 (C-9), 68.0 (C-10), 66.2 (C-3′), 59.2 (C-11), 44.5 (C-2′) (found C, 58.89; H, 5.95; N, 9.08; $C_{15}H_{18}N_2O_5$ requires C, 58.82; H, 5.92; N, 9.15).

*5.3.2.9. 7-(Benzyloxy)-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14i***. Similarly **7b** was allowed to react with benzyl bromide according to Method B to give **14i** in 85% yield, recrystallised from toluene, mp 174–176 °C; ν_{max} (KBr) 1672m (C=O), 1603s (C=C), 1572s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 7.9 (d, 1H, $J_{H5,H6} = 8.7$ Hz, H-5), 7.4 (m, 5H, H-11, H-12 & H-13), 6.9 (d, 1H, $J_{H6,H5} = 8.7$ Hz, H-6), 5.2 (s, 2H, H-9), 3.8 (bm, 8H, 4 × CH_2 of morpholine), 2.3 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.3 (C-4), 160.8 (C-7), 157.0 (C-2), 152.7 (C-8a), 136.2 (C-10), 128.6/128.2/127.2 (C-11/C-12/C-13), 126.2 (C-5), 112.7/110.8 (C-4a/C-8), 109.3 (C-6), 70.5 (C-9), 66.3 (C-3′), 44.4 (C-2′), 8.3 (8-CH_3) (found C, 68.04; H, 5.80; N, 7.89; $C_{20}H_{20}N_2O_4$ requires C, 68.17; H, 5.72; N, 7.95).

*5.3.2.10. 7-(2-Methoxyethoxy)-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14j***. Compound **7b** was allowed to react with 2-methoxy ethyl bromide (10 mmol) and heated to 60 °C for 20 h using Method B to give **14j** in 96% yield, recrystallised from toluene, mp 164–165 °C; ν_{max} (KBr) 1683m (C=O), 1605s (C=C), 1563s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 6.9 (d, 1H, $J_{H6,H5} = 9.0$ Hz, H-6), 4.2 (t, 2H, H-9), 3.8 (bm, 10H, 4 × CH_2 of morpholine & H-10), 3.5 (s, 3H, H-11), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.2 (C-4), 160.9 (C-7), 156.9 (C-2), 152.5 (C-8a), 126.1 (C-5), 112.6 (C-8), 110.6 (C-4a), 108.9 (C-6), 70.8 (C-9), 68.2 (C-10), 66.2 (C-3′), 59.2 (C-11), 44.3 (C-2′), 8.1 (8-CH_3) (found C, 59.95; H, 6.23; N, 8.81; $C_{16}H_{20}N_2O_5$ requires C, 59.99; H, 6.29; N, 8.74).

*5.3.2.11. 7-Methoxy-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **14k***. Compound **7b** was allowed to react with methyl iodide (10 mmol) and heated to 60 °C for 20 h according to Method B to give **14k** in 78% yield, recrystallised from ethyl acetate, mp 257–258 °C; ν_{max} (KBr) 1664m (C=O), 1602s (C=C), 1567s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.8$ Hz, H-5), 6.9 (d, 1H, $J_{H6,H5} = 8.8$ Hz, H-6), 3.9 (s, 3H, H-9), 3.8 (bm, 8H, 4 × CH_2 of morpholine), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.4 (C-4), 161.7 (C-7), 157.0 (C-2), 152.5 (C-8a), 126.3 (C-5), 112.2 (C-8), 110.5 (C-4a), 107.9 (C-6), 66.3 (C-3′), 56.0 (C-9), 44.5 (C-2′), 8.0 (8-CH_3) (found C, 60.94; H, 5.87; N, 10.18; $C_{14}H_{16}N_2O_4$ requires C, 60.86; H, 5.84; N, 10.14).

*5.3.2.12. 8-Methyl-2-morpholin-4-yl-7-(2-phenylethoxy)-4H-1,3-benzoxazin-4-one **14l***. Compound **7b** was reacted with (2-bromoethyl)benzene according to Method B to give **14l** in 61% yield, recrystallised from petroleum spirit (80–100)/toluene, mp 170–171 °C; ν_{max} (KBr) 1675m (C=O), 1601s (C=C), 1560s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 7.9 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 7.3 (bm, 5H, H-12, H-13 & H-14), 6.8 (d, 1H, $J_{H6,H5} = 9.0$ Hz, H-6), 4.3 (t, 2H, H-9), 3.8 (bm, 8H, 4 × CH_2 of morpholine), 3.2 (t, 2H, H-10), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.3 (C-4), 160.9 (C-7), 156.9 (C-2), 152.5 (C-8a), 137.8 (C-11), 128.9/128.4/126.6 (C-12/C-13/C-14), 126.1 (C-5), 112.3 (C-8), 110.4 (C-4a), 108.7 (C-6), 69.3 (C-9), 66.2 (C-3′), 44.4 (C-2′), 35.6 (C-10), 8.1 (8-CH_3) (found C, 68.91; H, 6.10; N, 7.64; $C_{21}H_{22}N_2O_4$ requires C, 68.84; H, 6.05; N, 7.65).

*5.3.2.13. 7-(2-Bromoethoxy)-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **15a***. Compound **7a** was added slowly in suspension to 1,2-dibromoethane (10 mmol) and cesium carbonate (6.2 mmol) in a modification to Method B to give **15a** in 86% yield, recrystallised from ethyl acetate, mp 201 °C decomp.; ν_{max} (KBr) 1666m (C=O), 1601s (C=C), 1557s

(C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H8} = 2.0$ Hz, $J_{H6,H5} = 9.0$ Hz, H-6), 6.7 (s, 1H, $J_{H8,H6} = 2.0$ Hz, H-8), 4.4 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 3.7 (t, 2H, H-10); ^{13}C ($CDCl_3$) δ 166.5 (C-4), 162.6 (C-7), 156.7 (C-2), 155.0 (C-8a), 129.4 (C-5), 113.3 (C-6), 111.2 (C-4a), 100.5 (C-8), 68.2 (C-9), 66.3 (C-3′), 44.6 (C-2′), 28.2 (C-10) (found C, 47.39; H, 4.16; N, 7.83; $C_{14}H_{15}BrN_2O_4$ requires C, 47.34; H, 4.26; N, 7.89).

*5.3.2.14. 7-(2-Bromoethoxy)-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **15b***. Similarly **7b** was added slowly in suspension to 1,2-dibromoethane (10 mmol) and cesium carbonate (6.2 mmol) in a modification to Method B to give **15b** in 80% yield, recrystallised from 95% ethanol, mp 223 °C decomp.; ν_{max} (KBr) 1678m (C=O), 1600s (C=C), 1576s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.0$ Hz, H-5), 6.8 (d, 1H, $J_{H6,H5} = 8.0$ Hz, H-6), 4.4 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 3.7 (t, 2H, H-10), 2.3 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.2 (C-4), 160.1 (C-7), 156.9 (C-2), 152.7 (C-8a), 126.3 (C-5), 113.0 (C-8), 111.1 (C-4a), 108.9 (C-6), 68.4 (C-9), 66.2 (C-3′), 44.4 (C-2′), 28.9 (C-10), 8.1 (8-CH_3) (found C, 48.91; H, 4.67; N, 7.54; $C_{15}H_{17}BrN_2O_4$ requires C, 48.80; H, 4.64; N, 7.59).

*5.3.2.15. 7-(2-Chloroethoxy)-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **15c***. Prepared when **7a** was allowed to react with 1-bromo-2-chloroethane according to Method B to give **15c** in 60% yield, recrystallised from ethyl acetate/toluene, mp 191–193 °C; ν_{max} (KBr) 1666m (C=O), 1602s (C=C), 1555s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H5} = 9.0$ Hz, $J_{H6,H8} = 2.0$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.0$ Hz, H-8), 4.3 (t, 2H, H-9), 3.9 (t, 2H, H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 162.7 (C-7), 156.7 (C-2), 154.9 (C-8a), 129.4 (C-5), 113.3 (C-6), 111.2 (C-4a), 100.4 (C-8), 68.4 (C-9), 66.2 (C-3′), 44.5 (C-2′), 41.3 (C-10) (found C, 54.07; H, 4.72; N, 8.98; $C_{14}H_{15}ClN_2O_4$ requires C, 54.11; H, 4.87; N, 9.02).

*5.3.2.16. 7-(2-Chloroethoxy)-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **15d***. Similarly **7b** was allowed to react with 1-bromo-2-chloroethane according to Method B to give **15d** in 55% yield, recrystallised from dioxane/acetic acid, mp 153 °C decomp.; ν_{max} (KBr) 1679m (C=O), 1602s (C=C), 1577s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9$ Hz, H-5), 6.8 (d, 1H, $J_{H6,H5} = 9$ Hz, H-6), 4.4 (t, 2H, H-9), 3.9 (t, 2H, H-10), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 2.3 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.2 (C-4), 160.2 (C-7), 156.9 (C-2), 152.7 (C-8a), 126.3 (C-5), 113.0 (C-8), 111.1 (C-4a), 108.9 (C-6), 68.6 (C-9), 66.2 (C-3′), 44.4 (C-2′), 41.8 (C-10), 8.1 (8-CH_3) (found C, 55.50; H, 5.31; N, 8.56; $C_{15}H_{17}N_2O_4$ requires C, 55.48; H, 5.28; N, 8.63).

*5.4. Preparation of 7-[2-(amino)ethoxy] derivatives **16a–d***

5.4.1. General method

A suspension of 7-(2-bromoethoxy) benzoxazine **15a** or **15b** (1 mmol) in 15 mL of DMF was treated with the appropriate amine (4 mmol) and heated at 80 °C for 20 h. At the completion of the reaction the mixture was cooled and the DMF was distilled off in vacuo. The resulting residue was taken up in 10 mL of $CHCl_3$ and then poured into 10 mL of 2 M NaOH and 10 mL of saturated NaCl and extracted. The mixture was extracted with a further 3×10 mL of $CHCl_3$ and the combined organics were dried over Mg_2SO_4 and evaporated in vacuo. The resulting residue was triturated with diethyl ether to give the crude solid.

*5.4.1.1. 2-Morpholin-4-yl-7-(2-morpholin-4-yl-ethoxy)-4H-1,3-benzoxazin-4-one **16a***. Compound **15a** was allowed to react with morpholine as described in the general method above to give **16a** in 70% yield, recrystallised from toluene, mp 190–192 °C; ν_{max} (KBr) 1671m (C=O), 1604s (C=C), 1559s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.7$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H8} = 2.3$ Hz, $J_{H6,H5} = 8.7$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.3$ Hz, H-8), 4.2 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 3.7 (t, 4H, C-12), 2.8 (t, 2H, H-10), 2.6 (t, 4H, C-11); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 163.3 (C-7), 156.7 (C-8a), 155.0 (C-2), 129.2 (C-5), 113.5 (C-6), 110.8 (C-4a), 100.1 (C-8), 66.8 (C-9), 66.5 (C-3′), 66.2 (C-12), 57.2 (C-11), 54.0 (C-10), 44.5 (C-2′) (found C, 59.80; H, 6.38; N, 11.51; $C_{18}H_{23}N_3O_5$ requires C, 59.82; H, 6.41; N, 11.63).

*5.4.1.2. 7-[2-(4-Methylpiperazin-1-yl)ethoxy]-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one **16b***. Compound **15a** was allowed to react with *N*-methylpiperazine as described in the general method to give **16b** in 81% yield, recrystallised from toluene, mp 151–154 °C decomp.; ν_{max} (KBr) 1673m (C=O), 1604s (C=C), 1560s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8.0$ Hz, H-5), 6.9 (dd, 1H, $J_{H6,H8} = 2.0$ Hz, $J_{H6,H5} = 8.0$ Hz, H-6), 6.7 (d, 1H, $J_{H8,H6} = 2.0$ Hz, H-8), 4.2 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 2.9 (t, 2H, H-10), 2.6 (bm, 4H, H-11), 2.5 (bm, 4H, H-12), 2.3 (s, 3H, H-13); ^{13}C ($CDCl_3$) δ 166.6 (C-4), 163.3 (C-7), 156.7 (C-2), 155.0 (C-8a), 129.1 (C-5), 113.5 (C-6), 110.6 (C-4a), 100.1 (C-8), 66.7 (C-9), 66.6 (C-3′), 56.7 (C-10), 55.0 (C-12), 53.5 (C-11), 45.9 (C-13), 44.4 (C-2′) (found C, 60.80; H, 6.95; N, 15.04; $C_{19}H_{26}N_4O_4$ requires C, 60.95; H, 7.00; N, 14.96).

*5.4.1.3. 2-Morpholin-4-yl-8-methyl-7-(2-morpholin-4-yl-ethoxy)-4H-1,3-benzoxazin-4-one **16c***. Compound **15b** was allowed to react with morpholine as described in the general method to give **16c** in 80% yield, recrystallised from cyclohexane/toluene, mp 127–129 °C; ν_{max} (KBr) 1672m (C=O), 1599s (C=C), 1561s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 9.0$ Hz, H-5), 6.9 (d, 1H, $J_{H6,H5} = 9.0$ Hz, H-6), 4.2 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 3.7 (t, 4H, H-12), 2.9 (t, 2H, H-10), 2.6 (t, 4H, H-11), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.3 (C-4), 160.8 (C-7), 156.9 (C-2), 152.6 (C-8a), 126.2 (C-5), 112.4 (C-8), 110.6 (C-4a), 108.8 (C-6), 66.9/66.9 (C-9/C-12), 66.2 (C-3′), 57.4 (C-11), 54.0 (C-10), 44.3 (C-2′), 8.2 (8-CH_3) (found C, 60.69; H, 6.65; N, 11.07; $C_{19}H_{25}N_3O_5$ requires C, 60.79; H, 6.71; N, 11.19).

5.4.1.4. 7-[2-(4-Methylpiperazin-1-yl)ethoxy]-8-methyl-2-morpholin-4-yl-4H-1,3-benzoxazin-4-one ***16d***. Compound **15b** was allowed to react with *N*-methylpiperazine as described in the general method to give **16d** in 71% yield, recrystallised from cyclohexane/toluene, mp 157–159 °C; ν_{max} (KBr) 1664m (C=O), 1598s (C=C), 1562s (C=N) cm^{-1}; ^1H ($CDCl_3$) δ 8.0 (d, 1H, $J_{H5,H6} = 8$ Hz, H-5), 6.9 (d, 1H, $J_{H6,H5} = 8$ Hz, H-6), 4.2 (t, 2H, H-9), 3.8 (bm, 8H, $4 \times CH_2$ of morpholine), 2.9 (t, 2H, H-10), 2.7 (bm, 4H, H-11), 2.5 (bm, 4H, H-12), 2.3 (s, 3H, H-13), 2.2 (s, 3H, 8-CH_3); ^{13}C ($CDCl_3$) δ 167.3 (C-4), 160.9 (C-7), 156.9 (C-2), 152.6 (C-8a), 126.2 (C-5), 112.4 (C-8), 110.6 (C-4a), 108.9 (C-6), 67.1 (C-9), 66.3 (C-3′), 57.0 (C-10), 55.1 (C-12), 53.6 (C-11), 46.0 (C-13), 44.5 (C-2′), 8.2 (8-CH_3) (found C, 61.71; H, 7.28; N, 14.34; $C_{20}H_{28}N_4O_4$ requires C, 61.84; H, 7.27; N, 14.42).

5.5. Platelet aggregometry

Venous blood was collected from drug free volunteers into trisodium citrate 22.0 g/L. Ethics approval was obtained from La Trobe University Human Ethics Committee (HREC Number 06-16). The whole blood was centrifuged at 130*g* for 15 min at room temperature to obtain platelet rich plasma (PRP). The remaining blood was centrifuged for a further 10 min at 820*g* in order to obtain platelet poor plasma (PPP). Platelet aggregation was determined by the optical method in a two-channel platelet aggregometer (Chrono-Log). Assays were carried out at 37 °C and had a total volume of 500 μL after the addition of the test compound and agonist. Stirring rate was 1000 rpm with the PRP and test compound being pre-incubated for 2 min before the addition of the appropriate agonist. The agonists used were ADP (final concentration 10 μM) and collagen (final concentration 4 μg/mL).

Test compounds were dissolved in either ethanol or DMSO depending on solubility and added in 3 μL volumes for ethanol and 2 μL volumes for DMSO. The samples dissolved in DMSO were added in 2 μL volumes to ensure that the final concentration of DMSO was kept below 0.5% (v/v) which made certain that the DMSO would not influence platelet aggregation [25]. Aggregation was recorded after the addition of the agonist and results were compared to platelet aggregation in the presence of an equivalent amount of test vehicle (ethanol or DMSO). The concentration of a compound at which the aggregation was inhibited by 50% (IC_{50}) was determined as the average of multiple determinations (three or more) where platelet aggregation was reduced by 50%.

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