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Design, synthesis, and biological testing of thiosalicylamides as a novel class of calcium channel blockers

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Abstract—The current research aimed to investigate the importance of the heterocyclic ring system in the structure of the cardiovascular drug diltiazem for its calcium channel blocking activity. The manuscript describes the design, synthesis, and biological testing of a total of 10 *S*-(*p*-methoxybenzyl), N-substituted thiosalicylamides as a series of non-cyclic compounds derived from diltiazem's structure. The new compounds maintained all diltiazem pharmacophores except the thiazepine ring system. In vitro evaluation of the new series for calcium channel blocking effects revealed moderate activities with IC_{50} values in the range of 4.8–56.0 μM. The data suggest that the ring system is not essential for activity; however, its absence leads to a considerable drop of activity relative to that of diltiazem (IC_{50} = 0.3 μM). Compounds of the current series showed optimum activity when the aliphatic alkyl chain on the salicylamide nitrogen is part of a piperidine or piperazine ring system substituted at the terminal nitrogen with a benzyl group.

1. Introduction

Calcium channel blockers constitute an important class of cardiovascular drugs. Members of the class are clinically useful in the treatment of cardiovascular disorders in which calcium plays a regulatory role. These disorders include hypertension, angina, cardiac arrhythmia, congestive heart failure, ischemic injury, stroke, migraine, tumor resistance to anti-neoplastic drugs, esophageal spasms, bronchial asthma, and dysmenorrhea.[1–4] Commercially available calcium channel blockers belong to one of three chemical classes: the dihydropyridines, the phenyl alkyl amines, and the benzothiazepines. Figure 1 depicts the structures of representative members of each of the three classes. The structure–activity relationships of the dihydropyridines and the phenyl alkyl amines are well defined and previously reviewed,[5,6] but the SAR of the benzothiazepine series has not been that extensively studied. Among the interesting structural modifications conducted for the benzothiazepine series are those aimed at changing the thiazepine hetero-cycle ring size of diltiazem molecule. Modifications included both ring contraction to the five-membered benzothiazole ring[7] or the six-membered benzothiazine ring[8] and ring expansion to the eight-membered naphthothiazocine ring.[9] Figure 2 illustrates examples of molecules with contracted and expanded heterocyclic nuclei. Changing the heterocyclic ring size generated derivatives that not only retained the calcium channel blocking activity but also resulted in several compounds that were more active than diltiazem itself.[7–9] To gain further insight into the structure–activity relationships of diltiazem and related molecules, the current research explores the importance of the heterocyclic ring for activity. A series of molecules that lacks the ring system but maintain all other diltiazem pharmacophores was synthesized and tested for activity. As depicted in Figure 3, diltiazem molecule features four major pharmacophores: the aromatic benzene ring fused with the heterocyclic thiazepine ring (area i), the methoxy aromatic ether (area ii), the stereo-chemical centers (area iii), and the aliphatic basic nitrogen separated from an amide group with a 2-carbon chain (area iv). Barrish et al.[10] identified two essential pharmacophores for diltiazem binding to calcium channels: the aryl ether (area ii, to act as hydrogen bond acceptor), and the basic nitrogen (area iv, for ionic bonding with a negative charge on the channel). Barrish et al. also suggested that the

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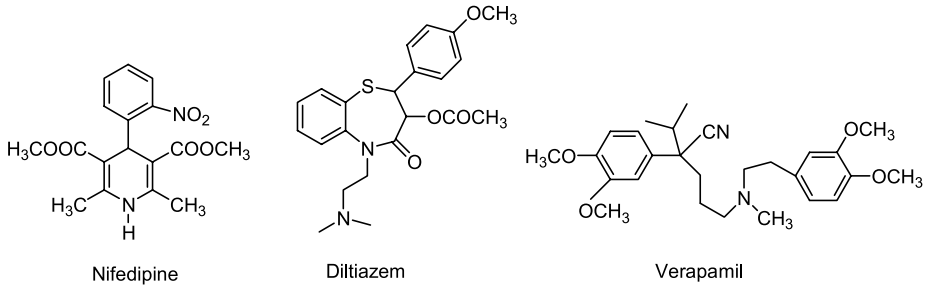


Figure 1. Examples of commercially available calcium channel blockers.

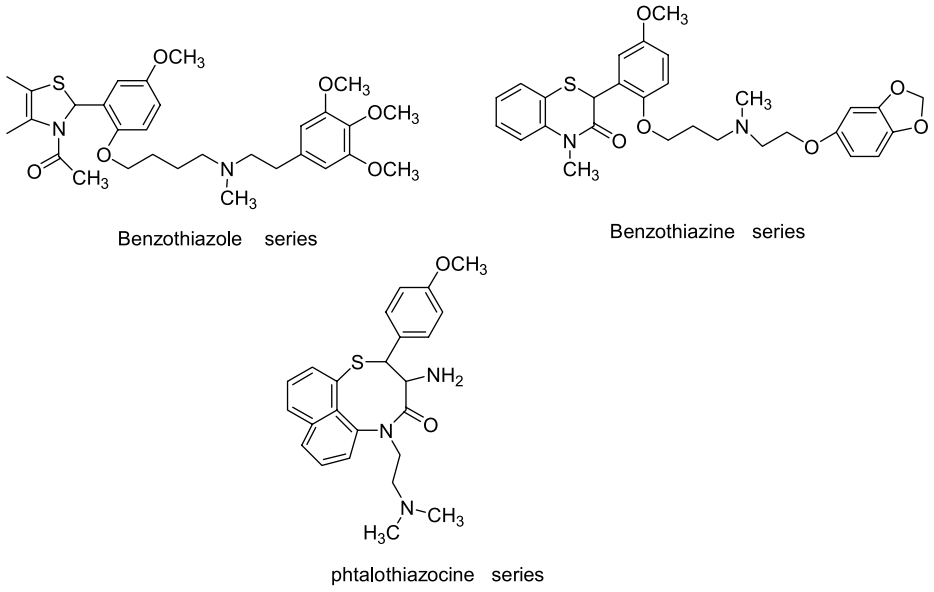


Figure 2. Examples of Diltiazem-like molecules with 5, 6 and 8-membered heterocyclic ring systems.

benzothiazepine nucleus may simply act as a spacer to hold the two pharmacophores at the required distance for binding to the receptors. Kimball et al.[11] developed a receptor-binding model identifying the benzene ring (area i) as a lipophilic group that facilitates transport into the channel, and the absolute stereochemistry (area iii) for the selective binding. The current series of compounds were designed to lack the ring system, and accordingly the stereo-chemical factors were eliminated. In spite of lacking the stereochemistry, the spatial conformational relationships of the remaining pharmacophores are expected to be maintained as suggested by Kimball et al.[11] for the fully extended methoxy-phenethylamines. Figure 3 illustrates different pharmacophoric groups and the relationship of the new thiosalicylamide derivatives (compounds **9**–**18**) to diltiazem.

2. Results and discussion

2.1. Chemistry and synthesis

Scheme 1 depicts the route to synthesize the target thiosalicylamide derivatives (compounds **9**–**18**, Fig. 3). Compounds **9** and **10** are thiosalicylamide derivatives that mimic dilatazem pharmacophoric group iv (Fig. 3) by separating the amide nitrogen from the tertiary one with a short dimethylamino aliphatic chain. Compound **11** has the aliphatic chain part of a piperidine ring system substituted with an *N*-benzyl group. Compounds **12**–**18** represent a series of derivative in which the amide nitrogen is incorporated into a piperazine ring system that is further substituted through the second nitrogen with various groups including methyl, piperonyl, benzyl, and different *p*-substituted benzyl groups. Scheme 1 depicts the synthetic route to prepare the target compounds. Reaction of thiosalicylic acid (**1**) with 4-methoxybenzyl chloride in dimethylformamide (DMF) provided *S*-(4-methoxybenzyl) thiosalicylic acid (**2**). Reaction of (**2**) with thionyl chloride provided the common intermediate acid chloride (**3**). Reaction of (**3**), without purification, with the corresponding precursor amine (Scheme 1) gave the corresponding final compounds **9**–**18**. The precursor amines were either commercially available or synthesized according to standard procedure.[12] Commercially available amines included *N*,*N*-dimethylaminoethylamine, *N*,*N*-dimethylaminopropylamine, 1-benzyl-4-aminopiperidine, 1-methylpiperazine, 1-benzylpiperazine, and 1-piperonylpiperazine. Reaction of the above listed amines with intermediate

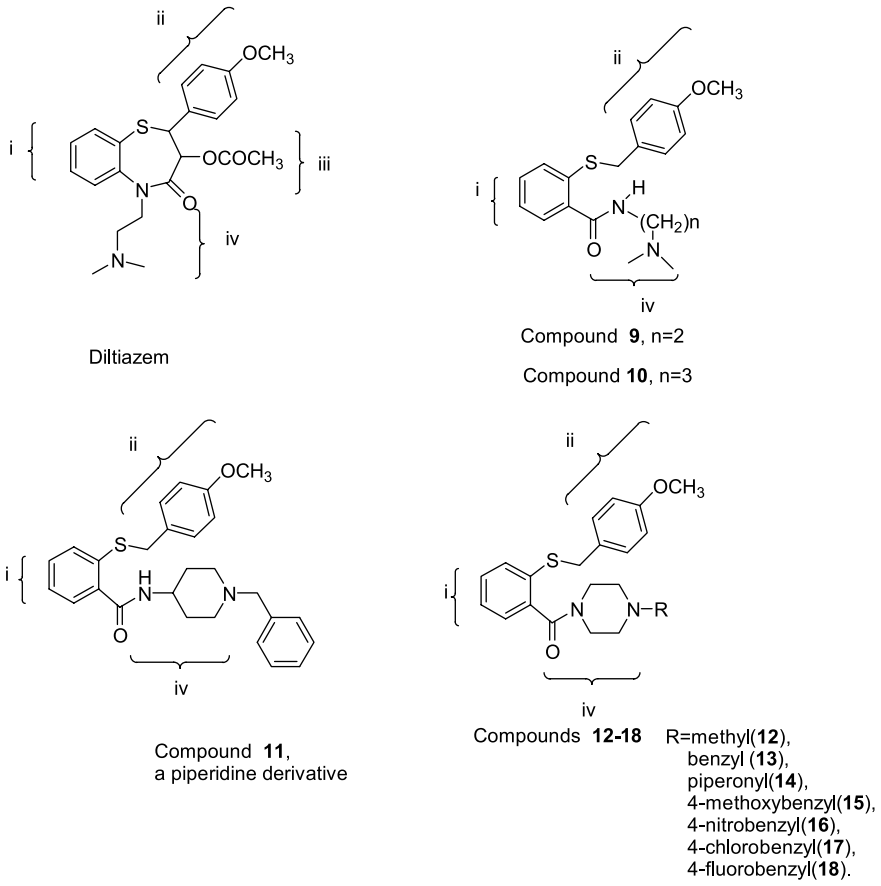
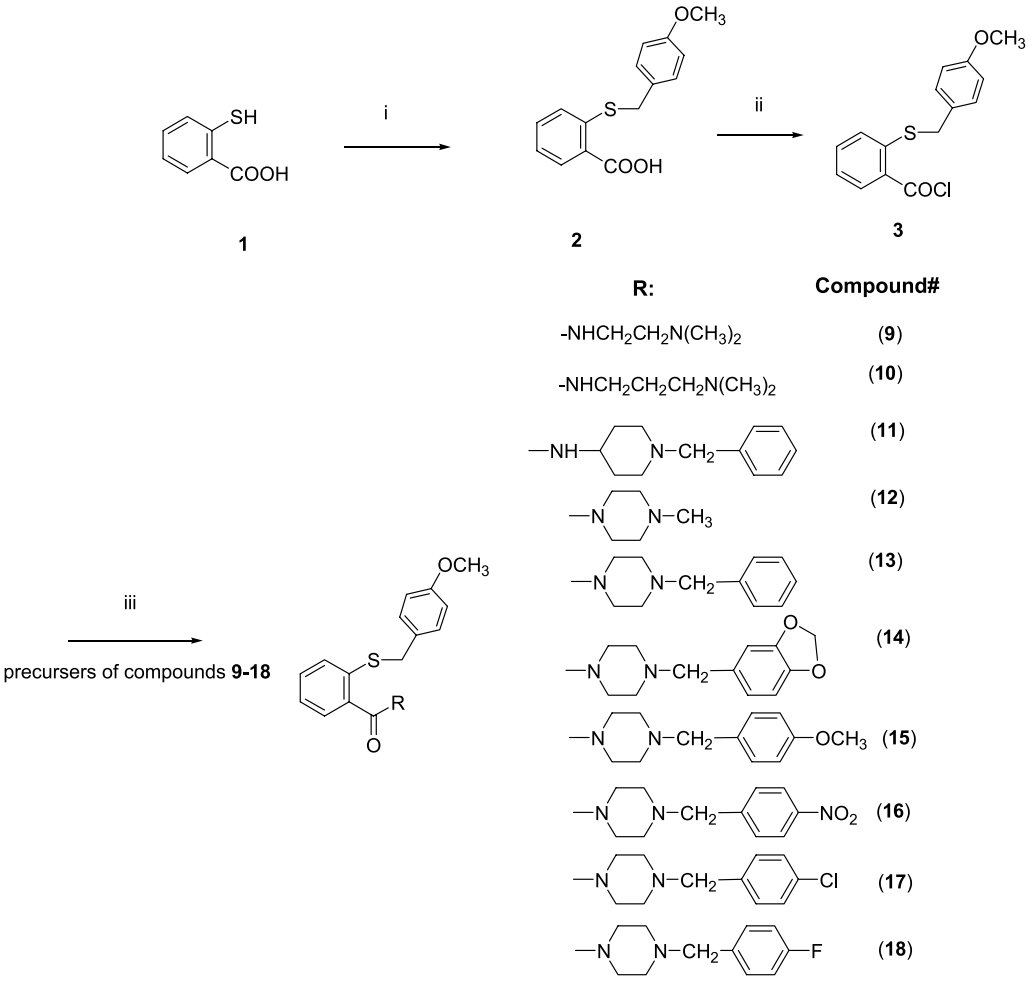


Figure 3. Comparison of the pharmacophoric groups of diltiazem and the new compounds. (i) lipophilic aromatic area, (ii) *p*-methoxyaryl group, (iii) stereo-chemical region and (iv) amide nitrogen linked to a tertiary amine with a spacer.

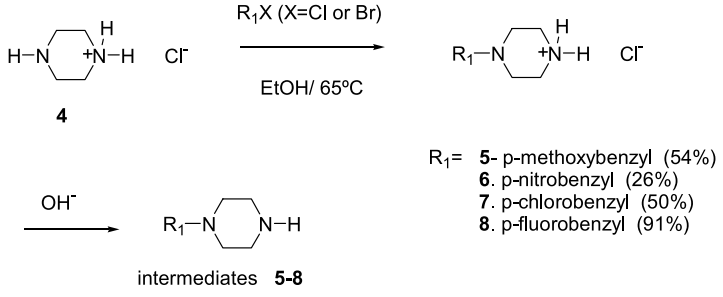
(**3**), gave compounds **9**–**14**, respectively. Commercially unavailable precursor amines required to prepare compounds **15**–**18** of Scheme 1 were synthesized according to a previously reported method[12] that is outlined in Scheme 2, and described below. Mixing of 1 equiv of piperazine with 1 equiv of piperazine dihydrochloride hydrate in warm absolute ethanol (65 °C) gave a solution containing 1 equiv of piperazine monohydrochloride (compound **4**, Scheme 2). Slow addition of 1 equiv of an alky halide to the above solution of **4** produced one equivalent of the corresponding monoalkylated piperazine (compounds **5**–**8**, Scheme 2) and 1 equiv of piperazine dihydrochloride dihydrate. The latter was insoluble in absolute ethanol and precipitated out of solution by further cooling in an ice bath. Filtration of the dihydrochloride salt followed by removal of ethanol left the desired crude intermediates 1-alkylpiperazinium chlorides (compounds **5**–**8**, Scheme 2) as either solid or oily residues. Reaction of the monosubstituted piperazine intermediates (**5**–**8**, Scheme 2) with intermediate **3** produced the desired final compounds **15**–**18** (Scheme 1), respectively. Reaction of **3** with precursor amines shown in Scheme 1 was accomplished through one of two general methods: A or B. According to method A, intermediate **3** reacted with either 2 equiv of the starting amine (in cases of inexpensive ones), or 1 equiv of the starting amine and one equivalent (or slight excess) of triethylamine. Compounds **9** and **12**–**15** were prepared using method A in yields ranging from 16% to 80% as indicated in the experimental section. Attempts to prepare compounds **10**, **11**, and **16**–**18** under method 'A' reaction conditions failed. The method was slightly modified into method B by using 10% sodium hydroxide solution as the neutralizing base instead of the extra equivalent of the precursor amine or the triethylamine. By applying method B, we successfully obtained compounds **10**, **11**, and **16**–**18** in yields ranging from 21% to 63% as detailed under experimental.

2.2. In vitro biological evaluation

All 10 compounds (**9**–**18**) were tested for inhibition of calcium-induced contractions of potassium depolarized isolated rat aorta strips according to a previously reported protocol.[13,14] The in vitro testing required incubation of the test compound with rat aorta strips in a calcium-free, potassium chloride-rich, Krebs-bicarbonate solution followed by addition of calcium chloride to elicit contraction. The contractile responses were recorded on a Grass Polygraph (model 7H, Grass Instruments, Quincy, MA), as described under experimental. Table 1 lists the calcium channel blocking activities for the new compounds, expressed as IC_{50} values, with diltiazem as the reference drug. Table 1 data indicate that in spite of lacking the heterocyclic ring system, the new compounds are moderately active as calcium channel



Scheme 1. Synthesis of thiosalicylamide derivatives. Reagents and conditions: (i) 4-methoxybenzyl chloride, K_2CO_3, DMF, reflux for 10 h; (ii) $SOCl_2$, reflux for 2 h; (iii) Method A: triethylamine, benzene, stir for 24 h at room temperature. Method B: 10% NaOH, 1,4-dioxane, stir for 24 h at room temperature.



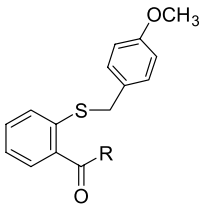
Scheme 2. Synthesis of commercially unavailable mono-substituted piperazine intermediates.

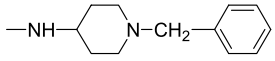
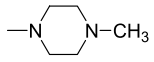
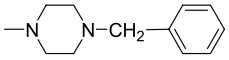
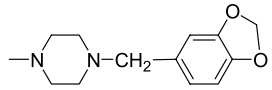
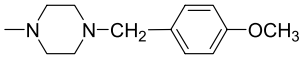
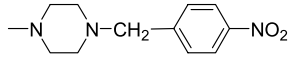
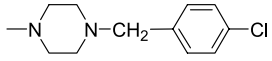
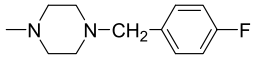
blockers. However, the high IC_{50} values of the new compounds (4.8–56.0 μM, Table 1) relative to that of diltiazem (0.3 μM) suggest that the absence of the heterocyclic ring results in a considerable drop in activity.

2.3. Structure–activity relationships (SAR)

As the IC_{50} values listed in Table 1 indicate, the new series of compounds has moderate calcium channel blocking activity. The activity of the current non-cyclic compounds clearly affirms that the heterocyclic ring system in the diltiazem structure is not essential for the calcium channel blocking activity. However, it is also evident from the IC_{50} values of the new compounds (4.8–56.2 μM, Table 1) that the absence of the ring structure leads to a significant drop in activity relative to that of diltizem (IC_{50} value of 0.3 μM). Although the new compounds have weaker calcium channel blocking activities than diltiazem, data in Table 1 provide the

Table 1. Calcium channel blocking activity of the new compounds expressed as IC_{50} in μM



Compound #	IC_{50} (μM)[a] ± S.D.	R
9	17.6 ± 8.0	–$NHCH_2CH_2N(CH_3)_2$
10	16.3 ± 3.8	–$NHCH_2CH_2CH_2N(CH_3)_2$
11	6.2 ± 1.5	
12	56.2 ± 8.5	
13	7.2 ± 3.2	
14	22.6 ± 5.7	
15	6.9 ± 1.5	
16	6.8 ± 4.1	
17	5.4 ± 2.6	
18	4.8 ± 1.2	
Diltiazem	0.3 ± 0.1	

[a] Using potassium depolarized rat aorta strips ($n = 4–5$) at 1.5 mM $CaCl_2$.

basis for the following tentative structure–activity relationships:

1. Compounds **9** and **10** showed moderate calcium channel blocking activity as reflected by the IC_{50} values of 17.6 and 16.3 μM, respectively. The two compounds have short spacer aliphatic chains (compound **9** $n = 2$, compound **10** $n = 3$) to mimic diltiazem dimethylaminoethyl pharmacophore. The closeness of the IC_{50} values indicates that the spacer length is not a crucial factor for activity.

2. Compound **12** has the aliphatic chain separating the two nitrogen atoms incorporated into a piperazine ring structure with *N*-methyl substitution. The IC_{50} value of compound **12** (56.0 μM) reflects a three-fold drop in activity from that of compounds **9** and **10** (open chain compounds with similar N-substitution). The data indicate that piperazine substituted at the terminal nitrogen with *N*-methyl group is not optimum for activity.

3. Compounds **11** and **13** are similar to compound **12** in having the side chain incorporated into a ring system but with *N*-benzyl substitution at the terminal nitrogen. Compound **11**, with the chain part of a piperidine ring, has an IC_{50} of 6.2 μM while compound **13**, with the side chain part of a piperazine ring, has an IC_{50} values of 7.2 μM. The piperidine and piperazine rings were chosen to provide compounds differing in the number of atoms separating the amide nitrogen from the basic one (three in case of compound **11** and two in case of compound **13**). The data reflect an overall 7–8 fold increase in activity over compound **12** with the *N*-methyl substitution and a 2–3 fold increase in activity over the open chain compounds **9** and **10** (IC_{50} values of 17.6 and 16.3 μM, respectively). The low IC_{50} values of compounds **11** and **13** suggest the importance of the benzyl group for enhancing activity. The close activities of the two compounds reinforce the previous conclusion that the distance separating the two nitrogen atoms is not crucial.

4. Compounds **15**–**18** have the benzyl group further substituted in the *para* position with an electron donating group (a methoxy group in compound **15**) or electron-withdrawing groups (nitro in compound **16**, chloro in compound **17**, or fluoro in compound **18**). The close IC_{50} value range of compounds **15**–**18** (4.8–6.9 μM) and that of compound **13** with unsubstituted benzyl (7.2 μM) indicates that the electronic properties of the substituent have no effect on the activity and that lipophilicity is the only determinant factor.

5. Compound **14**, with the piperazine ring substituted with a piperonyl group, showed an IC_{50} of 22.6 μM reflecting a 3–5-fold decrease of activity relative to the benzyl derivatives. If the piperonyl group is considered as a di-substituted benzyl, the weak activity of compound **14** relative to that of compounds **15**–**18** suggests that a mono substituted benzyl group is optimum for activity and di-substitution does not favor binding to the channel.

In summary of the structure–activity relationships, the calcium channel blocking activity of the new series is optimal when the alkyl side chain is part of a piperidine or piperazine ring structure further substituted with *N*-benzyl group. Neither the spacer chain length nor the type of substituent on the benzyl group plays role in the activity.

3. Conclusion

The current research reports the design, synthesis, and biological testing of 10 thiosalycilamide derivatives (**9**–**18**) as a new series of calcium channel blockers. All new compounds exhibited moderate activity in inhibiting calcium-induced contractions of rat aorta strips. The observed activities of the non-cyclic compounds suggest that the presence of a heterocyclic ring system is not essential for calcium channel blocking activity. However, the high IC_{50} values of the new compounds relative to that of diltiazem indicate that eliminating the ring structure results in a considerable drop in activity. The structure–activity relationships of the new compounds indicate optimum activity when the alkyl chain on the thiosalycylamide nitrogen is part of a piperazine

or piperidine ring system having a benzyl or para-substituted benzyl group at the terminal nitrogen. It is worthy to indicate that a United States patent has been issued, recognizing the new series of compounds as a new class of calcium channel blockers.[15]

4. Experimental

4.1. Chemistry

General information: Melting points were determined using a MEL-TEMP apparatus and are uncorrected. IR spectra were taken with a Perkin–Elmer 1420 Ratio Recording infrared spectrophotometer (Model 1420, Perkin–Elmer, Norwalk, CT) and Nicolet Impact 410 FT-IR spectrophotometers (Model: Impact, Nicolet Instrument Corporation, Madison, WI). ^{1}H NMR spectra were recorded on a Varian T-60 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The values of chemical shift (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). The reactions progress were monitored using TLC on silica gel plate (Whatman, PE SIL G/UV). Extracts were dried over $MgSO_4$ and the solvents removed under reduced pressure. Reported yields are for the purified products and not optimized. Elemental analyses were performed by Desert Analytics, Tucson, AZ.

4.2. *S*-(4-Methoxybenzyl) thiosalicylic acid (2)

To a solution of 15.42 g (0.1 mol) of thiosalicylic acid (**1**) and 41.4 g (0.3 mol) of K_2CO_3 in 150 mL of dimethylformamide (DMF), 15.6 g (0.1 mol) of 4-methoxybenzyl chloride was slowly added with stirring. The mixture was refluxed for 10 h, then cooled to room temperature. Addition of water (150 mL) and adjusting the pH to 3.0 with 3.0 M HCl resulted in the formation of white precipitate. The precipitate was collected by filtration and washed with acetone to give 27.364 g (99.7%) of intermediate (**2**) as a white powder. Mp 225–227 °C; IR (KBr) cm^{-1}: 3200–2500, 1673, 1510, 1234, 1027; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ ppm: 3.7 (s, 3H), 4.08 (s, 2H), 6.52 (s, 3H), 6.9 (d, 2H, J = 9 Hz), 7.4 (d, 2H, J = 9 Hz), 7.5 (s, 1H), 8.1 (d, H-6, J = 8 Hz).

4.3. *S*-(4-Methoxybenzyl) thiosalicyloyl chloride (3)

Compound **3** was obtained by refluxing a mixture of 822 mg (3.0 mmol) of *S*-(4-methoxybenzyl) thiosalicylic acid (compound **2**) and 10 mL thionyl chloride for two hours. Removal of excess thionyl chloride under reduced pressure left compound **3** as pale yellow solid, which was used in subsequent steps without further purification.

4.4. Piperazine monohydrochloride (4)

A solution of 1.08 g (12.5 mmol) of piperazine in 20 mL of absolute ethanol was heated to 65 °C (with continuous stirring) and 2.19 g (12.5 mmol) of piperazine dihydrochloride monohydrate was added while maintaining stirring and heating. At this point, the mixture contains 12.5 mmol of compound **4** that was used without further isolation.

4.5. 1-(4-Methoxybenzyl) piperazine (5)[12]

To the above mixture containing 12.5 mmol of compound **4**, 1.95 g (12.5 mmol) of 4-methoxybenzyl chloride were added over a 5 min period with vigorous stirring. The mixture was stirred for additional 25 min at 65 °C and was kept in an ice bath for about 30 min before filtering the precipitated piperazine dihydrochloride. The solid residue was washed with 3×5 mL of ice-cold absolute ethanol. The combined filtrates were evapo- rated to dryness to produce a solid crude product of 1-(4-methoxybenzyl)-4-piperazinium chloride. Heating the mixture with 20 mL absolute ethanol followed by rapid filtration removed any residual piperazine dihydrochloride from the solid. Concentration of the filtrate followed by cooling in ice produced 2.629 g (87%) of pure 1-(4-methoxybenzyl)-4-piperazinium chloride as white crystals having a melting point of 154–157 °C. The free base (compound **5**) was released from the above salt by dissolving it in 20 mL of water followed by alkalinization to pH 12 (with approximately 20 mL of 5.0 M sodium hydroxide solution). The mixture was extracted with methylene chloride and dried over $MgSO_4$ for 30 min. Filtration and solvent evaporation at reduced pressure left a white solid. Crystallization of the solid from petroleum ether gave 1.393 g (54%) of compound **5** as the free base. Mp: 99–101 °C; IR (KBr) cm^{-1}: 3392, 2943, 1509, 1301, 1034, 991; ^{1}H NMR ($CDCl_3$) δ ppm: 2.12 (s, 1H), 2.23–2.46 (m, 4H), 2.53–2.98 (m, 4H), 3.40 (s, 2H), 3.73 (s, 3H), 6.9 (d, 2H, J = 9 Hz), 7.1 (d, 2H, J = 9 Hz).

4.6. 1-(4-Nitrobenzyl) piperazine hydrochloride (6)

Compound **6** was obtained as a hydrochloride salt in a 26% yield using *p*-nitrobenzyl chloride in the same above procedure describing the synthesis of compound **5**. Mp: 209–212 °C; IR (KBr) cm^{-1}: 3513, 2930, 2815, 2720, 1604, 1514, 1349, 1099, 865, 743; ^{1}H NMR ($CDCl_3$) δ ppm: 2.60–2.95 (m, 4H), 3.0–3.36 (m, 4H), 3.60 (s, 2H), 7.5 (d, 2H, J = 9 Hz), 8.1 (d, 2H, J = 9 Hz).

4.7. 1-(4-Chlorobenzyl) piperazine (7)[16]

Compound **7** was obtained as free base in a 50% yield using *p*-chlorobenzyl chloride and the same procedure employed to prepare compound **5**, mp 93–97 °C; IR (KBr) cm^{-1}: 3383, 2807, 1489, 1472, 1423, 1299, 1000, 806; ^{1}H NMR ($CDCl_3$) δ ppm: 2.15–2.45 (m, 5H), 2.65–2.95 (m, 4H), 3.35 (s, 2H), 7.15 (s, 4H).

4.8. 1-(4-Fluorobenzyl) piperazine hydrochloride (8)

Compound **8** was prepared in a 91% yield, from *p*-fluorobenzyl chloride using the same procedure employed to prepare the hydrochloride salt of compound **5**. Mp 164–167 °C; IR (KBr) cm^{-1}: 3501, 2926, 2810, 2709, 2473, 1602, 1513, 1217, 1094, 765; ^{1}H NMR ($CDCl_3$) δ ppm: 2.4–2.72 (m, 4H), 2.8–3.24 (m, 4H), 3.45 (s, 2H), 7.7–7.4 (m, 4H).

4.9. *S*-(4-Methoxybenzyl), *N*-(2-(*N*′,*N*′-dimethylamino) ethyl) thiosalicylamide (9), method A, Scheme 1

Compound **9** was prepared by slowly adding a suspension of the acid chloride **3** (878 mg, 3.0 mmol) in 10 mL cold benzene to a solution of 528 mg (6 mmol) of *N*,*N*-dimethylethylenediamine in 10 mL ice cooled benzene. The mixture was stirred overnight at room temperature. Water (20 mL) was added and the aqueous layer separated. The benzene layer was further washed with 10% NaOH solution (10 mL) and the combined aqueous layers were extracted with two portions of chloroform (10 mL each) to recover any trapped product. The mixture of the combined chloroform extracts and original benzene layer were dried over anhydrous $MgSO_4$. Filtration of the drying material followed by solvent removal left compound **9** as an oily residue. Dissolving the oil in 10 mL methanol followed by the addition of 60 mL water and stirring for 24 h at room temperature gave white crystalline material. Filtration of the separated crystals and washing with water followed by petroleum ether gave 279 mg (27%) of compound **9** as pale yellow crystals. Mp of 83–84.5 °C; IR (KBr) cm^{-1}: 3322, 3257, 2943, 1633, 1511, 1029; ^1H NMR ($CDCl_3$) δ ppm: 2.2 (s, 6H), 2.4 (t, 2H, $J = 6$ Hz), 3.45 (q, 2H, $J = 5$ Hz), 3.7 (s, 3H), 4.0 (s, 2H), 6.75 (d, 2H, $J = 9$ Hz), 7.15 (d, 2H, $J = 9$ Hz), 7.25 (s, 3H) 7.5 (m, 1H); Anal. Calcd for $C_{19}H_{24}N_2O_2S$: C, 66.25; H, 7.02; N, 8.13. Found: C, 66.09; H, 7.14; N, 8.14.

4.10. *S*-(4-Methoxybenzyl) *N*-(3-(*N*′,*N*′dimethylamino) propyl) thiosalicylamide (10), method B, Scheme 1

A suspension of 1.45 g (5.0 mmol) of 3 in 10 mL warm dioxane was slowly added to an ice cooled solution of 511 mg (5.0 mmol) of 3-(dimethylamino) propylamine in 20 mL 10% NaOH. The mixture was stirred overnight at room temperature. Water (70 mL) was added and the mixture extracted with four 10 mL portions of chloroform. The extracts were dried over anhydrous $MgSO_4$. Filtration and solvent removal left an oily product that was dissolved in methanol (20 mL), followed by the addition of 100 mL water and stirring overnight at room temperature. Filtration of the separated crystals followed by washing with water, followed by petroleum ether gave 640 mg (35.7%) of pure compound **10** as white solid. Mp 97–98 °C; IR (KBr) cm^{-1}: 3254, 2936, 1634, 1510, 1437, 1250, 1028, 758; ^1H NMR ($CDCl_3$) δ ppm: 1.68 (t, 2H, $J = 9$ Hz), 2.22 (s, 6H), 2.30 (m, 2H, $J = 6$ Hz) 2.90 (d, 1H, $J = 6$ Hz), 3.4 (q, 2H, $J = 6$ Hz), 3.74 (s, 3H), 4.1 (s, 2H), 6.75 (d, 2H, $J = 9$ Hz), 7.15–7.6 (m, 6H). Anal. Calcd for $C_{20}H_{26}N_2O_2S$: C, 67.01; H, 7.31; N, 7.81. Found: C, 66.68; H, 7.47; N, 7.48.

4.11. *S*-(4-Methoxybenzyl)-(4-amino (1-benzyl) piperidinyl) thiosalicylamide (11)

Compound **11** was obtained as white solid by applying method B and using of 878 mg (3 mmol) of **3** and of 570 mg (3 mmol) of 4-amino-1-benzylpiperidine. Further purification by crystallization from ethanol/water gave 0.8 g (63%) of compound **11** as white crystals. Mp 121–122 °C; IR (KBr) cm^{-1}: 23294, 2934, 2788, 1629, 1533, 1510, 1250, 1025, 705; ^1H NMR ($CDCl_3$) δ ppm: 1.34–3.00 (m, 9H), 3.44 (s, 2H), 3.66 (s, 3H), 3.96 (s, 2H), 6.62–7.78 (m, 13H); Anal. Calcd for $C_{27}H_{30}N_2O_2S$: C, 72.61; H, 6.77; N, 6.27. Found: C, 72.28; H, 6.74; N, 6.14.

4.12. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-methylpiperazine (12)

Compound **12** was prepared using the same method described to prepare compound **9**. Reaction of 5.48 g (0.02 mol) of *S*-(4-methoxybenzyl) thiosalicylic acid and 20 mL of $SOCl_2$ produced 0.02 mol of intermediate **3** as a yellow residue. A suspension of **3** in warm benzene (30 mL) was slowly added to a 20 mL solution of 4.0 g (0.04 mol) 1-methylpiperazine in 20 mL benzene. The mixture was stirred overnight at room temperature. After the addition of 40 mL water the aqueous layer was extracted twice with 15 mL CH_2Cl_2. The combined methylene chloride extracts and the benzene layer washed with 10% NaOH were dried over anhydrous $MgSO_4$. Removal of the solvents under reduced pressure left an oily product that gave a solid upon crystallization from hot petroleum ether (10 mL). Collection of the separated solid by suction filtration followed by washing with cold petroleum ether gave 3.4 g (47%) of compound **12**. Mp 77–77.8 °C; IR (KBr) cm^{-1}: 2943, 1633, 1512, 1440, 1251, 1001; ^1H NMR ($CDCl_3$) δ ppm: 2.25 (s, 3H), 2.2–2.5 (m, 4H), 2.97–3.25 (m, 2H), 3.68 (s, 3H), 3.6–3.85 (m, 2H), 4.0 (s, 2H), 6.75 (d, 2H, $J = 9$ Hz), 7.0–7.3 (m, 6H); Anal. Calcd for $C_{20}H_{24}N_2O_2S$: C, 67.38; H, 6.79; N, 7.86; S, 9.00. Found: C, 67.15; H, 6.52; N, 7.90; S, 9.28.

4.13. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-benzylpiperazine (13)

Compound **13** was prepared using same procedure described above for compound **9** using 2.74 g (10 mmol) of intermediate **2**, 1.72 g (10 mmol) of 1-benzylpiperazine and 2.02 g (20 mmol) triethylamine. The oily product was dissolved in 5 mL of methylene chloride and purified by column chromatography on a silica gel column using ethyl acetate as an eluent to give 870 mg of compound **13** (20% yield). The product was further purified by crystallization from hot hexane to give white crystals with mp of 84–85 °C; IR (KBr) cm^{-1}: 2817, 1638, 1512, 1250, 1179, 1027; ^1H NMR ($CDCl_3$) δ ppm: 2.2–2.6 (m, 4H), 3.0–3.3 (m, 2H), 3.5 (s, 2H), 3.70 (s, 3H), 3.6–3.96 (m, 2H), 4.08 (s, 2H), 6.75 (d, 2H, $J = 8$ Hz), 7.0–7.38 (m, 11H); Anal. Calcd for $C_{26}H_{28}N_2O_2S$: C, 72.19; H, 6.52; N, 6.48. Found: C, 72.11; H, 6.69; N, 6.46.

4.14. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-piperonylpiperazine (14)

A suspension of 878 mg (3 mmol) of **3** in warm benzene (10 mL) was slowly added to an ice cooled benzene solution (10 mL) of 661 mg (3 mmol) of 1-piperonylpiperazine and 455 mg (4.5 mmol) triethylamine and

stirred overnight at room temperature. Addition of water (20 mL) formed an aqueous layer that was extracted with 2×15 mL portions of $CHCl_3$. The combined chloroform extracts were combined with the benzene layer, washed with 10% NaOH solution and dried over anhydrous $MgSO_4$. Removal of the solvents under reduced pressure left compound **14** as an oily product. The oil was dissolved in $CHCl_3$ (5 mL) then cooled in ice bath. Addition of petroleum ether precipitated compound **14** as white solid. Filtration followed by washing with acetone gave 1.144 g (80%) of the required product. Mp 126–127 °C; IR (KBr) cm^{-1}: 2929, 2817, 1634, 1610, 1517, 1488, 1254, 1035, 786; ^1H NMR ($CDCl_3$) δ ppm: 2.2–2.6 (m, 4H), 3.0–3.25 (m, 2H), 3.4 (s, 2H), 3.78 (s, 3H), 3.6–3.9 (m, 2H), 4.0 (s, 2H), 5.85 (s, 2H), 6.7 (s, 3H), 6.8 (s, 2H), 7.0–7.35 (m, 6H); Anal. Calcd for $C_{27}H_{28}N_2O_4S \cdot 1/2H_2O$: C, 66.78; H, 6.01; N, 5.77; S, 6.60. Found: C, 66.69; H, 6.02; N, 5.79; S, 6.75.

4.15. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-(4-methoxybenzyl) piperazine (15)

Compound **15** was prepared by the same method employed to prepare compound **14** using 823 mg (3 mmol) of **2**, 728 mg (3 mmol) 1-(4-methoxybenzyl) piperazine, and 1.36 g (13.5 mmol) triethylamine. The compound was obtained as a dark brown oil, which upon crystallization from acetone (5 mL)/water (20 mL) gave a solid material. Further purification by column chromatography on a silica gel column (ethyl acetate as eluent) gave 220 mg (16%) of compound **15**. Mp 99–101 °C; IR (KBr) cm^{-1}: 2950, 1635, 1513, 1430, 1251, 1178; ^1H NMR ($CDCl_3$) δ ppm: 2.2–2.6 (m, 4H), 3.0–3.25 (m, 2H), 3.4 (s, 2H), 3.75 (s, 3H), 3.78 (s, 3H), 3.6–3.9 (m, 2H), 4.0 (s, 2H), 6.65 (d, 2H, $J = 8$ Hz), 6.85 (d, 2H, $J = 8$ Hz), 7.0–7.3 (m, 8H); Anal. Calcd for $C_{27}H_{30}N_2O_3S$: C, 70.10; H, 6.54; N, 6.06. Found: C, 70.34; H, 6.80; N, 5.83.

4.16. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-(4-nitrobenzyl) piperazine (16)

Compound **16** was prepared according to method B and in a similar procedure to that used to prepare compound **11**. A suspension of 878 mg (3 mmol) of **3** in warm dioxane (10 mL), was slowly added to an ice cooled solution of 773 mg (3 mmol) of 1-(4-nitrobenzyl) piperazine in 10 mL 10% NaOH solution with stirring overnight at room temperature. The addition of water (70 mL) resulted in the separation of white precipitate. Collection of the precipitate by suction filtration followed by washing with acetone gave 602 mg (42%) of compound **16** as a white solid. Mp 122–123 °C; IR (KBr) cm^{-1}: 2913, 1622, 1508, 1439, 1179, 1350, 1241, 1024, 837; ^1H NMR ($CDCl_3$) δ ppm: 2.2–2.6 (m, 4H), 2.98–3.28 (m, 2H), 3.55 (s, 2H), 3.70 (s, 3H), 3.6–3.96 (m, 2H), 4.08 (s, 2H), 6.75 (d, 2H, $J = 8$ Hz), 6.9–7.25 (m, 6H), 7.4 (d, 2H, $J = 9$ Hz), 8.1 (d, 2H, $J = 9$ Hz); Anal. Calcd for $C_{26}H_{27}N_3O_4S$ as complex with acetone (CH_3COCH_3): C, 65.03; H, 6.21; N, 7.84. Found: C, 65.31; H, 6.02; N, 7.96; C, 64.94; H, 5.83; N, 8.22.

4.17. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-(4-chlorobenzyl) piperazine (17)

Compound **17** was prepared using the same procedure described above for compound **11** using 1.37 g (5 mmol) of **2**, and 1.05 g (5 mmol) of 1-(4 chlorobenzyl) piperazine. The product was filtered, washed with methanol, and further purified by column chromatography on a silica gel column (eluent: ethyl acetate) to give 496 mg (21%) of compound **17**. Mp 109–111.5 °C; IR (KBr) cm^{-1}: 2917, 1623, 1581, 1511, 1179, 1427, 1237, 1025, 846; ^1H NMR ($CDCl_3$) δ ppm: 2.0–2.6 (m, 4H), 2.98–3.28 (m, 2H), 3.38 (s, 2H), 3.68 (s, 3H), 3.6–3.96 (m, 2H), 4.0 (s, 2H), 6.7 (d, 2H, $J = 9$ Hz), 6.95–7.2 (m, 10H); Anal. Calcd for $C_{26}H_{27}ClN_2O_2S$: C, 66.87; H, 5.83; N, 6.00. Found: C, 66.64; H, 5.89; N, 6.07.

4.18. *N*1-(*S*-(4-Methoxybenzyl) thiosalicyloyl)-*N*4-(4-fluo- robenzyl) piperazine (18)

Compound **18** was prepared according to the same procedure described above for compound **11** using 1.36 g (5 mmol) of *S*-(4-methoxybenzyl) thiosalicylic acid, and 1.15 g (5 mmol) of 1-(4-fluorobenzyl) piperazine hydrochloride. The oily product was dissolved in 5 mL of acetone. Addition of 5 mL of 10% NaOH solution and 40 mL of water resulted in precipitating a white material that was collected by suction filtration to give 872 mg (39%) of the compound **18** with a melting point of 73–84 °C. Further purification by crystallization from chloroform/petroleum ether gave compound **18** as white crystals with mp of 84.2–85.8 °C; IR (KBr) cm^{-1}: 2917, 1623, 1581, 1511, 1179, 1427, 1237, 1025, 846; ^1H NMR ($CDCl_3$) δ ppm: 2.0–2.6 (m, 4H), 2.98–3.28 (m, 2H), 3.38 (s, 2H), 3.68 (s, 3H), 3.6–3.96 (m, 2H), 4.0 (s, 2H), 6.6 7.2 (m, 12H); Anal. Calcd for $C_{26}H_{27}FN_2O_2S$: C, 69.31; H, 6.04; N, 6.22. Found: C, 68.99; H, 6.02; N, 6.36.

4.19. In vitro testing for calcium channel blocking using isolated rat aorta strips

The new compounds were assessed for calcium channel blocking activities according to a previously reported protocol,[13,14] that is detailed below. Male Wistar rats (Charles River Laboratory) were housed in $12'' \times 24''$ plastic cages in the Massachusetts College of Pharmacy animal facility with a 12 h light and 12 h dark schedule. Animals had access to water and food ad lib. Rats (weighing approximately 350 g) were euthanized with sodium pentobarbital (100 mg/kg). The thoracic aorta was removed and placed in a 37 °C Krebs-bicarbonate solution containing NaCl, 112 mM; KCl, 5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1 mM; $NaHCO_3$, 25 mM; $CaCl_2$, 1.25 mM and glucose, 11.5 mM (pH 7.4). The thoracic aorta, after removal of connective tissues, was cut into rings that were approximately 2.5–3.0 mm in length and immersed in a 37 °C Krebs bicarbonate solution. The solution was prepared fresh on each day of an experiment and aerated with 95% O_2 and 5% CO_2. An Iso-temp Constant Temperature Circulator (Fisher Scientific model 801, Pittsburgh, PA) was used to maintain the bath temperature. Tissues were suspended between

two stainless steel loops. To measure tissue contraction and relaxation one of the loops was attached to a fixed glass tube that provided the gas mixture to the solution and the other loop was attached to a Grass force–displacement transducer via polyester thread to a Grass Polygraph (model 7H, Grass Instruments, Quincy, MA). Each preparation was stretched to an optimal resting tension of 2.0 g (previously determined by measuring tissue contraction to Ca^{2+} concentrations of 0.1–8.0 mM) that was maintained in all experiments. All test compounds were dissolved in absolute ethanol that was also used to prepare all other concentrations (0.01–10.0 mM) of the test compound. A 150 mM calcium chloride stock solution was prepared in de-ionized water. A group of 4–5 animals was used to evaluate each compound. After equilibration for 60 min with the above solution, tissues were washed every 15 min with fresh Krebs-bicarbonate solution. After the artery preparations were equilibrated in the normal Krebs-bicarbonate solution, the solution was replaced with a calcium-free, potassium-rich Krebs-bicarbonate solution (NaCl, 17 mM; KCl, 100 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1 mM; $NaHCO_3$, 25 mM and glucose, 11.5 mM (pH 7.4)) to induce depolarization. Tissues were washed every 15 with the calcium-free potassium-rich Krebs-bicarbonate solution every 15 min. Following equilibrium, 0.1 mL of absolute ethanol (vehicle control) was added to the tissue. After 10 min incubation of ethanol, the contractile base line response was obtained by adding 0.1 mL of a calcium chloride stock solution (to give 1.5 mM Ca^{2+} final concentration in the tissue bath). After obtaining the maximum responses with 1.5 mM of Ca^{2+} the tissues were washed every 15 min with Calcium-free, potassium-rich Krebs-bicarbonate solution for an hour. Once the tension had remained stable for at least 15 min, a second (control) response was obtained in the same manner. After monitoring the second response, the tissues were washed again every 15 min with calcium-free potassium-rich Krebs bicarbonate solution for one hour. Once tension had remained stable for at least 15 min tissues were treated (incubation period of 10 min) with 0.1 mL of the corresponding diltiazem solution in absolute ethanol to give the required final concentrations of 0.1–100 μM. The contractile response at each concentration was measured after adding 0.1 mL of $CaCl_2$ stock solution (150 mM, 1.5 mM in the tissue bath). Responses to all test compounds (at final concentrations of 0.1–100 μM in tissue bath) were obtained in the same manner. Each tissue received only one concentration of each test compound. The results of these experiments were expressed as percent inhibited contraction of the initial contraction. The percentage of contraction inhibition was calculated for each tissue as follows: % Inhibition = 100 − (contraction TC/contraction CC × 100), where contraction TC denotes the maximum response in the presence of the test compound and contraction CC denotes the maxi- mum response in under control condition. Dose response curves were constructed and IC_{50} values were calculated.

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