Role of 1,4-Benzothiazine Derivatives in Medicinal Chemistry

R. Fringuelli*, L. Milanese and F. Schiaffella

Department of Drug Chemistry and Technology, University of Perugia, Via del Liceo, 1, 06123 Perugia, Italy

Abstract: 1,4-Benzothiazine (1,4-BT) derivatives have been reported to exhibit a wide range of pharmacological properties including antifungal, immunostimulating, anti-aldoso-reductase, anti-rheumatic, anti-allergic, vasorelaxant, anti-arrhythmic, anti-hypertensive, neuroprotective and cytotoxic activities. These different effects indicate that 1,4-BT is a template potentially useful in medicinal chemistry research and therapeutic applications.

1,4-Benzothiazine (1,4-BT) derivatives are known to exert many effects *in vivo* and *in vitro*. A series of 1,4-BT analogues have shown good antifungal and immunostimulating activity; 4-substituted benzothiazolyl-1,4-BT derivatives are potent aldoso-reductase inhibitors; methotrexate derivatives bearing a 1,4-BT moiety exhibit anti-rheumatic effects; quinolinyl-piperazinyl-1,4-BT possess anti-allergic activity. 1,4-BTs are also active on the cardiovascular system and the vasorelaxant, anti-arrhythmic and anti-hypertensive effects have been reported. 1,4-BT-induced neurotoxic or neuroprotective effects have been described and a possible role in neurodegenerative diseases has been hypothesized. *In vivo* anti-tumor efficacy of 1,4-BT has been described and attributed to direct cytotoxic activity against neoplastic cells.

The focus of this review is to provide a comprehensive and up-to-date account on the most recent developments in the medicinal chemistry of 1,4-BT derivatives with significant biological activity starting from the last exhaustive publication in this field [1].

ANTIFUNGAL COMPOUNDS

During the past two decades the frequency of invasive and systemic fungal infections has increased dramatically in the population with altered immunity [2, 3]. Current therapy for fungal infections is affected by drug-related toxicity, hazardous drug-drug interactions, non-optimal pharmacokinetics and development of drug resistance [4].

Azoles are currently the most widely studied class of antifungal agents [5] and fluconazole (FLU) is the agent of choice in *Candida* infections. Enzymes in the ergosterol-biosynthesis pathway, specifically the cytochrome P450-dependent lanosterol 14α-demethylase ($P450_{14DM}$, CYP51), are targets for successfully marketed antifungal drugs. Azoles inhibit CYP51 causing accumulation of methylated sterols, depletion of ergosterol, and inhibition of cell growth [6].

1,4-BT nucleus shows, within itself, some antifungal activity [7]. A series of azole 1,4-BT derivatives structurally correlated with FLU and econazole was recently synthesized and *in vitro* and *in vivo* antifungal activity against *Candida albicans* was evaluated [8-10]. 1,4-BT azole derivatives differed with respect to, sulphur oxidation state, presence of the carbonyl group in C-3, insertion of the side chain on C-6, C-7 or C-8 of BT nucleus, and the nature of azole substituent: 1*H*-imidazol-1-yl (IMI) or 1*H*-1,2,4-triazol-1-yl (TRI) (Table **1**).

Structure-activity relationships (SAR) of 1,4-BT azole derivatives showed that the best activity correlated with the presence of a methyl group in N-4, the insertion of the side chain on the BT nucleus in C-6 or C-7 position, the presence of carbonyl group in C-3, sulphur as a thioether or sulfoxide and IMI as azole substituent in the side chain [9].

Among these derivatives, 7-[1-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (**2**) shows good efficacy against systemic candidiasis in a murine experimental model [8] but poor antifungal activity *in vitro* compared to FLU. A study was designed to evaluate whether the observed **2**-mediated anti-*Candida* effect could be due to the immunopotentiating properties of professional phagocytes such as macrophages [11]. The results showed that compound **2** promotes antifungal activity of macrophages in different anatomical districts and induces synthesis of tumor necrosis factor alpha (TNF-α) and nitric oxide secretion.

The IMI-1,4-BT derivatives reported in Table **1** showed mainly *in vivo* activity in a murine experimental model of candidiasis while very often *in vitro* activity was lacking. Building a 3D model of the cytochrome P450 14α-sterol demethylase of *Candida albicans* (CA-CYP51) on the basis of the sequence homology with the recently reported crystal structure of the cytochrome P450 14α-sterol demethylase of *Mycobacterium tuberculosis* (MT-CYP51) [12], the interactions of the more active compounds with target enzyme were studied. They adopt similar binding modes inside the catalytic site of CA-CYP51, however, subtle differences of interactions and log P values affect the *in vitro* antifungal activity of these compounds [13].

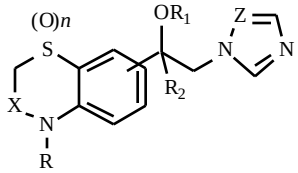
ANTI-RHEUMATIC AGENTS

Rheumatoid arthritis (RA) is a chronic progressive disease associated with systemic inflammation. The disease directly affects physical function and mobility and results in substantial short-term and long-term morbidity. Methotrexate (MTX), used as an anti-leukemic agent because of high anti-folate activity, exerts an anti-inflammatory effect

*Address correspondence to this author at the Department of Drug Chemistry and Technology, *Via* del Liceo, University of Perugia, 06123 Perugia, Italy; Tel: +39 075 585 5134; Fax: +39 075 585 5161; E-mail: fringuel@unipg.it

1389-5575/05 $50.00+.00

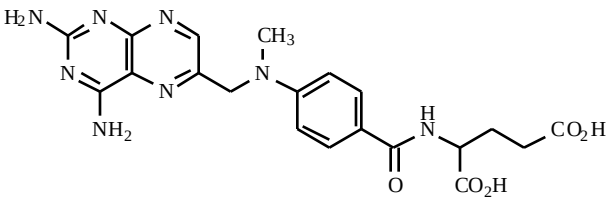
Table 1. ***In vitro*** **and** ***In vivo***[a] **Antifungal Activities of Compounds 1-22 Against** ***C. albicans*** **CA6**



Compd	Chain[b]	X	*n*	R	R_1	R_2	Z	MIC (μg/mL)	CFU (x 10^3)[c]	MST[d]	D/T[e]
Diluent[f]								>250	36.0 ± 4.0	12	10/10
1	7	CO	0	Me	H	H	CH	>250	18.5 ± 2.1	19[g]	9/10
2	7	CO	0	Me	4-Cl-benzyl	H	CH	46	8.5 ± 2.0	>60[g]	5/10
3	7	CO	0	Et	H	H	CH	>250	24.3 ± 3.2	18[g]	6/10
4	7	CO	0	*i*Pr	H	H	CH	>250	31.0 ± 5.3	11	10/10
5	7	CO	0	H	H	H	CH	>250	32.2 ± 4.8	10	10/10
6	7	CO	0	Me	H	CH_2-TRI	N	>250	49.6 ± 12.5	23[g]	9/10
7	7	CO	0	Me	H	CH_2-TRI	CH	>250	77.8 ± 15.7	13	10/10
8	7	CO	0	Me	H	H	N	>250	3.7 ± 0.8[g]	12	10/10
9	7	CO	0	Me	4-Cl-benzyl	H	N	>250	3.7 ± 2.8[g]	16[g]	8/10
10	6	CO	0	Me	H	H	CH	62.5	8.4 ± 3.1	19[g]	9/10
11	8	CO	0	Me	H	H	CH	>250	1.9 ± 1.0	12	10/10
12	7	CO	0	Et	4-Cl-benzyl	H	CH	15.7	24.3 ± 3.0	18[g]	6/10
13	6	CO	0	Me	4-Cl-benzyl	H	CH	62.5	8.5 ± 3.8	40[g]	6/10
14	7	CO	1	Me	H	H	CH	>250	25.9 ± 3.3	49[g]	6/10
15	7	CO	1	Et	H	H	CH	>250	4.0 ± 2.3	18[g]	7/10
16	6	CO	1	Me	H	H	CH	62.5	14.2 ± 2.1	29[g]	7/10
17	7	CO	2	Me	H	H	CH	>250	26.0 ± 2.9	12	10/10
18	7	CO	2	Et	H	H	CH	>250	7.3 ± 3.1	12	10/10
19	6	CO	2	Me	H	H	CH	>250	26.0 ± 3.3	12	10/10
20	7	CH_2	0	Me	H	H	CH	>250	20.0 ± 2.3	12	10/10
21	7	CH_2	0	Et	H	H	CH	>250	27.2 ± 3.7	12	10/10
22	6	CH_2	0	Me	H	H	CH	62.5	2.2 ± 1.8	15	10/10
FLU								0.9	0.0	>60	1/10

[a]Groups of 10 mice were inoculated iv with $2x10^5$ cells of *C. albicans* CA6. Diluent, azoles, and FLU were given ip at the dose of 10 mg/kg, 2h before and once daily for 7 consecutive days after infection. Data are from a representative experiment of 3 performed. The MST, D/T, and CFU results in *C. albicans*-treated mice were similar in all mice sacrificed 8 days after infection. [d]Median survival time (days). [e]Dead mice at 60 days over total number of animals tested. [f]Diluent = DMSO:H_2O, 1:4. [g]$p < 0.01$ (compounds-treated *versus* diluent-treated).

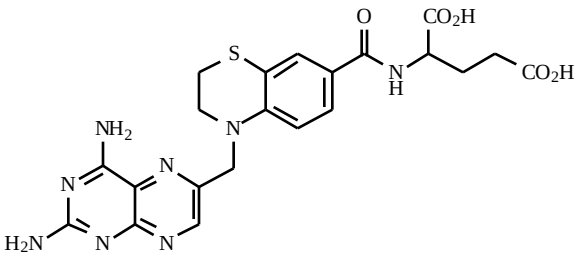
and low-dose intermittent therapy is effective in treatment of RA [14].



Methotrexate

Chart 1.

The mechanism of action of MTX in RA is not yet completely understood. MTX appears to inhibit dihydrofolate reductase (DHFR), to be readily polyglutamated by folylpolyglutamate synthetase (FPGS) and to be deposited intracellulary [15]. The intracellular storage of MTX as polyglutamate, with consequent decrease in available folates, may be crucial for the occurrence of adverse events [16]. To reduce these effects a new derivative, N-{[4-[(2,4-diaminopteridin-6-yl)methyl]-3,4-dihydro-2*H*-1,4-benzothiazin-7-yl]-carbonyl}-L-homoglutamic acid (MX-68), was synthesized. This derivative was designed to resist polyglutamation by replacing the glutamate residue of MTX with a homoglutamate and a 1,4-BT ring in place of the aminobenzoic acid [17].



MX-68

Chart 2.

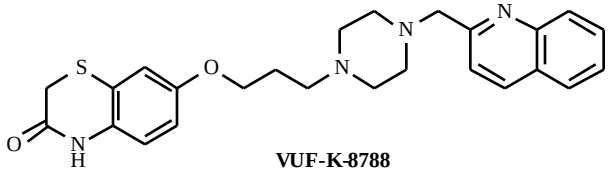
In vitro MX-68 inhibited the activity of DHFR to the same degree as MTX-polyglutamate. MX-68 treatment produced a similar antiproliferative effect to that of MTX. However, the intracellular concentration of MX-68 was much lower than the sum of the levels of MTX and its polyglutamate. The effects of MX-68 disappeared when removed from culture medium. These results indicate that polyglutamation is not essential for the anti-arthritic effect of antifolates [18, 19].

Weekly low-dose MXT therapy remains a mainstay in the treatment of inflammatory arthritis. Results demonstrate that adenosine, acting on one or more receptors, mediates the anti-inflammatory effects of MTX in animal models of acute and chronic inflammation. The effects of low-dose methotrexate, MX-68, dexamethasone, or vehicle control on acute inflammation were examined in an air-pouch model in A2A and A3 receptor knockout mice [20]. Weekly low-dose MTX treatment increased the adenosine concentration in exudates of all mice studied and reduced leukocyte and TNF-α accumulation in exudates of wild-type mice, but not in those of A2A or A3 receptor knockout mice. Dexamethasone, an agent that suppresses inflammation by a different mechanism, was equally effective at suppressing leukocyte accumulation in A2A knockout, A3 knockout, and wild-type mice, indicating that the lack of response was specific for MTX and MX-68. These studies confirm that adenosine, acting at A2A and A3 receptors, is a potent regulator of inflammation. Moreover, these results provide strong evidence that adenosine, acting on either or both receptors, mediates the anti-inflammatory effects of MTX and its analog MX-68.

MX-68 was also evaluated, in comparison to MTX, for the preventive effects on two experimental murine models of systemic lupus erythematosus: NBZxNZWF1 (BWF1) mice and chronic graft-versus-host disease mice [21]. Oral administration of MX-68 significantly delayed the onset of proteinuria and prolonged the life span of BWF1 mice. Increased serum blood urea nitrogen and cholesterol levels, resulting from the development of lupus nephritis, were also inhibited.

ANTI-ALLERGIC COMPOUNDS

Histamine is known to mediate allergic and inflammatory responses through histamine H_1-receptors and play an important role in atopic asthma, atopic dermatitis and allergic rhinitis. Consequently, many histamine H_1-receptors antagonist have been developed, however, the inhibitory action of such compounds on muscarine and serotonine receptors, in addition to the histamine receptors, and their permeation through the blood-brain barrier have the propensity to cause adverse effects on the central nervous system (CNS). A 1,4-BT derivative, 7-{3-[4-(2-quinolinylmethyl)-1-piperazinyl]propoxy}-2,3-dihydro-4*H*-1,4-benzothiazin-3-one (VUF-K-8788) has been synthesized [22] and its pharmacological properties were investigated *in vitro* and *in vivo* in guinea pigs and rats [23].



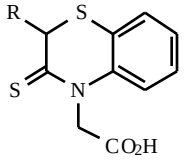
VUF-K-8788

Chart 3.

VUF-K-8788 was shown to be a potent and selective histamine H_1-receptor antagonist without anti-cholinergic or anti-serotonin activity. After systemic administration in guinea pigs, VUF-K-8788 barely antagonized the CNS H_1-receptors at the dose in which it showed anti-histaminergic effect suggesting that it could be useful in the treatment of allergic disorders such as atopic dermatitis and eczema. The same research group reported a new model for biphasic asthmatic response in lung parenchyma of guinea pigs in which late-phase asthmatic reactions (LAR) were caused by repeated airway inflammation with eosinophilia and chemical mediator release including histamine [24]. The effects of VUF-K-8788 on the histopathological changes during LAR resulted in inhibition of biphasic asthmatic reactions such as hyperplasia of airway epithelium, perivascular edema and infiltration by eosinophils, such that it should be used in treatment of asthmatic patients.

ALDOSE-REDUCTASE INHIBITORS

In hyperglycemia, aldose reductase catalized the conversion of excess glucose into sorbitol. The accumulation of intracellular sorbitol causes the development of diabetic complications such as neuropathy, retinopathy, nephropathy and cataracts [25]. N-Acetic acid derivatives of 2-substituted 1,4-BT were synthesized for evaluation as new aldose reductase inhibitors (ARI) [26]. Thiolactam derivatives of 1,4-BT, possessing benzyl moieties at the 2-position, showed potent ARI activities *in vitro* but were primarily inactive in the *in vivo* test. The activity of compounds bearing a branched substituent, such as isopropyl, at the 2-position were remarkable in the *in vivo* test.

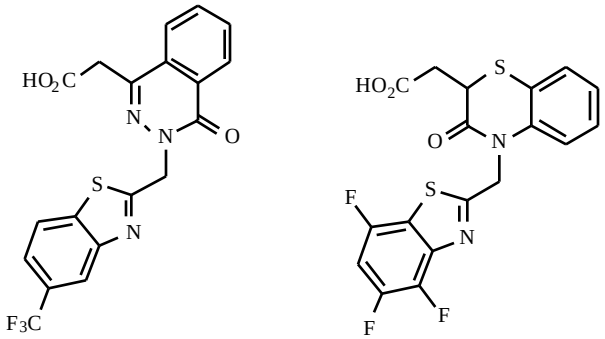


R = CH_2-C_6H_5, CH_2-*o*Cl-C_6H_4, CH_2-*m*Cl-C_6H_4, CH_2-*p*Cl-C_6H_4 $CH(CH_3)_2$

Chart 4.

The structure of zopolrestat, potent ARI, is characterized by a substituted benzothiazol-2-ylmethyl moiety suggesting the synthesis of a series of 4-substituted (benzothiazol-2-yl)methyl-1,4-benzothiazine-2-acetic acid derivatives

including a novel orally active ARI, SPR-210, and their bioisosteres [27].



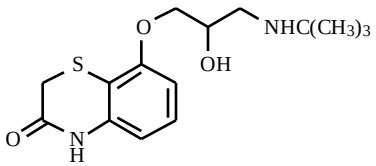
Zopolrestat **SPR-210**

Chart 5.

SPR-210 showed potent ARI activity *in vitro* (IC_{50} 9.5 x 10^{-9} M) and a significant reduction in sorbitol accumulation in rat sciatic nerve (ID_{50} 0.1 mg/kg) and lens (ID_{50} 9.8 mg/kg) of streptozotocin-diabetic rats. Preliminary SAR suggested that the carboxylic acid moiety and the benzothiazole ring were crucial for potent inhibitory activity. The benzothiazole moiety substituted with alogens, particulary fluorine, appears to be an effective pharmacophore.

β-ADRENOCEPTOR ACTIVITY

An oxypropanolamine side chain linked to an aromatic ring are the chemical features required for β-blocking activity. However, the alteration of these features as the intercalation of an imino group in the side chain does not abolish the interaction on β-adrenoceptors but can lead, in some cases, to potent β-antagonists [28, 29]. To evaluate the effect caused by a different type of insertion of pharmacophore oxypropanolamine chain in the 1,4-BT moiety that possesses short-lived blood pressure reducing effects in experimental animals [30], 6-, 7-, 8-oxypropanolamine and 6-, 7-, 8-acetimidoyloxypropanolamine of 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine were prepared [31]. The synthesized derivatives were first tested at the receptor level to determine their ability to displace the binding of [^{3}H] dihydroalprenolol from turkey erythrocyte membranes and then *in vivo* to evaluate β-blocking activity by inhibition of isoprenaline-induced tachycardia. The highest affinity and the *in vivo* antiadrenergic effects in inhibiting isoprenaline-induced tachycardia were observed with 8-oxypropanolamine derivatives. Among these derivatives, racemic 8-(*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine (**23**) showed remarkable β-blocking activity.



23

Chart 6.

Resolution of the racemate with (R)-(+)-methylbenzylisocianate and synthesis of (R)- and (S)-8-(*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine *via* Sharpless chiral epoxidation [32], showed modest enantioselectivity towards β_1- and β_2-adrenoceptors and no activity as α-adrenoceptor blockers.

In long-term treatment of essential hypertension, no single drug is yet entirely satisfactory. Combined treatment is necessary to evoke an optimal result and a β-blocker/diuretic combination is widely used as first line therapy in hypertension management. An anti-hypertensive drug with both β-blocker and moderate diuretic properties in the same molecule could be of great appeal [33]. An interesting approach to this symbiotic drug design, achieved by replacing the conventional alkyl substituent at the side chain nitrogen atom of β-blockers with a 2-(4-chloro-3-sulfamoylbenzamido)-ethyl group, has been reported [34, 35]. This type of substitution retained the structural requirements for the interaction with the β-adrenoceptor, due to the presence of a 2-amidoethyl group known to impart high β-blocking potency, and at the same time allowed the diuretic *o*-chlorobenzenesulfonamidic moiety to be incorporated into the molecule. This replacement was made on oxypropanolamine derivatives of 1,4-BT previously reported as β-adrenoceptor antagonists [31].

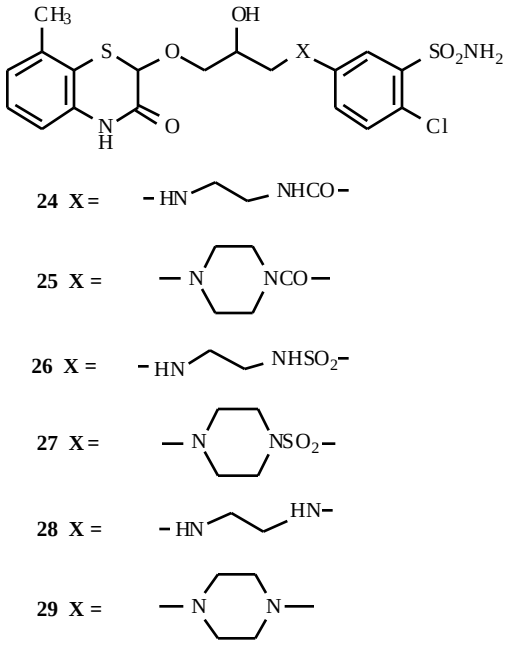
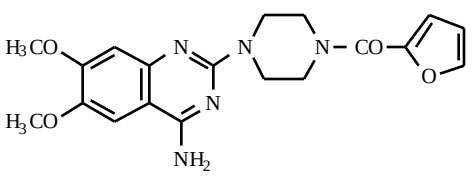


Chart 7.

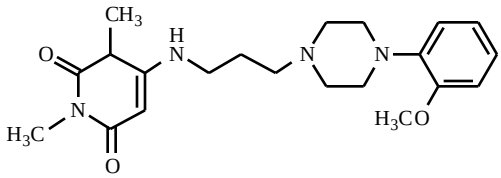
Pharmacological results indicate that the incorporation of *o*-chlorobenzenesulfonamidic moiety into the molecule by an amidoethylamino group allowed the desired diuretic activity and also maintained that of β-blockers in some cases. Indeed, compound **26** was found to exhibit differently modulated β-adrenoceptor blocking and diuretic activities.

α-ADRENOCEPTOR ACTIVITY

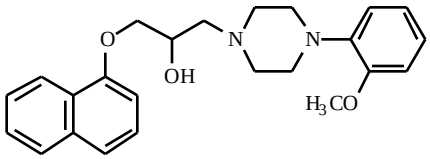
To expand the investigation on benzothiazine derivatives with ant-hypertensive properties, the 1,4-BT nucleus has been variously functionalized with phenylpiperazine (PP) or acylpiperazine (AP) moieties to drive the activity towards the α-andrenoceptor (α-AR) [36]. The rationale of this design is due to the high affinity for α-AR, and particularly for α1-AR, displayed by AP-containing products (e.g.



Prazosin



Urapidil

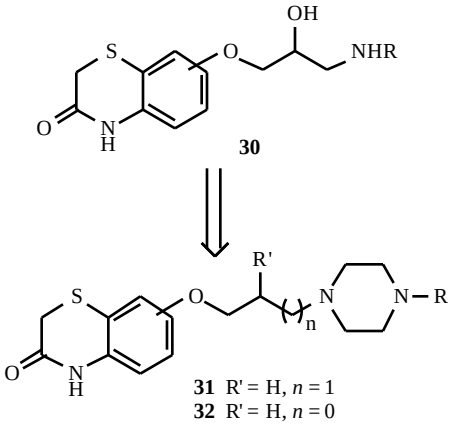


Naftopidil

Chart 8.

prazosin) or PP-containing products (e.g. urapidil, naftopidil).

In fact, these moieties furnish an electron-rich aromatic area coupled to a protonable nitrogen atom at a suitable distance that is one of the principal requirements of the ligand for binding to the α_1-AR protein [37-39]. Thus, considering naftopidil as a selective α1-AR blocking template, selected piperazine moieties, instead of a secondary amine, were inserted into the oxypropanolamine side chain of the 1,4-BT derivatives previously reported to be β-AR antagonists [40].



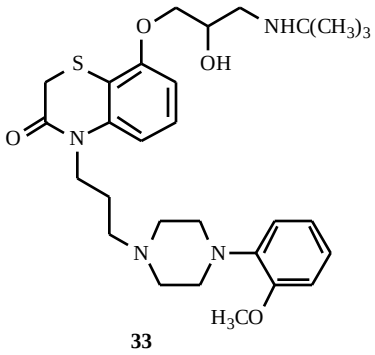
30

31 R' = H, *n* = 1
32 R' = H, *n* = 0

Chart 9

In the radioligand receptor binding assay some of these synthesized compounds showed notable affinity and selectivity for α1-AR while, as expected, no β-AR affinity was retained. Therefore, to evaluate how the bridge linking the 1,4-BT nucleus to piperazine moiety affects α-AR affinity, the oxypropanolpiperazine side chain was modified by eliminating the secondary alcoholic group and shortening it by one methylene unit to obtain (1,4-benzothiazinyloxy)-propyl- **31** and (1,4-benzothiazinyloxy)ethyl-piperazine derivatives **32**. These changes were made by fixing the 1-(2-methoxyphenyl)-piperazine moiety since it provided compounds with the highest α1-AR affinity.

Considering that combined therapy with α- and β-adrenergic blocking agents has synergic effectiveness in hypertension treatment [41], derivative **33** was synthesized according to a symbiotic approach.



33

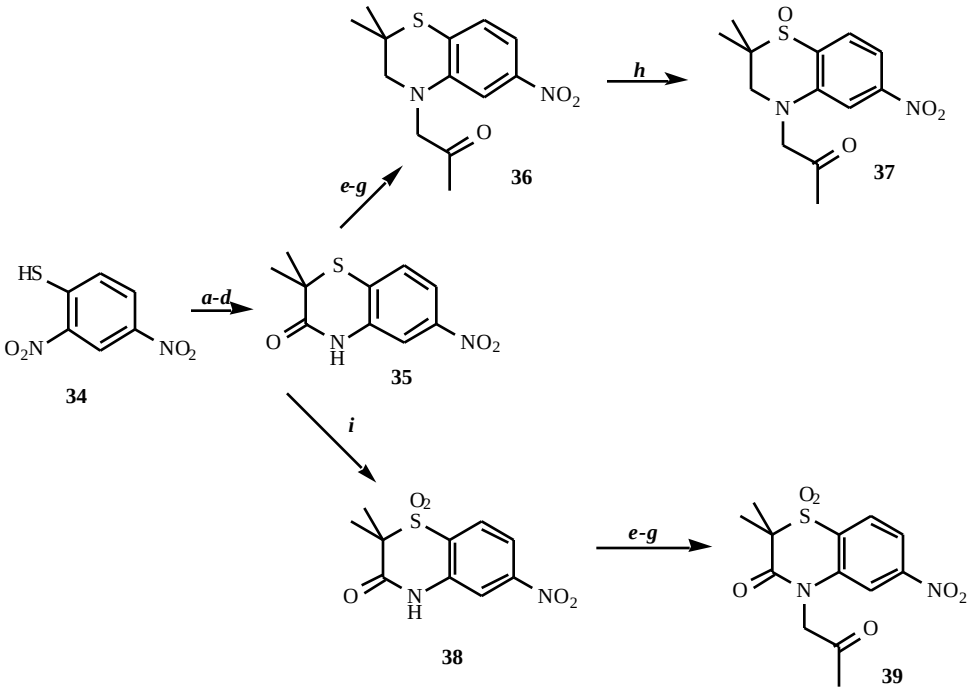
Chart 10.

It was obtained from the insertion of 3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl side chain, as α-AR activity carrier, at the N-4 position of the 8-[(3-*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-3-oxo-2H-1,4-benzothiazine derivative (**23**), a potent β-blocker previously synthesized [31].

It was confirmed that the addition of a (2-methoxyphenyl)piperazine side chain also onto 1,4-BT nucleus provides compounds with α affinity. This effect is only moderately influenced by the length of the alkyl spacer and by the insertion position on the BT vector. Moreover, the presence of this α-affinity-bearing side chain coupled with an oxypropanolamine β-blocker pharmacophore provided an interesting compound with a high affinity for both α and β-ARs that could be a new anti-hypertensive drug model.

K^+ CHANNEL OPENERS

The K_{ATP} channels are an important class of ionic channels whose function is regulated by changes in the intracellular level of adenosine triphosphate. These channels are closed when intracellular ATP levels are elevated and open when intracellular ATP levels decrease, thus linking membrane potential to the metabolic state of the cell [42]. Opening allows passage of potassium ions out of the cell causing transmembrane hyperpolarization and repolarization. These effects reduce intracellular calcium concentration through a blocking function of voltage-dependent calcium channels and inhibition of intracellular calcium release,

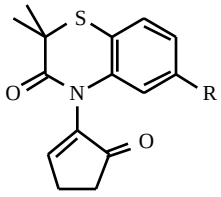


Scheme 1. ***a:*** $BrC(CH_3)_2CO_2C_2H_5$, K_2CO_3, DMF; ***b:*** Fe, HCl, EtOH; ***c:*** NaOH aq, reflux; ***d:*** $NaNO_2$, H_2SO_4, then $NaHCO_3$, $NaNO_2$, $CuSO_4$, Cu_2O; ***e:*** $ClCH_2COCH_3$, K_2CO_3, DMF; ***f:*** BH_3-THF; ***g:*** Swern oxidation; ***h;*** *m*CPBA; ***i:*** *m*CPBA excess.

resulting in smooth muscle relaxation and antispasmodic action [43]. Interest in potassium channel openers was triggered in the early 1980s when it was discovered that cromakalim, pinacidil and nicorandil relaxed smooth vascular muscle *via* a mechanism involving the opening of K_{ATP} channels. These agents were first indicated for the treatment of hypertension and angina pectoris, but this has since been extended to include other pathologies involving smooth muscle contraction such as asthma, urinary incontinence, and baldness. These agents are also thought to provide cellular protection against cardiac ischemia, regardless of their vasodilating actions, and to have anti-lipemic effects.

A great deal of research on structure modifications based on cromakalim, a benzopyran derivative, has been reported. Matsumoto's research group has been particularly active synthesizing a series of 1,4- benzoxazine derivatives variously substituted and finally trasforming the skeleton

Table 2. Vasorelaxant Activity of 1,4-BT Derivatives[a]



Compd	R	E_{max}[b] ± SEM[c], %	pIC_{50} ± SEM[c]
40	NO_2	98 ± 1 90 ± 5[d] 26 ± 19[e,g]	10.98 ± 0.020 6.38 ± 0.11[d,g] NC[e,f]
41	CF_3	99 ± 3 87 ± 15[d] 35 ± 4[e,g]	12.13 ± 0.13 6.36 ± 0.084[d,g] NC[e,f]
42	CN	95 ± 5 100[d] 18 ± 9[e,g]	11.3 ± 0.13 7.16 ± 0.12[d,g] NC[e,f]
LK		100	6.98 ± 0.11

[a]Vasorelaxant activity was evaluated in aortic ring precontracted with 20 mM KCl. [b]Maximal vasorelaxing response expressed as percentage of contractile tension. [c]Standard error of the mean of 5-10 separate experiments. [d]Parameter recorded in the presence of 1 M glibenclamide. [e]Parameter recorded in the presence of 60 mM KCl. [f]The parameter could not be calculated because of the low efficacy (≈ or < 50%). [g]Significantly different from the respective control.

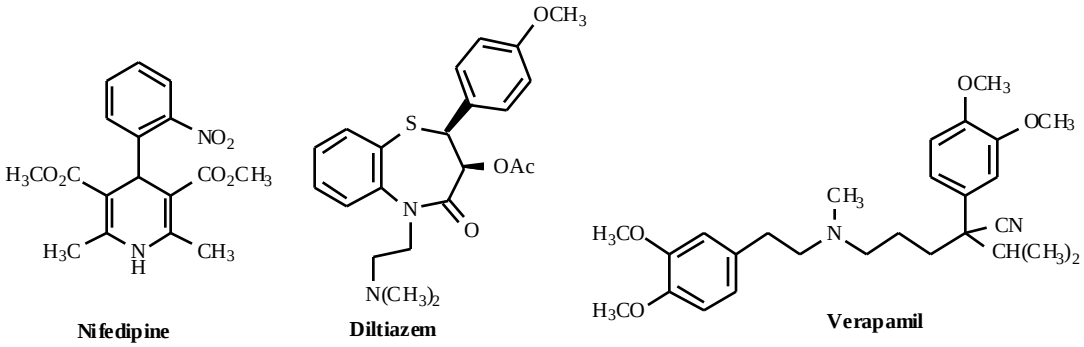


Chart 11.

into other fused rings [44]. Among them, 1,4-BT derivatives displayed more potent vasorelaxant activity. In particular, compound **36** was found to be approximately 20 times more potent *in vitro* than cromakalim.

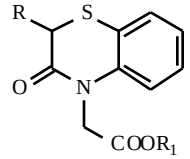
The study on 1,4-BT nucleus was amplified by Cecchetti *et al*. who suitably functionalized the nucleus with an electron-withdrawing group at C-6 position and lactam rings, acyclic amides, cyclopentenone, acyl chains, all bearing the usual oxo function at different distances from the 1,4-BT nucleus, as N-4 substituents [45]. All compounds were tested for vasorelaxing activity *in vitro* compared to levocromakalim, the biologically active enantiomer of cromakalim. The main observation that emerged from this study is that highly potent compounds can be obtained by replacing the benzopyran ring with a 1,4-BT nucleus. Potency increases when a suitable substitution pattern is present on the 1,4-BT nucleus: cyclopentenone as N-4 substituent coupled with a nitro, trifluoromethyl, or cyano group at the C-6 position. Indeed, these compounds have a vasorelaxant potency at least 10,000 times greater than levocromakalim which makes them the most potent potassium channel openers reported to date.

Ca^{2+} CHANNEL ANTAGONISTS

Calcium ion (Ca^{2+}) is an important cellular component involved in the regulation of many cell functions. The regulation of cytosolic Ca^{2+} levels occurs through various mechanisms. In particular, voltage-dependent Ca^{2+} channels are important in regulating the influx of Ca^{2+}, making Ca^{2+} channel blockers useful in the treatment of numerous diseases, such as angina pectoris, hypertension, ischemic heart disease and certain cardiac arrhythmias. The currently available Ca^{2+} channel blockers belong to three classes: 1,4-dihydropyridines (i.e. nifedipine), phenylalkylamines (i.e. verapamil) and benzothiazepines (i.e. diltiazem).

All these antagonists act on the L-type Ca^{2+} channel (skeletal muscle, cardiac muscle, neuroendocrine tissue), binding to different regions on the α1-subunit, as shown by photoaffinity labeling and molecular biological studies [46]. However, in some patients treatment with non-dihydropyridine Ca^{2+} channel blockers is related to adverse effects on the heart, such as cardiac failure, bradycardia, and asystole [47-50]. With the intent to reduce these effects and to obtain lower cardiac suppression, Fujita and co-workers changed the benzothiazepine nucleus into a benzothiazine nucleus [51].

A series of 1,4-BT was synthesized and Ca^{2+} antagonistic activities of the new compounds were evaluated through *in vitro* assay using isolated depolarized tenia cecum of guinea pigs. All the tested *N*-methyl-*N*-(phenoxyethyl)amino compounds showed potent Ca^{2+} antagonistic activities, with IC_{50} values in the order of 10^{-7} M, almost the same as diltiazem.



R = H, CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2C_6H_5$, CH_2CO_2Na

R_1 = H, Na

Chart 12.

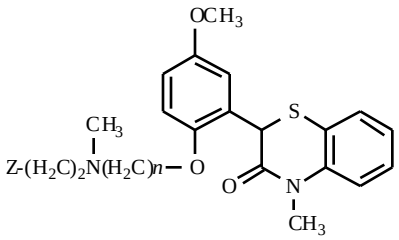
Six compounds of this series were selected to evaluate the vaso-cardio selectivity (expressed as ratios of IC_{50} values for Ca^{2+} antagonistic activity in aorta to IC_{50} values for the rate of contraction or contractile force in atria) and performed *in vitro* assay with guinea pig aorta and right atria. In light of the adverse effects of the Ca^{2+} antagonists due to cardio suppression, the lesser cardio selectivity is expected to be safer.

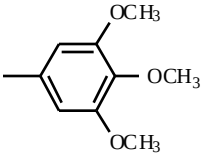
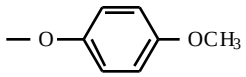
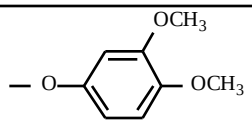
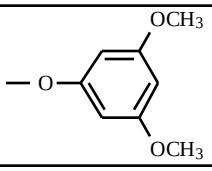
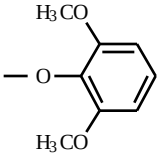
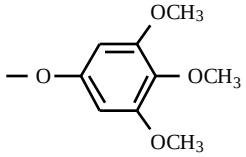
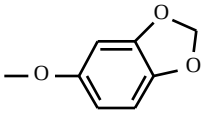
Compound **51** had the highest selectivity for vasodilatation among the tested compounds. The racemic mixture of (+)-**51** and (-)-**51** was then resolved by fractional crystallization, and the biological data for both enantiomers showed that Ca^{2+} blocking activity is to be ascribed chiefly to the R(+)-stereoisomer.

(+)-**51**, first named sesamodil, then SD-3211 and finally semotiadil, was selected as the new lead compound, structurally different from all known Ca^{2+} channel antagonists. The Ca^{2+} antagonistic activity of semotiadil depends, in part, on the long side chain at the C-3 position of the 1,4-BT nucleus, as suggested by the examination of the three-dimensional structure based on the conformational analysis [52, 53] and by the fact that similar structural components are present both in verapamil and in other phenylalkylamines. The 1,4-BT ring of semotiadil plays an important role in enhancing the potency.

Experimental [54] and clinical [55] studies have shown that Ca^{2+} antagonists can exert an anti-atherogenic action with several mechanisms acting on the regulation of LDL levels. Semotiadil significantly inhibits LDL oxidation, on

Table 3. Ca^{2+}-Antagonist Activity of Compounds 43-52



Compd	z	*n*	Ca^{2+} IC_{50} μM
43 **44**		3 4	0.27 ± 0.07 0.35 ± 0.06
45		4	0.53 ± 0.09
46		4	0.23 ± 0.04
47		3	0.55 ± 0.09
48		3	0.51 ± 0.15
49 **50**		3 4	0.23 ± 0.04 0.16 ± 0.03
51 **52**		3 4	0.18 ± 0.01 0.17 ± 0.03
Diltiazem			0.25 ± 0.08

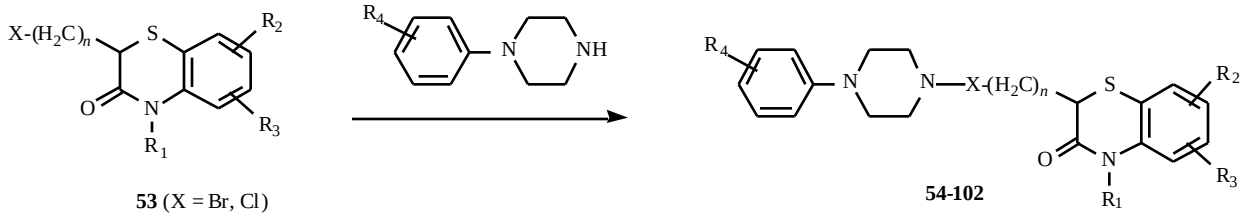
an *in vitro*-induced system, with greater potency than nifedipine, amlodipine and diltiazem. Taken together, these results suggest that the beneficial effects of Ca^{2+} antagonists, in particular semotiadil, may, in part, also be related to their anti-peroxidant activity. Semotiadil was also examined for its anti-platelet activity because platelets are known to be involved in the development and progression of human atherosclerotic lesions [56]. Several Ca^{2+} antagonists have been shown to exert anti-platelet activity [57, 58].

In a recent study *in vitro*, semotiadil inhibition of platelet aggregation was demonstrated in a dose-dependent manner using various known inducers of platelet aggregation such as adenosine diphosphate, collagen, arachidonic acid and platelet activating factor [59]. Comparing the effect of semotiadil to nifedipine, amlodipine and diltiazem, only the latter exhibited greater anti-aggregatory potency.

Table 4. Ca^{2+}-Antagonist Activity of Enantiomers of Compound 51

Compd	Ca^{2+} IC_{50} μM	Configuration of 2-position
(+)-**51**	0.089 ± 0.018	*R*
(-)-**51**	0.60 ± 0.06	*S*

Levosemotiadil (SD-3212), the S(-)-stereoisomer of semotiadil, is reported instead to have anti-arrhythmic



53 (X = Br, Cl) **54-102**

Scheme 2.

properties due to its activity primarily as Na^+ [60-62] and K^+ [63] channel blockers, rather than Ca^{2+} antagonist.

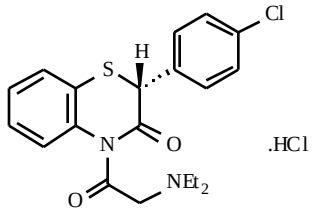
Calmodulin (CaM) is a ubiquitous intracellular Ca^{2+}-receptive protein employed in several mechanisms of stimulus transduction in cells, thus representing an alternative target to exert Ca^{2+} antagonistic activity. Several CaM antagonists have been reported [64], in particular, neuroleptic phenothiazines have shown potent CaM blocking activity but due to their potent CNS depressant action cannot be applied clinically as cardiovascular drugs [65, 66]. Since the tricyclic ring system in phenothiazines plays a crucial role in CNS activity, Kajino and co-workers [67] focused their attention on the BT skeleton, lacking one benzene ring of the phenothiazine structure, and synthesized a series of 2-[(4-phenyl-1-piperazinyl)alkyl]benzothiazines (**54-102**) and tested them for Ca^{2+} antagonistic and CaM antagonistic activity. Benzothiazine precursors wereprepared starting from various 2-aminothiophenols. Compounds **54-102** were synthesized by the reaction of benzothiazinic precursors with various phenylpiperazines with good yields.

Ca^{2+} antagonistic and CaM antagonistic activities of synthesized compounds are listed in Table **5**.

While all compounds showed only moderate Ca^{2+} blocking activity, some exerted CaM antagonistic activity comparable to that of trifluoperazine. Structurally, the R_4 position appears to be the most important for this type of action; in particular, 2-methoxy and 4-fluoro derivatives showed increased CaM antagonistic activity.

NEUROPROTECTIVE/NEUROTOXIC EFFECTS

Some Ca^{2+} channel antagonists, such as nimodipine [68] and nicardipine [69], can exert protective action against ischemia in neurons, but clinical application is limited by strong cardiovascular effects. T-477 is a benzothiazinic analogue of diltiazem, synthesized at the Discovery Research Laboratory of Tanabe Seiyaku Co. [70], and has shown significant differences in pharmacological activity with respect to the parent compound.



T-477

Chart 13.

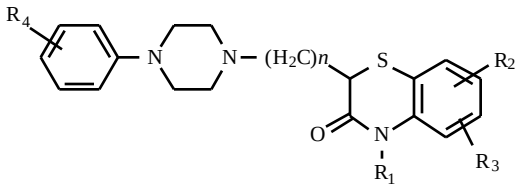
Ishii and co-workers showed that T-477 possesses protective effects on brain neurons of rats in ischemic models [71]. Experiments on cloned brain Ca^{2+} channels expressed in *Xenopus* oocytes showed that T-477 can exert this type of action due to its unique inhibitory spectrum among Ca^{2+} channel antagonists. T-477 blocks not only the L-type Ca^{2+} channels but also the P/Q-type BI, N-type BIII and R-type BII channels**.** It also shows selectivity for brain L-type Ca^{2+} channels, compared to cardiac L-type channels [72], and inhibits Na^+ channels thus reducing directly and indirectly Ca^{2+} influx in the cells [73].

Brain edema is the most common cause of death during the first week after stroke [74]. Swelling of the cells is mediated by a massive influx of Na^+ and Ca^{2+} and can lead to dysfunction and/or disruption of cellular membranes. T-477 may inhibit brain edema induced by cerebral ischemia. In particular, it is effective in the penumbra, the region between the ischemic core and the normal brain, suggesting that it could be used to limit the damaged area.

More recently, the same authors discovered that T-477 inhibits the fast Na^+ current of isolated rat brain neurons with a value of IC_{50} 7.8 μM [75], while IC_{50} of the same compound on cardiac and neuronal Ca^{2+} channels is about 50 μM [76]. An *in vitro* assay was performed to evaluate the capacity of T-477 to protect against neuronal injury induced by veratridine, a Na^+ channel site 2 agonist that determines both Na^+ and Ca^{2+} intracellular concentration increase. The results of this study suggest that T-477 could inhibit Ca^{2+} influx mainly through an indirect blocking action on neuronal Na^+ channels.

1,4-BT-induced neurotoxic effects may play a role in neurodegenerative disease such as Parkinson's disease. Parkinson's disease results from the rather selective degeneration of nigrostriatal dopaminergic neurons caused by pathological processes that take place in the cell bodies located in the substantia nigra pars compacta. This pathological process includes oxidative stress and a defect in complex I mitochondrial respiration. Based on firm evidence, Dryhurst *et al*. [76] proposed that in a very early stage in the pathogenesis of idiopathic Parkinson's disease there may be an elevated traslocation of L-cysteine into neuromelanin-pigmented dopaminergic cell bodies in the substantia nigra and that the influx of L-cysteine would divert the neuromelanin pathway by scavenging dopamine-*o*-quinone, the proximate autoxidation product of dopamine, to give 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2*H*-1,4-benzothiazine-3-carboxylic acid (DHBT-1) and other cysteinyldopamines and dihydrobenzothiazines. Successively the same authors advanced the possibility that DHBT-1 might be an endotoxin formed specifically in pigmented dopaminergic neurons that contribute to irreversible damage to mithocondrial complex I and substantia nigra cell death in Parkinson's disease [77] (Scheme **3**).

Table 5. Biological Activities of Compounds 54-102



Compd	R_1	R_2	R_3	n	R_4	Ca^{2+} blocking activity %[a]	CaM antagonistic activity IC_{50}
54	H	H	H	2	H	20	$>10^{-5}$
55·HCl	Me	H	H	2	H	40	$>10^{-5}$
56	H	H	H	3	H	70	1.1×10^{-6}
57	H	H	H	1	4-F	35	$>10^{-5}$
58	H	H	H	2	4-F	60	$>10^{-5}$
59	H	H	H	3	4-F	53	$>10^{-5}$
60	Me	H	H	3	4-F	-	$>10^{-5}$
61	H	H	H	4	4-F	30	$>10^{-5}$
62·HCl	Me	H	H	2	3-Cl	17	$>10^{-5}$
63	H	H	H	2	2-MeO	15	6.4×10^{-7}
64·2HCl	Me	H	H	2	2-MeO	0	$>10^{-5}$
65·2HCl	Et	H	H	2	2-MeO	15	8.1×10^{-6}
66·Oxalate	H	H	H	3	2-MeO	10	$>10^{-5}$
67·HCl	H	5-Me	H	3	H	40	$>10^{-5}$
68·2HCl	H	5-Me	H	3	2-F	28	2.5×10^{-6}
69·2HCl	H	5-Me	H	3	3-F	48	1.8×10^{-6}
70	H	5-Me	H	3	4-F	41	4.1×10^{-7}
71·2HCl	H	5-Me	H	3	3-Cl	50	$>10^{-5}$
72·2HCl	H	5-Me	H	3	4-Cl	40	$>10^{-5}$
73·2HCl	H	5-Me	H	3	3-CF_3	55	$>10^{-5}$
74·2HCl	H	5-Me	H	3	2-Me	11	8.7×10^{-7}
75·2HCl	H	5-Me	H	3	3-Me	50	1.5×10^{-6}
76	H	5-Me	H	3	4-Me	77	8.9×10^{-6}
77·2HCl	H	5-Me	H	3	2-MeO	33	3.6×10^{-6}
78	H	5-Me	6-Me	3	4-F	25	$>10^{-5}$
79	H	5-Me	7-Me	3	4-F	70	$>10^{-5}$
80·2HCl	H	5-Me	7-Me	3	2-MeO	72	$>10^{-5}$
81·2HCl	H	5-Me	8-Me	3	4-F	10	7.0×10^{-6}
82	H	5-Et	H	3	4-F	55	$>10^{-5}$
83	H	5-MeO	H	3	H	20	1.2×10^{-6}
84	H	5-MeO	H	3	2-MeO	18	1.2×10^{-6}
85	H	6-Cl	H	3	2-MeO	40	1.2×10^{-8}
86	H	6-CF_3	H	3	4-F	45	5.0×10^{-6}

(Table 5). contd.....

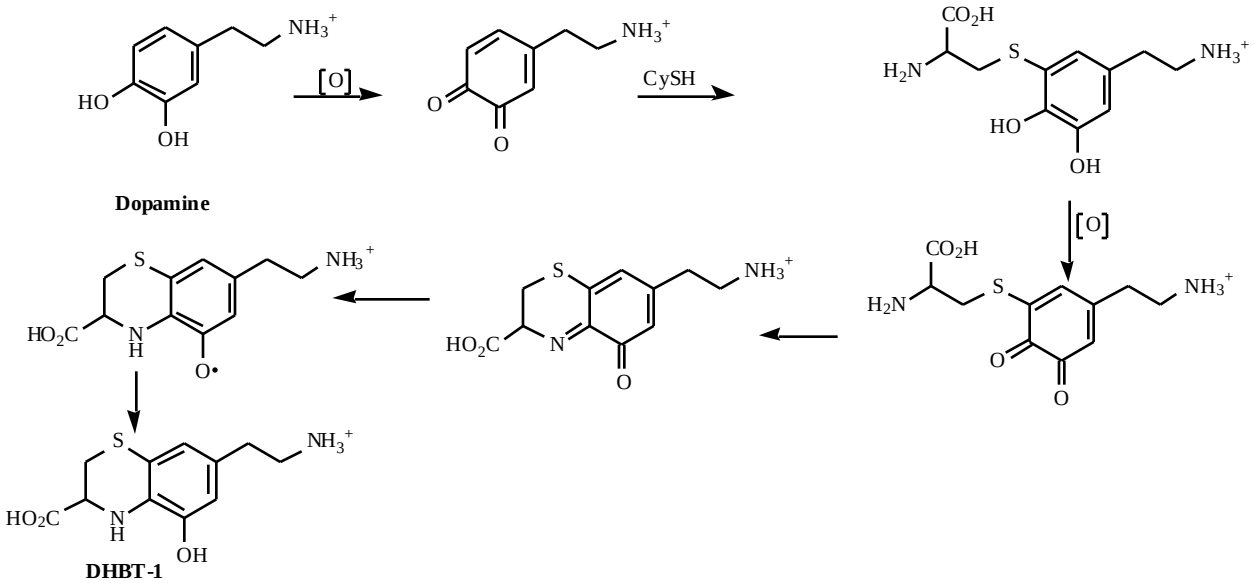
Compd	R_1	R_2	R_3	n	R_4	Ca^{2+} blocking activity %[a]	CaM antagonistic activity IC_{50}
87·2HCl	H	6-Me	H	3	4-F	16	3.9×10^{-6}
88	H	6-Me	7-Me	3	4-F	50	$>10^{-5}$
89·2HCl	H	6-Me	7-Me	3	2-MeO	40	$>10^{-5}$
90·2HCl	H	6-Me	8-Me	3	4-F	40 (10^{-6} M)	5.3×10^{-6}
91	H	7-Cl	H	3	2-MeO	34	4.0×10^{-7}
92·2HCl	H	7-Me	H	3	4-F	20	8.1×10^{-7}
93·2HCl	H	7-Me	H	3	2-MeO	32	6.5×10^{-6}
94	H	7-MeO	H	2	69	15	2.4×10^{-6}
95	H	7-MeO	H	3	70	10	$>10^{-5}$
96	H	7-MeO	H	3	2-MeO	26	1.0×10^{-5}
97.2HCl	H	5,6-$(CH_2)_4$-		3	4-F	35	4.5×10^{-7}
98	H	5,6-$(CH{=}CH)_2$-		3	4-F	21	3.9×10^{-6}
99	H	6,7-$(CH_2)_3$-		3	4-F	14	5.2×10^{-7}
100	H	6,7-$(CH_2)_4$-		3	4-F	19	5.9×10^{-7}
101·2HCl	H	6,7-OCH_2O-		3	4-F	22	3.5×10^{-6}
102·HCl	H	7,8-$(CH_2)_4$-		3	4-F	-	3.6×10^{-6}
Trifluoroperazine							4.1×10^{-6}

[a]% of inhibition of muscle contraction induced by KCl 60 mM; drugs tested at 10^{-5} M.

ANTI-TUMOR COMPOUNDS

In vivo anti-tumor efficacy of 1,4-BT has been described and attributed to direct cytotoxic activity against neoplastic cells. Inoue *et al*. [78] showed that dihydro-1,4-benzothiazine-6,7-dione is the ultimate toxic metabolite produced by tyrosinase oxidation of 4-S-cysteaminylphenol, a phenolic thioether evaluated for melanocytotoxicity. Riccardi and co-workers analyzed the mechanisms underlying 1,4-BT-mediated cytotoxicity and observed that 1,4-BT derivatives induce thymocyte apoptosis through a complex signalling pathway requiring the activation of different biochemical events that include rapid activation of phosphatidylcholine specific-phospholipase C and acidic sphingomyelinase, loss of mitochondrial membrane potential, cytochrome *c* release and caspase activation [79].

A set of representative 1,4-BT derivatives **1**, **2**, **6-8**, **10**, **11**, **13**, **14**, **17**, **20** (see Table **1**), previously synthesized [8, 9], was assessed for apoptotic activity. Preliminary SAR



Scheme 3. Simplified biosynthetic pathway of DBHT-1.

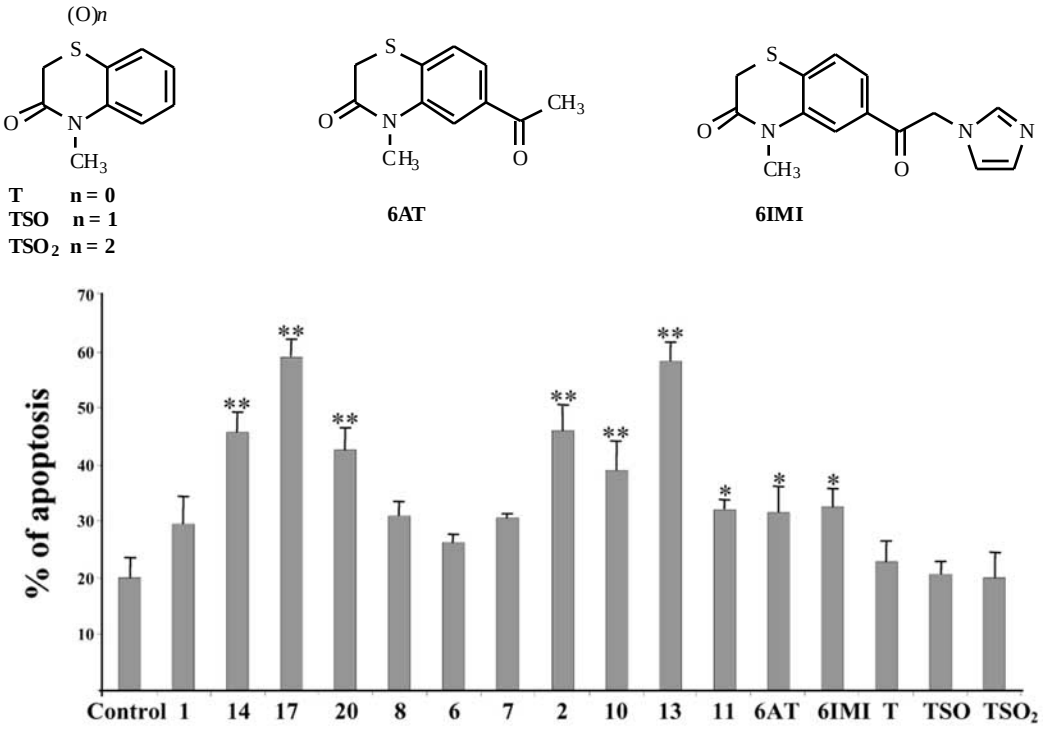


Fig. (1). 1,4-BT *in vitro* apoptotic activity. Apoptosis was tested as DNA fragmentation by PI assay, using thymocytes treated with different 1,4-BT analogs (10 µg/mL) for 18 h. * $p < 0.05$; ** $p < 0.001$ compared with untreated thymocytes.

analysis focused attention on the sulphur oxidation state because an increase in the oxidation state resulted in a significant increase in apoptosis. Insertion of the side chain at different positions on the aromatic ring also influenced apoptosis and suggested that substitution at position 6 yielded higher activity. Neither replacing IMI with a TRI nor introducing a second CH_2-TRI group or a CH_2-IMI group modified apoptotic activity. Transformation of the alcoholic group into an ether group clearly increased activity. Side chain length also appeared to be involved as apoptotic activity decreased as the side chain shortened.

Further studies focused on structural modifications such as a different skeleton (1,4-benzoxazine or 1,2,3,4-tetrahydroquinoline), azolic substituent in the side chain (1*H*-benzimidazole, 1-(2-methoxyphenyl)piperazine, 2-methylpiperazine and 1-methylpiperazine) and side chain (presence, absence or transformation of alcoholic group) [80]. Replacing the 1,4-BT skeleton with 1,2,3,4-tetrahydroquinoline results in slight changes while replacement with 1,4-benzoxazine significantly decreases activity. Replacing imidazole in the side chain with different piperazines significantly decreases activity, while replacing imidazole with benzimidazole causes only slight changes in apoptotic activity. Removing the side chain alcoholic group by dehydration to olefin significantly increases activity. These findings could account for the pharmacological effects associated with 1,4-BT treatment, including its well-described neurotoxicity and anti-tumor activity.

REFERENCES

[1] Gupta, R.R. Phenothiazines and Benzothiazines Chemical and Biomedical Aspects, Elsevier: Amsterdam, **1988**.

[2] Imwidthaya, P.; Poungvarin, N. *Postgrad. Med. J.,* **2000**, *76*, 85.

[3] Steenbergen, J.N.; Casadevall, A. *J. Clin. Microbiol.,* **2000**, *38*, 1974.

[4] Maertens, J.A.; Boogaerts M.A. *Curr. Pharm. Des.,* **2000**, *6*, 225.

[5] Sheehan, D.J.; Hitchcock, C.A.; Sibley, C.M. *Clin. Microbiol. Rev.*, **1999**, *12*, 40.

[6] Lamb, D.; Kelly, D.; Kelly, S. *Drug Res. Updates*, **1999**, *2*, 390.

[7] Baruffini, A.; Pagani, G.; Amoretti, L. *Il Farmaco*, **1967**, *22*, 528.

[8] Fringuelli, R.; Schiaffella, F.; Bistoni, F.; Pitzurra, L.; Vecchiarelli, A. *Bioorg. Med. Chem*., **1998**, *6*, 103.

[9] Schiaffella, F.; Guarraci, A.; Fringuelli, R.; Pitzurra, L.; Bistoni, F.; Vecchiarelli, A. *Med. Chem. Res.*, **1999**, *9*, 291.

[10] Fringuelli, R.; Schiaffella, F.; Vecchiarelli, A. *J. Chemother*., **2001**, *13*, 9.

[11] Pitzurra, L.; Fringuelli, R.; Perito, S.; Schiaffella, F.; Barluzzi, R.; Bistoni, F.; Vecchiarelli, A. *Antimicrob. Agents Chemother.,* **1999**, *43*, 2170.

[12] Podust, L. M.; Poulos, T. L.; Waterman, M.R. *PNAS*, **2001**, *98*, 3068.

[13] Macchiarulo, A.; Costantino, G.; Fringuelli, D.; Vecchiarelli, A.; Schiaffella, F.; Fringuelli, R. *Bioorg. Med. Chem.,* **2002**, *10*, 3415.

[14] Gubner, R.; August, S.; Ginsberg, V. *Am. J. Med. Sci.*, **1951**, *221*, 176.

[15] Chabner, B.A.; Allegra, C.J.; Curt, G.A.; Clendeninn, N.J.; Baram, J.; Koizumi, S.; Drake, J.C.; Jolivet, J. *J. Clin. Invest*., **1985**, *76*, 907.

[16] Ahern, M.J.; Kevat, S.; Hill, W.; Hayball, P.J.; Harley, H.; Hall, P. *Ann. Rheum. Dis*., **1991**, *50*, 477.

[17] Matsuoka, H.; Ohi, N.; Mihara, M.; Suzuki, H.; Miyamoto, K.; Maruyama, N.; Tsuji, K.; Kato, N.; Akimoto, T.; Takeda, Y.; Yano, K.; Kuroki, T. *J. Med. Chem.*, **1997,** *40*, 105.

[18] Urakawa, K.; Mihara, M.; Takagi, N.; Kawamura, A.; Akamatsu, K.; Takeda, Y. *Eur. J. Pharmacol.,* **2002**, *435*, 237.

[19] Urakawa, K.; Mihara, M.; Suzuki, T.; Kawamura, A. *Immunopharmacology*, **2000**,*48*, 137.

[20] Montesinos, M.C.; Desai, A.; Delano, D.; Chen, J.F.; Fink, J.S.; Jacobson, M.A.; Cronstein, B.N. *Arthritis Rheum.*, **2003**, *48*, 240.

[21] Mihara, M.; Takagi, N.; Urokawa, K.; Morya. Y.; Takeda, Y. *Int. J. Immunopharmacol.*, **1997**, *19*, 67.

[22] Timmerman, H.; Onogi, K.; Tamura, M.; Tohma, T.; Wada, Y. *PCT Int. Appl.* **1999**, WO 9902520

[23] Toshiaki, T.; Matsumoto, J.; Tohma, T.; Kanke, T.; Wada, Y.; Nagao, M.; Inagaki, N.; Nagai, H.; Zhang, M.; Timmerman, H. *Jpn. J. Pharmacol.*, **2001**, *86*, 55.
[24] Toshiaki, T.; Watanabe, C.; Saiki, I.; Tohma, T.; Nagai, H. *Biol. Pharm. Bull.*, **2001**, *24*, 1127.
[25] Kador, P.F.; Kinoshita, J.H.; Sharpless, E. *J. Med. Chem.*, **1985**, *28*, 841.
[26] Tawada, H.; Sugiyama, Y.; Ikeda, H.; Yamamoto, Y.; Meguro, K. *Chem. Pharm. Bull.*, **1990**, *38*, 1238.
[27] Aotsuka, T.; Hosono. H.; Kurihara, T.; Nakamura, Y.; Matsui, T.; Kobayashi, F. *Chem. Pharm. Bull.*, **1994**, *42*, 1264.
[28] Bouzoubaa, M.; Leclerc, G.; Decker, N.; Andermann, G. *J. Med. Chem.*, **1984**, *27*,1291.
[29] Imbs, J.L.; Miesch, F.; Schwartz, J.; Velley, J.; Leclerc, G.; Mann. A.; Wermuth, C. G. *Br. J. Pharmacol*., **1977**, *60*, 357.
[30] Prasad, N.R. *J. Med. Chem*., **1969**, *12*, 290.
[31] Cecchetti, V.; Fringuelli, R.; Schiaffella, F.; Fravolini, A.; Bruni, G.; Fiaschi, A. I.; Segre, G. *Eur. J. Med. Chem.*, **1989**, *24*, 479.
[32] Schiaffella, F.; Fringuelli, R.; Cecchetti, V.; Fravolini, A.; Angeli, P.; Marucci, G. *Il Farmaco*, **1990**, *45*, 1299.
[33] Gifford, R.W.; Borazanian, R.A. *Hypertension*, **1989**, *13*, 119.
[34] Cecchetti, V.; Schiaffella, F.; Tabarrini, O.; Zhou, W.; Fravolini, A.; Goi, A.; Bruni, G.; Segre, G. *Eur. J. Med. Chem*., **1991**, *26*, 381.
[35] Cecchetti, V.; Fravolini, A.; Schiaffella, F.; Tabarrini, O.; Bruni, G.; Segre, G. *J. Med. Chem.*, **1993**, *36*, 157.
[36] Cecchetti, V.; Schiaffella, F.; Tabarrini, O.; Fravolini, A. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 465.
[37] De Marinis, R.M.; Wise, M.; Hieble, J.P.; Ruffolo, Jr. R.R. In *The α-1 Adrenergic Receptors*; Ruffolo, Jr. R. R., Ed.; Humana Press: Clifton, NJ, **1987**; pp. 241-258.
[38] Russo, F.; Romeo, G.; Guccione, S.; De Blasi, A. *J. Med. Chem*., **1991**, *34*, 1850.
[39] Chern, J.W.; Tao, P.L.; Wang, K.C.; Gutcait, A.; Liu, S.W.; Yen, M.H.; Chien, S.L.; Rong, J.K. *J. Med. Chem*., **1998**, *41*, 3128.
[40] Cecchetti, V.; Fravolini, A.; Fringuelli, R.; Schiaffella, F.; Mascellani, G.; Pagella, P.G.; Rugarli, P.L. *Il Farmaco*, **1987**, *42*, 61.
[41] Quadri, L.; Gobblini, M.; Monti, L. *Curr. Pharm. Des*., **1998**, *4*, 489.
[42] Gopalakrishnan, M.; Janis, R.A.; Triggle, D.J. *Drug Dev. Res.*, **1993**, *28*, 95.
[43] Ulrich, Q. *Trends Pharmacol. Sci.*, **1993**, *14*, 332.
[44] Matsumoto, Y.; Tsuzuki, R.; Matsuhisa, A.; Yoden, T.; Yamagiwa, Y.; Yanagisawa, I.; Shibanuma, T.; Nohira, H. *Bioorg. Med. Chem.*, **2000**, *8*, 393.
[45] Cecchetti, V.; Calderone, V.; Tabarrini, O.; Sabatini, S.; Filipponi, E.; Testai, L.; Spogli, R.; Martinotti, E.; Fravolini, A. *J. Med. Chem.*, **2003**, *46*, 3670.
[46] Varadi, G.; Mori, Y.; Mikala, G.; Schwartz, A. *Trends Pharmacol. Sci.*, **1995**, *16*, 43.
[47] Singh, B.N.; Ellrodt, G.; Peter, C.T. *Drugs*, **1978**, *15*, 169.
[48] Opie, L.H. *Lancet*, **1980**, *I*, 806.
[49] Klieman, R.L.; Stephenson, S.H. *Q. Rev. Drug Metab. Drug Interact.*, **1985**, *5*, 193.
[50] Gibson, R.S.; Boden, W.E.; Theroux, P.; Strauss, H.D.; Pratt, C.M.; Gheorghiade, M.; Capone, R.J.; Crawford, M.H.; Schlant, R.C.; Kleiger, R.E.; Young, P.M.; Schechtman, K.; Perryman, M.B.; Roberts, R. *N. Engl. J. Med.*, **1986**, *315*, 423.
[51] Fujita, M.; Ito, S.; Ota, A.; Kato, N.; Yamamoto, K.; Kawashima, Y.; Yamauchi, H.; Iwao, J. *J. Med. Chem*., **1990**, *33*, 1898.
[52] Kojic-Prodic, B.; Ruzic-Toros, Z.; Sunjic, V.; Decorte, E.; Moimas, F. *Helv. Chim. Acta*, **1984**, *67*, 916.
[53] Ota, A.; Kawashima, Y.; Ohishi, H.; Ishida, T. *Chem. Pharm. Bull*., **1993**, *41*, 1681.
[54] Kritz, H.; Underwood, S.R.; Sinzinger, H. *Wr. Klin. Wschr*., **1996**, *108*, 98.
[55] Kritz, H.; Underwood, S.R.; Sinzinger, H. *Wr. Klin. Wschr*., **1996**, *108*, 123.
[56] Bouvier, C.A. *Drug Res.*, **1983**, *33*, 1401.
[57] Holzgreve, H.; Burkle, B. *J. Hypertens*., **1993**, *11*, 55.
[58] Weinstein, D.B.; Heider, J.G. *Am. J. Cardiol*., **1987**, *59*, B163.
[59] Wolfram, R.M.; Kritz, H.; Oguogho, A.; Sinzinger, H. *Thromb. Res.*, **2002**, *106*, 187.
[60] Miyawaki, N.; Yamazaki, T.; Furuta, T.; Shigei, T.; Yamauchi, H. *Drug Dev. Res.*, **1991**, *22*, 293.
[61] Kodama, I.; Suzuki, R.; Maruyama, K.; Toyama, J. *Br. J. Pharmacol.*, **1995**, *114*, 503.
[62] Yoshikawa, H.; Shimizu, E.; Kawahara, K.; Kuniyasu, A.; Shibano, T.; Nakayama, H. *Heterocycles*, **2003**, *59*, 613.
[63] Hara, Y.; Nakaya, H. *Br. J. Pharmacol.*, **1995**, *116*, 2750.
[64] Tanaka, T. *Jpn. J. Pharmacol.*, **1988**, *46*, 101.
[65] Levin, R.M.; Weiss, B. *Mol. Pharmacol.*, **1977**, *13*, 690.
[66] Ingagki, M.; Tanaka, T.; Hidaka, H. *Pharmacoogy*, **1983**, *27*, 125.
[67] Kajino, M.; Mizuno, K.; Tawada, H.; Shibouta, Y.; Nishikawa, K.; Meguro, K. *Chem. Pharm. Bull.*, **1991**, *39*, 2888.
[68] Steen, P.A.; Newberg, L.A.; Milde, J.H.; Michenfelder, J.D. *J. Cereb. Blood Flow Metab.*, **1983**, *3*, 38.
[69] Alps, B.J.; Calder, C.; Hass, W.K.; Wilson, A.D. *Br. J. Pharmacol.*, **1987**, *91*, 312P.
[70] Maeda, K.; Ozeki, M.; Yasuda, K.; Kodato, S.; Kudo, Y. *Eur. Pat. Appl.* EP0441539, **1991**.
[71] Ishii, T.; Okamoto, M.; Kume, T.; Narita, H.; Kudo, Y.; Matsuoka, Y. *Jpn. J. Pharmacol.*, **1996**, *71*, 175P.
[72] Kobayashi, T.; Strobeck, M.; Schwartz, A.; Mori, Y. *Eur. J. Pharmacol.*, **1997**, *332*, 313.
[73] Okuyama, K.; Kiuchi, S.; Okamoto, M.; Narita, H.; Kudo, Y. *Jpn. J. Pharmacol.*, **1999**, *81*, 170.
[74] White, O.B.; Norris, J.W.; Hachinski, V.C.; Lewis, A. *Stroke*, **1979**, *10*, 743.
[75] Okuyama, K.; Kiuchi, S.; Okamoto, M.; Narita, H.; Kudo, Y. *Eur. J. Pharmacol.*, **2000**, *398*, 209.
[76] Shen, X. M.; Dryhurst, G. *Chem. Res. Toxicol.*, **1996**, *9*, 751.
[77] Li, H.; Dryhurst, G. *J. Neurochem*., **1997**, *69*, 1530.
[78] Inoue, S.; Hasegawa, K.; Wakamatsu, K.; Ito, S. *Melanoma Res.*, **1998**, *8*, 105.
[79] Marchetti, C.; Ulisse, S.; Bruscoli, S.; Migliorati, G.; Schiaffella, F.; Cifone, G.; Riccardi, C.; Fringuelli, R. *J. Pharmacol. Exp. Ther.*, **2002**, *300*, 1.
[80] Fringuelli, R.; Schiaffella, F.; Utrilla Navarro, M.P.; Milanese, L.; Santini, C.; Rapucci, M.; Marchetti, C.; Riccardi, C. *Bioorg. Med. Chem.*, **2003**, *11*, 3245.