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1,5-Benzoxazepines vs 1,5-Benzodiazepines. One-Pot Microwave-Assisted Synthesis and Evaluation for Antioxidant Activity and Lipid Peroxidation Inhibition

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Amino-1,5-benzoxazepines **2** and **5** and hydroxyl-1,5-benzodiazepines **3** and **6** have been synthesized in one-pot solvent-free conditions from 2,3-diaminophenol and ketones through microwave assisted acid catalysis, the benzoxazepine/benzodiazepine ratio depending on the R^1 and R^3 aryl substituents. The otherwise inaccessible and unknown 2,2-dimethyl-4-aryl-1,5-benzodiazepines **8** were also prepared in an analogous manner. The reaction mechanism was investigated by means of DFT calculations. Structural assignments of the new compounds as well as complete assignment of ^{1}H and ^{13}C NMR signals have been unambiguously achieved on the basis of the analysis of their ^{1}H and ^{13}C NMR (1D and 2D), IR, MS, and elemental analysis data, whereas the presence of an amino group in **5** and of a hydroxyl in **6** was confirmed by derivatization. Compounds **2**, **3**, **5f**, **6a**, **6c**, **6d**, **6f**, **6h**, **8c**, and **12** were evaluated as antioxidants and lipid peroxidation inhibitors in vitro. Compound **6f** was also evaluated as anti-inflammatory agent in vivo. Compounds **2** and **6f** were found to be the most potent as inhibitors of lipoxygenase and of lipid peroxidation, respectively.

Introduction

Heterocyclic compounds are highly ranked among pharmaceutically important natural and synthetic materials. The remarkable ability of heterocyclic nuclei to serve as both biomimetics and active pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs. Heterocyclic derivatives such as morphine alkaloids, β-lactam antibiotics, and benzodiazepines are just a few examples from various pharmaceuticals featuring a heterocyclic component.[1]

The benzodiazepine nucleus is a pharmacophoric scaffold, and many benzodiazepines have recently received great attention because of their wide range of therapeutic and pharmacological properties. Many members of the benzodiazepine family are nowadays widely used as antianxiety, antidepressant, sedative, hypnotic, tranquilizing, anticonvulsant, antihistaminic, analgesic, and anti-inflammatory agents.[2,3] Because of their wide range of biological applications, the development of mild, efficient, and environmentally friendly protocols continues to be a challenging endeavor in synthetic organic chemistry. As a result, considerable attention has been drawn recently to new improved methods for the preparation of 1,5-benzodiazepines[4] also by means of three component reactions.[5]

Concerning their counterparts, the one nitrogen benzoannelated seven-membered ring heterocycles (the oxaza derivatives), although much less studied than benzodiazepines, became increasingly interesting because of their biological activity.[6]

Some time ago, we described a facile synthesis of 2,3-dihydro-1*H*-1,5-benzodiazepines by condensation of ketones with *o*-phenylenediamines by application of microwave irradiation.[7] In the meantime, although many methods for the preparation of benzodiazepines using *o*-phenylenediamines as starting materials appeared in the literature,[8] the possibility of benzodiazepine formation bearing a hydroxyl in the phenylenediamine moiety was never investigated. The use of 2,3-diaminophenol, instead of *o*-phenylenediamine, could lead to the formation of either hydroxybenzodiazepines or aminobenzoxazepines, so the whole project looked very interesting.

Moreover, taking into consideration that diazepam causes a decrease in peroxidative decomposition of polyunsaturated fatty acids of membranes on withdrawal, which could be due to stabilization of membranes after long-term binding of diazepam,[9] it looked promising to examine the possibility of hydroxybenzodiazepines and/or aminobenzoxazepines to act as inhibitors of lipoxygenase (LOX[a]) activity and of peroxidation

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[a] Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; AM1, Austin model 1; au, atomic unit of energy (1 au = 627.51 kcal/mol); B3LYP, Becke three-parameter Lee−Yang−Parr hybrid functional; 6-31G*, a type of valence double-ζ polarized basis set; ClogP, calculated logarithm of partition coeficient log($C_{octanol}/C_{water}$); COLOC, two-dimensional C−H correlation via long-range coupling $^2J_{CH}$ or $^3J_{CH}$; H−H COSY, two-dimensional H−H correlation spectroscopy via $^nJ_{HH}$; C−H COSY or HETCOR, two-dimentional heteronuclear correlation via $^1J_{CH}$; DFT, density functional theory; DPPH, 1,l-diphenyl-2-picrylhydrazyl radical; ICPE, Inhibition of carrageenin-induced rat paw edema; IMA, indomethacin; LOX, lipoxygenase; LPO, lipid peroxidation; MW, microwave irradiation; NDGA, nordihydroguaiaretic acid; NOESY, two-dimensional H−H nuclear Overhauser effect correlation spectroscopy; NSAIDs, nonsteroidal anti-inflammatory drugs; Trolox, trade name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TS, transition state; *p*-TsA, *p*-toluenesulfonic acid; ZPE, zero point energy.

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of biological membranes as well as to present anti-inflammatory activity.

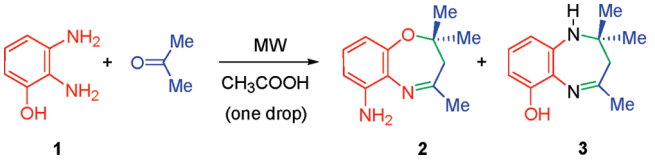
Results and Discussion

Chemistry. Very recently, we have investigated the synthesis and antioxidant as well as the aldose reductase inhibition of some 1,5-benzodiazepine derivatives[10] and also their cytogenetic activity in vitro in normal lymphocyte cultures.[11] In continuation of our previous work concerning benzodiazepines[7,12] this paper describes a study of the synthesis of 6-hydroxy-2,3-dihydro-1*H*-1,5-benzodiazepines versus 6-amino-2,3-dihydro-1,5-benzoxazepines by condensation of ketones with 2,3-diaminophenol through one-pot microwave assisted acid catalyzed reaction without solvent and the testing of the novel compounds with regard to their antioxidant ability as well as to their potent lipid peroxidation (LPO) inhibitory activity.

Initially, the reaction was attempted simply by mixing 2,3-diaminophenol **1** with acetone, in a 1.5:2 molar ratio, in the presence of a catalytic amount of acetic acid and irradiating in a Biotage Initiator 2.0 microwave oven, whereupon a mixture of aminobenzoxazepine **2** and hydroxybenzodiazepine **3** in a 3:4 ratio was formed (Scheme 1). Separation between **2** and **3** became feasible, when immediately after irradiation the reaction mixture was quenched with a 10% sodium bicarbonate solution. Otherwise, complete transformation of **2** to **3** was observed by the traces of acetic acid, which remained in the reaction mixture after microwave irradiation. The reaction proceeded analogously to give a mixture of **2** and **3** (7:3 ratio), when *p*-TsA was used instead of acetic acid. These results indicated that the oxazepine derivative **2** constitutes the kinetic product, whereas the benzodiazepine derivative **3** the thermodynamic one.

After the selectivity of the reaction with acetone was established, in order to investigate the possibility of a more general application of the method, the reaction was repeated with a number of substituted acetophenones **4**, and the results are shown in Scheme 2 and Table 1.

Scheme 1. Reaction of 2,3-Diaminophenol with Acetone



From Scheme 2 and Table 1 it can be concluded that the presence of electron-withdrawing substituents in the acetophenone moiety stabilizes the initially formed 1,5-benzoxazepines **5** and consequently renders their isolation more possible. It is characteristic that in the case of the *p*-nitro-substituted acetophenone **4g** only the benzoxazepine derivative **5g** was formed, which could not be completely transformed to benzodiazepine **6g** even after prolonged refluxing with acetic acid (after 5 days of reflux **5g**/**6g** = 1:5 and then decomposition had occurred). To the contrary, in the presence of electron-releasing substituents, the electron rich oxygen is easily protonated resulting in spontaneous transformation of benzoxazepines **5** to benzodiazepines **6**. As a result, in the case of the methoxy-substituted acetophenones **4b**−**d** only the corresponding benzodiazepine derivatives **6b**−**d** were isolated. Moreover, it should also be noticed that the 9-hydroxybenzodiazepines **7** were never traced.

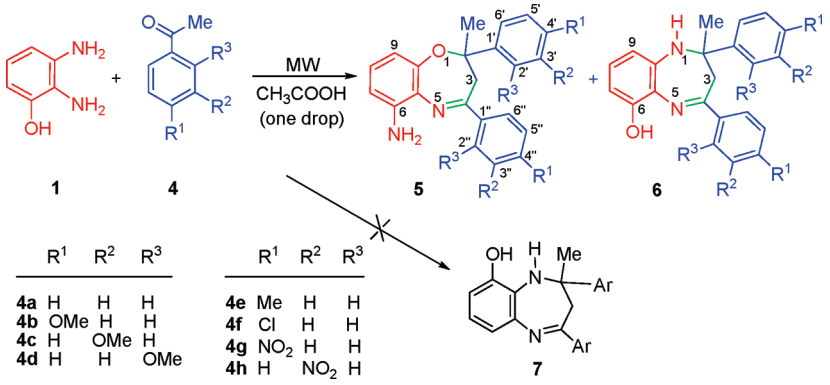
The possibility of formation of the otherwise inaccessible and unknown 2,2-dimethyl-4-arylbenzodiazepines **8** was also examined (Scheme 3 and Table 2). Thus, when *o*-phenylenediamine **1b** was mixed with acetophenone **4a** and acetone in a 1:2:0.5 molar ratio in the presence of catalytic amount of acetic acid and then irradiated with microwaves, 2,2-dimethyl-4-phenylbenzodiazepine **8c** was formed in 92% yield. This reaction seems to be very sensitive to the molar ratio of the starting materials. Indeed, when the amount of acetone was increased to 1 molar ratio, a mixture of all three possible products, namely, **8c** and the known[10] **9** and **10**, was formed in 45%, 16%, and 31% yield, respectively. Analogously, from **4f** the product **8d** was isolated in 93% yield. 2,3-Diaminophenol behaved analogously, affording with acetophenones **4b** and **4e** the corresponding 6-hydroxy-2,2-dimethyl substituted benzodiazepines **8a** and **8b** in 56% and 95% yield, respectively.

Table 1. Reaction Conditions and Products for the Reaction of Scheme 2

ketone	time (min)	power (W)	**5** (%)	**6** (%)	**5**/**6**[a] ratio	total yield (%)
4a	2	80	35	59	37:63	94
4b	3	240		88	0:100	88
4b[b]	2	80	trace	80	0:100	80
4c	2	80		90	0:100	90
4d	2	80		93	0:100	93
4e	3	240	25	61	29:71	86
4e[b]	2	80	81	trace	100:0	81
4f	2	80	41	56	42:58	97
4g	3	240	81	trace	100:0	81
4h	5	80	47	30	61:39	77

[a] Quenched immediately after irradiation. [b] With *p*-TsA as catalyst.

Scheme 2. Reaction of 2,3-Diaminophenol with Substituted Acetophenones



Scheme 3. Reaction of Diamines **1** with a Mixture of Acetone and Acetophenone

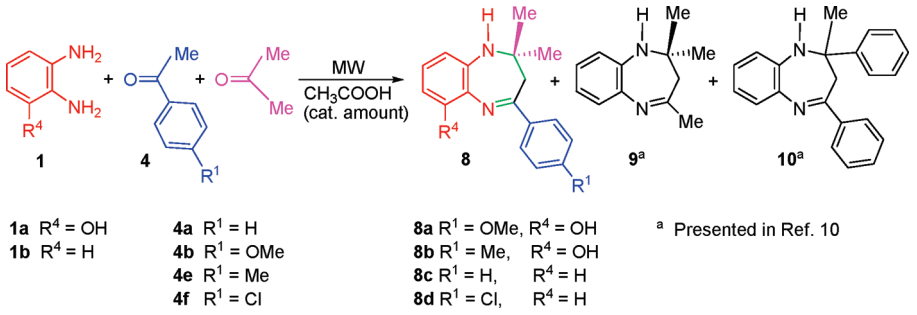
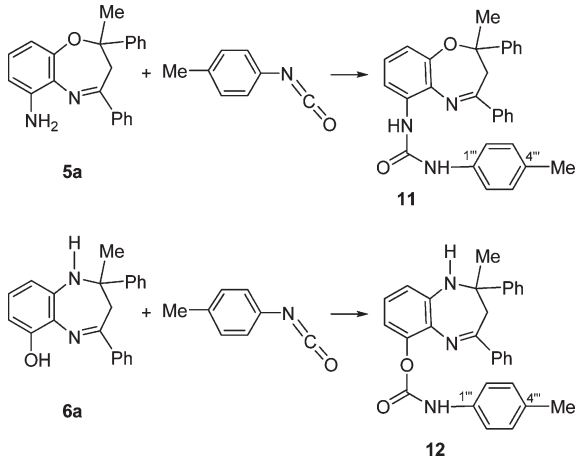


Table 2. Reaction Conditions and Products for the Reaction of Scheme 3

					products (%)		
amine	acetophenone	molar ratio[a]	time (min)	power (W)			
1a	**4e**	1:2:1	2	80	**3** (10)	**6e** (21)	**8b** (51)
1a	**4e**	1:2:0.5	2	80			**8b** (95)
1a	**4b**	1:2:0.5	2	80		**6b** (20)	**8a** (56)
1b	**4a**	1:2:1	2	80	**8c** (45)	**9** (16)	**10** (31)
1b	**4a**	1:2:0.5	2	80			**8c** (92)
1b	**4f**	1:2:0.5	2	80			**8d** (93)

[a] Amine/acetophenone/acetone.

Scheme 4. Derivatization of **5a** and **6a** by Reacting with *p*-Tolyl Isocyanate



Finally, the differentiation between aminobenzoxazoles **5** and hydroxybenzodiazepines **6** was also established beyond doubt by their derivatization. For this reason, **5a** was transformed to the arylurea derivative **11**, whereas **6a** was transformed to the carbamate one **12** (Scheme 4), easily differentiated from their carbonyl IR absorptions[13,14] at 1665 and 1738 cm^{-1}, respectively.

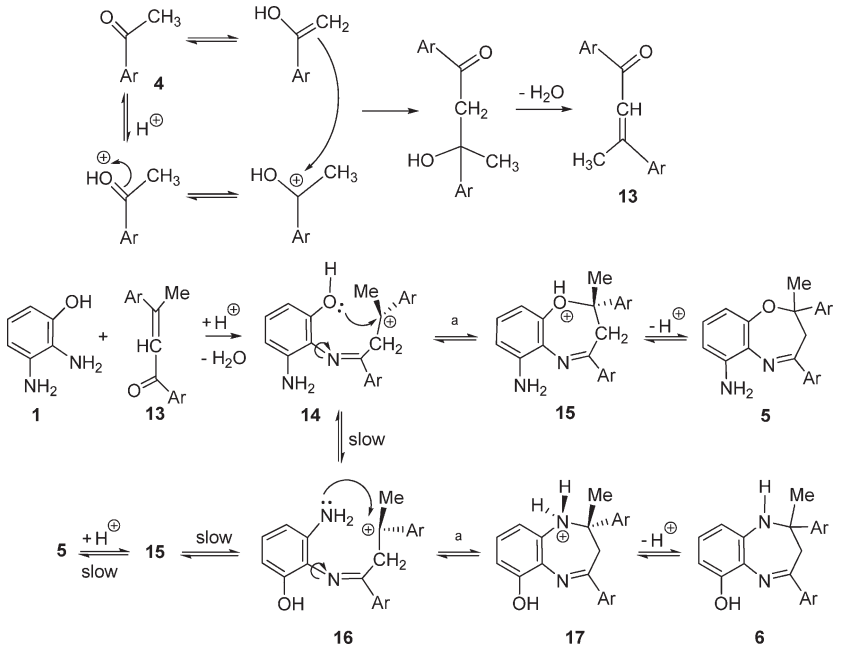
For the formation of benzoxazepines **5** and benzodiazepines **6** a plausible mechanistic scheme (Scheme 5) involving aldol condensation of two acetophenone molecules to **13** could be implicated, whereupon the initially formed conformers **14** and **16** could finally cyclize to the seven-membered ring products **5** and **6**, respectively. The fact that 9-hydroxybenzodiazepines **7** (Scheme 1) were never traced offers decisive proof in favor of the proposed mechanism.

To further support the above mechanism, some DFT calculations have been carried out on the first protonation site of aminophenol **1** and on oxa- or aza-protonation selectivity of **5** or **6** as well as on the stability of intermediates **15** and **17**. For this purpose, in order to investigate the possible influence of aryl substituents, the unsubstituted and the *p*-OMe and *p*-NO_2 substituted aryl derivatives were examined, and the results are presented in Tables 3 and 4.

As already mentioned, according to the proposed plausible mechanism shown in Scheme 5, after the first aldol condensation of the ketone moiety, intermediate **13** is formed. The more electronic charge computed on N-5 of diaminophenol **1** suggests that most possibly this is the favored amino group that reacts with the carbonyl carbon of **13** to afford the intermediate imine **14**. In the next step, the seven-membered ring closes by reaction of the hydroxyl or the amino group of the intermediate conformer **14** or **16**, respectively, leading to the protonated intermediate **15** or **17**. From the calculated values of total energies (E_{total}, Table 4) it is concluded that benzodiazepines **6** are slightly more stable than benzoxazepines **5**, constituting thus the thermodynamic products. Even though compounds **6** are thermodynamically favored over **5**, in most cases a mixture of both products with varied ratios is obtained. This fact can be attributed to the slow interconversion of the intermediate conformers **14** and **16** via the hindered rotation of the aromatic ring around the Ar−N bond, due to the two substituents ortho to the imino nitrogen. Moreover, the formation of compounds **5** can be explained by studying the thermochemical data of the transition state. According to these results (Table 4) considering the intermediate conformers **14** and **16** as transition states to **15** and **17**, respectively, the relative activation energy of **14** was found slightly lower than that of **16** (1.3−3.2 kcal/mol), thus favoring the formation of the protonated intermediate **15** over that of **17**; therefore, intermediate **15** can be considered as the kinetic product, whereas intermediate **17** is the thermodynamic one. Although this energy difference of 1.3−3.2 kcal/mol seems to be rather small in absolute value, it always points toward the same direction and is decisive for the kinetic/thermodynamic pathway.

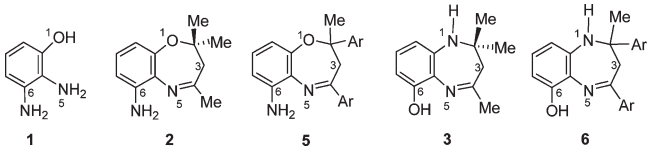
On the other hand, in the exploration of the mechanism of conversion of **5** to **6**, the traces of acetic acid from the initial condensation reaction were implicated. Hence, the protonation site of **5** and **6** and the stability of the protonated

Scheme 5. Plausible Mechanism Leading to Benzoxazepine **5** and Benzodiazepine Derivatives **6**, as Well as for Conversion of **5** to **6**[a]



[a] These intermediates do not depict the absolute configuration of the transition state and the final products **5** and **6** are mixtures of their *R* and *S* enantiomers.

Table 3. Net Atomic Charges (Mulliken) on Heteroatoms for the Neutral Species **1**, **2**, **3**, and Some Selected Substituted **5** and **6**



atoms	**1**[a]	**2**	**5a**	**5b**	**5g**	atoms	**3**	**6a**	**6b**	**6g**
				Q_{net} Atomic Charges (Electrons) in Compounds						
O1	−0.6565	−0.5619	−0.5675	−0.5686	−0.5639	N1	−0.6609	−0.6962	−0.7007	−0.7091
N5	−0.8393	−0.5187	−0.5873	−0.5964	−0.5813	N5	−0.5463	−0.6193	−0.6345	−0.6186
6-N	−0.7913	−0.7946	−0.7925	−0.7627	−0.7923	6-O	−0.6546	−0.6558	−0.6549	−0.6431
				Hydrogen Charges Summed up on Heteroatoms						
O1	−0.2369	−0.5619	−0.5675	−0.5686	−0.5639	N1	−0.3538	−0.3764	−0.3845	−0.3443
N5	−0.1946	−0.5187	−0.5873	−0.5964	−0.5813	N5	−0.5463	−0.6193	−0.6345	−0.6186
6-N	−0.1680	−0.1421	−0.1294	−0.1322	−0.1173	6-O	−0.2384	−0.2323	−0.2338	−0.2202

[a] The atom numbering in **1** is arbitrary, analogous as in the rest of compounds, for simplicity reasons.

intermediates **15** and **17** were investigated. The computational results indicate that the favored protonation sites of **5** and **6** are the heteroatoms in position 1 of the seven-membered ring, since these atoms have more electronic charge than the exocyclic amino nitrogen and hydroxyl group, respectively (Table 3). After protonation, the resulting intermediates **17** are computed to be more stable than **15** by 7.3−24.2 kcal/mol. Protonated intermediates **15** are computed to have the tendency to open the benzoxazepine ring, since the final O1−C2 bond length is computed to vary between 1.70 and 1.80 Å. Consequently, the protonated compounds **5** are gradually converted again to the open form **14**, which may recyclize to **17** and finally transformed to **6**.

Structure Assignment of the New Compounds. The assigned molecular structures of all new compounds **2**, **3**, **5**, **6**, **8**, **11**, and **12** were based on rigorous spectroscopic analysis including IR, MS, NMR (^{1}H, ^{13}C, H−H COSY, C−H COSY, and C−H COLOC), and elemental analysis data.

The 3-methylene protons of compounds **5** and **6** constitute an AB spin system giving two distinct doublets with $^2J \approx$ 13−14 Hz at about 2.9−3.1 δ and 3.2−3.5 δ, respectively. The *endo*-proton being in pseudoaxial configuration in the diazepine ring is shielded by the C=N anisotropy field, whereas the *exo*-proton being in the pseudoequatorial configuration is deshielded by the two adjacent phenyls.[12c] In the COSY spectrum of **6f** there is a weak cross-peak between the 3-H_{exo} and the N1−H via a ^4J coupling through a W, almost coplanar conformation. In most ^{1}H NMR spectra this 3-H_{exo} proton signal is split by a small coupling of ~1.2 Hz or is slightly broadened. In compound **6f** the above 3-H_{endo} has COLOC correlation via $^3J_{CH}$ with the C-1′ of the 2-phenyl group. In practice, this finding led us to the acceptance that in solution this proton has a significant coupling with C-1′

Table 4. Total DFT Energies of Formation (B3LYP/6-31G*) for the Neutral Compounds **2** and **3** and for Their Protonated Intermediates **2H** and **3H** for Some Substituted Derivatives of **5** and **6**, Their Protonated Intermediates **15** and **17**, and Their Transition Intermediates **14** and **16**

	2		**3**	**2H**		**3H**
E_{total}[a]	−651.373833		−651.373265	−651.697902		−651.727397
ΔE[b]			1.52			−18.51
	5a	**6a**	**5b**	**6b**	**5g**	**6g**
E_{total}[a]	−1034.735771	−1034.738193	−1263.716398	−1263.719183	−1443.734070	−1443.735835
ΔE[b]		−1.52		−1.75		−1.11
	15a	**17a**	**15b**	**17b**	**15g**	**17g**
E_{total}[a]	−1035.061874	−1035.100457	−1264.078311	−1264.089926	−1444.043362	−1444.074656
ΔE[b]		−24.21		−7.29		−19.63
	14a	**16a**	**14b**	**16b**	**14g**	**16g**
E_{total}[a]	−1035.071508	−1035.068739	−1264.069061	−1264.067026	−1444.043340	−1444.038226
ΔE[b]		1.74		1.28		3.21
TS bond[c]	2.786	3.148	2.919	3.481	2.635	3.142
ν[d]	−59.01	−70.68	−89.32	−25.18	−18.14	−118.40

[a] Sum of electronic energy and zero-point energy (ZPE, the oscilator's vibrational energy at absolute zero) correction (au); 1 au = 627.51 kcal/mol. [b] Relative stabilization energy: $\Delta E = E_{benzodiazepine} - E_{benzoxazepine}$ (kcal/mol). [c] Interatomic distance for the new forming bond in TS (Å). [d] Imaginary frequency (cm^{-1}).

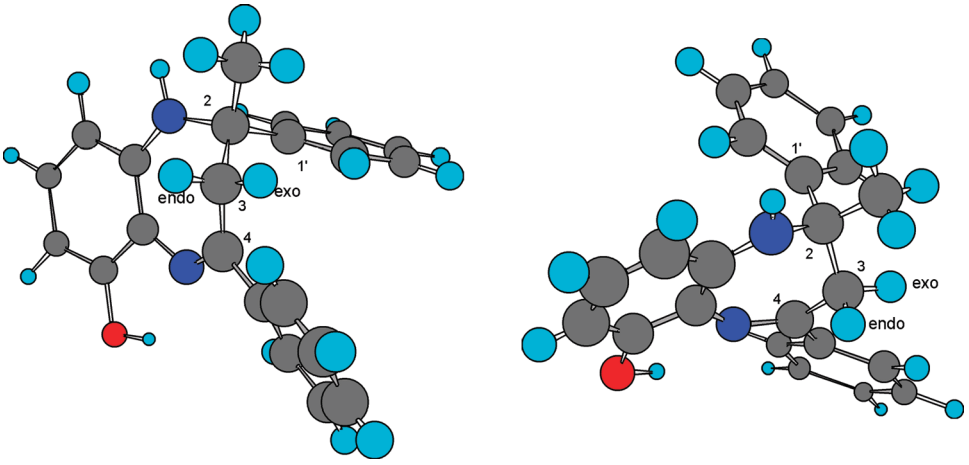


Figure 1. Global lower energy conformation of compound **6a** (from two different views) that fits COLOC and NOESY data (by DFT).

carbon; hence, the dihedral angle H_{endo}−C3−C2−C1′ is near 180° or 0° in almost coplanar configuration. In Figure 1, the favored conformation calculated by DFT that fits with the previous observations is shown, where the two phenyl groups are arranged on the same side of diazepine ring and in almost parallel configuration.

In ^{1}H NMR of many members of 6-aminobenzoxazepines the two amino protons are differentiated, giving two absorptions. One signal at ∼7−8 ppm is assigned to a proton forming a hydrogen bond with N-5, whereas the remaining proton is resonating at ∼4−5 ppm.

In Figure 2 the COLOC correlations observed via $^2J_{CH}$ and $^3J_{CH}$ in compounds **5a** and **6f** are depicted. Some important NOESY correlations in compound **6f** are also included, confirming the proposed structure.

Biological Evaluation. In the present investigation, compounds **2**, **3**, **5f**, **6a**,**c**,**d**,**f**,**h**, **8c**, and **12** were studied in vitro with regard to their antioxidant ability in comparison to well-known antioxidant agents such as nordihydroguaiaretic acid (NDGA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). For estimation of the antioxidative potential of chemical components, different experimental approaches were used.[15] To evaluate the in vitro antioxidant activity of the synthesized compounds two different

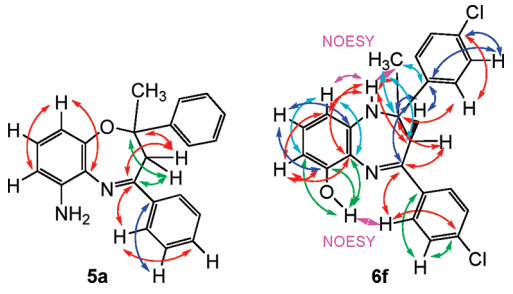


Figure 2. COLOC correlations via $^2J_{CH}$ and $^3J_{CH}$ observed in compounds **5a** and **6f**.

antioxidant assays have been used: (a) Interaction with the stable free radical 1,l-diphenyl-2-picrylhydrazyl radical (DPPH) and (b) Interaction with the water-soluble azo compound 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), used as a source of peroxyl radicals (ROO·). Both methods require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results.[16] The DPPH method is described as a simple, rapid, and convenient method independent of sample polarity for screening many samples for radical scavenging activity.[17] These advantages made the DPPH method interesting for testing our compounds.

Table 5. % Interaction of the Tested Compounds (at 100 and 50 μM) with DPPH (DPPH %) after 20 and 60 min, Inhibition Values (IC_{50}) of Lipid Peroxidation (LPO) Induced by AAPH, in Vitro Inhibition of Soybean Lipoxygenase (LOX), in Vivo % Inhibition of Carrageenin-Induced Rat Paw Edema (ICPE %) at 0.01 mmol/kg Body Weight[a]

		DPPH %, 20 min		DPPH %, 60 min				
compds	ClogP	100 μM	50 μM	100 μM	50 μM	LPO inhib IC_{50} (μM)	% LOX inhib 100 μM	ICPE
2	1.75	86	87	86	88	70	98	
3	2.08	66	44	65	67	77	NA[b]	
5f	6.69	86	87	86	86	47.5	NA[b]	
6a	4.91	30		29		50	28	
6c	4.65	27		27		60	37	
6d	4.51	36		36		46	3	
6f	6.37	54		53		2.5	NA[b]	35*
6h	4.39	32		32		46	NA[b]	
8c	4.05	15		14		45	6	
12	7.39	10		9		50	3	
NDGA		93		96			84	
Trolox						63		
IMA[c]								47**

[a] Each in vitro experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean. Each in vivo result represents the mean obtained from 6−15 animals in two independent experiments. In all cases, significant difference from control was as follows: (*) $p < 0.1$, (**) $p < 0.01$ (Student's t test). [b] NA: no action detectable under the reported experimental conditions. [c] IMA: indomethacin.

The use of the AAPH peroxyl radical is recommended as more appropriate for measuring radical-scavenging activity in vitro because the activity of the AAPH peroxyl radical shows a greater similarity to cellular activities, such as lipid peroxidation (LPO).[18] The water-soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals.[19] The interaction of the examined compounds with the stable free radical DPPH is shown in Table 5. This interaction indicates their radical scavenging ability in an iron-free system. The above-mentioned compounds were examined for their DPPH interaction at 100 and 50 μM after 20 and 60 min. Compounds **2**, **3**, **5f**, and **6f**, showed the best DPPH interaction percentage values, some of them displaying similar values (86%) to that of the reference compound NDGA (93%) at the same concentration. These compounds have either a phenolic group or a free aromatic amino group in their structure, so they are able to donate a hydrogen atom. The presence of substituents with low lipophilicity, expressed as π values (π value expresses the hydrophobic contribution of a substituent), like OCH_3 ($\pi = -0.02$) or NO_2 group ($\pi = -0.28$) diminishes reducing ability (**6c**, **6d**, **6h**). On the contrary, a substituent with high π value, e.g., Cl (0.71), as in compound **6f**, increases the scavenging result. The role of the electronic effect of the substituents is not well-defined, and it looks contradictory. Thus, compound **6h** with a $R^2 = NO_2$ group with a negative inductive effect ($-I$) presents similar results with the unsubstituted derivative **6a**, whereas **6f** with a chloro substituent, with a negative inductive effect ($-I$) too, is more potent than **6a**. The most interesting derivative is the unsubstituted compound **2** combining an aromatic amino moiety and an oxazepine ring. The second more significant is the benzoxazepine **5f** with a chloro substituent. The unsubstituted diazepine **3** with a phenylhydroxyl group instead of a free aromatic amino group is less potent than **2**. Lower calculated[20] lipophilicity values seem to be important, since **2** is less lipophilic than **3** (ClogP for **2** is 1.75, and for **3** it is 2.08). For the sake of structure comparison, benzoxazepine **5f** presents higher interaction with DPPH compared to benzodiazepine **6f**, indicating that the derivative with a free aromatic amino group in its structure is more able to donate a hydrogen atom and to act as an antioxidant. For compound **3** the interaction values were found to be time and concentration dependent, whereas for compounds **6a**, **6c**, **6d**, **6f**, **6h**, **8c**, and **12** no results were detectable at 50 μM. The disappearance of the phenolic hydroxyl in compound **12**, due to the transformation of **6a** to the carbamate **12**, supports the decrease of the reducing ability. Each in vitro experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies in vitro.[19] The results (Table 5) showed that benzodiazepine **6f**, having a free phenolic group, a benzodiazepine ring, and lipophilic ($R^1 = Cl$) substituents, is the best inhibitor of lipid peroxidation, displaying an IC_{50} value of 2.5 μM. No significant differences are observed between the oxazepine **2** and diazepine **3**. Benzoxazepine **5f** also presents lower anti-lipid peroxidation (anti-LPO) activity compared to benzodiazepine **6f**. Compounds **6d**, **5f**, and **6h** presented similar IC_{50} values (46−47.5 μM), whereas compounds **6a** and **12** follow with IC_{50} values of 50 μM. It seems that a free phenolic hydroxyl group in combination with a benzodiazepine ring leads to better inhibition of lipid peroxidation. The transformation of **6a** to carbamate **12** did not offer any advantage in anti-LPO activity (both present similar IC_{50} values). Between the methoxybenzodiazepines, **6d** is a more potent inhibitor than **6c**, indicating that $R^3 = OCH_3 > R^2 = OCH_3$. From our results it seems that higher inhibition of lipid peroxidation (lower IC_{50} values) is correlated with higher lipophilicity values (ClogP values, Table 5). These data are supported by literature findings, which revealed that lipophilicity (ClogP) is the main physicochemical parameter influencing the anti-LPO activity.[21]

Lipoxygenases (LOXs) play a significant role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer[22] from the biotransformation of arachidonic acid catalyzed by LOX. Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, certain types of cancer, and cardiovascular diseases.[23]

In this context, we decided to further evaluate the synthesized derivatives for their ability to inhibit soybean LOX by the UV absorbance based enzyme assay.[24] It has been

shown that inhibition of plant LOX activity by nonsteroidal anti-inflammatory drugs (NSAIDs) is qualitatively similar to their inhibition of the rat mast cell LOX and may be used as a simple qualitative screen for such activity. Most of the LOX inhibitors are antioxidants or free radical scavengers.[25] LOXs contain a "non-heme" iron per molecule in the enzyme active site as high-spin Fe^{2+} in the native state and the high spin Fe^{3+} in the activated state. Some studies suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+}. This inhibition is related to their ability to reduce the iron species in the active site to the catalytically inactive ferrous form,[25] whereas several LOX inhibitors are excellent ligands for Fe^{3+}. From the tested derivatives only compound **2** presented 98% inhibition at 100 μM, a value considerable better than that of the reference compound NDGA (Table 5). Although lipophilicity is referred to as an important physicochemical property for LOX inhibitors,[26] herein the most potent compound, namely, the oxazepine analogue **2** (98%) with a ClogP of 1.75, does not follow this concept. For the sake of comparison oxazepine **2** is potent whereas the diazepine **3** is inactive, under our experimental conditions. A methoxy substitution on **6a** leading to **6c** does not increase significantly the inhibition in **6c**. The transformation also of **6a** to the carbamate derivative **12** diminishes the LOX inhibition.

Our study indicates that lipid peroxidation inhibitory activity is not always accompanied by DPPH radical scavenging activity. Thus, although compounds such as **6f**, **5f**, **6d**, **6a**, **6h**, and **12** inhibit lipid peroxidation potently, they present low DPPH scavenging activity. This is in accordance with the finding of Curini et al.,[27] who have studied the antioxidant and LOX inhibitory activity of five natural prenyloxycarboxylic acids. They showed that boropinic acid was the most efficient lipid peroxidation inhibitor (LOX), although it was not the most active DPPH radical scavenger.

Compound **6f**, which was the most potent lipid peroxidation inhibitor, was tested as an in vivo anti-inflammatory agent. In acute toxicity experiments, the in vivo examined compound did not present toxic effects in doses up to 0.2 mmol/kg body weight. Ulcerogenicity was not found. The in vivo anti-inflammarory effect of compound **6f** was assessed by using the carrageenin-induced rat paw edema (ICPE) model. Acute inflammation is due to the release of chemical mediators, which cause edema as a result of extravasations of fluid and proteins from the local microvasculature and accumulation of polymorphonuclear leukocytes at the inflammatory site. The induced edema is a nonspecific inflammation highly sensitive to NSAIDs. Thus, it has been accepted as a useful tool for studying new anti-inflammatory agents.[28] It reliably predicts the anti-inflammatory potency of the NSAIDs, as a result of inhibition of prostaglandin amplification. Compound **6f** showed 35% percentage of protection, while the reference drug indomethacin (IMA) induced 47% protection at an equivalent dose.

Physicochemical Studies. Determination of Lipophilicity as ClogP Values. Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic activity, and elimination, we tried to calculate theoretically the lipophilicity values of benzoxazepines/benzodiazepines as ClogP values in *n*-octanol-buffer by the ClogP programme of Biobyte Corp.[20]

Conclusions

In the present study several new benzodiazepines and benzoxazepines have been synthesized with the aim of studying their antioxidant and anti-inflammatory activities. The synthesis of 6-hydroxybenzodiazepines versus their 6-aminobenzoxazepine counterparts was investigated, and it was established that benzoxazepines constitute the kinetic products, whereas benzodiazepines constitute the thermodynamic ones. In addition, the otherwise inaccessible and unknown 2,2-dimethyl-4-arylbenzodiazepines were also synthesized.

In terms of biological activity, the most interesting antioxidant derivatives were those with the benzoxazepine moiety and the amino group substituent. Improved inhibitory activities on lipid peroxidation were observed by retaining a phenolic group and a benzodiazepine ring. Benzodiazepine **6f** was the best inhibitor of lipid peroxidation, displaying an IC_{50} value of 2.5 μM against the value of 63 μM of the reference compound Trolox, whereas the trimethyl-substituted benzo xazepine **2** showed the best LOX inhibition (98%), much better than the reference compound NDGA (84%). Compounds **2** and **6f** can be used as lead molecules for the design of agents with excellent LOX and LPO inhibitory activities.

Experimental Section

General. DPPH and NDGA were purchased from Aldrich Chemical Co. (Milwaukee, WI). Soybean LOX, linoleic acid sodium salt, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO), and carrageenin, type K, was commercially available. For the in vivo experiments male and female Fischer-344 rats (180−240 g) were used. For the in vitro tests a Lambda 20 (Perkin−Elmer) UV−visible double beam spectrophotometer was used. Melting points were measured on a Kofler hot stage and are uncorrected. Column chromatography was carried out using Merck silica gel (70−230 mesh). Thin layer chromatography (TLC) was performed using precoated silica gel 0.25 mm glass plates containing fluorescent indicator UV_{254} (Macherey-Nagel) using a 3:1 mixture of petroleum ether−ethyl acetate. Petroleum ether refers to the fraction boiling between 60 and 80 °C. NMR spectra were recorded at room temperature on a Bruker AM 300 or AVANCE III 300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using $CDCl_3$ as solvent, unless otherwise is indicated. The chemical shifts are expressed in δ values (ppm) relative to TMS as internal standard for ^{1}H and relative to TMS (0.00 ppm) or to $CDCl_3$ (77.05 ppm) for ^{13}C NMR spectra. Coupling constants ^nJ are reported in Hz. Second order ^{1}H spectra, where it was possible, were analyzed by simulation.[29] IR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer and are reported in wave numbers (cm^{-1}). LC−MS (ESI, 1.65 eV) spectra were recorded on LCMS-2010 EV system (Shimadzu). Low-resolution electron impact mass spectra (EIMS) were obtained on a 6890N GC/MS instrument (Agilent Technology); results are reported as m/z (rel intensity in %) at ionization energy of 70 eV. The purity of all novel compounds was confirmed to exceed 95% by elemental analysis performed with a Perkin-Elmer 2400-II CHN analyzer. Structural assignments of the derived compounds were established by analysis of their IR, MS, and NMR spectra (^{1}H, ^{13}C, COSY, NOESY, HETCOR, and COLOC). For the computational analysis, transition states and products were built by ChemDraw (ChemDraw7) and optimized by AM1 as implemented in MOPAC package[30] and subsequently optimized by density functional theory (DFT) using B3LYP level with 6-31G-(d) basis set as implemented in the Gaussian 03 package.[31,32] The corresponding transition states of the reactions were located as described in our previous work.[33]

Reaction of 2,3-Diaminophenol with Acetone under Microwave Irradiation. Acetone (2.0 mmol), 2,3-diaminophenol **1** (1.5 mmol),

and a drop of acetic acid were mixed thoroughly and were irradiated in a Biotage Initiator 2.0 microwave oven at a power of 80 W for 2 min. The crude product was dissolved in dichloromethane (30 mL) and washed with 5% aqueous $NaHCO_3$ and with water. The organic layer was dried over Na_2SO_4, and after filtration the solvent was evaporated and the residue was purified by column chromatography on silica gel using petroleum ether−ethyl acetate (10:1) of slowly increasing polarity to afford in elution order compounds **3** and **2**.

6-Hydroxy-2,2,4-trimethyl-2,3-dihydro-1*H*-1,5-benzodiazepine (3). Light yellow crystals (0.114 g, 56%), mp 145−147 °C. IR (KBr) ν_{max}: 3448, 3345, 1639, 1584 cm^{-1}. ^1H NMR: 1.28 (s, 6H, 2-CH_3), 2.29 (s, 3H, 4-CH_3), 2.52 (s, 2H, 3-H), 3.69 (br s, 1H, NH), 6.10 (dd, J = 8.1, 1.1 Hz, 1H, 9-H), 6.43 (dd, J = 8.0, 1.4 Hz, 1H, 7-H), 6.89 (dd, J = 8.1, 8.0 Hz, 1H, 8-H), 8.10 (br s, 1H, OH). ^{13}C NMR: 30.1 (2-CH_3), 30.7 (4-CH_3), 49.4 (C-3), 57.0 (C-2), 103.9 (C-7), 109.6 (C-9), 121.6 (C-6a), 128.2 (C-8), 139.6 (C-9a), 154.2 (C-6), 167.0 (C-4). MS (LCMS) m/z (%) 205 (100, M^+ + H). Anal. Calcd for $C_{12}H_{16}N_2O$ (204.27): C, 70.56; H, 7.90; N, 13.71. Found: C, 70.51; H, 7.81; N, 13.68.

6-Amino-2,2,4-trimethyl-2,3-dihydro-1,5-benzoxazepine (2). Brown crystals (0.086 g, 42%), mp 135−136 °C. IR (KBr) ν_{max}: 3437, 3358, 3333, 1632, 1578 cm^{-1}. ^1H NMR: 1.35 (s, 6H, 2-CH_3), 2.28 (s, 2H, 3-H), 2.38 (s, 3H, 4-CH_3), 3.7 (br s, 1H, NH_2), 6.56 (dd, J = 7.9, 1.1 Hz, 1H, 9-H), 6.70 (dd, J = 8.0, 1.2 Hz, 1H, 7-H), 6.87 (dd, J = 8.0, 7.9 Hz, 1H, 8-H), 6.9 (br, 1H, NH_2). ^{13}C NMR: 29.3 (4-CH_3), 30.1 (2-CH_3), 46.3 (C-3), 68.2 (C-2), 111.1 (C-7), 116.9 (C-7), 123.5 (C-8), 125.4 (C-6a), 143.3 (C-9a), 150.9 (C-6), 170.6 (C-4). MS (LCMS) m/z (%) 205 (100, M^+ + H). Anal. Calcd for $C_{12}H_{16}N_2O$ (204.27): C, 70.56; H, 7.90; N, 13.71. Found: C, 70.49; H, 7.95; N, 13.64.

General Procedure for the Reaction of 2,3-Diaminophenol with Acetophenones under Microwave Irradiation. Acetophenone **4** (2.0 mmol), 2,3-diaminophenol **1** (1.5 mmol), and a drop of acetic acid were mixed thoroughly and were irradiated in the microwave oven at a power and time indicated in Table 1. The crude product was worked up as above to afford in elution order compounds **6** and **5**.

From Acetophenone. 6-Hydroxy-2-methyl-2,4-diphenyl-2,3-dihydro-1*H*-1,5-benzodiazepine (6a). Yellow crystals (0.194 g, 59%), mp 88−90 °C. IR (KBr) ν_{max}: 3446, 3360, 1610, 1578 cm^{-1}. ^1H NMR: 1.60 (s, 3H, 2-CH_3), 2.98 (d, J = 14.3 Hz, 1H, 3-H_{endo}), 3.45 (d, J = 14.6 Hz, 1H, 3-H_{exo}), 4.32 (br s, 1H, NH), 6.22 (dd, J = 7.9, 1.1 Hz, 1H, 9-H), 6.45 (dd, J = 7.9, 1.1 Hz, 1H, 7-H), 6.94 (dd, J = 7.9, 7.9 Hz, 1H, 8-H), 7.04−7.09 (m, 3H, 2′,4′,6′-H), 7.20−7.30 (m, 5H, 3′, 5′,3″,4″,5″-H), 7.53 (dd, J = 7.5, 1.4, 2H, 2″,6″-H), 8.07, (br s, 1H, OH). ^{13}C NMR: 30.6 (2-CH_3), 45.9 (C-3), 64.6 (C-2), 103.4 (C-7), 109.4 (C-9), 121.9 (C-5a), 125.1 (C-2′,6′), 126.7 (C-2″,6″), 126.9 (C-4′), 128.1 (C-3′,5′), 128.2 (C-3″,5″), 129.0 (C-4″), 129.5 (C-8), 139.5 (C-1″), 140.4 (C-9a), 146.9 (C-1′), 154.9 (C-6), 162.8 (C-4). MS (LCMS) m/z (%) 329 (100, M^+ + H). Anal. Calcd for $C_{22}H_{20}N_2O$ (328.41): C, 80.46; H, 6.14; N, 8.53. Found: C, 80.61; H, 6.23; N, 8.65.

6-Amino-2-methyl-2,4-diphenyl-2,3-dihydro-1,5-benzoxazepine (5a). Green crystals (0.115 g, 35%), mp 155−157 °C. IR (KBr) ν_{max}: 3360, 1627, 1610 cm^{-1}. ^1H NMR: 1.72 (s, 3H, 2-CH_3), 3.02 (d, J = 13.0 Hz, 1H, 3-H_{endo}), 3.20 (d, J = 13.0 Hz, 1H, 3-H_{exo}), 6.58 (d, J = 7.8, 1.4 Hz, 1H, 9-H), 6.83 (dd, J = 7.8, 8.1 Hz, 1H, 8-H), 6.90 (dd, J = 8.1, 1.4 Hz, 1H, 7-H), 7.15−7.35 (m, 8H, 2′, 3′,4′,5′,6′,3″,4″,5″-H), 7.61 (dd, J = 6.9, 2.2 Hz, 2H, 2″,6″-H), 7.65 (br s, 2H, NH_2). ^{13}C NMR: 30.5 (2-CH_3), 43.3 (C-3), 73.2 (C-2), 111.4 (C-9), 119.9 (C-7), 122.0 (C-8), 125.4 (C-2′,6′), 125.9 (C-5a), 127.1 (C-4′), 127.2 (C-2″,6″), 128.1 (C-3″,5″), 128.3 (C-3′,5′), 130.1 (C-4″), 139.5 (C-1″), 142.8 (C-9a), 147.3 (C-1′), 148.6 (C-6), 168.8 (C-4). MS (LCMS) m/z (%) 329 (91, M^+ + H), 301 (100). Anal. Calcd for $C_{22}H_{20}N_2O$ (328.41): C, 80.46; H, 6.14; N, 8.53. Found: C, 80.30; H, 6.05; N, 8.61.

From 4-Methoxyacetophenone. 6-Hydroxy-2,4-bis(4-methoxyphenyl)-2-methyl-2,3-dihydro-1*H*-1,5-benzodiazepine (6b). Brown crystals (0.342 g, 88%), mp 193−195 °C. IR (KBr) ν_{max}: 3360, 3210, 1627, 1610 cm^{-1}. ^1H NMR: 1.68 (s, 3H, 2-CH_3), 3.07 (d, J = 14.0 Hz, 1H, 3-H_{endo}), 3.39 (d, J = 14.0 Hz, 1H, 3-H_{exo}), 3.74 (s, 3H, 4″-OCH_3), 3.83 (s, 3H, 4′-OCH_3), 4.16 (br s, 1H, NH), 6.28 (dd, J = 8.1, 1.2 Hz, 1H, 9-H), 6.49 (dd, J = 7.9, 1.2 Hz, 1H, 7-H), 6.76 (d, J = 8.7 Hz, 2H, 3′,5′-H), 6.84 (d, J = 8.7 Hz, 2H, 3″,5″-H), 6.98 (dd, J = 7.9, 8.1 Hz, 1H, 8-H), 7.33 (d, J = 8.7 Hz, 2H, 2′,6′-H), 7.63 (d, J = 8.7 Hz, 2H, 2″,6″-H), 7.90 (br s, 1H, OH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 30.6 (2-CH_3), 45.7 (C-3), 55.3 (4′-OCH_3), 55.4 (4″-OCH_3), 65.6 (C-2), 104.0 (C-7), 109.8 (C-9), 113.6 (C-3″,5″),* 113.7 (C-3′,5′),* 122.8 (C-5a), 126.4 (C-2′,6′), 128.5 (C-8), 128.6 (C-2″,6″), 133.1 (C-1″), 139.5 (C-9a),* 139.7 (C-1′),* 154.6 (C-6), 158.5 (C-4′), 161.1 (C-4″), 163.1 (C-4). MS (LCMS) m/z (%) 389 (100, M^+ + H). Anal. Calcd for $C_{24}H_{24}N_2O_3$ (388.46): C, 74.21; H, 6.23; N, 7.21. Found: C, 74.09; H, 6.15; N, 7.30.

From 3-Methoxyacetophenone. 6-Hydroxy-2,4-bis(3-methoxyphenyl)-2-methyl-2,3-dihydro-1*H*-1,5-benzodiazepine (6c). Light yellow crystals (0.350 g, 90%), mp 165−167 °C. IR (KBr) ν_{max}: 3363, 3221, 1629, 1598 cm^{-1}. ^1H NMR: 1.68 (s, 3H, 2-CH_3), 3.07 (d, J = 14.2 Hz, 1H, 3-H_{endo}), 3.49 (d, J = 14.2 Hz, 1H, 3-H_{exo}), 3.62 (s, 3H, 3′-OCH_3), 3.77 (s, 3H, 3″-OCH_3), 4.31 (br s, 1H, NH), 6.30 (dd, J = 8.3, 1.4 Hz, 1H, 9-H), 6.48 (dd, J = 7.6, 1.4 Hz, 1H, 7-H), 6.69 (dd, J = 8.2, 1.5 Hz, 1H, 4′-H), 6.88−6.96 (m, 3H, 2′,6′,4″-H), 7.00 (dd, J = 7.6, 8.3 Hz, 1H, 8-H), 7.10−7.17 (m, 2H, 2″,6″-H), 7.17−7.25 (m, 2H, 5′,5″-H), 7.90 (br s, 1H, OH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 30.8 (2-CH_3), 46.2 (C-3), 55.1 (4″-OCH_3), 55.3 (4′-OCH_3), 65.1 (C-2), 103.7 (C-7), 109.6 (C-9), 111.4 (C-2′), 112.1 (C-2″), 112.4 (C-4′), 115.7 (C-4″), 119.6 (C-6″), 122.1 (C-5a), 129.2 (C-5′), 129.2 (C-5″), 129.5 (C-8), 139.5 (C-9a), 142.1 (C-1″), 148.8 (C-1′), 154.9 (C-6), 159.49 (C-3″),* 159.55 (C-3′),* 162.9 (C-4). MS (LCMS) m/z (%) 389 (100, M^+ + H). Anal. Calcd for $C_{24}H_{24}N_2O_3$ (388.46): C, 74.21; H, 6.23; N, 7.21. Found: C, 74.29; H, 6.30; N, 7.15.

From 2-Methoxyacetophenone. 6-Hydroxy-2,4-bis(2-methoxyphenyl)-2-methyl-2,3-dihydro-1*H*-1,5-benzodiazepine (6d). Light yellow crystals (0.361 g, 93%), mp 64−66 °C. IR (KBr) ν_{max}: 3393, 3200, 1625, 1596, 1578 cm^{-1}. ^1H NMR: 1.68 (s, 3H, 2-CH_3), 2.77 (d, J = 13.9 Hz, 1H, 3-H_{endo}), 3.07 (s, 3H, 4′-OCH_3), 3.83 (s, 3H, 4″-OCH_3), 4.39 (d, J = 13.9 Hz, 1H, 3-H_{exo}), 4.64 (br s, 1H, NH), 6.25−6.32 (m, 2H, 3′,3″-H), 6.34 (dd, J = 7.8, 1.1 Hz, 1H, 9-H), 6.44 (dd, J = 8.0, 1.1 Hz, 1H, 7-H), 6.66 (ddd, J = 7.0, 7.0, 1.1 Hz, 1H, 5′-H), 6.75 (ddd, J = 7.0, 7.0, 1.1 Hz, 1H, 5″-H), 6.85 (dd, J = 7.0, 1.2 Hz, 1H, 6′-H), 6.96 (dd, J = 8.0, 7.8 Hz, 1H, 8-H), 7.08 (ddd, J = 7.1, 7.0, 1.1 Hz, 1H, 4′-H), 7.13 (dd, J = 7.0, 1.1 Hz, 1H, 6″-H), 7.21 (ddd, J = 7.5, 7.0, 1.1 Hz, 1H, 4″-H), 8.10 (br s, 1H, OH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 30.0 (2-CH_3), 46.9 (C-3), 53.8 (4′-OCH_3), 55.5 (4″-OCH_3), 62.5 (C-2), 101.4 (C-7), 108.1 (C-9), 110.2 (C-3″),* 110.7 (C-3′),* 120.13 (C-5″),* 120.15 (C-5′),* 120.9 (C-5a), 127.9 (C-6″),* 128.0 (C-6′),* 128.7 (C-8), 128.7 (C-4′),* 129.8 (C-4″),* 132.5 (C-1″),* 133.0 (C-1′),* 139.2 (C-9a), 155.2 (C-6),* 155.3 (C-2′),* 157.4 (C-2″), 165.3 (C-4). MS (LCMS) m/z (%) 389 (100, M^+ + H). Anal. Calcd for $C_{24}H_{24}N_2O_3$ (388.46): C, 74.21; H, 6.23; N, 7.21. Found: C, 74.30; H, 6.19; N, 7.14.

From 4-Methylacetophenone. 6-Hydroxy-2-methyl-2,4-bis(4-methylphenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (6e). Green crystals (0.217 g, 61%), mp 68−70 °C. IR (KBr) ν_{max}: 3379 (NH), 3210 (br, OH), 1626, 1583 cm^{-1}. ^1H NMR: 1.67 (s, 3H, 2-CH_3), 2.28 (s, 3H, 4′-CH_3), 2.37 (s, 3H, 4″-CH_3), 3.14 (d, J = 14.3 Hz, 1H, 3-H_{endo}), 3.44 (d, J = 14.3 Hz, 1H, 3-H_{exo}), 4.41 (br s, 1H, NH), 6.27 (dd, J = 8.2, 1.4 Hz, 1H, 9-H), 6.48 (dd, J = 7.9, 1.4 Hz, 1H, 7-H), 7.00 (dd, J = 8.2, 7.9 Hz, 1H, 8-H), 7.05 (d, J = 7.9 Hz, 2H, 3′,5′-H), 7.15 (d, J = 7.9 Hz, 2H, 3″,5″-H), 7.28 (d, J = 7.9 Hz, 2H, 2′,6′-H), 7.58 (d, J = 7.9 Hz, 2H, 2″,6″-H), 8.23 (br s, 1H, OH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 20.7 (4′-CH_3), 21.1 (4″-CH_3), 30.4 (2-CH_3), 45.8 (C-3), 64.2 (C-2), 103.4 (C-7), 109.5 (C-9), 122.1 (C-5a), 124.9

(C-2′,6′), 126.8 (C-2″,6″), 128.7 (C-8), 128.86 (C-3′,5′),* 128.86 (C-3″,5″),* 137.0 (C-4′), 137.8 (C-1″), 139.7 (C-9a), 139.7 (C-4″), 144.3 (C-1′), 154.8 (C-6), 162.7 (C-4). MS (LCMS) *m*/*z* (%) 379 (100, $M^+ + Na$), 357 (70, $M^+ + H$). Anal. Calcd for $C_{24}H_{24}N_2O$ (356.46): C, 80.87; H, 6.79; N, 7.86. Found: C, 80.95; H, 6.75; N, 7.80.

6-Amino-2-methyl-2,4-bis(4-methylphenyl)-2,3-dihydro-1,5-benzoxazepine (5e). Oil (0.089 g, 25%. IR (KBr) ν_{max}: 3350, 3207, 1679, 1607, 1581 cm^{-1}. ^1H NMR: 1.67 (s, 3H, 2-CH_3), 2.29 (s, 3H, 4′-CH_3), 2.34 (s, 3H, 4″-CH_3), 3.03 (d, J = 14.3 Hz, 1H, 3-H_{endo}), 3.16 (d, J = 14.3 Hz, 1H, 3-H_{exo}), 6.65 (dd, J = 7.7, 1.6 Hz, 1H, 9-H), 6.88 (dd, J = 8.0, 1.6 Hz, 1H, 7-H), 6.95 (dd, J = 8.0, 7.7 Hz, 1H, 8-H), 7.09 (d, J = 8.3 Hz, 2H, 3′,5′-H), 7.11 (d, J = 8.3 Hz, 2H, 3″,5″-H), 7.1 (br s, 2H, NH_2), 7.47 (d, J = 8.3 Hz, 2H, 2′,6′-H), 7.63 (d, J = 8.3 Hz, 2H, 2″,6″-H). ^{13}C NMR-(∗ indicates that the assignments may be interchanged): 20.9 (4′-CH_3), 21.4 (4″-CH_3), 30.4 (2-CH_3), 42.6 (C-3), 72.6 (C-2), 111.0 (C-9), 119.4 (C-7), 123.1 (C-8), 125.1 (C-5a), 125.2 (C-2′,6′), 127.3 (C-2″,6″), 128.9 (C-3″,5″),* 129.1 (C-3′,5′),* 136.76 (C-1″),* 136.81 (C-4′),* 140.5 (C-4″), 144.4 (C-1′),* 144.5 (C-9a),* 149.5 (C-6), 168.8 (C-4). MS (LCMS) *m*/*z* (%) 357 (100, $M^+ + H$). Anal. Calcd for $C_{24}H_{24}N_2O$ (356.46): C, 80.87; H, 6.79; N, 7.86. Found: C, 80.80; H, 6.72; N, 7.94.

From 4-Chloroacetophenone. 6-Hydroxy-2-methyl-2,4-bis(4-chlorophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (6f). Green crystals (0.222 g, 56%), mp 118−120 °C. IR (KBr) ν_{max}: 3381, 3375, 1624, 1584 cm^{-1}. ^1H NMR: 1.67 (s, 3H, 2-CH_3), 2.95 (d, J = 14.2 Hz, 1H, 3-H_{endo}), 3.52 (d, J = 14.2 Hz, 1H, 3-H_{exo}), 4.29 (br s, 1H, NH), 6.28 (dd, J = 8.2, 1.2 Hz, 1H, 9-H), 6.48 (dd, J = 8.0, 1.2 Hz, 1H, 7-H), 7.00 (dd, J = 8.2, 8.0 Hz, 1H, 8-H), 7.11 (d, J = 8.9 Hz, 2H, 3′,5′-H), 7.20 (d, J = 8.7 Hz, 2H, 2′,6′-H), 7.26 (d, J = 8.7 Hz, 2H, 3″,5″-H), 7.47 (d, J = 8.9 Hz, 2H, 2″,6″-H), 7.86 (br s, 1H, OH). ^{13}C NMR: 31.3 (2-CH_3), 45.7 (C-3), 64.9 (C-2), 104.0 (C-7), 109.5 (C-9), 122.0 (C-5a), 126.7 (C-2′,6′), 128.1 (C-2″,6″), 128.4 (C-3′,5′), 128.5 (C-3″,5″), 129.5 (C-8), 132.8 (C-4′), 136.0 (C-4″), 138.7 (C-1″), 139.2 (C-9a), 145.2 (C-1′), 154.9 (C-6), 161.5 (C-4). MS (LCMS) *m*/*z* (%) 397/399/401 (100, $M^+ + H$). Anal. Calcd for $C_{22}H_{18}Cl_2N_2O$ (397.30): C, 66.51; H, 4.57; N, 7.05. Found: C, 66.41; H, 4.27; N, 6.98.

6-Amino-2-methyl-2,4-bis(4-chlorophenyl)-2,3-dihydro-1,5-benzoxazepine (5f). Green crystals (0.163 g, 41%), mp 187−189 °C. IR (KBr) ν_{max}: 3450, 3340, 1576 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6): 1.73 (s, 3H, 2-CH_3), 2.90 (d, J = 13.4 Hz, 1H, 3-H_{endo}), 3.13 (d, J = 13.4 Hz, 1H, 3-H_{exo}), 4.40 (br s, 1H, NH_2), 6.73−6.80 (m, 3H, 7,8,9-H), 7.17 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.20 (d, J = 8.4 Hz, 2H, 3″,5″-H), 7.48 (d, J = 8.8 Hz, 2H, 2′,6′-H), 7.54 (d, J = 8.4 Hz, 2H, 2″,6″-H), 9.26 (br s, 1H, NH_2). ^{13}C NMR ($CDCl_3$ + DMSO-d_6) (∗ indicates that the assignments may be interchanged): 30.3 (2-CH_3), 43.1 (C-3), 72.6 (C-2), 111.2 (C-9), 119.2 (C-7),* 119.6 (C-8),* 126.7 (C-5a), 126.8 (C-2′,6′), 127.6 (C-2″,6″),* 127.7 (C-3″,5″),* 127.9 (C-3′,5′), 132.0 (C-4′), 135.2 (C-4″), 137.6 (C-1″), 139.2 (C-9a), 146.0 (C-1′), 147.3 (C-6), 165.2 (C-4). MS (LCMS) *m*/*z* (%) 397/399/401 (100, $M^+ + H$). Anal. Calcd for $C_{22}H_{18}Cl_2N_2O$ (397.30): C, 66.51; H, 4.57; N, 7.05. Found: C, 66.33; H, 4.40; N, 6. 97.

From 4-Nitroacetophenone. 6-Amino-2-methyl-2,4-bis(4-nitrophenyl)-2,3-dihydro-1,5-benzoxazepine (5g). Red crystals (0.339 g, 81%), mp 180−182 °C. IR (KBr) ν_{max}: 3423, 1597, 1514, 1349 cm^{-1}. ^1H NMR: 1.83 (s, 3H, 2-CH_3), 3.05 (d, J = 13.6 Hz, 1H, 3-H_{endo}), 3.35 (dd, J = 13.6, 1.3 Hz, 1H, 3-H_{exo}), 5.19 (br s, 1H, NH_2), 6.73 (dd, J = 7.9, 1.5 Hz, 1H, 9-H), 6.91 (t, J = 7.9 Hz, 1H, 8-H), 6.97 (dd, J = 7.9, 1.5 Hz, 1H, 7-H), 7.72 (d, J = 9.0 Hz, 2H, 2′,6′-H), 7.75 (d, J = 9.0 Hz, 2H, 2″,6″-H), 7.70−7.78 (br, 1H, NH_2), 8.08 (d, J = 9.0 Hz, 2H, 3′,5′-H), 8.09 (d, J = 9.0 Hz, 2H, 3″,5″-H). ^{13}C NMR: 31.0 (2-CH_3), 43.4 (C-3), 73.0 (C-2), 112.5 (C-9), 120.9 (C-7), 121.8 (C-8), 123.5 (C-3″,5″), 123.6 (C-3′,5′), 126.4 (C-5a), 126.8 (C-2″,6″), 127.7 (C-2′,6′), 139.7 (C-6), 144.8 (C-1′), 146.4 (C-1″), 146.9 (C-4″), 148.5 (C-4′), 154.3 (C-9a), 164.5 (C-4). MS (LCMS) *m*/*z* (negative polarity, %) 417 (100, $M^+ - H$), 387 (90). Anal. Calcd for $C_{22}H_{18}N_4O_5$ (418.40): C, 63.15; H, 4.34; N, 13.39. Found: C, 63.05; H, 4.29; N, 13.47.

6-Hydroxy-2-methyl-2,4-bis(4-nitrophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (6g). This compound was isolated from **5g** after acid catalysis. Red crystals, mp 235−237 °C. IR (KBr) ν_{max}: 3450 br, 3399, 1624, 1597, 1518, 1346 cm^{-1}. ^1H NMR: 1.81 (s, 3H, 2-CH_3), 3.09 (d, J = 14.4 Hz, 1H, 3-H_{endo}), 3.82 (d, J = 14.4 Hz, 1H, 3-H_{exo}), 4.48 (br s, 1H, NH), 6.35 (dd, J = 8.2, 1.3 Hz, 1H, 9-H), 6.52 (dd, J = 8.0, 1.3 Hz, 1H, 7-H), 7.00 (dd, J = 8.2, 8.0 Hz, 1H, 8-H), 7.43 (d, J = 8.8 Hz, 2H, 2′,6′-H), 7.67 (d, J = 8.7 Hz, 2H, 2″,6″-H), 7.69 (s, 1H, OH), 8.01 (d, J = 8.8 Hz, 2H, 3′,5′-H), 8.16 (d, J = 8.7 Hz, 2H, 3″,5″-H). ^{13}C NMR: 32.0 (2-CH_3), 45.9 (C-3), 64.8 (C-2), 104.4 (C-7), 109.3 (C-9), 121.5 (C-5a), 123.8 (C-3′,5′,3″,5″), 126.4 (C-2′,6′), 127.4 (C-2″,6″), 130.9 (C-8), 139.0 (C-9a), 145.7 (C-1″), 146.9 (C-4′), 148.4 (C-4″), 153.2 (C-1′), 155.5 (C-6), 159.2 (C-4). MS (LCMS) *m*/*z* (%) 417 (100, $M^+ - H$). Anal. Calcd for $C_{22}H_{18}N_4O_5$ (418.40): C, 63.15; H, 4.34; N, 13.39. Found: C, 63.28; H, 4.21; N, 13.17.

From 3-Nitroacetophenone. 6-Hydroxy-2-methyl-2,4-bis(3-nitrophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (6h). Yellow crystals (0.126 g, 30%), mp 152−154 °C. ^1H NMR: 1.85 (s, 3H, 2-CH_3), 3.08 (d, J = 14.0 Hz, 1H, 3-H_{endo}), 3.77 (d, J = 14.0 Hz, 1H, 3-H_{exo}), 4.37 (br s, 1H, NH), 6.41 (dd, J = 8.0, 1.1 Hz, 1H, 9-H), 6.53 (dd, J = 8.0, 1.1 Hz, 1H, 7-H), 7.09 (t, J = 8.0 Hz, 1H, 8-H), 7.35 (dd, J = 8.0, 7.6 Hz, 1H, 5′-H), 7.47 (dd, J = 7.6, 7.2 Hz, 1H, 5″-H), 7.58 (br s, 1H, OH), 7.68 (d, J = 7.6 Hz, 1H, 6′-H), 7.89 (d, J = 7.6 Hz, 1H, 6″-H), 7.96 (d, J = 8.3 Hz, 1H, 4′-H), 8.16 (s, 1H, 2′-H), 8.17 (d, J = 7.2 Hz, 1H, 4″-H), 8.28 (s, 1H, 2″-H). ^{13}C NMR: 31.6 (2-CH_3), 45.4 (C-3), 66.6 (C-2), 104.9 (C-7), 109.9 (C-9), 120.6 (C-2″), 122.2 (C-2′), 124.4 (C-8), 127.5 (C-5a), 129.5 (C-4′), 129.5 (C-4″), 129.6 (C-5′), 130.4 (C-5″), 131.7 (C-6″), 132.3 (C-6′), 138.0 (C-1′), 141.4 (C-9a), 148.1 (C-3″), 148.1 (C-1″), 148.2 (C-3′), 154.9 (C-6), 161.2 (C-4). MS (LCMS) *m*/*z* (%) 441 (100, $M^+ + Na$). Anal. Calcd for $C_{22}H_{18}N_4O_5$ (418.40): C, 63.15; H, 4.34; N, 13.39. Found: C, 63.08; H, 4.40; N, 13.19.

6-Amino-2-methyl-2,4-bis(3-nitrophenyl)-2,3-dihydro-1,5-benzoxazepine (5h). Yellow crystals (0.197 g, 47%), mp 193−195 °C. ^1H NMR: 1.83 (s, 3H, 2-CH_3), 3.05 (d, J = 13.2 Hz, 1H, 3-H_{endo}), 3.35 (d, J = 13.2 Hz, 1H, 3-H_{exo}), 6.73 (dd, J = 7.2, 1.5 Hz, 1H, 9-H), 6.91 (dd, J = 7.9, 7.2 Hz, 1H, 8-H), 6.97 (dd, J = 7.9, 1.5 Hz, 1H, 7-H), 7.39−7.50 (m, 3H, 5′,5″-H, NH), 7.94−8.05 (m, 3H, 4′,6′,6″-H), 8.14 (dd, J = 9.1, 1.9 Hz, 1H, 4″-H), 8.26 (t, J = 1.9 Hz, 1H, 2′-H), 8.52 (t, J = 1.9 Hz, 1H, 2″-H). ^{13}C NMR: 30.8 (2-CH_3), 43.2 (C-3), 73.8 (C-2), 112.2 (C-9), 120.9 (C-7), 121.2 (C-8), 121.3 (C-2′), 121.8 (C-4′), 122.2 (C-2″), 124.5 (C-4″), 126.2 (C-5a), 129.3 (C-5′), 129.5 (C-5″), 132.0 (C-6″), 132.7 (C-6′), 140.5 (C-1″), 140.7 (C-9a), 146.7 (2 × C-3′,3″), 148.3 (C-1′), 149.4 (C-6), 164.5 (C-4). MS (LCMS) *m*/*z* (%) 441 (100, $M^+ + Na$). Anal. Calcd for $C_{22}H_{18}N_4O_5$ (418.40): C, 63.15; H, 4.34; N, 13.39. Found: C, 63.28; H, 4.46; N, 13.24.

General Procedure for the Reaction of 2,3-Diaminophenol with Acetophenones and Acetone under Microwave Irradiation. Acetophenone **4** (2.0 mmol), 2,3-diaminophenol **1** (1.0 mmol), acetone (0.5 mmol), and four drops of acetic acid were mixed thoroughly and were irradiated in the microwave oven at a power and time indicated in Table 2. The crude product was worked up as above to afford compounds **8**, **9**, and **10**.

From 2,3-Diaminophenol and 4-Methoxyacetophenone. 6-Hydroxy-4-(4-methoxyphenyl-2,2-dimethyl-2,3-dihydro-1*H*-1,5-benzodiazepine (8a). Green crystals (0.166 g, 56%), mp 75−77 °C. ^1H NMR: 1.34 (s, 6H, 2 × 2-CH_3), 2.96 (s, 2H, 3-CH_2), 3.80 (br s, 1H, NH), 3.89 (s, 3H, OCH_3), 6.21 (dd, J = 8.0, 1.0 Hz, 1H, 9-H), 6.53 (dd, J = 8.0, 1.0 Hz, 1H, 7-H), 6.96 (t, J = 8.0 Hz, 1H, 8-H), 6.98 (d, J = 7.0 Hz, 2H, 3′,5′-H), 7.70 (br s, 1H, OH), 7.92 (d, J = 7.0 Hz, 2H, 2′,6′-H). ^{13}C NMR: 30.7 (2 × 2-CH_3), 44.1 (C-3), 55.4 (4′-OCH_3), 60.5 (C-2), 104.1 (C-7), 110.1 (C-9), 113.8 (C-3′,5′), 123.1 (C-5a), 128.3 (C-8), 128.7 (C-2′,6′), 133.1 (C-1′), 139.3 (C-9a), 154.4 (C-6), 161.3 (C-4′), 163.3 (C-4). MS (LCMS) *m*/*z* (%) 319 (65, $M^+ + Na$), 297

(100, $M^+ + H$). Anal. Calcd for $C_{18}H_{20}N_2O_2$ (296.36): C, 72.95; H, 6.80; N, 9.45. Found: C, 73.07; H, 6.91; N, 9.32.

From 2,3-Diaminophenol and 4-Methylacetophenone. 6-Hydroxy-4-(4-methylphenyl-2,2-dimethyl-2,3-dihydro-1*H*-1,5-benzodiazepine (8b). Oil (0.266 g, 95%). IR (KBr) ν_{max}: 3383, 3400−3200 (br), 1626, 1211 cm^{-1}. ^1H NMR: 1.31 (s, 6H, 2 × 2-CH_3), 2.40 (s, 3H, 4′-CH_3), 2.96 (s, 2H, 3-CH_2), 3.00 (br s, 1H, NH), 6.17 (dd, J = 7.9, 1.4 Hz, 1H, 9-H), 6.48 (dd, J = 7.9, 1.4 Hz, 1H, 7-H), 6.94 (t, J = 7.9 Hz, 1H, 8-H), 7.24 (d, J = 8.0 Hz, 2H, 3′,5′-H), 7.60 (br s, 1H, OH), 7.81 (d, J = 8.0 Hz, 2H, 2′,6′-H). ^{13}C NMR: 21.3 (4′-CH_3), 30.6 (2 × 2-CH_3), 44.7 (C-3), 59.3 (C-2), 103.8 (C-7), 109.8 (C-9), 122.5 (C-5a), 127.0 (C-3′,5′), 128.7 (C-8), 129.2 (C-2′,6′), 137.9 (C-1′), 139.4 (C-9a), 140.3 (C-4′), 154.7 (C-6), 163.2 (C-4). MS (LCMS) m/z (%) 281 (35, $M^+ + H$), 266 (10), 171 (22), 155 (25), 153 (100). Anal. Calcd for $C_{18}H_{20}N_2O$ (280.36): C, 77.11; H, 7.19; N, 9.99. Found: C, 77.07; H, 7.26; N, 10.08.

From *o*-Phenylenediamine and Acetophenone. 2,2-Dimethyl-4-phenyl-2,3-dihydro-1*H*-1,5-benzodiazepine (8c). Yellow crystals (0.230 g, 92%), mp 110−112 °C. ^1H NMR: 1.34 (s, 6H, 2 × 2-CH_3), 2.74 (s, 2H, 3-CH_2), 3.00 (br s, 1H, NH), 6.77−6.82 (m, 1H, 9-H), 7.01−7.08 (m, 2H, 7,8-H), 7.28−7.31 (m, 1H, 6-H), 7.45−7.50 (m, 3H, 3′,4′,5′-H), 8.01−8.06 (m, 2H, 2′,6′-H). ^{13}C NMR: 30.8 (2 × 2-CH_3), 40.8 (C-3), 69.4 (C-2), 121.7 (C-9), 122.1 (C-7), 126.0 (C-8), 127.3 (C-2′,6′), 128.0 (C-6), 128.5 (C-3′,5′), 130.2 (C-4′), 137.7 (C-9a), 139.9 (C-1′), 141.2 (C-5a), 168.6 (C-4). MS (GCMS) m/z (%) 250 (41, M^+), 235 (100), 194 (30), 133 (38). Anal. Calcd for $C_{17}H_{18}N_2$ (250.34): C, 81.56; H, 7.25; N, 11.19. Found: C, 81.41; H, 7.17; N, 11.09.

From *o*-Phenylenediamine and 4-Chloroacetophenone. 2,2-Dimethyl-4-(4-chlorophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (8d). Yellow crystals (0.265 g, 93%), mp 77−79 °C. ^1H NMR: 1.32 (s, 6H, 2 × 2-CH_3), 2.69 (s, 2H, 3-H), 3.10 (br s, 1H, NH), 6.75−6.80 (m, 1H, 9-H), 7.00−7.15 (m, 2H, 7,8-H), 7.25−7.30 (m, 1H, 6-H), 7.41 (d, J = 8.8 Hz, 2H, 3′,5′-H), 7.96 (d, J = 8.8 Hz, 2H, 2′,6′-H). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 30.7 (2 × 2-CH_3), 40.7 (C-3), 69.3 (C-2), 121.7 (C-9), 122.2 (C-7), 126.2 (C-8), 127.9 (C-6), 128.6 (C-2′,6′),* 128.7 (C-3′,5′),* 136.4 (C-4′), 137.7 (C-9a), 138.2 (C-1′), 140.9 (C-5a), 167.2 (C-4). MS (LCMS) m/z (%) 284/286 (100, M^+). Anal. Calcd for $C_{17}H_{17}ClN_2$ (284.78): C, 71.70; H, 6.02; N, 9.84. Found: C, 71.51; H, 6.17; N, 10.01.

Reaction of 5a and 6a with *p*-Tolyl Isocyanate. A mixture of **5a** (or **6a**) (1 mmol) and *p*-tolyl isocyanate (0.146 g, 1.1 mmol) was refluxed in dry toluene (10 mL) for 10 h. The solvent was evaporated from the crude reaction mixture and the remainder was purified by column chromatography on silica gel using petroleum ether−ethyl acetate (5:1) of slowly increasing polarity to afford 1-(2-methyl-2,4-diphenyl-2,3-dihydro-1,5-benzoxazepin-6-yl)-3-(4-tolyl)urea (**11**) [or 2-methyl-2,4-diphenyl-2,3-dihydro-1*H*-1,5-benzodiazepin-6-yl(4-tolyl)carbamate (**12**)].

1-(2-Methyl-2,4-diphenyl-2,3-dihydro-1,5-benzoxazepin-6-yl)-3-(4-tolyl)urea (11). Yellow crystals (0.355 g, 77%), mp 117−118 °C. IR (KBr) ν_{max}: 3272, 3196, 1654 cm^{-1}. ^1H NMR: 1.62 (s, 3H, 2-CH_3), 2.36 (s, 3H, 4‴-CH_3), 2.79 (d, J = 16.3 Hz, 1H, 3-H_{endo}), 3.36 (d, J = 16.3 Hz, 1H, 3-H_{exo}), 6.82 (d, J = 8.1 Hz, 1H, 9-H), 6.91 (d, J = 8.6 Hz, 1H, 7-H), 7.10−7.23 (m, 10H, aromatics), 7.28−7.31 (m, 2H, aromatics), 7.41 (tt, J = 7.6, 1.4 Hz, 1H, 4″-H), 7.50−7.54 (m, 2H, 2″,6″-H), 9.01 (br s, 1H, NH), 10.70 (br s, 1H, NH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 20.7 (4‴-CH_3), 29.6 (2-CH_3), 43.9 (C-3), 74.3 (C-2), 115.4 (C-9), 119.0 (C-8), 119.2 (C-7), 125.5 (C-2′,6′), 126.4 (C-4′), 126.5 (C-5a), 127.2 (C-2″,6″),* 127.4 (C-2‴,6‴),* 127.9 (C-3″,5″), 128.4 (C-3′,5′), 129.2 (C-3‴,5‴), 130.0 (C-4″), 133.3 (C-4‴), 135.1 (C-1‴), 139.1 (C-9a), 139.3 (C-1″), 146.1 (C-1′), 147.5 (C-6), 152.0 (NHCONH), 169.5 (C-4). MS (LCMS) m/z (%) 484 (30, $M^+ + Na$), 459 (70), 333 (75), 301 (100). Anal. Calcd for $C_{30}H_{27}N_3O_2$ (461.55): C, 78.07; H, 5.90; N, 9.10. Found: C, 78.30; H, 6.05; N, 8.99.

2-Methyl 2,4-Diphenyl-2,3-dihydro-1*H*-1,5-benzodiazepin-6-yl(4-tolyl)carbamate (12). Yellow crystals (0.318 g, 69%), mp 154−156 °C. IR (KBr) ν_{max}: 3303, 1738 cm^{-1}. ^1H NMR: 1.74 (s, 3H, 2-CH_3), 2.21 (s, 3H, 4‴-CH_3), 2.99 (d, J = 13.4 Hz, 1H, 3-H_{endo}), 3.07 (d, J = 13.4 Hz, 1H, 3-H_{exo}), 3.53 (br s, 1H, 1-H), 6.71 (dd, J = 8.0, 1.1 Hz, 1H, 9-H), 6.85 (dd, J = 9.0, 1.1 Hz, 1H, 7-H), 6.95 (d, J = 7.6 Hz, 2H, 3‴,5‴-H), 7.00−7.08 (m, 1H, 8-H), 7.00−7.08 (m, 2H, 2′,6′-H), 7.12−7.28 (m, 6H, 3′,4′,5′,3″,4″,5″-H), 7.41 (d, J = 7.6, 2H, 2‴,6‴-H), 7.64 (d, J = 8.3, 2H, 2″,6″-H), 7.8 (br s, 1H, 6-CONH). ^{13}C NMR (∗ indicates that the assignments may be interchanged): 21.2 (4‴-CH_3), 27.3 (2-CH_3), 40.4 (C-3), 60.4 (C-2), 118.5 (C-7), 121.9 (C-9), 124.7 (C-2″,6″),* 125.3 (C-2′,6′),* 126.4 (C-5a), 126.8 (C-4′), 128.3 (C-3′,5′,3″,5″), 128.4 (C-8), 129.1 (C-2‴,3‴,5‴,6‴), 130.6 (C-4″), 133.8 (C-4‴), 134.3 (C-1‴), 137.9 (C-1″), 146.4 (C-9a), 146.9 (C-1′), 151.9 (br, NHC=O), 155.7 (C-6), 164.8 (C-4). MS (LCMS) m/z (%) 484 (100, $M^+ +$ Na). Anal. Calcd for $C_{30}H_{27}N_3O_2$ (461.55): C, 78.07; H, 5.90; N, 9.10. Found: C, 78.20; H, 6.13; N, 8.97.

Biological Assays. Each in vitro experiment was performed at least in triplicate. The results were averaged, and the standard deviation of absorbance was less than 10% of the mean. The results are presented in Table 5.

In Vitro Assays. Determination of the Reducing Activity of the Stable Radical DPPH.[34] To an ethanolic solution of DPPH (0.05 mM) in absolute ethanol an equal volume of the compounds (final concentrations of 50 and 100 μM) dissolved in DMSO was added. The mixture was shaken vigorously and allowed to stand for 20 or 60 min. Absorbance at 517 nm was determined spectrophotometrically, and the percentage of activity was calculated. All tests were undertaken on three replicates, and the results were averaged (Table 5).

Inhibition of Linoleic Acid Lipid Peroxidation.[19] The water-soluble azo compound AAPH is used as a free radical initiator for in vitro studies of free radical production. Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution is monitored at 234 nm. This assay can be used to follow oxidative changes and to understand the contribution of each tested compound.

An amount of 10 μL of the 16 mM linoleic acid sodium salt solution was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4, prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) in the assay without antioxidant, and lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides.

Soybean LOX Inhibition Study in Vitro.[34] The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 mL) and 0.2 mL of enzyme solution (1 part of enzyme 1×10^{-4} w/v in saline, and 9 parts of saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor.

In Vivo Assays: Inhibition of the Carrageenin-Induced Edema..[34] Edema was induced in the right hind paw of Fisher 344 rats (150−200 g) by the intradermal injection of 0.1 mL of 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. Each group was composed of 6−15 animals. The animals, which have been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance, but they were entirely fasted during the experiment period. Our studies were in accordance with recognized guidelines on animal experimentation. The tested compound, 0.01 mmol/kg body weight, was diluted in water with a few drops of Tween 80 and ground in a mortar before use, and it was given intraperitoneally simultaneously with the carrageenin injection. The rats were euthanized 3.5 h after carrageenin administration. The difference between the weight of the injected and uninjected paws was

calculated for each animal. The change in paw weight was compared with that in control animals (treated with water) and expressed as a percent inhibition of the edema (% ICPE values). Indomethacin was tested as a reference compound in 0.01 mmol/kg (47%). Values of ICPE (%) are the mean from two different experiments with a standard error of the mean of less than 10%.

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References

(1) Roth, H. J.; Kleemann, A. *Drug Synthesis in Pharmaceutical Chemistry*; Wiley: New York, 1988; Vol. 1.

(2) (a) Schutz, H. *Benzodiazepines*; Springer: Heidelberg, Germany, 1982. (b) Smalley, R. K. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Pergamon: Oxford, U.K., 1979; Vol. 4, p 600. (c) Landquist, J. K. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, U.K, 1984; Vol. 1, pp 166−170. (d) De Baun, J. R.; Pallos, F. M.; Baker, D. R. U.S. Patent 3,978,227, 1976; *Chem. Abstr.* **1977**, *86*, 5498d. (e) Archer, G. A.; Sternbach, L. H. The chemistry of benzodiazepines. *Chem. Rev.* **1968**, *68*, 747–784.

(3) (a) Zellou, A.; Cherrah, Y.; Hassar, M.; Essassi, E.-M. Synthesis and pharmacological study of 1,5-benzodiazepin-2,4-diones and alkyl derivatives. *Ann. Pharm. Fr.* **1998**, *56*, 169–174. (b) Savelli, F.; Boido, A.; Mule, A.; Piu, L.; Alamanni, M. C.; Pirisino, G.; Satta, M.; Peana, A. 1,4-Disubstituted 1,3-dihydro-2H-1,5-benzo- and chlorobenzodiazepin-2-ones with activity on the central nervous system (CNS). *Farmaco* **1989**, *44*, 125–140. (c) Srivastava, V. K.; Satsangi, R. K.; Kishore, K. 2-(2′-Hydroxyphenyl)-4-aryl-1,5-benzodiazepines as CNS active agents. *Arzneim.-Forsch.* **1982**, *32*, 1512–1514. (d) Parker, K. A.; Dermatakis, A. Tetrahydro imidazo[1,5,4-ef][1,5]benzodiazepinones (isoTIBO's): synthesis and evaluation as HIV-1 non-nucleoside reverse transcriptase inhibitors. *J. Org. Chem.* **1997**, *62*, 4164–4167. (e) Failli, A. A.; Shumsky, J. S.; Steffan, R. J.; Caggiano, T. J.; Williams, D. K.; Trybulski, E. J.; Ning, X.; Lock, Y.; Tanikella, T.; Hartmann, D.; Chan, P. S.; Park, C. H. Pyridobenzodiazepines: a novel class of orally active, vasopressin V 2 receptor selective agonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 954–959. (f) Hadac, E. M.; Dawson, E. S.; Darrow, J. W.; Sugg, E. E.; Lybrand, T. P.; Miller, L. J. Novel benzodiazepine photo affinity probe stereoselectively labels a site deep within the membrane-spanning domain of the cholecystokinin receptor. *J. Med. Chem.* **2006**, *49*, 850–863.

(4) (a) Yadav, J. S.; Reddy, B. V. S.; Praveenkumar, S.; Nagaiah, K.; Lingaiah, N.; Saiprasad, P. S. $Ag_3PW_{12}O_{40}$: a novel and recyclable heteropoly acid for the synthesis of 1,5-benzodiazepines under solvent-free conditions. *Synthesis* **2004**, 901–904. (b) Guzen, K. P.; Cella, R.; Stefani, H. A. Ultrasound enhanced synthesis of 1,5-benzodiazepinic heterocyclic rings. *Tetrahedron Lett.* **2006**, *47*, 8133–8136. (c) Polshettiwar, V.; Varma, R. S. Greener and rapid access to bio-active heterocycles: room temperature synthesis of pyrazoles and diazepines in aqueous medium. *Tetrahedron Lett.* **2008**, *49*, 397–400. (d) Ghorbani-Vaghei, R.; Veisi, H. The Michael addition of indoles and pyrrole to α,β-unsaturated ketones and double-conjugate 1,4-addition of indoles to symmetric enones promoted by pulverization−activation method and thia-Michael addition catalyzed by wet cyanuric chloride. *Mol. Diversity* **2010**, *14*, 385–391. (e) Madhav, J. V.; Rajitha, B. Expeditious synthesis of thiadiazolobenzodiazepines under conventional method and microwave irradiation. *Phosphorus, Sulfur Silicon Relat. Elem.* **2008**, *183*, 2984–2989. (f) Che, X.; Zheng, L.; Dang, Q.; Bai, X. Pyrrolodihydropteridines via a cascade reaction consisting of iminium cyclization and O-N Smiles rearrangement. *Tetrahedron* **2006**, *62*, 2563–2568. (g) Insuasty, B.; Orozco, F.; Lizarazo, C.; Quiroga, J.; Abonia, R.; Hursthouse, M.; Nogueras, M.; Cobo, J. Synthesis of new indeno[1,2-e]pyrimido[4,5-b][1,4]diazepine-5,11-diones as potential antitumor agents. *Bioorg. Med. Chem.* **2008**, *16*, 8492–8500.

(5) (a) Murai, K.; Nakatani, R.; Kita, Y.; Fujioka, H. One-pot three-component reaction providing 1,5-benzodiazepine derivatives. *Tetrahedron* **2008**, *64*, 11034–11040. (b) Willy, B.; Dallos, T.; Rominger, F.; Schönhaber, J.; Müller, T. J. J. Three-component synthesis of cryofluorescent 2,4-disubstituted 3H-1,5-benzodiazepines. Conformational control of emission properties. *Eur. J. Org. Chem.* **2008**, 4796–4805. (c) Alizadeh, A.; Zohreh, N.; Zhu, L.-G. One-pot and stereoselective synthesis of 2,3-dihydro-1,5-benzodiazepin-2-one with a phosphanylidene or phosphono-succinate substituent. *Tetrahedron* **2009**, *65*, 2684–2688. (d) Shaabani, A.; Rezayan, A. H.; Keshipour, S.; Sarvary, A.; Ng, S. W. A novel one-pot three-(in situ five-)component condensation reaction: an unexpected approach for the synthesis of tetrahydro-2,4-dioxo-1H-benzo[b][1,5]diazepine-3-yl-2-methylpropanamide derivatives. *Org. Lett.* **2009**, *11*, 3342–3345. (e) Sañudo, M.; Garcia-Valverde, M.; Marcaccini, S.; Delgado, J. J.; Rojo, J.; Torroba, T. Synthesis of benzodiazepine β-turn mimetics by an Ugi 4CC/Staudinger/aza-Wittig sequence. Solving the conformational behavior of the Ugi 4CC adducts. *J. Org. Chem.* **2009**, *74*, 2189–2192.

(6) (a) Meng, Q.; Bai, H.; Li, Z.; Wang., Q; Tao, F. A facile approach to 4,5-dihydro[1,2,4]triazolo[3,2-*d*][1,5]benzoxazepines. *Synthesis* **2007**, 1629–1634. (b) Erker, T. Studies on the chemistry of O,N- and S,N-containing heterocycles. 9. Investigations of the formation of pyrido[2,1-d][1,5]benzoxazepines and pyrido[2,1-d][1,5]benzothiazepines. *Liebigs Ann. Chem.* **1989**, 601–603. (c) Campiani, G.; Nacci., V.; Fiorini, I.; De Filippis, M. P.; Garofalo, A.; Greco, G.; Novellino, E.; Altamura, S.; Di Renzo, L. Pyrrolobenzothiazepinones and pyrrolobenzoxazepinones: novel and specific non-nucleoside HIV-1 reverse transcriptase inhibitors with antiviral activity. *J. Med. Chem.* **1996**, *39*, 2672–2680.

(7) Pozarentzi, M.; Stephanidou-Stephanatou, J.; Tsoleridis, C. A. An efficient method for the synthesis of 1,5-benzodiazepine derivatives under microwave irradiation without solvent. *Tetrahedron Lett.* **2002**, *43*, 1755–1758.

(8) (a) Srinivas, U.; Srinivas, C.; Narender, P.; Rao, V. J.; Palaniappan, S. Polyaniline-sulfate salt as an efficient and reusable catalyst for the synthesis of 1,5-benzodiazepines and 2-phenyl benzimidazoles. *Catal. Commun.* **2007**, *8*, 107–110. (b) Saini, R. K.; Joshi, Y. C.; Joshi, P. Solvent-free synthesis of some 1,5-benzothiazepines and benzodiazepines and their antibacterial activity. *Phosphorus, Sulfur Silicon Relat. Elem.* **2008**, *183*, 2181–2190. (c) Hekmatshoar, R.; Sadjadi, S.; Shiri, S.; Heravi, M. M.; Beheshtiha, Y. S. Green protocol for synthesis of 1,5-benzodiazepines and 1,5-benzothiazepines in the presence of nanocrystalline aluminum oxide. *Synth. Commun.* **2009**, *39*, 2549–2559. (c) Escobar, C. A.; Donoso-Tauda, O.; Araya-Maturana, R.; Sicker, D. Synthesis of 1,5-benzodiazepines with unusual substitution pattern from chalcones under solvent-free microwave irradiation conditions. *Synth. Commun.* **2009**, *39*, 166–174.

(9) Musavi, S.; Kakkar, P. Effect of diazepam treatment and its withdrawal on pro/antioxidative processes in rat brain. *Mol. Cell. Biochem.* **2003**, *245*, 51–56.

(10) Pozarentzi, M.; Stephanidou-Stephanatou, J.; Tsoleridis, C. A.; Zika, C.; Demopoulos, V. A combinatorial access to 1,5-benzodiazepine derivatives and their evaluation for aldose reductase inhibition. *Tetrahedron* **2009**, *65*, 7741–7751.

(11) Ekonomopoulou, M. T.; Tsoleridis, C. A.; Argyraki, M.; Polatoglou, E.; Stephanidou-Stephanatou, J.; Iakovidou-Kritsi, Z. Cytogenetic activity of newly synthesized 1,5-benzodiazepines in normal human lymphocyte cultures. *Genet. Test. Mol. Biomarkers* **2010**, *14*, 377–383.

(12) (a) Pozarentzi, M.; Stephanidou-Stephanatou, J.; Tsoleridis, C. A. The first benzodiazepine *o*-quinodimethane: generation and Diels−Alder reactions. *Tetrahedron Lett.* **2003**, *44*, 2007–2009. (b) Neochoritis, C.; Pozarentzi, M.; Stephanidou-Stephanatou, J.; Tsoleridis, C. A. Unexpected opening of the benzodiazepine ring during acetylation. *Lett. Org. Chem.* **2008**, *5*, 22–25. (c) Tsoleridis, C. A.; Pozarentzi, M.; Mitkidou, S.; Stephanidou-Stephanatou, J. An experimental and theoretical study on the regioselectivity of successive bromination sites of 7,8-dimethyl-2,4-diphenyl-3H-1,5-benzodiazepine. Efficient microwave assisted solventless synthesis of 4-phenyl-3H-1,5-benzodiazepines. *ARKIVOC* **2008**, No. xv, 193–209.

(13) Burgess, H.; Donelly, J. A. The reactions of halogenated phenylnitromethanes with triethyl phosphate. *Tetrahedron* **1991**, *47*, 111–120.

(14) Aresta, M.; Berloco, C.; Quaranta, E. Biomimetic building-up of the carbamic moiety: the intermediacy of carboxyphosphate analogues in the synthesis of *N*-aryl carbamate esters from arylamines and organic carbonates promoted by phosphorus acids. *Tetrahedron* **1995**, *51*, 8073–8088.

(15) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity of phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4303.

(16) Kulisic, T.; Radonic, A.; Katalinic, V.; Milos, M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem.* **2004**, *85*, 633–640.

(17) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.

(18) Niki, E. Lipoxygenase inhibitory activity of boropinic acid, active principle of *Boronia pinnata*. *Chem. Phys. Lipids* **1987**, *44*, 227–253.

(19) Liégeois, C.; Lermusieau, G.; Colin, S. Measuring antioxidant efficiency of wort, malt, and hops against the 2,2′-azobis(2-amidinopropane) dihydrochloride-induced oxidation of an aqueous

dispersion of linoleic acid. *J. Agric. Food Chem.* **2000**, *48*, 1129–1134.
(20) C-QSAR Database: Biobyte Corp., 201 West 4th Street, Suite 204, Claremont, CA 91711.
(21) Hadjipavlou-Litina, D.; Magoulas, G. E.; Krokidis, M.; Papaioannou, D. Syntheses and evaluation of the antioxidant activity of acitretin analogs with amide bond(s) in the polyene spacer. *Eur. J. Med. Chem.* **2010**, *45*, 298–310.
(22) (a) Kühn, H.; Belkner, J.; Wiesner, R.; Brash, A. R. Oxygenation of biological membranes by the pure reticulocyte lipoxygenase. *J. Biol. Chem.* **1990**, *265*, 18351–18361. (b) Maccarrone, M.; Baroni, A.; Finazzi-Agro, A. Natural polyamines inhibit soybean (glycine max) lipoxygenase-1, but not the lipoxygenase-2 isozyme. *Arch. Biochem. Biophys.* **1998**, *356*, 35–40.
(23) (a) Shureiqi, I.; Lippman, S. M. Lipoxygenase modulation to reverse carcinogenesis. *Cancer Res.* **2001**, *61*, 6307–6312. (b) Zhao, L.; Funk, C. D. Lipoxygenase pathways in atherogenesis. *Trends Cardiovasc. Med.* **2004**, *14*, 191–195.
(24) Taraporewala, I. B.; Kauffman, J. M. Synthesis and structure activity relationships of anti-inflammatory 9,10-dihydro-9-oxo-2-acridine alkanoic acids and 4-(2-carboxyphenyl)aminobenzene alkanoic acids. *J. Pharm. Sci.* **1990**, *79*, 173–178.
(25) Müller, K. 5-Lipoxygenase and 12-lipoxygenase: attractive targets for the development of novel antipsoriatic drugs. *Arch. Pharm.* **1994**, *327*, 3–19.
(26) Pontiki, E.; Hadjipavlou-Litina, D. Lipoxygenase inhibitors: a comparative QSAR study review and evaluation of new QSARs. *Med. Res. Rev.* **2008**, *28*, 39–117.
(27) Curini, M.; Epifano, F.; Genovese, S.; Menghini, L.; Ricci, D.; Fraternale, D.; Giamperi, L.; Bucchini, A.; Bellacchio, E. Lipoxygenase inhibitory activity of boropinic acid, active principle of *Boronia pinnata*. *Nat. Prod. Commun.* **2006**, *1*, 1141–1145.
(28) Shen, T. Y. Non Steroidal Anti-Inflammatory Agents. In *Burger's Medicinal Chemistry*; Wolf, M. E., Ed.; Wiley. New York, 1980; pp 1217−1219.
(29) SpinWorks, version 2.5 (simulation program), available from http://davinci.chem.umanitoba.ca/pub/marat/SpinWorks/.
(30) (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909. (b) MOPAC 2000, version 1.11, and Fujitsu in Chem3D Ultra 7.0.
(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Peterson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, Y. C.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.02; Gaussian: Pittsburgh, PA, 2003.
(32) (a) Halgren, T. A.; Lipscomp, W. N. The synchronous-transit method for determining reaction pathways and locating molecular transition states. *Chem. Phys. Lett.* **1977**, *49*, 225–232. (b) Peng, C.; Ayala, P. Y.; Schlegel, H. B.; Frisch, M. J. Using redundant internal coordinates to optimize equilibrium geometries and transition states. *J. Comput. Chem.* **1996**, *17*, 49–56. (c) Peng, C.; Schlegel, H. B. Combining synchronous transit and quasi-Newton methods for finding transition states. *Isr. J. Chem.* **1994**, *33*, 449–454.
(33) Terzidis, M. A.; Tsoleridis, C. A.; Stephanidou-Stephanatou, J. One-pot synthesis of chromenylfurandicarboxylates and cyclopenta[*b*]chromenedicarboxylates involving zwitterionic intermediates. A DFT investigation on the regioselectivity of the reaction. *J. Org. Chem.* **2010**, *75*, 1948–1955.
(34) Kontogiorgis, C.; Hadjipavlou-Litina, D. Biological evaluation of several coumarin derivatives designed as possible anti-inflammatory/antioxidants agents. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 63–69.