Cardiovascular Characterization of [1,4]Thiazino[3,4-c][1,2,4]oxadiazol-1-one Derivatives: Selective Myocardial Calcium Channel Modulators

Roberta Budriesi,[†] Barbara Cosimelli,[‡] Pierfranco Ioan,[†] Camilla Zaira Lanza,[‡] Domenico Spinelli,[‡] and Alberto Chiarini*,[†]

Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, and Dipartimento di Chimica Organica "A. Mangini", Università di Bologna, Via S. Donato 15, 40127 Bologna, Italy

As an extension of previous investigations (*Tetrahedron* **1999**, *55*, 5433−5440; *J. Heterocycl. Chem.* **2000**, *37*, 875−878), a series of 21 [1,4]thiazino[3,4-c][1,2,4]oxadiazolones, which has already been synthesized (except for compounds **5a**, **5b**, **6**), was evaluated as calcium entry blockers by functional studies, namely, in isolated guinea-pig left and right atria and K+-depolarized aortic strips. With the aim of investigating the effect of a condensed benzene ring on the molecular structure and the influence of substituents on the 8-phenyl ring of **4a**, ab initio computations (RHF/3-21*G) were performed on compounds **3**, **4a**−**d**, **4f**, and **4k**. The results obtained show that many of the compounds studied are potent and selective negative inotropic agents; in particular, compounds **4e** and **4f** are about 3- and 2-fold more potent than diltiazem, respectively.

Introduction

In the last 20 years, heart disorders have derived much benefit from the development of compounds capable of blocking calcium channels. Calcium entry blockers (CEBs) are used in the treatment of hypertension as well as in ischemic heart diseases mainly for their vascular properties.[1,2] On the other hand, for their ability in blocking myocardial calcium channels, CEBs reduce the slow calcium current with a consequently direct negative inotropic effect.[3] However, negative inotropic activity is usually masked because the vasodilatory response may affect cardiac performance by reflex-mediated changes. The best-known CEBs which are widely used as therapeutic agents in cardiovascular diseases are dihydropyridines (DHPs), such as nifedipine, and non-DHPs, represented by verapamil and diltiazem. Diltiazem (**1**), a 1,5-benzothiazepine, like the other CEBs, has affinity for the L-type calcium channel but is less potent on the peripheral smooth muscle than on myocardial tissue. This weak selectivity produces negative chronotropic and inotropic effects that reduce the myocardial oxygen demand.[4]

Although much information has been reported about the structure−activity relationships (SARs) of DHPs and verapamil, there is little information about compounds structurally related to diltiazem. Some years ago, Campiani et al.[5−8] described a new series of diltiazem analogues that were potent calcium-selective antagonists for cardiac tissue over vascular tissue. Most pyrrolo[2,1-c][1,4]benzothiazine derivatives (**2**) showed high affinity and selectivity for cardiac parameter inotropism with respect to diltiazem (Chart 1).[7,8]

Recently, we described the synthesis of one benzo-[5,6][1,4]thiazino[3,4-c][1,2,4]oxadiazol-1-one (**3**)[9] and

Chart 1

Diltiazem (**1**)   pyrrolo[2,1-c][1,4]benzothiazines (**2**)
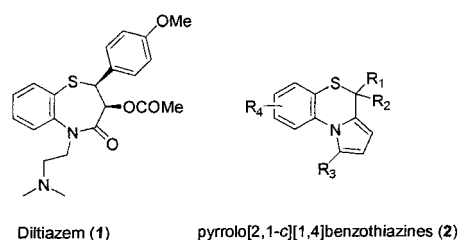
several [1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-ones (**4a**−**d**[9] and **4e**−**q**[10]) which should be correlated to the cited diltiazem (**1**) and pyrrolobenzothiazines (**2**).

To obtain a better understanding of the SARs of diltiazem-type CEBs, we have functionally tested the cardiovascular activity of these compounds as well as of other new derivatives (**5a**, **5b**, **6**) using diltiazem as the reference standard. In particular, preliminary assays for the cardiovascular activity of 4-(4-chlorophenyl)-4-hydroxy-4H-benzo[5,6][1,4]thiazino[3,4-c][1,2,4]oxadiazol-1-one (**3**) show very interesting selective negative inotropic activity without showing effects on chronotropism and vascular smooth muscle. Therefore, we thought it would be of interest to study the cardiovascular profile of compounds structurally correlated to **3** (Chart 2).
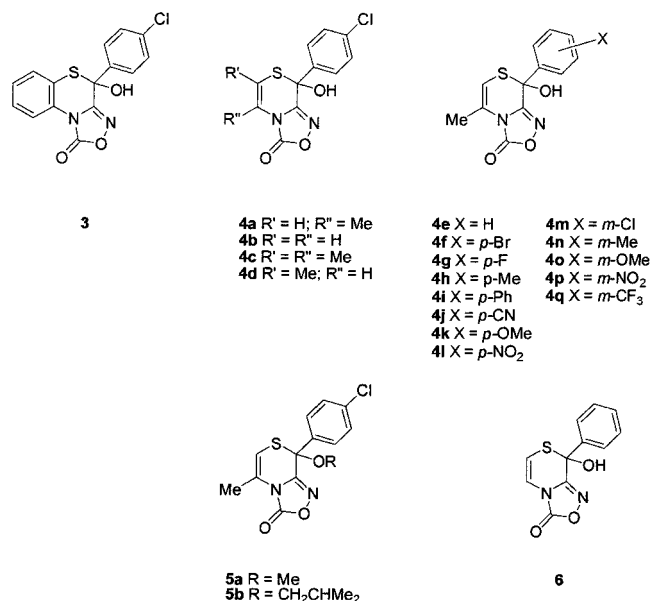
Compounds **4a**−**d** may be considered to be derivatives of **3**, in which the benzo-fused ring constraint is lost. This should allow us to evaluate the influence of a benzo-condensed ring on the activity and selectivity of such molecules as the blockers of calcium channels. With the aim also of investigating the influence of substitution on the phenyl ring, we have assayed compounds **4e**−**q**, in light of the fact that **4a** had resulted in the most promising molecule. It should be pointed out that, although the hydroxy group at C-8 should be important for the activity of compound **4a**, to the best of our knowledge, the literature reports no SAR study about similar structures containing an OH group.[7]

* Corresponding author. Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy. Tel: +39-051-2099721. Fax: +39-051-2099721. E-mail: alchi@alma.unibo.it.
† Dipartimento di Scienze Farmaceutiche.
‡ Dipartimento di Chimica Organica "A. Mangini".

Chart 2

3

4a R' = H; R" = Me
4b R' = R" = H
4c R' = R" = Me
4d R' = Me; R" = H

4e X = H   **4m** X = *m*-Cl
4f X = *p*-Br   **4n** X = *m*-Me
4g X = *p*-F   **4o** X = *m*-OMe
4h X = p-Me   **4p** X = *m*-NO₂
4i X = *p*-Ph   **4q** X = *m*-CF₃
4j X = *p*-CN
4k X = *p*-OMe
4l X = *p*-NO₂

5a R = Me
5b R = CH₂CHMe₂

6

To widen the analysis, we have also modified the hydroxylic function synthesizing the alkoxy derivatives **5a** and **5b**. Moreover, to also evaluate the influence of the presence of a methyl group, we have compared **4a**, which bears a methyl at C-5, with **4b**, **4c**, and **4d**, which contain no methyl at C-5 and C-6, a methyl at both C-5 and C-6, and a methyl at C-6, respectively.

The hydroxy emithioacetal group should be important in the formation of an equilibrium with the open chain structure (only the cyclic structure has been evidenced in both the solid state and in solution[9,10]); furthermore, it can give specific interactions with the active sites. Finally, we have synthesized **6** which does not bear substituents on either the thiazino or the phenyl ring and can be regarded as the parent compound of both compound groups **4a**−**q** and **5a** and **5b**.

Synthesis, biological evaluation, and SARs of these new derivatives are described in this paper. The results obtained show that many of the compounds studied are endowed with selective negative inotropic activity and are weak vasorelaxant agents.

Chemistry

The synthesis of compounds **3** and **4a**−**q** has been previously reported.[9,10] We obtained the new 8-hydroxy-8-phenyl-8*H*-[1,4]thiazino[3,4-*c*][1,2,4]oxadiazol-3-one (**6**) in a similar manner from 5-nitroso-6-phenylimidazo[2,1-*b*][1,3]thiazole and dilute hydrochloric acid (Scheme 1).
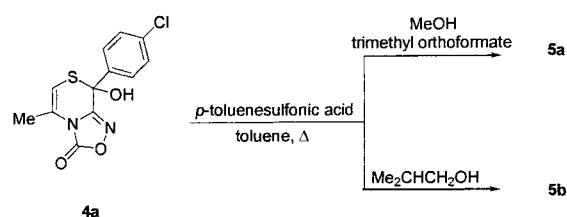
Scheme 1

7
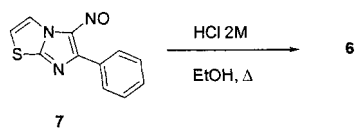
Compounds **5a** and **5b** were prepared from **4a** and the appropriate primary alcohol in the presence of catalytic amounts of *p*-toluenesulfonic acid in refluxing toluene (Scheme 2).

Pharmacology. The pharmacological profile of compounds was tested on guinea-pig isolated left and right atria to evaluate their inotropic and chronotropic effects,

Scheme 2

4a

respectively, and on K⁺-depolarized guinea-pig aortic strips to assess calcium antagonist activity. At first, all compounds were checked at increasing doses to evaluate the percent decrease of the developed tension on the isolated left atrium driven at 1 Hz (negative inotropic activity), the percent decrease in the atrial rate on the spontaneously beating right atrium (negative chronotropic activity), and the percent inhibition of calcium-induced contraction on K⁺-depolarized aortic strips (vasorelaxant activity). Data were analyzed by the Student's *t* test. The potency of the drugs defined as EC_{50}, EC_{30}, and IC_{50} was evaluated from log concentration−response curves (Probit analysis by Litchfield and Wilcoxon, $n = 6$−8) in the appropriate pharmacological preparations. All data are presented as mean ± SEM.[11]

Results and Discussion

The biological activities of the various compounds (**3**, **4a**−**q**, **5a**, **5b**, and **6**) have been evaluated in comparison with those of diltiazem. The efficacy and potency on the driven left atria, spontaneously beating right atria, and smooth muscle aortic vessel are reported in Table 1.

From an inspection of the biological data, it clearly emerges that all of the compounds exhibit very good activity as negative inotropic agents. Most of them show better intrinsic activity and potency than diltiazem in decreasing the developed tension of the driven left atria. On the contrary, the negative chronotropic and vasorelaxant activities of the compounds are lower than those of the reference compound. In particular, none of the compounds tested elicit negative chronotropic and vasorelaxant activities over 40% at the maximum concentration used (50 μM).

Structure−activity and structure−affinity relationships have long been used to characterize the interaction between non-dihydropyridino CEBs, such as diltiazem,[5−8] and calcium channels; therefore, calcium antagonistic activities of diltiazem and the benzothiazine derivatives[12] are well-known.

Here, we report the cardiovascular characterization of some [1,4]thiazino[3,4-*c*][1,2,4]oxadiazol-1-one derivatives in an attempt to better understand the relevance of structural features, such as the benzo-condensed ring, the phenyl substituent X, and the emithioacetalic OH.

Compound **3**, bearing a benzothiazine moiety, has been the starting point of our study. Its backbone was chosen because the contraction of the seven-member ring of diltiazem had proved to be a useful structural manipulation to improve CEB activity.[6,7] Compound **3** is more active, as an inotropic agent, and more selective, with regard to the chronotropic and vascular activity, than diltiazem (Table 1); thus, its interesting pharmacological activity discloses the way to the discovery of a new class of CEBs with an interestingly high selectivity. The clear-cut affinity and selectivity of **3** as compared

Table 1. Cardiovascular Activity of Tested Compounds

comp	% decrease ($M \pm SEM$)		EC_{50} of inotropic negative activity		EC_{30} of chronotropic negative activity		vasorelaxant activity		
	negative inotropic activity[a]	negative chronotropic activity[b]	$EC_{50}{}^{c}$ (μM)	95% conf. lim ($\times 10^{-6}$)	$EC_{30}{}^{c}$ (μM)	95% conf. lim ($\times 10^{-6}$)	activity[d] ($M \pm SEM$)	$IC_{50}{}^{c}$ (μM)	95% conf. lim ($\times 10^{-6}$)
1	78 ± 3.5^{e}	94 ± 5.6^{g}	0.79	0.70–0.85	0.07	0.064–0.075	88 ± 2.3^{h}	2.6	2.2–3.1
3	94 ± 2.3^{f}	21 ± 1.3^{e}	0.52	0.41–0.65			40 ± 3.1		
4a	80 ± 4.1^{e}	18 ± 1.8	0.80	0.65–1.05			26 ± 1.4		
4b	78 ± 4.8	7 ± 0.9	4.07	3.67–4.52			10 ± 0.4		
4c	70 ± 1.9	19 ± 0.4	1.42	1.02–1.98			28 ± 1.3		
4d	71 ± 2.6	11 ± 0.7	1.72	1.34–2.19			15 ± 0.8		
4e	95 ± 3.2^{e}	14 ± 0.5	0.23	0.18–0.30			4 ± 0.2		
4f	97 ± 3.7	18 ± 1.0	0.32	0.23–0.43			12 ± 0.6		
4g	74 ± 3.6	10 ± 0.6	1.21	1.03–1.45			8 ± 0.7		
4h	82 ± 1.5^{e}	13 ± 0.7	0.57	0.53–0.64			5 ± 0.3		
4i	71 ± 3.2	18 ± 1.3	2.65	2.35–3.10			9 ± 0.6		
4j	76 ± 2.7	30 ± 1.4^{f}	0.76	0.55–0.93			13 ± 0.8		
4k	76 ± 3.7	12 ± 0.9	0.52	0.41–0.72			18 ± 1.5		
4l	73 ± 2.4	16 ± 0.6	1.65	1.35–2.05			19 ± 1.2		
4m	88 ± 3.6	34 ± 1.4	0.65	0.46–0.92			17 ± 0.9		
4n	89 ± 3.4^{e}	10 ± 0.5	0.72	0.61–0.85			12 ± 0.6		
4o	86 ± 4.8	16 ± 0.7	0.49	0.43–0.56			10 ± 0.6		
4p	68 ± 3.2	13 ± 0.8	1.76	1.35–2.23			19 ± 0.4		
4q	96 ± 3.8^{e}	20 ± 1.8	2.17	1.67–2.83			37 ± 3.2		
5a	62 ± 2.5	22 ± 1.1	1.54	1.21–1.88			14 ± 0.9		
5b	83 ± 1.2	15 ± 0.5	0.56	0.39–0.79			37 ± 1.5		
6	74 ± 1.7	14 ± 0.4	6.58	5.99–7.13			11 ± 0.6		

[a] Decrease in the developed tension in isolated guinea-pig left atrium at 5×10^{-5} M, expressed as percent changes from the control ($n = 4$–6). The left atria were driven at 1 Hz. The 5×10^{-5} M concentration gave the maximum effect for most compounds. [b] Decrease in the atrial rate of guinea-pig spontaneously beating isolated right atria at 5×10^{-5} M, expressed as percent changes from the control ($n = 6$–8). The pretreatment heart rate ranged from 170 to 195 beats/min. The 5×10^{-5} M concentration gave the maximum effect for most compounds. [c] Calculated from log concentration–response curves (Probit analysis according to Litchfield and Wilcoxon[11] with $n = 6$–8). When the maximum effect was <50%, the EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} values were not calculated. [d] Percent inhibition of calcium-induced contraction on K^+-depolarized guinea-pig aortic strip at 5×10^{-5} M. The 5×10^{-5} M concentration gave the maximum effect for most compounds. [e] At 10^{-5} M. [f] At 5×10^{-6} M. [g] At 10^{-6} M. [h] At 10^{-4} M.

to those of diltiazem are probably due to the simultaneous presence of the fused [1,2,4]oxadiazolonic and para-substituted benzene ring at C-8. Compound **3** seems to possess the necessary features to bind more preferentially to the cardiac rather than to the vascular calcium channel binding sites. To investigate the influence of the benzo-condensed ring, we studied **4a–d**. If the ring constraint is completely removed (i.e., **4b**), the cardiovascular activity is significantly reduced, and although the selectivity trend is maintained, a large drop in the inotropic negative potency occurs. This result should be attributed to the absence of a hydrophobic link which is able to interact with the hydrophobic region of the binding site.

The insertion of two methyl groups into the 5 and 6 positions of the thiazinoxadiazolo ring improves the inotropic negative potency of **4c** ($EC_{50} = 1.42$ μM) in comparison with that of **4b** ($EC_{50} = 4.07$ μM). This finding has prompted us to evaluate the influence of a single methyl group, assaying **4a** and **4d**. Interestingly, the latter has shown a different behavior, displaying a slight decrease of inotropic negative potency ($EC_{50} = 1.72$ μM), while **4a** shows an inotropic negative potency ($EC_{50} = 0.80$ μM) comparable to that of diltiazem. Therefore, we may conclude that the hydrophobic pocket could be better reached by the 5-Me group than by the 6-Me group, and the resulting order of potency is the following: **4a** > **4c** ≥ **4d** ≫ **4b**. In particular, compound **4a**, besides showing the best negative inotropic potency among the **4a–d** series, is also 2-fold less active than **3** as a vasorelaxant on K^+-depolarized guinea-pig aortic strips (Table 1).

For a long time, the knowledge of geometric constraints of biologically active compounds has been used to understand their activity.[7,13] Thus, in an attempt to rationalize the biological activity of compounds **3** and **4a–d**, we have performed ab initio computations (RHF/3-21G*) on these products. The optimized structure for compound **4a** is schematically represented in Figure 1 in three different orientations.

The values of the most relevant geometrical parameters (bond distances (Å) and angles (deg)) are also reported. The sulfur atom is severely bent out of the average molecular plane (which contains the frame of the two condensed rings). A measure of the pyramidalization of the sulfur atom is given by the torsion angle ω (the angle between the two planes S–C8–C3 and C8–C3–N) which is 44.2°. The inspection of Figure 1 also shows that the phenyl ring is approximately orthogonal to the molecular plane to lower the steric repulsion with the sulfur lone pairs.

The optimized structures for compounds **4b–d** evidenced only small differences in angle and bond length values (Supporting Information). An examination of the electronic density values reveals the presence of a negative area located on the heteroatoms (nitrogens and oxygens) of the oxadiazolone ring.

The most striking geometrical difference among the compounds examined, which could be related to the biological activity observed, concerns the molecular length where **4b** is appreciably shorter (9.90 Å) than **4a**, **4c**, or **4d** (10.9 Å). This result seems to confirm the importance of a hydrophobic link on the thiazine ring
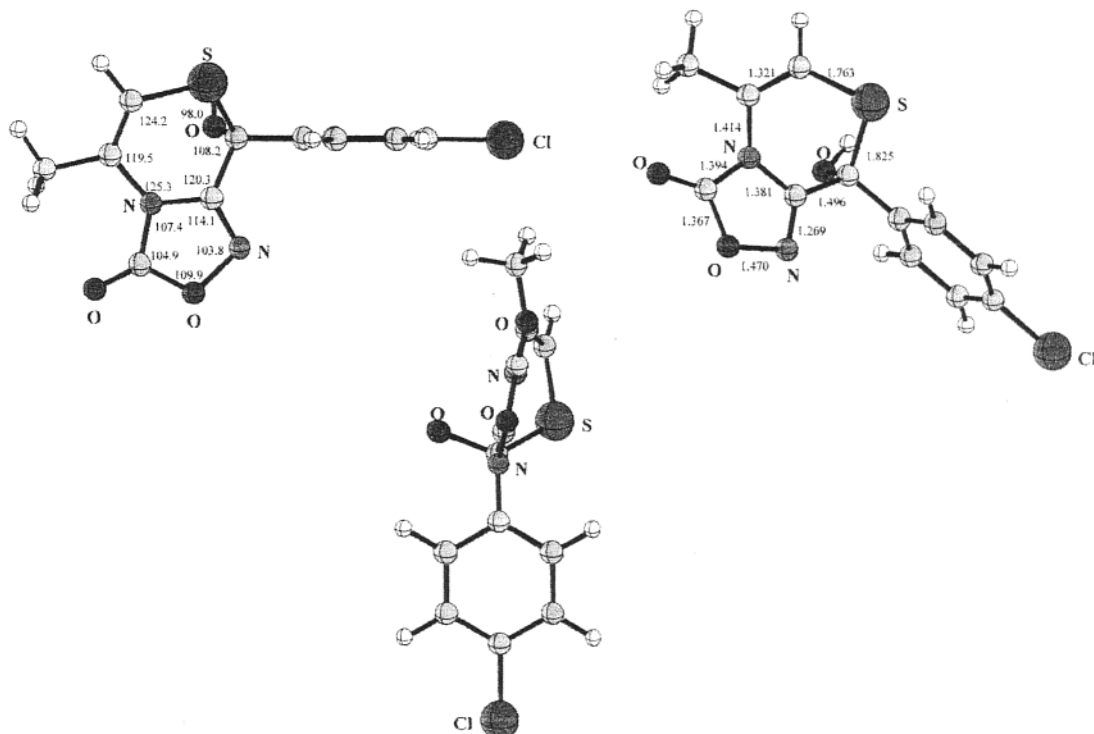
**Figure 1.** Optimized geometries for **4a** at the RHF/3-21G* level. Three orientations are shown.
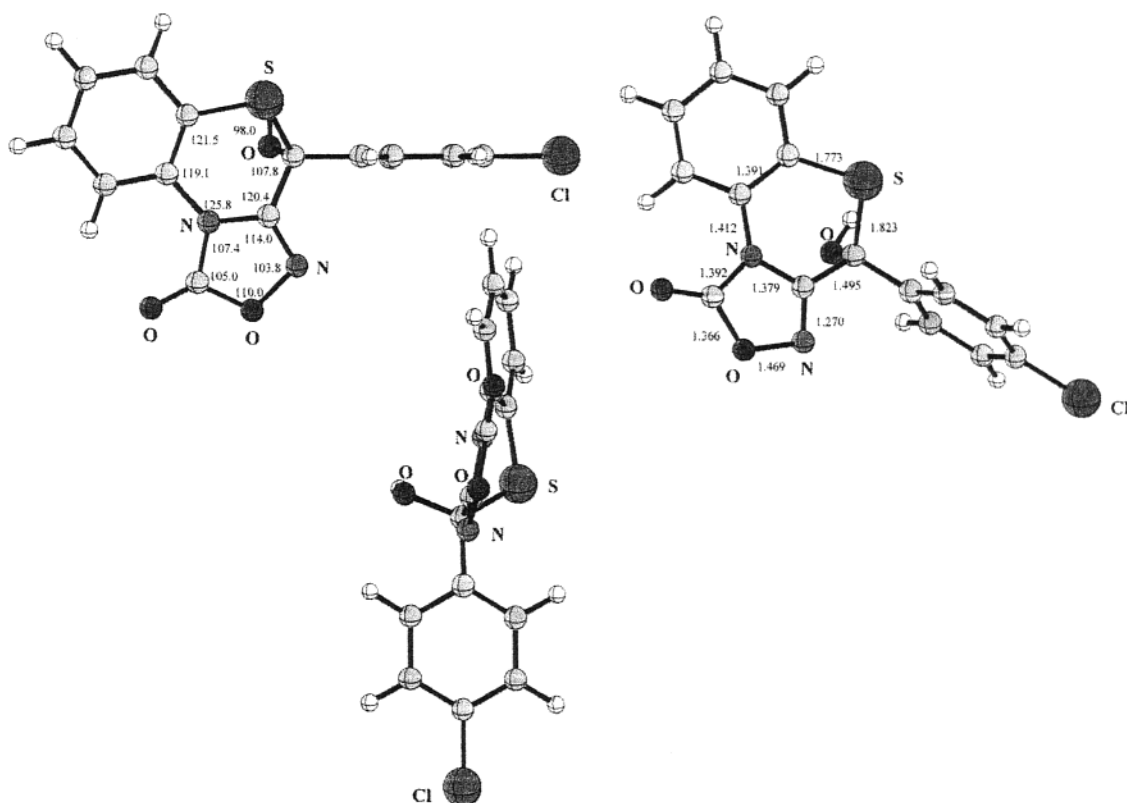
Figure 2. Optimized geometries for **3** at the RHF/3-21G* level. Three orientations are shown.

which could allow the insertion into the hydrophobic region of the binding site.

In Figure 2, the structure of compound **3** is shown. In this case, we report three different orientations of the molecular frame to outline the degree of pyramidalization of the sulfur atom.

It is interesting to note that the inclusion of a benzene ring condensed at the two carbons in the α and β positions with respect to sulfur has negligible effects on the entire molecular structure. The dihedral angle ω changes to 45.2°, and the length of the various bonds of the five- and six-member rings remains (except for the C5–C6 bond) almost unchanged. The C5–C6 bond shows a significant variation (from 1.321 Å in **4a** to 1.391 Å in **3**) because it becomes a true aromatic bond as a part of the benzene ring.
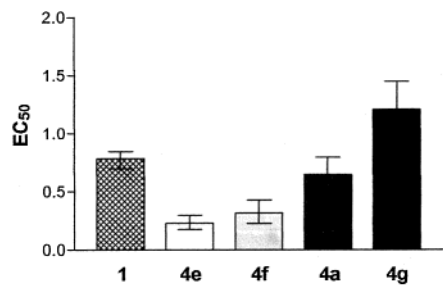
Figure 3. Graph showing negative inotropic activity, expressed as EC_{50} values, in guinea-pig left atria driven at 1 Hz of **4a**, **4e**, **4f**, and **4g** in comparison with that of **1**.
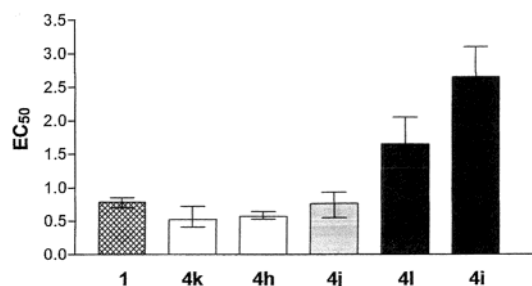
Figure 4. Graph showing negative inotropic activity, expressed as EC_{50} values, in guinea-pig left atria driven at 1 Hz of **4h**−**l** in comparison with that of **1**.

Because **4a** (X = Cl) shows the best inotropic versus vasorelaxant selectivity, we chose this compound as a starting point to evaluate the influence of the substituent X in **4e**−**q** on the blocking activity of L-type calcium channels. Such an analysis was also judged to be of interest on the basis of the report, by Campiani et al.,[6] that the calcium channel activity of pyrrolo[2,1-*c*][1,4]-benzothiazines is dependent upon the nature of the substituent present on the phenyl ring at C-4 (comparable with the phenyl ring at C-8 in **4a**−**q**).

Compound **4e** (X = H) showed very good negative inotropic activity ($EC_{50} = 0.23 \ \mu$M); the substitution of the chlorine atom in **4a** with fluorine (**4g**) or bromine (**4f**) caused a potency decrease ($EC_{50} = 1.21 \ \mu$M) or increase ($EC_{50} = 0.32 \ \mu$M), respectively. Compounds **4f** and **4e** (X = H) show similar negative inotropic activity (Figure 3) probably because bromine is the least electronegative halogen.

We then tested compounds **4h**, **4j**, **4k**, and **4l**, in which X = Me, CN, OMe, and NO_2, respectively, because these groups are always present in known CEBs, while **4i** (X = Ph) served to evaluate the effect of a large substituent. Analysis of the data reveals that the most-active compound is **4k**, whereas the least-active compound is **4i**.

In **4i**, the second phenyl ring probably does not allow a correct insertion of the molecule (whose length exceeds 14 Å) into the receptor site. This steric effect is a confirmation of the trend observed for compound **4h** (X = OMe) versus that of compound **4e** (X = H), the former being more than 2-fold less active. Therefore, the resulting activity order is **4k** ≥ **4h** ≥ **4j** > **4l** > **4i**. Perhaps the electronic effect of the substituent is responsible for these results because they range from a strong +M effect to a strong −I, −M effect (Figure 4).

Ab initio computations for **4f** (X = Br, a very large atom) and **4k** (X = OMe, a polyatomic substituent) (Supporting Information) showed that the most stable
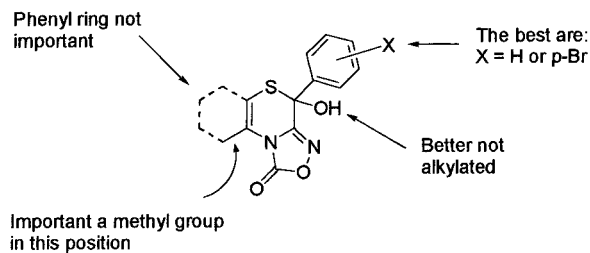
Figure 5. Schematic summary of the structure−activity relationships for selective negative inotropism of [1,4]thiazino-[3,4-*c*][1,2,4]oxadiazol-1-one derivatives related to diltiazem.

conformation of the two molecules is not influenced by the para substituent; in fact, the results obtained for **4f** as well as those for **4k** are similar to those obtained for **4a** (X = Cl) and are reported in Figure 1. As for compounds **4m**−**q**, it is to be noted that the meta substitution displays effects not much different from those of the para substitution (Table 1).

Furthermore, we assayed the influence of the OH group, changing it into OR (with R = methyl or isobutyl), that is, two groups with very different steric requirements. Interestingly, when one goes from **4a** to **5a** ($EC_{50} = 1.54 \ \mu$M), the potency is cut by more than one-half (probably because a hydrogen bond between the ligand and the receptor is now missing), whereas when one goes from **4a** to **5b** ($EC_{50} = 0.56 \ \mu$M), it remains almost unaltered (probably because the large alkyl group can give a new kind of hydrophobic interaction).

Thus, the body of this work has led to the clarification of the structure−activity relationships for this class of calcium entry blockers and allows us to propose some structural requirements in order to differentiate the activity on the cardiac tissue with respect to that of the vascular tissue. It is interesting to note that the benzo-fused ring is not a necessary moiety if a methyl group is present at the 5 position of the thiazinooxadiazolone system. Thus, 8-(4-chlorophenyl)-8-hydroxy-5-methyl-8*H*-[1,4]thiazino[3,4-*c*][1,2,4]oxadiazol-3-one (**4a**) shows good negative inotropic activity while the negative chronotropic activity and the vasorelaxant effect are completely reduced. Furthermore, a halogen atom at position 4 of the phenyl ring seems to improve the negative inotropism.

Finally, the functional studies on compound **6** have confirmed ($EC_{50} = 6.58 \ \mu$M) that the activity strongly depends on the presence of both a substituent in the phenyl ring and a methyl group at the 5 position.

Conclusions

In conclusion, the present results, together with previous SARs and information from computational data, enlighten some structural features which improve negative inotropic activity and reduce the chronotropic and vasorelaxant effects of [1,4]thiazino[3,4-*c*][1,2,4]-oxadiazolones, namely, a methyl group at position 5 of the thiazinoxadiazolo ring, a free OH at C-8, and an unsubstituted or *p*-bromo-substituted 8-phenyl ring (Figure 5).

In particular, compounds **3**, **4a**, **4e**, and **4f** became negative inotropic agents which are more active than diltiazem (**1**).

Experimental Section

Chemistry. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 instrument in the Fourier transform mode at 21 ± 0.5 °C in DMSO-d_6. Chemical shifts (δ) are in parts per million (ppm) from tetramethylsilane, and coupling constants are in hertz (Hz). Mass spectra were recorded on a VG70 70E apparatus. Melting points are uncorrected. All new compounds gave satisfactory microanalyses (C, H, N). Solvents were removed under reduced pressure. Silica gel plates (Merck F$_{254}$) and silica gel 60 (Merck 230−400 mesh) were used for analytical TLC and for column chromatography, respectively.

8-(4-Chlorophenyl)-8-methoxy-5-methyl-8H-[1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-one (5a). Trimethylorthoformate (0.4 mL, 3.2 mM) and p-toluenesulfonic acid (100 mg) were added to a solution of 8-(4-chlorophenyl)-8-hydroxy-5-methyl-8H-[1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-one (**4a**) (250 mg, 0.8 mM) in MeOH (5 mL) and anhydrous toluene (5 mL). The solution was refluxed for 24 h, cooled to room temperature, and then poured into a saturated solution of NaHCO$_3$ (50 mL). The organic layer was separated, and the water was extracted with CH$_2$Cl$_2$ (3 × 10 mL). The extracts were dried over Na$_2$SO$_4$ and evaporated. Pure **5a** was obtained (200 mg, 80%) by flash chromatography (ethyl acetate/petroleum ether, 1:5 v/v as eluant) as white crystals. Mp: 131−132 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.41 (3H, d, ^3J = 1.0 Hz, 5-Me), 3.26 (3H, s, OMe), 6.21 (1H, q, ^3J = 1.0 Hz, H-6), 7.56 (4H, ps, H−Ar). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.54 (qd, ^1J = 131.0 Hz, ^3J = 4.3 Hz, Me-5), 52.13 (q, ^1J = 144.9 Hz, OMe), 82.63 (m, C-8), 102.91 (dq, ^1J = 184.7 Hz, ^3J = 6.3 Hz, C-6), 128.82 (dd, ^1J = 169.0 Hz, ^3J = 3.0 Hz, C-3′ and C-5′), 129.45 (dd, ^1J = 164.0 Hz, ^3J = 4.9 Hz, C-2′ and C-6′), 129.75 (qd, $^2J_{Me}$ = 7.3 Hz, ^2J = 5.3 Hz, C-5), 131.69 (m, C-1′), 134.65 (tt, ^2J = ^3J = 7.5 Hz, C-4′), 154.15 (s, C-8a), 154.60 (s, C-3). MS: m/z 310 (M$^+$, 60), 279 (48), 266 (13), 251 (14), 235 (50), 139 (100), 111 (75), 75 (36), 72 (21), 71 (30), 67 (10), 50 (13), 45 (32). HRMS: calcd for C$_{13}$H$_{11}$ClN$_2$O$_3$S, 310.01789; found, 310.01845.

8-(4-Chlorophenyl)-8-(2-methylpropoxy)-5-methyl-8H-[1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-one (5b). 2-Methylpropanol (600 mg, 8 mM) and p-toluenesulfonic acid (20 mg) were added to a suspension of 8-(4-chlorophenyl)-8-hydroxy-5-methyl-8H-[1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-one (**4a**) (250 mg, 0.8 mM) in anhydrous toluene (25 mL). The mixture was refluxed for 3 h, cooled to room temperature, and then poured into a saturated solution of NaHCO$_3$ (50 mL). The organic layer was separated, and the water was extracted with toluene (3 × 20 mL). The extracts were dried over Na$_2$SO$_4$ and evaporated. Pure **5b** was obtained (203 mg, 72%) by flash chromatography (ethyl acetate/petroleum ether, 1:5 v/v as eluant) as white crystals. Mp: 122−123 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (6H, d, ^3J = 6.7 Hz, 2 × Me), 1.84 (1H, m, CH), 2.39 (3H, d, ^3J = 1.2 Hz, 5-Me), 3.11 (1H, dd, ^2J = 8.8 Hz, ^3J = 6.1 Hz, OCH$_2$), 3.32 (1H, dd, ^2J = 8.8 Hz, ^3J = 6.1 Hz, OCH$_2$), 6.22 (1H, q, ^3J = 1.2 Hz, H-6), 7.59 (4H, ps, H−Ar). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.14 (qd, ^1J = 130.8 Hz, ^3J = 4.2 Hz, Me-5), 18.77 (m, Me), 18.75 (m, Me), 27.54 (m, CH), 70.41 (m, CH$_2$), 81.90 (s, C-8), 103.42 (dq, ^1J = 184.6 Hz, ^3J = 6.4 Hz, C-6), 128.59 (dd, ^1J = 168.8 Hz, ^3J = 3.8 Hz, C-3′ and C-5′), 129.17 (dd, ^1J = 163.8 Hz, ^3J = 5.4 Hz, C-2′ and C-6′), 129.60 (qd, $^2J_{Me}$ = 7.2 Hz, ^2J = 5.3 Hz, C-5), 132.13 (t, ^3J = 6.3 Hz, C-1′), 134.47 (tt, ^2J = 7.7 Hz, ^3J = 8.6 Hz, C-4′), 154.21 (s, C-8a), 154.46 (s, C-3). MS: m/z 352 (M$^+$, 20), 279 (10), 157 (52), 139 (100), 111 (25), 57 (80). HRMS: calcd for C$_{16}$H$_{17}$ClN$_2$O$_3$S, 352.06484; found, 352.06540.

8-Hydroxy-8-phenyl-8H-[1,4]thiazino[3,4-c][1,2,4]oxadiazol-3-one (6). A suspension of 5-nitroso-6-phenylimidazo[2,1-b][1,3]thiazole (7)[13] (0.23 g, 1 mM) in ethanol (40 mL) was refluxed and stirred with 2 M HCl (1 mL) for 30 min. Removal of the solvent left a solid which was purified by chromatography (ethyl acetate/petroleum ether, 1:3 v/v as eluant) to give the colorless compound **6** (0.15 g, 61%). Mp: 133−136 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.60 (1H, d, ^3J = 7.5 Hz, H-6), 7.25 (1H, d, ^3J = 7.5 Hz, H-5), 7.49−7.43 (3H, BB′C part of the AA′BB′C system, H−Ar), 7.70−7.67 (2H, AA′ part of the AA′BB′C system, H−Ar), 8.31 (1H, br s, exch. OH). ^{13}C NMR

(75 MHz, DMSO-d_6): δ 77.45 (dt, ^3J = ^3J = 4.0 Hz, C-8), 109.93 (dd, ^1J = 185.4 Hz, ^2J = 6.9 Hz, C-6), 115.99 (dd, ^1J = 194.8 Hz, ^2J = 5.5 Hz, C-5), 126.28 (ddd, ^1J = 162.0 Hz, ^3J = ^3J = 6.2 Hz, C-2′ and C-6′), 128.04 (dm, ^1J = 163.2 Hz, C-3′ and C-5′), 129.13 (dt, ^1J = 161.2 Hz, ^3J = 7.3 Hz, C-4′), 136.75 (t, ^3J = 7.4 Hz, C-1′), 154.35 (d, C-8a), 154.66 (s, C-3). MS: m/z 248 (M$^+$, 2), 204 (5), 190 (6), 189 (5), 172 (80), 161 (7), 144 (15), 117 (6), 105 (100), 77 (64), 51 (24), 44 (81). HRMS: calcd for C$_{11}$H$_8$N$_2$O$_3$S, 348.02556; found, 248.02606.

Computational Procedure. Computation was performed at the Hartree−Fock (HF) level with the Gaussian 98[15] series of programs. The 3-21G* basis set was used. This basis set has a double-ζ character in the valence shell and includes polarization functions (3d orbitals) for the third-row atoms. In each case, the various molecular structures were fully optimized with the gradient method available in Gaussian 98. A computation of the harmonic vibrational frequencies demonstrated that all the optimized structures correspond to a minimum of the potential surface.

Function Studies. Guinea-Pig Atrial Preparations. Guinea pigs (300−400 g females) were sacrificed by cervical dislocation. After thoracotomy, the heart was immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution with the following composition: 136.9 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl$_2$, 1.0 mM MgCl$_2$, 0.4 mM NaH$_2$PO$_4$·H$_2$O, 11.9 mM NaHCO$_3$, and 5.5 mM glucose. The physiological salt solution (PSS) was buffered at pH 7.4 by saturation with 95% O$_2$/5% CO$_2$ gas, and the temperature was maintained at 35 °C. The isolated guinea-pig heart preparations of the spontaneously beating right atria and driven left atria were used at 1 Hz. For each preparation, the entire left and right atria were dissected from the ventricles, cleaned of excess tissue, and hung vertically in a 15 mL organ bath containing the PSS which was continuously bubbled with 95% O$_2$/5% CO$_2$ gas at 35 °C (pH 7.4). The contractile activity was recorded isometrically by means of a force transducer (FT 0.3, Grass Instruments, Quincy, MA) using Power Lab software (Basile, Italy). The left atria were stimulated by rectangular pulses of 0.6−0.8 ms duration and about 50% threshold voltage through two platinum contact electrodes in the lower holding clamp (Grass S88 stimulator). The right atrium was spontaneously beating. Once the tissue had been beating for several minutes, a length−tension curve was determined, and the muscle length was maintained which elicited 90% of the maximum contractile force observed at the optimal length. A stabilization period of 45−60 min was allowed before the atria were used to test compounds. During the equilibration period, the bathing solution was changed every 15 min and the threshold voltage was ascertained for the left atria. Atrial muscle preparations were used to examine the inotropic and chronotropic activity of the compounds (0.1, 0.5, 1, 5, 10, 50, and 100 μM first dissolved in DMSO, and then diluted with PSS. According to this procedure, the concentration of DMSO in the bath solution never exceeded 0.3%, a concentration which did not produce appreciable inotropic and chronotropic effects. During the construction of the cumulative dose−response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state.

Guinea-Pig Aortic Strips. The thoracic aorta was removed and placed in Tyrode solution with the following composition: 118 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl$_2$, 1.20 mM MgSO$_4$, 1.19 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, and 11 mM glucose equilibrated with 95% O$_2$/5% CO$_2$ gas at pH 7.4. The vessel was cleaned of extraneous connective tissue. Two helicoidal strips (10 × 1 mm) were cut from each aorta beginning from the end most proximal to the heart. Vascular strips were then tied with surgical thread (6-0) and suspended in a jacketed tissue bath (15 mL) containing aerated physiological salt solution (PSS) at 35 °C. Strips were secured at one end to a force displacement (FT 0.3) transducer for monitoring changes in isometric contraction. Aortic strips were subjected to a resting force of 1g and were washed every 20 min with fresh PSS for 1 h after the equilibration period; guinea-pig aortic

strips were contracted by washing them in PSS containing 80 mM KCl (equimolar substitution of K^+ for Na^+). After the contraction reached a plateau (about 45 min), the compounds (0.1, 0.5, 1, 5, 10, 50, and 100 μM) were added cumulatively to the bath, allowing for any relaxation, to obtain an equilibrated level of force. Addition of the drug vehicle had no appreciable effect on K^+-induced contraction (DMSO for all compounds).

Supporting Information Available: Ab initio computation (RHF/3-21*G) structures in three different orientations for **4b**−**d**, **4f**, and **4k**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dustan, H. P. Calcium Channel Blockers, Potential Medical Benefits and Side Effects. *Hypertension* **1989**, *13*, I137−I140.

(2) Wilson, S.; Colucci, M. D. Usefulness of Calcium Antagonists for Congestive Heart Failure. *Am. J. Cardiol.* **1987**, *59*, 52B−58B.

(3) Schwinger, R. H. G.; Böhm, M.; Erdmann, E. Different Negative Inotropic Activity of Ca^{2+}-Antagonists in Human Myocardial Tissue. *Klin. Wochenschr.* **1990**, *68*, 797−805.

(4) Triulzi, M. O.; Mattioli, R.; Signorini, G.; Cirino, D.; Esposti, D.; Aguggini, G.; Maggi, G. C. Effects of low-dose diltiazem on isovolumetric relaxation and contraction. *G. Ital. Cardiol.* **1990**, *20*, 1137−1143.

(5) Campiani, G.; Nacci, V.; Garofalo, A.; Botta, M.; Fiorini, I.; Tafi, A. Synthesis and Preliminary Biological Evaluation of 1-Aminomethyl-4-substituted-4*H*-pyrrolo[2,1-*c*][1,4]benzothiazines, A New Class of Calcium Antagonists. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1193−1198.

(6) Campiani, G.; Garofalo, A.; Fiorini, I.; Nacci, V.; Botta, M.; Tafi, A. Synthesis and "In Vitro" Cardiovascular Activity of 4-Aryl-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazin-3-ones and 7-Acetoxy-6-phenyl-7a,8,9,10-tetrahydropyrrolo[2,1-*d*][1,5]benzothiazepin-10-one. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1235−1240.

(7) Campiani, G.; Garofalo, A.; Fiorini, I.; Botta, M.; Nacci, V.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R. Pyrrolo-[2,1-*c*][1,4]benzothiazines: Synthesis, Structure−Activity Relationships, Molecular Modeling Studies and Cardiovascular Activity. *J. Med. Chem.* **1995**, *38*, 4393−4410.

(8) Corelli, F.; Manetti, F.; Tafi, A.; Campiani, G.; Nacci, V.; Botta, M. Diltiazem-like Calcium Entry Blockers: A Hypothesis of the Receptor-Binding Site Based on a Comparative Molecular Field Analysis Model. *J. Med. Chem.* **1997**, *40*, 125−131.

(9) Billi, R.; Cosimelli, B.; Leoni, A.; Spinelli, D. Ring−ring interconversion. Part 3. On the Effect of the Substituents on the Thiazole Moiety in the Ring-Opening/Ring-Closing Reactions of Nitrosoimidazo[2,1-*b*][1,3]thiazoles with Hydrochloric Acid. *J. Heterocycl. Chem.* **2000**, *37*, 875−878.

(10) Billi, R.; Cosimelli, B.; Spinelli, D.; Rambaldi, M. Ring−ring interconversion. Part 2. Effect of the Substituents on the Rearrangement of 6-Aryl-3-methyl-5-nitrosoimidazo[2,1-*b*][1,3]-thiazoles into 8-Aryl-8-hydroxy-5-methyl-8*H*-[1,4][3,4-*c*][1,2,4]-oxadiazol-3-ones. A Novel Class of Potential Antitumor Agents. *Tetrahedron* **1999**, *55*, 5433−5440.

(11) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs,* 2nd ed.; Springer-Verlag: New York, 1987.

(12) Fujita, M.; Ito, S.; Ota, A.; Kato, N.; Yamamoto, K.; Kawashima, Y.; Yamauchi, H.; Iwao, J. Synthesis and Ca^{2+} antagonist activity of 2-[2-(aminoalkyl)oxy]-5-methoxyphenyl-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzothiazines. *J. Med. Chem.* **1990**, *33*, 1898−1905.

(13) Li, H.; Goldstein, B. M. Carboxamide group conformation in the nicotinamide and thiazole-4-carboxamide rings: implications for enzyme binding. *J. Med. Chem.* **1992**, *35*, 3560−3567.

(14) Andreani, A.; Rambaldi, M.; Andreani, F.; Hrelia, P.; Cantelli Forti, G. Synthesis and mutagenic activity of imidazo[2,1-*b*]-thiazoles bearing at least one nitro or nitroso group. *Arch. Pharm. Chemi., Sci. Ed.* **1987**, *15*, 41−49.

(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.

JM020815D