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2-Amino-Benzo[d]isothiazol-3-one Derivatives: Synthesis and Assessment of their Antiplatelet/Spasmolytic Effects

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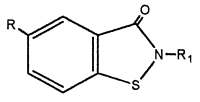
Abstract—We describe a series of 2-amino-benzo[d]isothiazol-3-one derivatives (**2**–**8**), which were synthesized and screened in vitro for inhibition of platelet aggregation and for their spasmolytic activity, with the awareness that the development of antiplatelet agents with additional vasodilation activity could be beneficial in the treatment of various vaso-occlusive disorders. The tested compounds show a powerful antiplatelet activity and various modifications resulted in molecules possessing antiaggregating effects as well as spasmolytic actions. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

It is well known that platelet aggregation plays an important role in the pathogenesis of thromboembolic diseases[1] and various platelet inhibitors are commonly used in treating ischemic illness.[2]

We have been engaged, for a long time, in a program aimed to synthesize diverse benzo[d]isothiazole derivatives and to describe their pharmacological profile.[3] Recently we identified 2-amino-benzo[d]isothiazol-3-one (**1**) as a highly potent platelet aggregation inhibitor against different agonists, in vitro and ex vivo.[4] This result encouraged us to synthesize new 2-amino-benzo[d]isothiazol-3-one derivatives **2**–**8**, in order to extend the study of the antiplatelet activity and to establish the structure–activity relationship. The choice of the 2-amino substitution (**3**–**8**) was made on the basis of the description in the literature of some 2-substituted benzo[d]isothiazol-3-ones, as promising inhibitors of platelet aggregation[5] and for the importance, recently demonstrated by us,[6] of the 2-amino group, missing in the compounds described in the literature. Indeed the introduction of the 2-amino substituents as well as the 5-methyl substitution on the benzo[d]isothiazole system were directed to modulate molecular features of the compounds, in particular lipophilicity, which seems to be directly related to the antiaggregating activity.[7]

In this paper we report the synthesis and the in vitro antiplatelet aggregation action of compounds **1–8.** Pharmacological in vitro experiments were also performed to investigate the spasmolytic activity of the compounds with the awareness that the development of antiplatelet agents with additional vasodilation activity could, indeed, be beneficial in the treatment of various vaso-occlusive disorders (Fig. 1).[8]



Compound	R	R_1
1	H	NH_2
2	CH_3	NH_2
3	H	NH-C_6H_5
4	CH_3	NH-C_6H_5
5	H	NH-C_6H_4-Cl(4)
6	CH_3	NH-C_6H_4-Cl(4)
7	H	N=CH-C_6H_5
8	CH_3	N=CH-C_6H_5

Figure 1.

We demonstrate here that 2-amino-benzo[d]isothiazol-3-one moiety is responsible for a powerful antiplatelet activity and that various modifications resulted in molecules possessing antiaggregating effects as well as spasmolytic actions.

Chemistry

The preparative routes to compounds designed for this study are outlined in Scheme 1.

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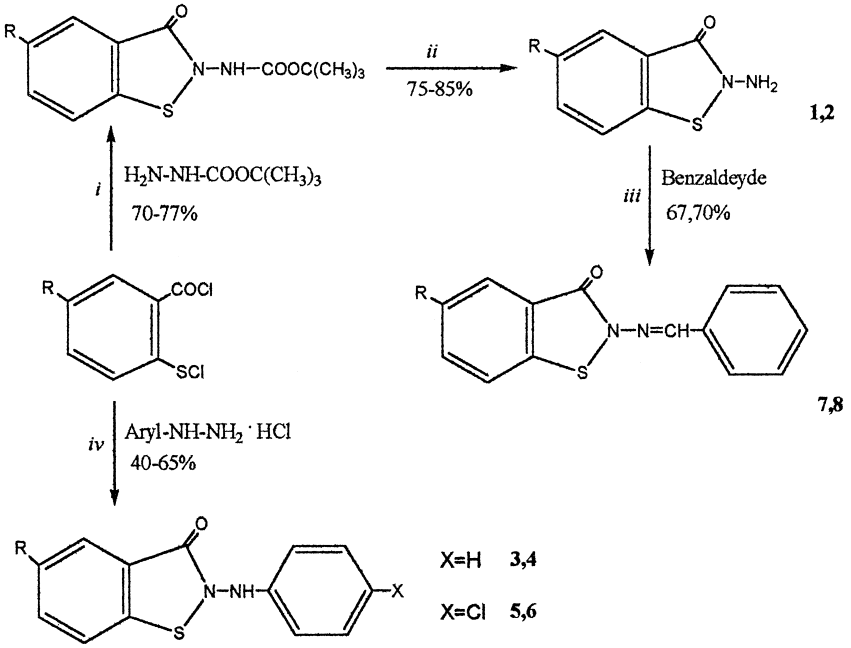
The compounds **1,2** were obtained in two steps through synthetic routes *i* and *ii*, starting from chlorocarbonylphenylsulfenylchloride (R=H, CH_3) according to the method previously reported by us for compound **1**.[4] Compound **2** is here described for the first time. The compounds **3–6** were prepared by direct reaction of the common intermediate (sulfenylchloride) with appropriate hydrazine derivatives. The compounds **1** and **2** are also intermediates for the synthesis of compounds **7** and **8**, which were obtained by reaction with benzaldehyde in buffered (HCl, CH_3COONa) ethanolic solution, at room temperature. The experimental details for these procedures and the characterization data for novel compounds **2**,**4**,**6**,**7**,**8** and compounds **3** and **5**, previously reported in a Japanese patent[9] as antibacterial and anticarcinogenic, are given in the Experimental section.

Results and Discussion

The 2-amino-benzo[d]isothiazoles under study (compounds **1–8**) displayed in vitro antiplatelet activity when tested (0.5–1000 μM) against adenosine diphosphate (ADP) (3 μM) and arachidonic acid (AA) (50 μM) mediated aggregation on guinea-pig platelet-rich plasma (PRP). The synthesized compounds exhibited similar inhibitory activity (expressed as IC_{50} values, μM) toward the platelet responses to both the aggregating agents ADP and AA following the rank order of potency benzylideneamino (**7,8**) ≥ *p*-chlorophenylamino (**5,6**)≅amino (**1,2**) > phenylamino (**3,4**) derivatives. This finding diverged from that obtained in the present study for acetylsalicylic acid (ASA), which prevented the AA stimulated aggregation but produced only 40% inhibition of the ADP-induced response also when tested at the maximal concentration of 300 μM. However, this latter result was consistent with previous data obtained for this antithrombotic agent in comparable experimental conditions.[10,11] As concerns AA-induced aggregation, the test compounds proved to be over 3–20-fold more potent than ASA (Table 1).

The antiaggregating activity shown by the 2-amino-benzo[d]isothiazol-3-one derivatives was coupled with a relaxing effect on guinea-pig vasal and extravasal smooth muscle. Such spasmolytic effect was assessed on isolated preparations of guinea-pig aorta and ileum precontracted with high potassium concentration (40 mM) before the application of the drugs under study. Most compounds relaxed isolated aortic rings only at concentrations from 4 to 100 times greater than those required to prevent platelet aggregation. Amongst the test molecules, the phenyl derivatives (**3, 4**) showed the highest vasorelaxing potency while the compound **6** failed to relax vascular smooth muscle, precontracted by high potassium concentration up to 300 μM. Additionally, all the compounds have been proved to be effective in relaxing high potassium-induced hypertone in guinea-pig ileum. Both ileal and aortic contractile responses to KCl addition were refractory to ASA up to 300 μM.

In conclusion, these outcomes confirm that the 2-amino-benzo[d]isothiazol-3-one moiety is responsible for the in vitro antiplatelet activity,[6] since the introduction of various lipophilic substituents resulted in molecules possessing slightly modified antiaggregating effects. Indeed the insertion of lipophilic R_1 substituents generated the compounds **5** and **7** endowed with slightly higher antiplatelet potency than the parent compound **1**. On the other hand, the additional presence of the quite lipophilic methyl group in the R position (compounds **2**,**4**,**6**,**8**) weakly impaired the antiaggregating activity with respect to the unmethylated analogues. Thus it can be said that the antiplatelet activity of the test



Scheme 1. (i) Et_2O, Py, 10 °C; then 1 h, rt; (ii) CCl_3COOH/H_2O, 150 min, rt; (iii) $HCl/H_2O/CH_3COONa$, EtOH, 1 h, 100 °C; (iv) MeOH, Et_3N, CCl_4, 10 °C; then 1 h, rt.

Table 1. In vitro potency (IC_{50}±SD μM; $n = 6$–8 experiments) in inhibition of ADP (3 μM) and arachidonic acid (AA, 50 μM) platelet aggregation and in the relaxation of KCl (40 mM) smooth muscle hypertone

	Antiplatelet activity in guinea-pig PRP		Spasmolytic activity in guinea-pig isolated tissues	
	ADP-induced aggregation IC_{50}±SD	AA-induced aggregation IC_{50}±SD	KCl precontracted aorta IC_{50}±SD	KCl precontracted ileum IC_{50}±SD
1	7.4±2.2	4.1±1.3	77.6±47.2	28.2±7.2
2	9.5±2.9	4.8±0.4	138.0±58.9	45.7±7.4
3	9.5±4.2	10.2±0.4	38.9±36.2	33.8±5.5
4	11.3±2.7	11.3±4.0	45.7±26.9	8.7±5.1
5	6.3±2.9	2.9±0.9	269.0±39.3	11.7±3.6
6	7.9±0.4	5.4±2.9	> 300	16.9±1.2
7	2.8±1.9	3.1±0.3	263.4±46.5	19.5±0.9
8	4.4±2.2	4.6±2.7	100.4±13.9	83.2±9.1
ASA	> 300	60.9±7.8	> 300	> 300

compounds does not depend on their lipophilicity as no significantly different effect from compound **1** was detected.

The ability of such molecules to prevent ADP/AA induced aggregation as well as to exert a spasmolytic action was different from that detected for ASA. This enables us to speculate upon the involvement of distinct mechanisms of action between these 2-amino-benzo[d]-isothiazol-3-one substituted derivatives and the conventional drug ASA acting as cyclooxygenase inhibitor. Such availability of novel antiplatelet 2-amino-benzo[d]-isothiazol-3-one derivatives endowed with a certain vasodilating property and exhibiting a non ASA-like mode of action is actually suggestive of the development of attractive antithrombotic agents devoid of gastrointestinal adverse side effects.

Experimental

Chemistry

Melting points (°C) were determined with a Buchi 512 apparatus and are uncorrected. New compounds were analyzed in our analytical laboratory, on a Carlo Erba 1106 Elemental Analyzer, for C H N. IR spectra were recorded, as KBr pellets, on a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Tokyo, Japan). All reactions were monitored by TLC on F_{254} silica-gel precoated sheets (Merck) using ethyl acetate:petroleum ether = 1:1 for compounds **2,5,8** and ethyl acetate:hexane = 2:3 for compounds **3** and **4**, as eluents. The purified compounds each showed a single spot. Spectral IR data were consistent with the assigned structure in all cases and the reported wavenumbers are given in cm^{-1}. The found values for C H N elemental analysis were ±0.4% of the theoretical ones.

Solvents, unless otherwise specified, were of analytical reagent grade or of the highest quality commercially available. Synthetic starting material, reagents and solvents were purchased from Aldrich Chemical Co.

Characterization data for 5-methyl-2-amino-benzo[d]isothiazol-3-one (2). Crystallization solvent: EtOH–H_2O; mp 154–156 °C; yield: 75%; IR (KBr): 3320, 3190, 2940–2880, 1665 cm^{-1}. Anal. calcd for $C_8H_8N_2OS$ (180.24): C, 53.32; H, 4.47; N, 15.54. Found: C, 53.06; H, 4.58; N, 15.15.

General procedure for synthesis of compounds 3–6. Triethylamine (40 mmol) was added gradually to a solution of appropriate arylhydrazine hydrochloride (20 mmol) in MeOH (20 mL) and then appropriate chlorocarbonylsulfenyl chloride (20 mmol) in dried CCl_4 (50 mL) was added dropwise with stirring at 10 °C. The mixture was kept overnight at room temperature, the precipitate removed by filtration, and reaction adduct was evaporated in vacuo, yielding the title compounds which were recrystallized from EtOH.

2-Phenylamino-benzo[d]isothiazol-3-one (3). Mp 168–169 °C; yield: 60%; IR (KBr): 3223, 3122–2909, 1655 cm^{-1}. Anal. calcd for $C_{13}H_{10}N_2OS$ (242.30): C, 64.44; H, 4.16; N, 11.56. Found: C, 64.85; H, 4.09; N, 11.34.

5-Methyl-2-phenylamino-benzo[d]isothiazol-3-one (4). Mp 171–172 °C; yield: 62%; IR (KBr): 3218, 3115–2843, 1653 cm^{-1}. Anal. calcd for $C_{14}H_{12}N_2OS$ (256.32): C, 65.59; H, 4.71; N, 10.92. Found: C, 65.95; H, 4.85; N, 10.51.

2-(4-Chlorophenylamino)-benzo[d]isothiazol-3-one (5). Mp 158–159 °C; yield: 42%; IR (KBr): 3208, 3180–2892, 1655 cm^{-1}. Anal. calcd for $C_{13}H_9ClN_2OS$ (276.74): C, 56.42; H, 3.28; N, 10.12. Found: C, 56.77; H, 3.22; N, 9.93.

5-Methyl-2-(4-chlorophenylamino)-benzo[d]isothiazol-3-one (6). Mp 161–162 °C; yield: 64%; IR (KBr): 3252, 3105–2830, 1656 cm^{-1}. Anal. calcd for $C_{14}H_{11}ClN_2OS$ (290.77): C, 57.83; H, 3.81; N, 9.63. Found: C, 58.05; H, 3.78; N, 9.37.

General procedure for preparation of compounds 7,8. The appropriate 2-amino-benzo[d]isothiazol-3-one (**1**,**2**) (5 mmol) was poured in water (40 mL) under stirring and hydrochloric acid added until acidic pH; then the suspension was buffered with sodium acetate and ethanol was added (15 mL). Benzaldeyde (5.7 mmol) dissolved in ethanol (15 mL) was dropped into the

mixture under stirring, and then heated for 30 min at 100 °C. The resulting crude product was filtered, washed with water and recrystallized from ethanol.

2-Benzylideneamino-benzo[d]isothiazol-3-one (7). Mp 195–196 °C; yield: 67%; IR (KBr): (C=O) 1682 cm^{-1}. Anal. calcd for $C_{14}H_{10}N_2OS$ (254.31): C, 66.12; H, 3.96; N, 11.02. Found: C, 66.45; H, 4.00; N, 10.67.

5-Methyl-2-benzylideneamino-benzo[d]isothiazol-3-one (8). Mp 214–215 °C; yield: 70%; IR (KBr): 2960–2840, (C=O) 1685 cm^{-1}. Anal. calcd for $C_{15}H_{12}N_2OS$ (268.33): C, 67.14; H, 4.51; N, 10.44. Found: C, 67.31; H, 4.57; N, 10.11.

Pharmacology

The 2-amino-benzisothiazoles **1**–**8** were screened for their in vitro antiplatelet property in guinea-pig platelet-rich plasma inducing the aggregation by ADP or arachidonic acid (AA). Other experiments were performed in the guinea-pig isolated aorta and ileum to evaluate their spasmolytic activity on the vasal/extravasal smooth muscle.

Antiplatelet activity

Male guinea-pig (Morini, S.Polo, RE, Italy) blood obtained by cardiac puncture after CO_2 euthanasia was collected in plastic tubes containing sodium citrate (3.8% w/v; 9 parts blood:1 part sodium citrate). Platelet-rich plasma (PRP) was prepared by centrifugation for 10 min at 2000 *g*. Platelet aggregation was performed in an Aggrecorder PA 3220 aggregometer (A. Menarini, Firenze, Italy) following the Born turbidimetric method.[12] Aggregation was recorded as percent change in light transmission: the baseline was set by using PRP and full transmission (100%) was set by using platelet-poor plasma. PRP (250 μL) was preincubated at 37 °C for 5 min with solvent dimethyl sulfoxide (DMSO) and the compounds under study or the reference drug ASA (from 5×10^{-5} to 1×10^{-3} M) before the addition of the platelet aggregatory agent. PRP aggregation was induced by 3 μM ADP (25 μL) (Sigma) or 50 μM arachidonic acid (25 μL) (Menarini) and using concentrations sufficient to achieve maximum aggregation. Tests were performed within 3 h to avoid platelet inactivation. The effects of test compounds were determined as percent inhibition calculated from the total aggregation in 5 min. Control samples received the same volume addition of DMSO at the final concentration of 0.5%. This concentration of solvent did not interfere with platelet assay.

Spasmolytic activity

According to Low et al.[13] segments of terminal ileum (3 cm length) or a chain of six rings (dissected from guinea-pig thoracic aorta) were set up in organ bath containing modified Krebs solution (mM composition: NaCl 134, KCl 3.4, $CaCl_2$ 2.8, KH_2PO_4 1.3, $NaHCO_3$ 16, $MgSO_4$ 0.6, glucose 7.7) bubbled with 95% O_2–5% CO_2 and warmed to 37 °C. The contractile response was isometrically recorded and concentration–response curves for the test compounds were obtained cumulatively adding the test molecules (1–100 mM) to the tissues stretched to the optimal resting force (2 g for aorta, 1 g for ileum) and precontracted with 40 mM KCl to produce half maximal contraction. The tested compounds were dissolved in DMSO at the final concentration of 0.1%, ineffective in muscular responsiveness.

Statistical analysis

The results were expressed as mean±SD. Antiplatelet as well as spasmolytic potency was expressed as IC_{50} value (the concentration inhibiting the pharmacological responses by 50%) and it was calculated in relation to control values by linear regression analysis.

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