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Efficient Approach to Androstene-Fused Arylpyrazolines as Potent Antiproliferative Agents. Experimental and Theoretical Studies of Substituent Effects on BF_3-Catalyzed Intramolecular [3 + 2] Cycloadditions of Olefinic Phenylhydrazones

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Abstract: Highly diastereoselective Lewis acid induced intramolecular 1,3-dipolar cycloadditions of alkenyl phenylhydrazones (containing various substituents on the aromatic ring) obtained from a D-secopregnene aldehyde were carried out under fairly mild conditions to furnish androst-5-ene-fused arylpyrazolines in good to excellent yields. The ability of phenylhydrazones to undergo cyclization was found to be affected significantly by the electronic features of the substituents on the aromatic moiety. The rates of the ring-closure reactions were observed to be increased by electron-donating and decreased by electron-withdrawing groups. The experimental findings on the BF_3-catalyzed transformations were supported by calculations of the proposed mechanism at the BLYP/6−31G(d) level of theory, indicating a noteworthy dependence, mainly of the initial complexation step, and hence of the whole process, on the character of the substituent. The cycloaddition was estimated to occur via a zwitterionic intermediate rather than involving a pure concerted mechanism. The antiproliferative activities of the structurally related pyrazoline derivatives were tested *in vitro* on three malignant human cell lines (HeLa, MCF7, and A431): the microculture tetrazolium assay revealed that several compounds exerted marked cell growth-inhibitory effects. The highest cytotoxic activities, displayed by the *p*-methoxyphenylpyrazoline derivative **7d** (IC_{50} values: 2.01, 2.16, and 1.41 μM on HeLa, MCF7, and A341 cells, respectively), were better than those of cisplatin (IC_{50} values: 12.43, 9.63, and 2.84 μM, respectively).

1. Introduction

The construction of five-membered heterocycles containing two or more ring N atoms via the reactions of azomethine imines with multiple-bond systems is one of the synthetic applications of the well-known and exhaustively studied 1,3-dipolar cycloadditions.[1] However, these transformations have attracted less attention as compared with those of other 1,3-dipoles (e.g., nitrones, nitrile oxides, and azomethine ylides) with unsaturated dipolarophiles. The condensation of aldehydes with 1,2-disubstituted hydrazines is a method frequently applied for the *in situ* generation of azomethine imines,[2] though monosubstituted hydrazones as potential azomethine imine precursors are also known to undergo inter- or intramolecular cycloadditions under thermal[3] or strongly acidic conditions.[4] An azomethine imine tautomer is suggested to be formed from the corresponding hydrazone during these conversions by an *N*,*N*′-prototropic shift.

The development of 1,3-dipolar cycloaddition chemistry has entered a new stage in recent years, with control of stereose-

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lectivity by choosing appropriate chiral substrates or applying a chiral Lewis acid catalyst becoming the most important challenge. Lewis acid catalysis allows the cycloaddition pathway to proceed under milder conditions with improved diastereoselectivity and rate acceleration relative to those in conventional protocols. Furthermore, in contrast with intermolecular reactions, the intramolecular version has the advantage of higher degrees of regio- and diastereoselectivity due to entropy factors and limited conformational mobility in the transition state. Several Lewis acid induced stereoselective 1,3-dipolar cycloadditions of nitrones,[5] azomethine ylides,[6] and nitrile oxides,[7] leading to optically active five-membered ring systems, have previously been reported. Similar catalytic reactions of azomethine imines, however, have been less intensively studied.[8] [3 + 2] cycloadditions of acylhydrazones as azomethine imine equivalents with different Lewis acids were recently described by Kobayashi et al.[9] 4-Nitrobenzoylhydrazone systems underwent intra- and intermolecular ring closures with olefins with high stereoselectivity in the presence of $Zr(OTf)_4$, $Sc(OTf)_3$, or chiral Zr catalysts. The experimental findings suggested that the intermolecular reaction mechanism involved a [3 + 2]-concerted pathway.[9c] Lower diastereoselectivity was attained in the cycloaddition of an acylhydrazone to cyclopentadiene when a stoichiometric amount of $BF_3 \cdot OEt_2$ was used.[9a] Nevertheless, even a catalytic amount of $BF_3 \cdot OEt_2$ can be suitable for promoting stereoselective intramolecular 1,3-dipolar cycloadditions, as we reported earlier in connection with a D-secoestrone phenylhydrazone containing a propenyl side chain.[10] In the presence of this Lewis acid, the chiral sterane skeleton bearing both a 1,3-dipole and a dipolarophile moiety afforded an excellent possibility for control of the stereochemistry of the process. Since unsubstituted phenylhydrazone was found to be an appropriate precursor for the *in situ* generation of the *quasi*-azomethine imine 1,3-dipole in Lewis acid media, the synthesis of analogous derivatives was planned with a view to investigate their behavior under similar catalytic conditions. Therefore, a series of olefinic D-secopregnene phenylhydrazones containing different substituents on the aromatic moiety were synthetized, and their $BF_3 \cdot OEt_2$-induced intramolecular 1,3-dipolar cycloadditions were carried out. The proposed reaction mechanism was subjected to theoretical analysis by DFT (B3LYP) calculations with the intention of supporting the experimental findings. Moreover, the synthetized androst-5-ene-fused pyrazoline derivatives were applied in *in vitro* pharmacological studies to investigate their antiproliferative effects on three human malignant cell lines. Due to the structural diversity of the tested compounds, structure−activity relationships were also examined.

2. Results and Discussion

2.1. Synthetic Studies. During preliminary experiments, D-secopregnene aldehyde **2** was synthetized in a multistep pathway from commercially available pregnadienolone acetate **1** (Scheme 1).[11] The presence of the formyl group and the unsaturated side chain makes **2** an excellent starting material for condensation and subsequent cyclization to give fused heteroatom-containing frameworks via intramolecular sequences.[12] Thus, the phenylhydrazones of **2** as olefinic azomethine imine precursors were expected to undergo Lewis acid mediated 1,3-dipolar cycloadditions. Aldehyde **2** was therefore initially reacted with phenylhydrazines **3a**−**l** to furnish the corresponding phenylhydrazones **4a**−**l**, respectively, which were isolated from the reaction mixtures in crystalline form in high yields.[13] The ^{1}H NMR spectra recorded at different times for each compound, however, revealed that *E*/*Z* isomerization occurred in solution,[14a] which can be catalyzed by traces of acid[14b] or free phenylhydrazine.[14c] The same equilibration was observed on acidic silica-based TLC plates. To determine the configuration of the products arising from the condensations, the chemical shifts of the 16-H signals of the related isomers were compared. In all cases, 16-H resonated at lower fields (∼7.00 ppm) in the originally formed isomer, and at higher fields (∼6.5 ppm) for the stereomer that appeared after a period in solution. This is in good agreement with the Karabatsos rule,[14a,d] which states that the proton attached to the C=N group in aldehyde phenylhydrazones is more deshielded when it is *cis* than when it is *trans* to the anilino moiety. Geometry optimization carried out on some selected derivatives (**4a**,**b**,**d**,**f**,**k**) to compare the relative stabilities of the related isomers indicated that the *E* isomers are theoretically

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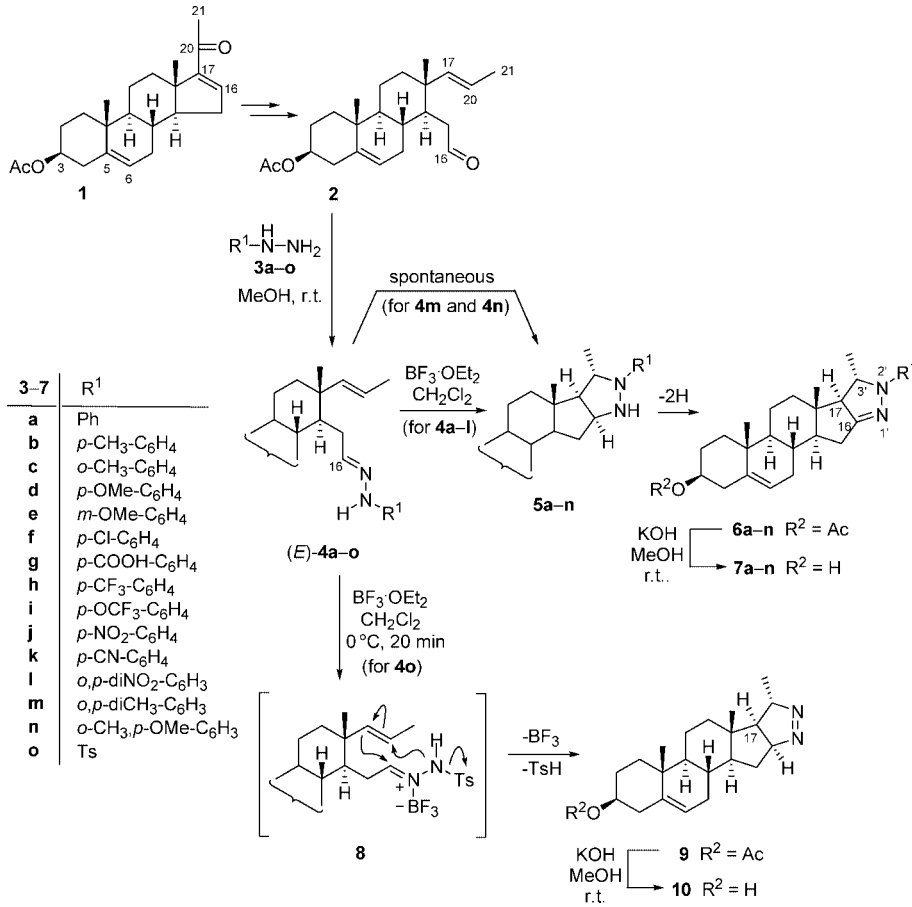
Scheme 1. Synthesis of Novel D Ring-Fused Steroidal Azacycles by 1,3-Dipolar Cycloaddition of Olefin-Substituted Hydrazones



5−6 kJ mol^{-1} more stable than the corresponding *Z* isomers. With regard to the *E*/*Z* isomerism of **4a**−**l** and the instability even in the solid state of those containing an electron-donating group (EDG) on the aromatic ring, the crude (*E*)-phenylhydrazones (**4a**−**l**) were subjected to intramolecular cyclization in the presence of a catalytic amount of $BF_3 \cdot OEt_2$ directly, without purification. The ring closures of **4a**−**j** proceeded in a stereoselective manner to furnish androstene-fused pyrazoline derivatives **6a**−**j** as single diastereomers, with 17-H in the α and 3′-H in the *β* position. No traces of the primarily formed pyrazolidines **5a**−**j** were observed, which is not surprising as such hererocyclic compounds are known to be often unstable and to undergo facile dehydrogenation under the reaction conditions or during the workup.[3e,10a,15] Interestingly, **5k** could be entrapped from the reaction mixture as a product of the catalytic ring closure of **4k**, but it transformed slowly to pyrazoline **6k** on standing in the air. In contrast, the 2′,4′-dinitrophenylpyrazolidine **5l** obtained from **4l** by Lewis acid treatment displayed high stability, with no subsequent dehydrogenation, probably as a consequence of internal H-bond formation between the hetero ring NH and the *ortho* NO_2 group on the Ph ring. Nevertheless, these transformations furnished evidence relating to the stereostructures of the primary products **(5)** via which pyrazoline formation conceivably occurs. The reaction rates depended strongly on the electronic character of the substituents on the Ph, in correlation with the observed stability of the starting phenylhydrazones **4a**−**l**. Compounds **4b**−**e** containing an EDG readily underwent heterocycle formation within 20 min at 0 °C to give **6b**−**e** in excellent yields. The *ortho* CH_3 group in **4c** seemed to have no steric influence at all on the intramolecular ring closure. In contrast, the unsubstituted phenylhydrazone **4a** and those containing an electron-withdrawing group (EWG) on the aromatic moiety (**4f**−**k**) proved to be more stable, and a longer reaction time (3 h) was needed at the same temperature for appreciable conversion. The residual phenylhydrazones reduced the yields of the desired products **6a** and **6f**−**k** in these latter cases. The strong dependence of the process on the nature of the substitution is supported by the fact that increased reaction temperature (40 °C) was required for the catalytic cyclization of 2′,4′-dinitrophenylhydrazone **4l** to afford **5l**, but only a low yield was achieved. Besides the presence of the two unfavorable EWGs on the Ph ring in **4l**, steric hindrance of the *ortho* NO_2 group (larger than *ortho* CH_3) can also hamper the cycloaddition. Compounds **4m** and **4n**, with two EDGs on the Ph ring, were so reactive that they were only detected by TLC and underwent *in situ* cyclization in the absence of the Lewis acid under the conditions of their formation from **2** with **3m** or **3n**. However, in the absence of $BF_3 \cdot OEt_2$, the stereoselectivity of both reactions proved to be the same as that observed for the catalytic reactions, i.e. probably only one (**5m** or **5n**) of the four possible pyrazolidine diastereomers was produced, which was further converted to **6m** or **6n** by oxidation. Similarly to phenylhydra-

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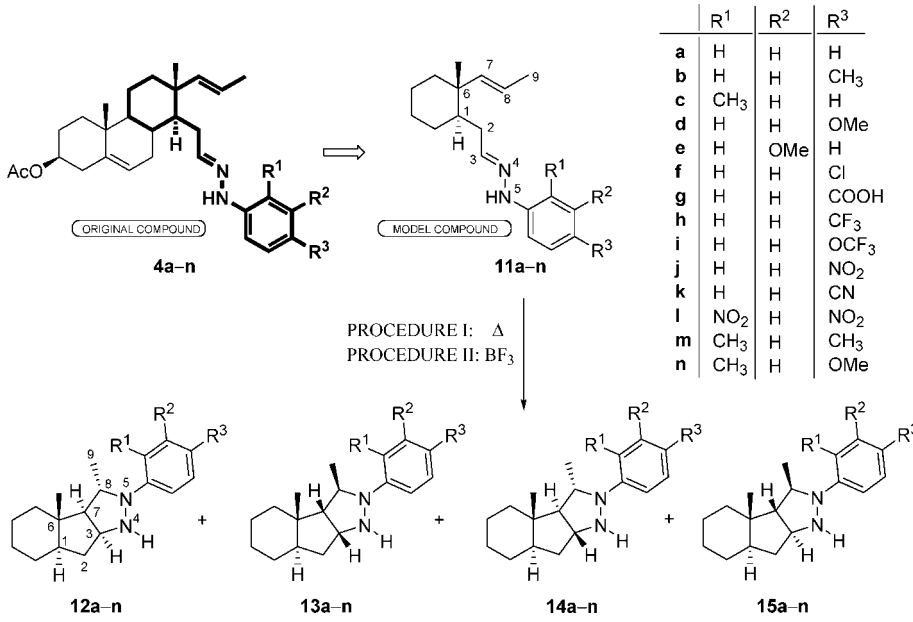
Scheme 2. A Simplified Model (**11**) of the Starting Sterane Skeleton (**4**), and the Four Possible Products (**12**, **13**, **14**, and **15**), with the Atom Numbering



	R^1	R^2	R^3
a	H	H	H
b	H	H	CH_3
c	CH_3	H	H
d	H	H	OMe
e	H	OMe	H
f	H	H	Cl
g	H	H	COOH
h	H	H	CF_3
i	H	H	OCF_3
j	H	H	NO_2
k	H	H	CN
l	NO_2	H	NO_2
m	CH_3	H	CH_3
n	CH_3	H	OMe

zones **4a**−**l**, tosylhydrazone **4o** was synthetized by the condensation of **2** with tosylhydrazide **3o**, the cyclization of which furnished **9** regio- and diastereoselectively through the presumed ionic intermediate **8**. Although the base-induced 1,3-dipolar cycloaddition of olefinic tosylhydrazones has been reported to occur under thermal conditions via a diazoalkane 1,3-dipole generated in situ, which further reacts with the internal dipolarophile in a concerted mechanism, a stepwise carbocation pathway has been presumed for the Lewis acid-catalyzed ring closure of such systems.[16] The stereostructure of compound **9**, involving an N═N double bond in its pyrazoline ring, is quite similar to those of the primary products **5a**−**n** derived from phenylhydrazones **4a**−**n**, which suggests a similar reaction mechanism for their formation. For pharmacological studies, the synthetized cycloadducts **6a**−**k,m,n** and **9**, but not **5l**, because of its low isolated yield from **4l**, were deacetylated in alkaline MeOH to furnish the corresponding 3-OH derivatives **7a**−**k,m,n** and **10**, respectively.

2.2. Theoretical Studies. To understand the reasons for the observed substituent effect during the $BF_3 \cdot OEt_2$-promoted cycloadditions of phenylhydrazones **4a**−**l** and to explain the high diastereofacial selectivity of the transformations, including those from **4m** and **4n**, which were carried out without the Lewis acid catalyst, computational analysis of the possible thermally induced (PROCEDURE I) and BF_3-catalyzed reaction (PROCEDURE II) mechanisms was performed to compare the energy profiles of the different processes. For the calculations, a simplified alkenyl phenylhydrazone model structure **11** (derived from the original sterane framework **4**) was used, assuming that the part ignored does not have a significant effect on the reaction (Scheme 2). The atoms directly involved in the cycloaddition or situated near the reaction center in **11** are arbitrarily numbered. Theoretically, the ring closure of **11** can lead to four pyrazolidine diastereomers (**12**, **13**, **14**, and **15**) in both PROCEDURES I and II: the *trans* configuration of the olefinic dipolarophile moiety is conserved during the reaction, and thus the opposite spatial orientation of the protons on C-7 and C-8 is seen in the products.[17]

2.2.1. Thermally vs Lewis Acid Induced Ring Closures. The two kinds of procedure were investigated first for the unsubstituted phenylhydrazone **11a**, which permitted a comparison of the different pathways. During PROCEDURE I, an initial isomerization is presumed to occur around the C-1−C-2 σ-bond, with equilibration between the *anti* (**11a**) and *gauche* isomers (**16a**), the latter existing in four conformers (I, II, III, and IV) by twisting around the C-2−C-3 and/or C-6−C-7 axis (Scheme 3). From this point, two possible routes (*Routes A* and *B*) must be considered via which the thermally induced ring closure can take place. In *Route A*, the equilibrium step is immediately followed by cyclization to yield four zwitterionic intermediates (**17a**, **18a**, **19a**, and **20a**) with different stereostrucures with respect to the configurations at C-3, C-7, and C-8. The subsequent rapid deprotonation−protonation equilibrium step can lead finally to the corresponding pyrazolidine diastereomers (**12a**, **13a**, **14a**, and **15a**). A rather different mechanism predominates for *Route B*, however, where isomer **16a** first rearranges by tautomerization to **21a**, which also has four conformational states (**21a**-I, **21a**-II, **21a**-III, and **21a**-IV). The direct ring closure of **21a** eventually results in the four possible diastereomeric products (**12a**, **13a**, **14a**, and **15a**). The energy profiles of *Routes A* and *B* are significantly different as each transition state in *Route B* possesses lower energy than those in *Route A* (Figure 1A). *Route B*, involving the *in situ* formation of an azomethine imine 1,3-dipolar intermediate **21a** from the starting phenylhydrazone **11a**, can therefore be suggested to be the active path, in accordance with the Curtin−Hammet principle.[18] This is also in good agreement with the recommendations of other authors[3] for reactions of this type under thermal conditions. Nevertheless, even in *Route B* all the activation energies computed for the reactions leading to any of the possible products are relatively high ($\Delta G^{\ddagger} = 155-220$ kJ mol^{-1}), and the transformations are therefore predicted to require an elevated reaction temperature to proceed. Similarly as in PROCEDURE I, there are two alternative mechanisms

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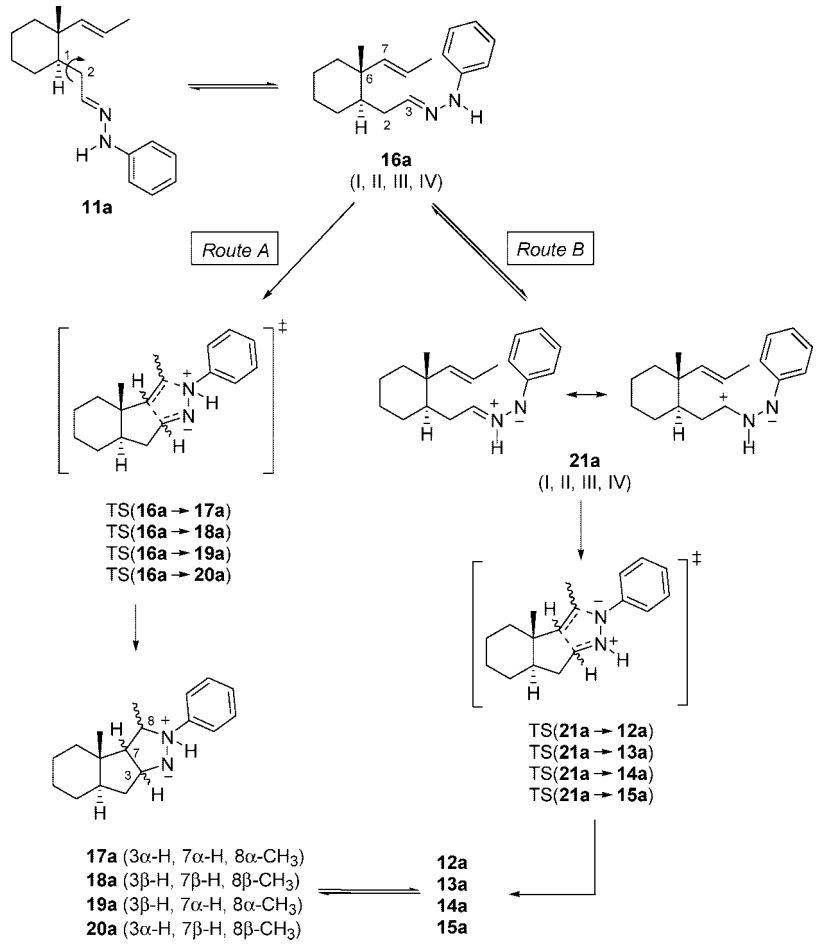
Scheme 3. Two Possible Mechanisms (*Route A* and *Route B*) Suggested for the Formation of Pyrazolidines (**12a**−**15a**) under Thermal Conditions (PROCEDURE I); TS = Transition State



(*Routes C* and *D*) for the formation of the four possible products (**12a**, **13a**, **14a**, and **15a**) via the $BF_3 \cdot OEt_2$-promoted intramolecular cyclization (PROCEDURE II) of model compound **11a** (Scheme 4). The initiating steps in both *Routes C* and *D* include two fast equilibria, i.e., the complexation of N-4 in **11a** with BF_3 to furnish **22a**, and a subsequent conformational change around the C-1−C-2 bond to give **23a**. Complex formation at N-5 is less preferable by 18.56 kJ mol^{-1}. Intermediates **22a** and **23a** can exist in four conformers (I, II, III, and IV) by rotation around the C-2−C-3 and/or C-6−C-7 bond axis. The direct ring closure of **23a** (*Route C*) can lead to cyclic intermediates **24a**, **25a**, **26a**, and **27a**, differing in their configurations at C-3, C-7, and C-8. The subsequent deprotonation−protonation and decomplexation result in the four possible pyrazolidines (**12a**, **13a**, **14a**, and **15a**). In contrast, *Route D* in PROCEDURE II supposes an additional isomerization step similarly as in thermally induced *Route B*, during which a proton/BF_3 interchange between N-4 and N-5 can occur to afford a *quasi*-azomethine imine **32a** with four conformational states (**32a**-I, **32a**-II, **32a**-III, and **32a**-IV). The following cyclization leads to intermediates **28a**, **29a**, **30a**, and **31a**, which can further transform to the isomeric products (**12a**, **13a**, **14a**, and **15a**) by loss of BF_3 during the workup. The computed energy profiles of the hypothetical *Routes C* and *D* in PROCEDURE II (Figure 1B) suggest that the latter would be more favorable, due to the lower energies of all the transition states via which the final products (**12a**, **13a**, **14a**, and **15a**) can be produced. Comparison of the preferred *Route B* in PROCEDURE I and *Route D* in PROCEDURE II reveals that

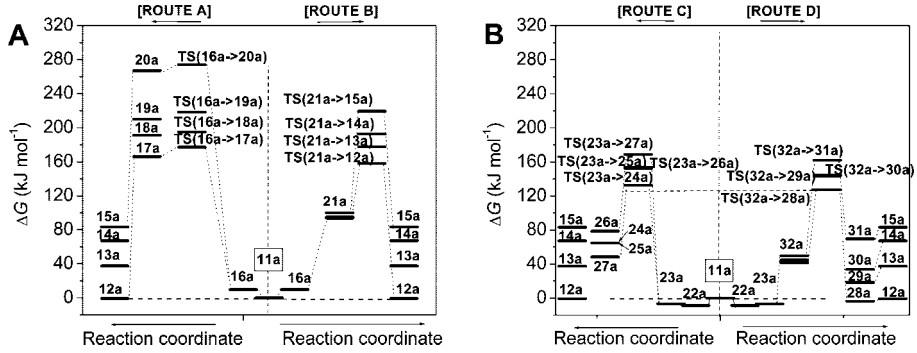
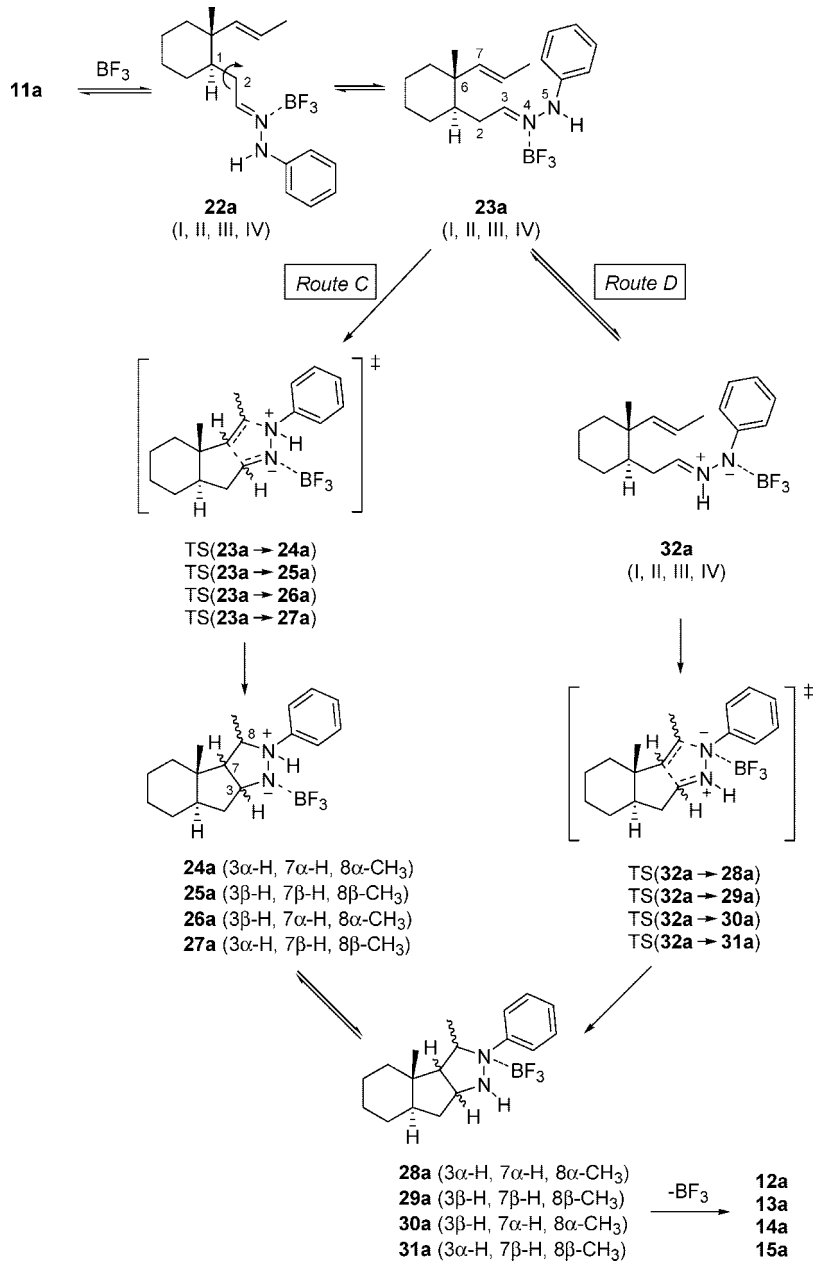


Figure 1. Energy diagrams of the possible routes for PROCEDURE I (**A**) and PROCEDURE II (**B**).

Scheme 4. Two Possible Mechanisms (*Route C* and *Route D*) Suggested for the $BF_3 \cdot OEt_2$-Induced Formation (PROCEDURE II) of Pyrazolidines (**12a**−**15a**); TS = Transition State



the lowest threshold energy is required for the formation of **12a**, which means that the reaction may be expected to take place in a stereoselective manner even under controlled thermal conditions. This is in agreement with the experimental finding that the ring closures of **4l** and **4m**, derived from the condensations of **2** with **3l** and **3m** (Scheme 1), were diastereoselective. These cycloadditions, which proceeded spontaneously at room temperature to give **6l** and **6m**, can be regarded as thermally induced transformations, as compared with most of those carried out at 0 °C with the Lewis acid catalyst. Additionally, the energy diagram of *Route D* indicates that the transition states which can be assigned to the other three diastereomers (**13a**, **14a**, and or **15a**) have relatively high energies and the reactions leading to these products are endothermic; hence, their formation is negligible at room temperature or below. The overall activation energy difference between *Route B* and *Route D* for **12a** is ∼30 kJ mol^{-1}, which makes the latter more favorable and allows it to occur under milder conditions.

2.2.2. Computational Studies of Transition States Leading to 12a in Routes B and D. Since the mechanisms of several electrocyclic reactions during which a heteroatom (N or O) is involved in ring formation have been reported to include nucleophilic features,[18] a further detailed characterization of the transition states relating to **12a** was carried out for both *Route B* and *Route D*. The cyclization of **11a** via *Route B* is expected to follow a concerted mechanism, which means that the corresponding transition state should possess an aromatic character.[19] The calculated nucleus-independent chemical shift

(19) (a) Cossio, F. P.; Morao, I.; Jiao, H.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1999**, *121*, 6737. (b) Morao, I.; Lecea, B.; Cossio, F. P. *J. Org. Chem.* **1997**, *62*, 7033.

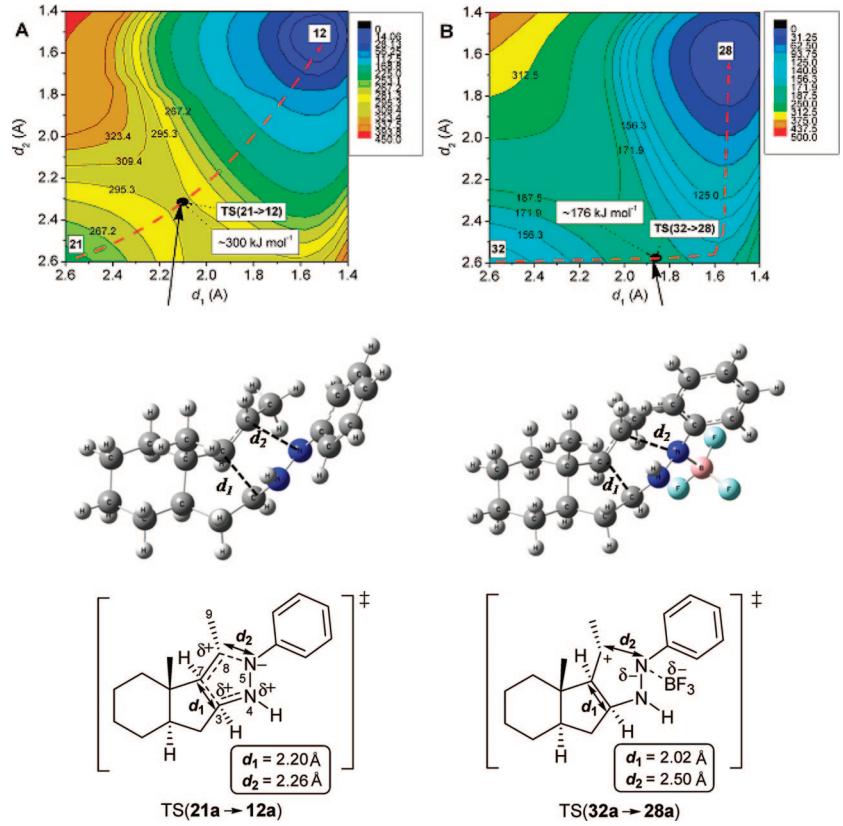


Figure 2. 2D cross sections of the potential energy surface and the structures of the two transition states (TS) for *Route B* (A) and *Route D* (B)

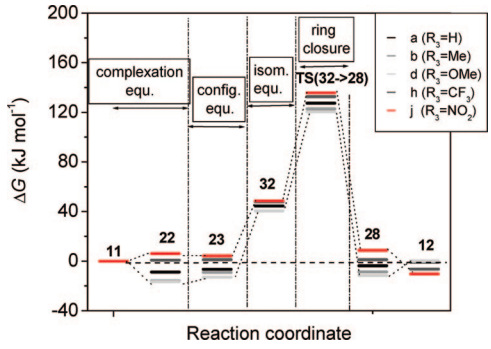


Figure 3. Energy diagram of *Route D* for the intramolecular [3 + 2] cycloadditions of some selected alkenyl phenylhydrazones (**11b**,**d**,**h**,**j**) compared with that of **11a**.

Table 1. Computed Gibbs Free Energies (kJ mol^{-1}) of the Individual Intermediates Relative to **11** and $\Delta G^{\ddagger}$[overall] of *Route D* for the Intramolecular [3 + 2] Cycloaddition of **11**→**12**

	11	22	23	32	$\Delta G^{\ddagger}$[overall]	28	12
a	0.00	−8.78	−6.64	44.54	127.46	−3.71	−0.73
b	0.00	−15.86	−9.05	40.44	122.87	−8.7	−0.31
c	0.00	−12.69	8.96	49.80	120.67	33.95	6.25
d	0.00	−16.53	−13.03	40.39	120.43	−11.56	0.26
e	0.00	−3.20	−0.55	46.98	129.76	−0.34	2.23
f	0.00	−4.11	−2.99	46.06	128.64	−3.43	0.00
g	0.00	2.46	0.74	47.59	132.66	3.22	−3.50
h	0.00	0.72	1.09	47.87	132.61	1.19	−6.50
i	0.00	−2.30	0.01	45.12	127.12	4.42	−6.17
j	0.00	6.14	4.27	48.61	135.70	8.76	−10.25
k	0.00	4.32	2.91	47.98	134.11	4.98	−4.50
l	0.00	17.88	15.97	65.47	188.01	59.03	31.89

(NICS),[20] which can be used as a descriptor of the aromatic or antiaromatic character of a transition state, is −17.7 ppm for that intermediate between **21a** and **12a**. The distances between the C-3 and C-7 (d_1 = 2.20 Å) and N-5 and C-8 (d_1 = 2.26 Å) are very similar in this transition state, and the minimum energy pathway, which develops as an almost diagonal line on the *ab initio* potential energy surface, supports the concerted reaction theory for the thermally induced formation of **12a** in *Route B* (Figure 2A). In contrast, the ring closure of **11a** via *Route D* seems to proceed in a significantly different pathway as the distances C-3−C-7 and N-5−C-8 are appreciably different and the minimum energy pathway is notably bent in this case; hence, the transition state between **32a** and **28a** can be regarded rather as a zwitterionic transition state (Figure 2B). This assumption is supported by the calculated NICS, which is considerably lower in magnitude (−13.8 ppm) for the transition state between **32a** and **28a** in *Route D* than for that between **21a** and **12a** in *Route B*. Accordingly, the Lewis acid catalyzed *Route D* is suggested to take place in a stepwise manner with zwitterionic character rather than in a synchronous mechanism; i.e., electrophilic attack of the electron-deprived C-3 to C-7 precedes the N-5−C-8 bond formation.

2.2.3. Quantitative Analysis of Substituent Effects on Route D. With regard to the experimental findings observed during the $BF_3 \cdot OEt_2$-induced cyclization of **4a**−**l**, the reactivity of the structurally related olefinic phenylhydrazone **11a** was predicted to change on substitution on the Ph ring and this was presumed to be manifested in the energy profile of the theoretically more preferable *Route D* of PROCEDURE II. The overall Gibbs free energy of activation of the reaction from

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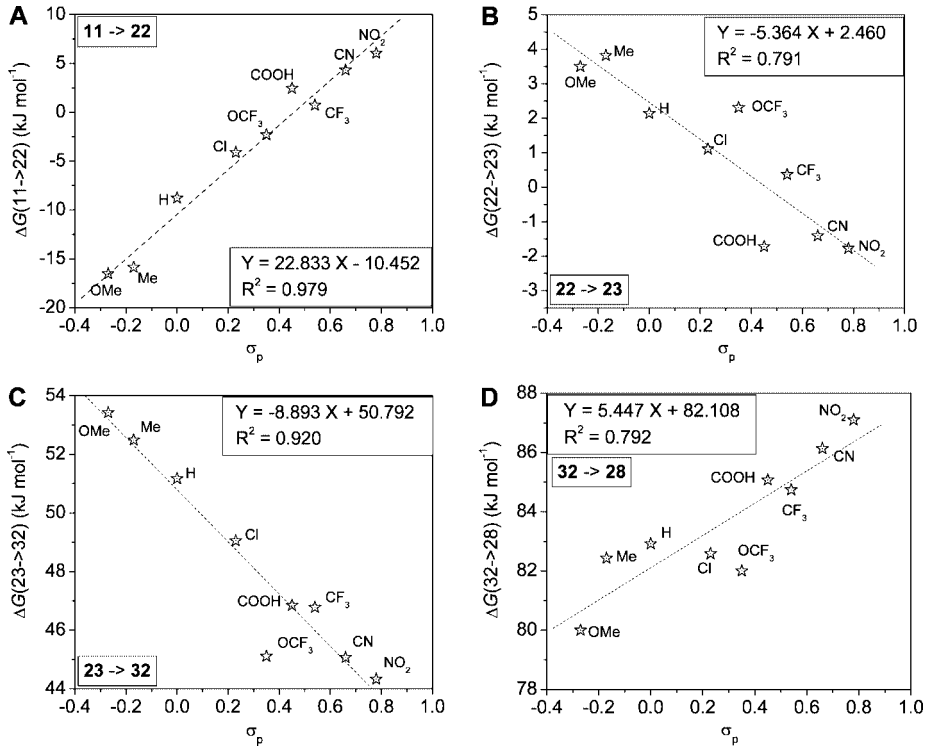


Figure 4. Correlation of the ΔG values of the individual subprocesses with the Hammett parameters σ_p of the different substituents on the Ph in the reactions of alkenyl *para*-substituted phenylhydrazones (**11a**,**b**,**d**,**f**−**k**): (A) complexation equilibrium, (B) configurational equilibrium, (C) isomerization equilibrium, (D) ring-closure step.

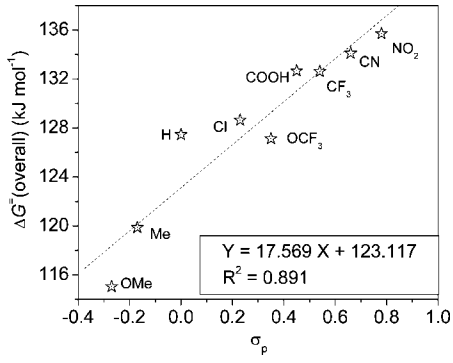


Figure 5. Overall Gibbs free energies [$\Delta G^{\ddagger}$(overall)] for the ring closures of **11a**,**b**,**d**,**f**−**k** correlated with the Hammett parameters σ_p of the different Ph substituents.

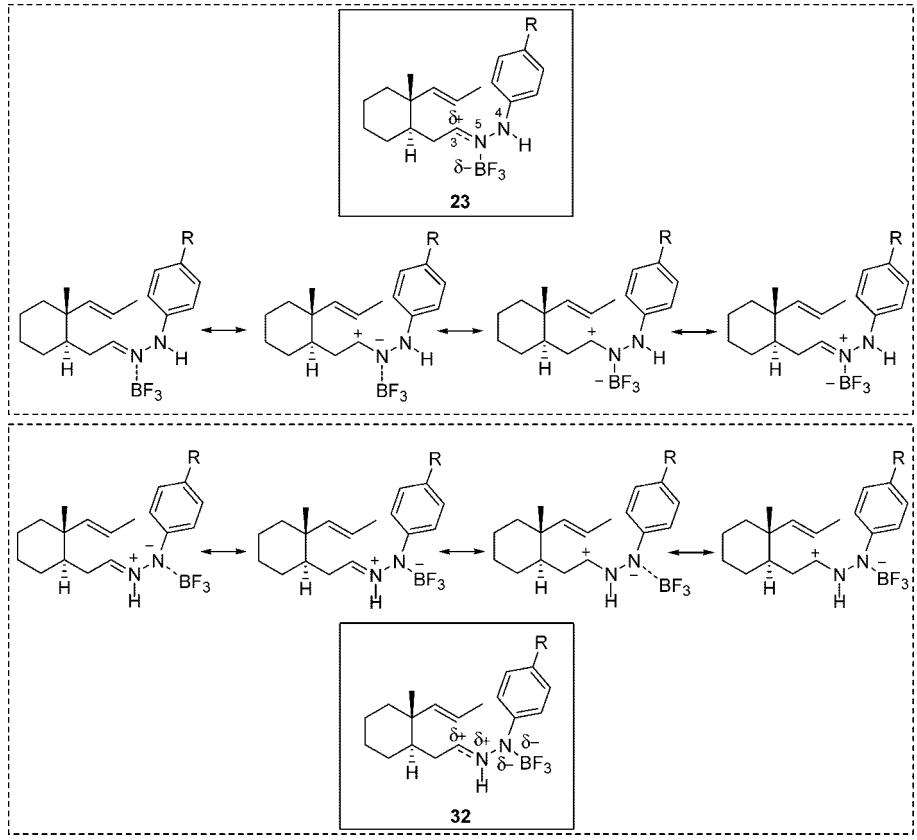
11ato the transition state between **32a** and **28a** consists of the energies of four subprocesses (Scheme 4, Figure 1B, eq 1), which were computed for the reactions of 11 other phenylhydrazone derivatives (**11b**−**l**) containing substituents at similar positions as in the experimental compounds **4b**−**l**. The detailed analysis of the four subprocesses indicated that the changes in Gibbs free energy during the individual steps differed considerably, depending on the electronic character of the substituents on the Ph (Figure 3). The overall Gibbs free energy, which is the resultant of the energies of the single steps, is higher for EWGs than for EDGs on the aromatic ring (Table 1). Accordingly, in agreement with the experimental observations, an EDG seems to favor cycloaddition, whereas a higher activation energy is needed when the Ph bears an EWG. The computed activation energies of the four subprocesses for the cycloaddition of 2′,4′-dinitrophenylhydrazone **11l** are extremely high, as an indication of the unfavorable nature of the transformation in complete accordance with the experimental observations during the ring closure of **4l**. The Hammet constants σ_p,[21] which are widely used to describe the electronic effects of different substituents and hence to measure the reactivities of substituted benzenes, were found to display a relatively good linear correlation[22] with the activation energies of the individual subprocesses and also with $\Delta G^{\ddagger}$[overall] for the reactions of the *para*-substituted derivatives (**11a**,**b**,**d**,**f**−**k**) (Figures 4 and 5). The fitted lines exhibit positive slopes for the first complexation equilibrium (Figure 4A) and for the final ring-closure steps (Figure 4D), which means that EWGs make these subprocesses less favorable as compared with EDGs. The negative slopes of the fitted lines for the conformation (Figure 4B) and isomerization equilibrium steps (Figure 4C), however, show that these subprocesses are somewhat more preferable when there are EWGs on the Ph ring.

Of the various subprocesses, the initial complex-formation step (A) exhibits the largest substituent dependence (the highest absolute value of the slope), which means that the electron density and therefore the Lewis basicity of N-4 are mainly determined by the electronic features of the substituent on the Ph. Accordingly, EDGs facilitate complexation, whereas EWGs make the BF_3 complex less stable. A smaller but opposite effect prevails for the isomerization subprocess (C): EWGs promote H/BF_3 interchange between N-4 and N-5. This can be interpreted by means of the resonance structures

(21) (a) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165. (b) Hammett, L. P. *J. Am. Chem. Soc.* **1937**, *59*, 96. (c) Hammett, L. P.; *Physical Organic Chemistry*, 2nd ed.; McGraw-Hill: New York, 1970; pp 347−390. (d) Wells, P. R. *Linear Free Energy Relationships*; Academic Press: New York, 1968. (e) Wells, P. R. *Chem. Rev.* **1963**, *63*, 171. (f) Johnson, C. D. *The Hammett Equation*; Cambridge University Press: Cambridge, 1973.

(22) Mucsi, Z.; Viskolcz, B.; Hermecz, I.; Csizmadia, I. G.; Keglevich, G. *Tetrahedron* **2008**, *64*, 1868.

Scheme 5. Some Relevant Resonance Structures of **23** and **32**



of **23** and **32** (Scheme 5), which show that, while N-5 in **23** can be regarded as roughly neutral, it possesses a significant negative character in structure **32**, which is more preferable when there are EWGs on the Ph ring. As expected, the conformational equilibrium (B) is not highly affected by the different substituents, which is manifested as a small slope of the correlation line. However, it is somewhat surprising that the terminal ring-closure step (D) is likewise almost insensitive to the nature of the Ph group. This small substituent dependence is also understandable in view of the resonance structure of **32** (Scheme 5), where an EWG increases the positive charge (electrophilicity) on C-3, but at the same time and to roughly the same extent it decreases the negative charge (nucleophilicity) on N-5. The opposite effect is observed for EDGs. The atomic distances d_1 and d_2 in the transition state and the natural bond orbital charges on C-3 and N-5 further support this concept. Finally, the resultant of the two electronic effects is manifested in a small slope (Figure 4D).

$$\Delta G^{\ddagger}[\text{overall}] = \Delta G[\mathbf{11a} \rightarrow \mathbf{22a}] + \Delta G[\mathbf{22a} \rightarrow \mathbf{23a}] + \Delta G[\mathbf{23a} \rightarrow \mathbf{32a}] + \Delta G^{\ddagger}[\mathbf{32a} \rightarrow \mathrm{TS}(\mathbf{32a} \rightarrow \mathbf{28a})] \quad (1)$$

2.3. Pharmacological Studies. In recent years, considerable interest has been focused on steroidal azacycles due to the broad spectrum of their biological activities. However, D-ring-fused pyrazolines[23] like most of the products (**6**, **7**, **9**, and **10**) have received less attention, at least from a pharmacological aspect. Several different compounds containing a pyrazoline structural moiety have been reported to exert antiproliferative activity[24] even on drug-resistant cancer cells, and the synthetized derivatives are structurally related to natural alkaloid analogues (Figure 6) which also have cytotoxic properties[25a,c] or act as chemosensitizers in multidrug resistance;[25d,e] consequently, investigation of the cycloadducts for cytotoxic efficacy appeared obvious. The activities were determined by the microplate-based MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-

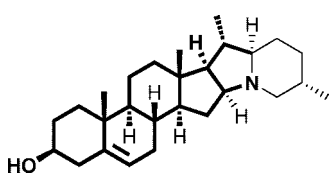


Figure 6. Structure of the natural steroid alkaloid solanidine; the part structurally identical with the synthetized compounds is shown in bold.

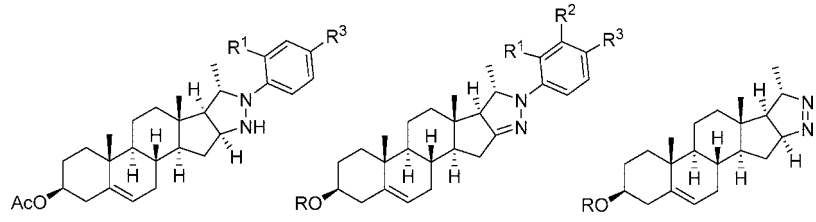
(23) (a) Amr, A. E-G. E.; Abdel-Latif, N. A.; Abdalla, M. M. *Bioorg. Med. Chem.* **2006**, *14*, 373. (b) Amr, A. E-G. E.; Abdel-Latif, N. A.; Abdalla, M. M. *Acta Pharm.* **2006**, *56*, 203.

(24) (a) Johnson, M.; Younglove, B.; Lee, L.; LeBlanc, R.; Holt, H.; Hills, P.; Mackay, H.; Brown, T.; Mooberry, S. L.; Lee, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5897. (b) Rostom, S. A. F. *Bioorg. Med. Chem.* **2006**, *14*, 6475. (c) Brzozowski, Z.; Sączewski, F.; Gdaniec, M. *Eur. J. Med. Chem.* **2000**, *35*, 1053. (d) Manna, F.; Chimenti, F.; Fioravanti, R.; Bolasco, A.; Secci, D.; Chimenti, P.; Ferlini, C.; Scambia, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4632. (e) Distefano, M.; Scambia, G.; Ferlini, C.; Gallo, D.; De Vincenzo, R.; Filippini, P.; Riva, A.; Bombardelli, E.; Mancuso, S. *Anti-Cancer Drug Des.* **1998**, *13*, 489.

(25) (a) Lee, K. R.; Kozukue, N.; Han, J. S.; Park, J. H.; Chang, E. Y.; Baek, E. J.; Chang, J. S.; Friedman, M. *J. Agric. Food Chem.* **2004**, *52*, 2832. (b) Koduru, S.; Grierson, D. S.; van de Venter, M.; Afolayan, A. J. *Pharm. Biol. (Lisse, Neth)* **2007**, *45*, 613. (c) US Patent 2003 /0114393 A1. (d) Lavie, Y.; Harel-Orbital, T.; Gaffield, W; Liscovitch, M. *Anticancer Res.* **2001**, *21*, 1189.

(26) (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55. (b) Csomós, P.; Zupkó, I.; Réthy, B.; Fodor, L.; Falkay, G.; Bernáth, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6273. (c) Réthy, B.; Zupkó, I.; Minorics, R.; Hohmann, J.; Ocsovszki, I.; Falkay, G. *Planta Med.* **2007**, *73*, 41. (d) Kovács, A.; Forgó, P.; Zupkó, I.; Réthy, B.; Falkay, G.; Szabó, P.; Hohmann, J. *Phytochemistry* **2007**, *68*, 687.

Table 2. Cytotoxic Activities of the Tested Compounds



5 **6** R = Ac, **7** R = H, **9** R = Ac, **10** R = H

				IC_{50} (μM)		
compound	R^1	R^2	R^3	HeLa	MCF-7	A431
6a	H	H	H	>30	>30	>30
7a	H	H	H	>30	>30	27.90
7b	H	H	CH_3	2.14	>30	7.34
7c	CH_3	H	H	22.11	>30	26.12
6d	H	H	OMe	4.47	15.60	3.34
7d	H	H	OMe	2.01	2.16	1.42
7e	H	OMe	H	2.63	2.89	8.92
7f	H	H	Cl	2.23	5.11	13.96
7g	H	H	COOK	>30	>30	>30
7h	H	H	CF_3	6.28	8.34	18.00
6i	H	H	OCF_3	3.98	>30	>30
7i	H	H	OCF_3	3.16	24.68	>30
7j	H	H	NO_2	3.17	7.30	6.89
5k	H	H	CN	>30	>30	>30
7k	H	H	CN	2.45	3.51	7.87
5l	NO_2	H	NO_2	9.71	>30	>30
6m	CH_3	H	CH_3	26.09	>30	>30
7m	CH_3	H	CH_3	7.39	11.88	7.93
6n	CH_3	H	OMe	>30	>30	>30
7n	CH_3	H	OMe	10.48	3.48	22.30
9				>30	>30	>30
10				14.00	29.37	7.57
doxorubicin				0.15	0.28	0.15
cisplatin				12.43	9.63	2.84

mide] colorimetric assay,[26] using the HeLa, MCF7, and A431 cell lines, in comparison with doxorubicin and cisplatin as positive controls. The cell growth-inhibitory potencies of the investigated compounds, expressed as IC_{50} values in Table 2, demonstrate that several of the derivatives exert pronounced antiproliferative effects on all three cell lines, these effects being similar to or higher than those of the reference cisplatin.

In some cases, the pyrazoline 3-acetates (**6d**,**i**,**m**,**n** and **9**) were also tested for comparison with the corresponding 3-OH-containing analogues (**7d**,**i**,**m**,**n** and **10**); as expected, the latter proved to be more potent. The esters containing a pyrazolidine substructure (**5k** and **5l**) did not display any substantial activity up to the final concentration of 30 μM, with the only exception of **5l** on the HeLa cells. The introduction of a Ph group into the pyrazoline moiety (**7**) and substitution on the Ph ring in at least the *meta* or *para* positions (**7b**, **7d**−**f**, and **7h**−**k**) enhanced the activity, while the absence of Ph (**10**) or the presence of unsubstituted Ph (**7a**) or *ortho*-substituted Ph (**7c**, **7m**, and **7n**) resulted in a decrease in potency. The *p*-methoxyphenylpyrazoline **7d** proved to be an outstandingly potent cytotoxic compound on all three cell lines, with IC_{50} values lower than those of cisplatin and close to those of doxorubicin. The 3-acetate analogue **6d** may also be of interest because of its marked antiproliferative effect, especially on the HeLa and A431 cells. The currently known structure−activity relationships offer a promising possibility for the design and screening of further derivatives.

3. Summary

Androst-5-ene-fused arylpyrazoline derivatives were prepared via the $BF_3 \cdot OEt_2$-catalyzed intramolecular [3 + 2] cycloaddition of steroidal alkenyl tosyl- and phenylhydrazones under fairly mild conditions. The rates of the ring closures depended significantly on both the electronic features and the positions of the substituents on the Ph moiety, although all transformations, including those in the absence of the Lewis acid, proved highly diastereoselective. The experimental findings were supported by computational calculations performed on simplified model compounds structurally related to the experimentally used steroidal alkenyl phenylhydrazones. These revealed that mainly the initial complexation with the Lewis acid depends on the substituent character and can therefore determine the overall Gibbs free energy of the process. The diastereofacial selectivity of the cyclizations was confirmed theoretically, since only one of the four possible diastereomers may be expected to form under catalytic conditions; however, this single isomer was also predicted to predominate even in the absence of the Lewis acid, in accord with the experimental findings. For the $BF_3 \cdot OEt_2$-promoted reactions, a stepwise rather than a pure concerted mechanism may be suggested. The synthetized androst-5-ene arylpyrazoline derivatives are of interest from a pharmacological

(27) Frisch, M. J. et al. Gaussian03 6.0; Gaussian, Inc.: Pittsburgh, PA, 2003; seeSupporting Information.

aspect, since several analogues exerted marked *in vitro* cytotoxic activity on three malignant human cell lines.

Experimental Section

Theoretical Studies. All computations were carried out with the Gaussian03 program package.[27] Geometry optimizations and subsequent frequency calculations were performed by applying the B3LYP/6−31G(d) basis set.[28,29] Thermodynamic parameters (*U*, *H*, *G*, and *S*, listed in the Supporting Information, Tables S2−S4) were computed at 298.15 K, applying a quantum chemical rather than a conventional thermodynamic scale.

Bioassays. Cytotoxic effects were measured *in vitro* on three human cell lines (ECACC; Salisbury, UK): HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), and A431 (skin epidermoid carcinoma). The cells were cultivated in a minimal essential medium (Gibco BRL; Paisley, UK) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and an antibiotic−antimycotic mixture. The cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. Near-confluent cells were seeded into a 96-well plate (5000 cells/well), and after overnight standing, the medium containing the tested compound was added. Following a 72-h incubation the cytotoxicity was measured with the MTT assay.[26] The precipitated formazan crystals were solubilized in dimethyl sulfoxide (DMSO), and the absorbance was read at 545 nm with a microplate reader. Sigmoidal concentration−response curves were fitted to the measured points, and the IC_{50} values were calculated with GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). Reported values are the averages of the results of two independent experiments with four parallels. The highest DMSO concentration of the medium, 0.1%, did not have any significant effect on cell proliferation. Doxorubicin and cisplatin were used as reference compounds.

Acknowledgment. The financial support by the Hungarian Scientific Research Fund (OTKA PD-72403, T-49366, and K-72309) is gratefully acknowledged. É. Frank's work was supported by the award of a Bolyai János Research Fellowship.

Supporting Information Available: Experimental procedures for the preparation and NMR spectra of all synthetized compounds; the full author list for ref 27; calculated stereostructures of selected (*E*)- and (*Z*)-phenylhydrazones; Table S1 contains the natural bond orbital charges on C-3 and N-5 and the d_1 and d_2 bond distances for compounds TS(**32a−l→28a−l**); Tables S2−S4 contain the computed energies (*E*), zero-point energies (E_{ZPE}), internal energies (*U*), enthalpies (*H*), and Gibbs free energies (*G*) in hartree at the B3LYP/6−31G(d) level of theory for compounds **11−32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) Mucsi, Z.; Szabó, A.; Hermecz, I.; Kucsman, Á.; Csizmadia, I. *J. Am. Chem. Soc.* **2005**, *127*, 7615.