Pure Appl. Chem., Vol. 83, No. 3, pp. 645–653, 2011.
doi:10.1351/PAC-CON-10-09-25

2-Substituted agelasine analogs: Synthesis and biological activity, and structure and reactivity of synthetic intermediates*

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Abstract: 2-Substituted *N*-methoxy-9-methyl-9*H*-purin-6-amines were synthesized either from their corresponding 6-chloro-9-methyl-9*H*-purines or 2-chloro-*N*-methoxy-9-methyl-9*H*-purin-6-amine. Great diversity in the amino/imino tautomeric ratios was observed and calculated based on ^{1}H NMR. The tautomers were identified by 1D and 2D ^{1}H, ^{13}C, and ^{15}N NMR techniques, and showed significant variation both in ^{13}C and ^{15}N shift values. Comparison of the tautomeric ratios with Hammett F values revealed that as the field/inductive withdrawing abilities of the 2-substituent increased, the ratio of amino:imino tautomers was shifted toward the amino tautomer. Computational chemistry exposed the significance of hydrogen bonding between solvent and the compound in question to reach accurate predictions for tautomeric ratios. B3LYP/def2-TZVP density functional theory (DFT) calculations resulted in quantitatively more accurate predictions than when employing the less expensive BP86 functional. N-7-Alkylation of the 2-substituted *N*-methoxy-9-methyl-9*H*-purin-6-amines showed that when the field/inductive withdrawing ability of the 2-substituent reached a certain point the reactivity drastically dropped. This correlated with the atomic charges on N-7 calculated using a natural bond orbital (NBO) analysis. Biological screening of the final 2-substituted agelasine analogs indicated that the introduction of a methyl group in the 2-position is advantageous for antimycobacterial and antiprotozoal activity, and that an amino function may improve activity against several cancer cell lines.

Keywords: agelasine; biological activity; density functional theory; purine; tautomerism; X-ray crystallography.

INTRODUCTION

Natural products are an important source for new drugs, either as the active component, or as inspiration [1]. Since the 1970s, a group of purine compounds called agelasines (Fig. 1) have been isolated

*Paper based on a presentation made at the 18th International Conference on Organic Synthesis (ICOS-18), Bergen, Norway, 1–6 August 2010. Other presentations are published in this issue, pp. 411–731.

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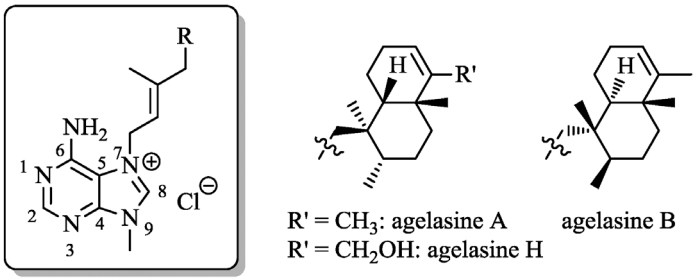


Fig. 1 General structure of agelasines (left) and examples of substituents R (right) of three naturally occurring agelasines.

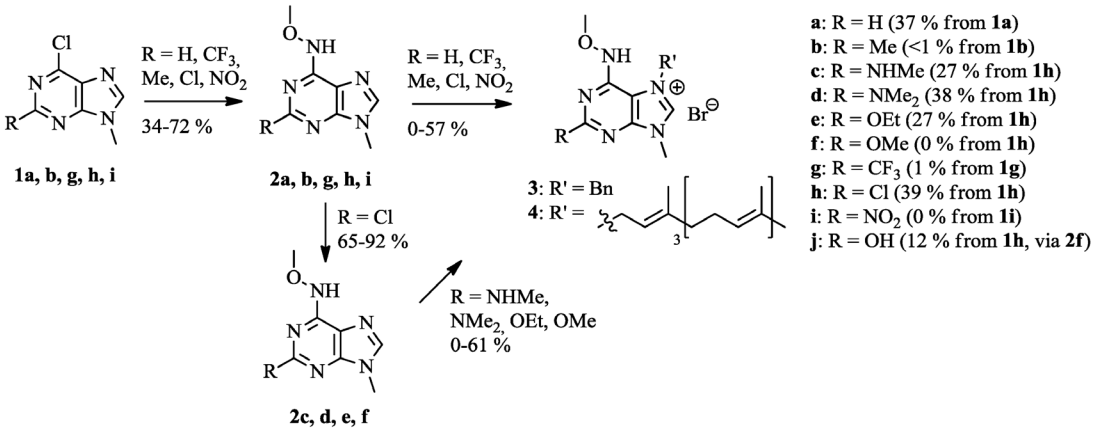
from marine sponges (*Agelas sp*.) [1–3]. They have shown bioactivities such as antifouling activity, cytotoxic effects, and antimicrobial activity, amongst others against *Mycobacterium tuberculosis*, the main cause of tuberculosis in humans [1].

Previously, we have reported the total synthesis of agelasines D [4] and E [5], and a range of agelasine analogs with variation in substituents on N^6, N-7, and N-9 of the purine scaffold [6]. We would now like to give a short review on our recently reported work related to 2-substituted agelasine analogs, and the structure and reactivity of certain interesting synthetic intermediates [7–9].

As trisubstituted purines, agelasines require regioselective syntheses. Unfortunately, alkylation of a 9-substituted adenine gives mainly N-1- or N^6-alkylation [10,11]. Inversing the introduction of the 7- and 9-substituents does not help, because a 7-substituted adenine will most likely be alkylated on N-3 (see Fig. 1 for numbering of the purine ring system) [12]. However, introducing an alkoxy group on N^6 of a 9-substituted purine results mainly in the desired 7-alkylation, and the alkoxy group can be removed after the alkylation. To date, all agelasines and analogs have been prepared by this method [13,14].

SYNTHESIS

The substituents on N^6, N-7, and N-9 for the target 2-substituted agelasine analogs (**4**) were chosen based on previous studies to achieve high bioactivity [4,5,10]. Nine *N*-methoxy-9-methyl-9*H*-purin-6-amines (**2**) were then prepared as intermediates for the synthesis of a series of 2-substituted agelasine analogs (**3**) (Scheme 1). Preparation of these synthetic intermediates was achieved either by introduc-



Scheme 1 General synthetic route to compounds **2**, **3**, and **4**, and substituents R used in the study.

tion of a methoxyamino group on C-6 from corresponding 2-substituted 6-chloro-9-methyl-9*H*-purines (**1**) or by nucleophilic aromatic substitution on C-2 of the 2-chloro-*N*-methoxy-9-methyl-9*H*-purin-6-amines (**2h**) [7].

To study the reactivity of the synthetic intermediates **2** in alkylation reactions, compounds **2a–i** were treated with benzyl bromide in DMA at 50 °C for 25 h (Scheme 1, Table 1). The reactions resulted in the expected N-7- and N^6-alkylated products in most cases, with the former as the major product, but also revealed a significant variation in reactivity for the nine analogs. Benzylation of the methoxy compound **2f** resulted in the formation of a range of products; the O^2-methyl group appeared to be unstable under the reaction conditions either in starting material **2f** and/or product **3f** [7]. Demethylation of other methoxypurines has previously been observed in the Gundersen research group at the University of Oslo [15]. 2-Ethoxypurine **2e** showed no detectable cleavage of the alkoxy group. A comparison of the field/inductive withdrawing ability (Hammett F values were employed) of the 2-substituents with the reactivity of the compounds was then conducted. Compounds **2** with substituents with Hammett F values lower than approximately 0.3 displayed good reactivity toward N-7-alkylation, whereas Hammett F values higher than this resulted in very low or no formation of the desired compounds **3** [7].

Table 1 Field/inductive parameter (F) for the substituents R, calculated and experimental tautomeric ratios of the compounds **2**, calculated NBO atomic charges on N-7 in the amino tautomers **2**, and isolated yields from N-7-alkylation of compounds **2** by treatment with R'Br.

Compound	R	F[a]	% Amino tautomer						NBO atomic charge on *N*7[c]	% Yield from 2	
			Single molecule			DMSO H-bonded complex		NMR[b]			
			BP/ SV(P)	BP86/ def2-TZVP	B3LYP/ def2-TZVP	BP86/ def2-TZVP	B3LYP/ def2-TZVP				
2a	H	0.03	5	2	13	9	30	20	–0.481	**3a**: 51	**4a**: 49
2b	Me	0.01	2	0	2	2	7	18	–0.488	**b**: 57	**4b**: 44
2c	NHMe	0.03	39	10	36	1	2	28	–0.487	**33c**: 56	**4c**: 42
2d	NMe_2	0.15	76	21	55	88	98	100	–0.479	**3d**: 61	**4d**: 26
2e	OEt	0.26	30	21	55	90	96	94	–0.483	**3e**: 52	**4e**: 39
2f	OMe	0.29	28	19	42	69	86	92	n.d.[d]	**3f**: –[e]	**4j**: 55[g,i]
2g	CF_3	0.38	96	95	95	100	100	100	–0.476	**3g**: <2[f]	**4g**: n.d.[d]
2h	Cl	0.42	97	91	91	100	100	100	–0.473	**3h**: 4[g]	**4h**: 18
2i	NO_2	0.65	96	90	90	100	100	100	–0.473	**3i**: n.o.[h]	**4i**: n.d.[d]

[a]Taken from ref. [16].
[b]Determined by ^{1}H NMR data obtained in DMSO-d_6 at 25 °C.
[c]For amino tautomers **2**. Based on the B3LYP/def2-TZVP calculations in solution phase where hydrogen bonding is included. Similar results were seen when results from BP86/def2-TZVP calculations were used, and also when no hydrogen bonding to a solvent molecule was included.
[d]Not determined.
[e]Formed, but not isolated in pure form.
[f]Calculated from ^{1}H NMR of a mixture.
[g]Isolated as betaine.
[h]Not observed (n.o.).
[i]R = OH

To determine the atomic charges on N-7 (which should function as the nucleophile in the alkylation of compounds **2**), a partial natural bond orbital (NBO) analysis [17] was performed (Table 1) as a part of a computation study of the compounds **2** [9]. The results indicated that a minimum negative charge was required on N-7 for the alkylation to occur. If this negative charge was too low, poor yields were observed.

The chloro analog **2h** was then treated with geranylgeranyl bromide, in DMA at 50 °C for 25 h to prepare its corresponding N-7-alkylated agelasine analog. An increase in reactivity from 4 to 18 %

was observed compared with the test reaction using benzyl bromide as the alkylating agent (Scheme 1, Table 1). Although this yield was a significant improvement, it is still a poor yield, and the two other low-yielding synthetic intermediates **2g,i** were excluded in the agelasine analog synthesis. The remaining six compounds **2a–f** were treated with geranylgeranyl bromide in the same manner as compound **2h**. Agelasine analogs **4** were isolated in low to acceptable yields, and the undesired N^6-alkylated products were isolated in lesser amounts in all reactions. As for the benzylation of compound **2j**, the treatment with geranylgeranyl bromide caused demethylation of the 2-methoxy group; the 2-oxo compound was isolated in 55 % yield. With the highest isolated yield in the series, the demethylation appeared to be quite facile [8].

STRUCTURE ANALYSES OF *N*-METHOXY-9-METHYL-9*H*-PURIN-6-AMINES (2)

Structure elucidation

In 1987, Fuji et al. reported that *N*-methoxy-9-methyl-9*H*-purin-6-amine (**2a**) existed as a mixture of two tautomers in DMSO-d_6 solution with its NH hydrogen on either N-1 or N^6, as shown in Fig. 2 [18]. During the synthesis of 2-substituted *N*-methoxy-9-methyl-9*H*-purin-6-amines **2**, we discovered that these synthetic intermediates showed great diversity in tautomeric ratios depending on the substituent in the 2-position (Table 1) [7].

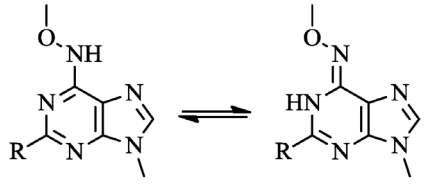


Fig. 2 Tautomerism of *N*-methoxy-9-methyl-9*H*-purin-6-amines **2**.

For the 2-substituted compounds **2**, no couplings in 2D ^1H-^{13}C or ^1H-^{15}N NMR supported nor contradicted whether the NH hydrogen was situated on N-1 or not, although chemical shifts indicated similarity with the *N*1-protonated **2a'**. Because of the uncertainty surrounding the protonation site, the possibility of different placements of the NH hydrogen was tested by computational chemistry. Imino tautomers of the methoxy compound **2k** with their NH on either N-1, N-3, or N-7 were all checked using the BP86 density functional and the def2-TZVP basis set. The N-3- and N-7-protonated imino tautomers turned out to have 46 and 36 kJ/mol higher energy than the *N*1-protonated imino tautomer, respectively. Imino tautomers with its NH hydrogen at either of these two sites therefore seemed unlikely, and in subsequent work it was assumed that the NH is situated on N1 for all imino structures [9].

A study of the NMR data acquired for compounds **2** revealed several interesting trends in chemical shifts depending on the identity of the tautomer for the compounds. ^1H NMR shift values for our system varied a fair bit depending on the C-2 substituent, and were quite close for the two tautomers. The ^{13}C NMR shifts showed a much clearer trend; as was indicated for ^1H NMR, lower shift values were clearly observed for the imino tautomers compared with the amino tautomers. This trend was especially clear for C-4, C-6, and OCH_3 whose peaks for the amino tautomers appeared at 150–153, 155–156, and 63–64 ppm, respectively, but at 142–144, 141–142, and 61 ppm for the imino tautomers. ^{15}N NMR shift values (extracted from 2D ^1H-^{15}N HSQC and HMBC spectra) showed a considerable difference for N^6 for the two tautomers, as expected based on the major difference in structure for these nitrogens; one having sp^3, the other sp^2 hybridization. In the amino tautomers N^6 resonated at ca.

–210 ppm, compared with ca. –95 ppm in imines (relative to $MeNO_2$ at 0 ppm). The opposite was seen for N-1 whose peaks appeared at –186 ppm for amines vs. –245 ppm for imines. These NMR results indicated that ^{13}C and ^{15}N NMR may be used to determine the identity of the major tautomer in these systems [7].

Tautomeric ratios

Tautomeric ratios for compounds **2** were calculated from ^1H NMR, and are shown in Table 1 as a percentage of the amino tautomer **2** out of the total of amino tautomer **2** + imino tautomer **2'**. ^1H NMR spectra for all compounds **2** were also acquired in CD_3OD solution at 25 °C, and calculated tautomeric ratios were consistent with the results from DMSO-d_6 solution with only small variations. Comparing the Hammett F values of the substituents in the study with the tautomeric ratio of compounds **2**, the compounds appeared to divide into two groups; all the compounds in the series with substituents with a significant field/inductive withdrawing ability showed the tautomeric ratio being shifted toward the amino tautomer. For the substituents with a small field/inductive withdrawing ability on the other hand, the imino tautomer dominated [7].

The experimental findings were backed up by a computational study. Accurately predicting the tautomeric ratio of a chemical compound is considered a challenge for contemporary quantum chemistry [19]. Our approach to prediction of tautomeric equilibria was based on quantum-chemical computation of the energy differences between the relevant tautomeric forms. These energy differences were then used in the standard Boltzmann distribution formula to predict the tautomeric ratios [9].

Initial density functional theory (DFT) calculations using the low-cost functional BP86 with the SV(P) basis set and a continuum solvation model predicted the correct major tautomer for only seven out of nine compounds (Table 1). In addition, the quantitative predictions for the seven qualitatively correctly predicted compounds were far from the experimental observations. The imino tautomers appeared to be predicted too stable compared to the amino tautomers, relative to what was observed experimentally. Re-optimizing the geometries with a larger basis set def2-TZVP resulted in an additional compound being wrongly predicted (Table 1). The necessity of inclusion of continuum solvation effects, zero-point vibrational energy and/or thermal corrections to the total energy was also checked, and their utility confirmed for accurate predictions. Switching to the B3LYP functional, still employing the def2-TZVP basis set, the predicted tautomeric ratios were somewhat improved, but the methoxy compound **2f** was still predicted as the imino tautomer, in addition to qualitative predictions being far from the experimentally observed results (Table 1) [9].

Hydrogen bonding to the solvent, DMSO, may be responsible for the discrepancy between the calculated and observed tautomeric ratios. ^1H NMR of compound **2e** was acquired in six different solvents. The results showed that as the dipole moment of the solvent increased, the amount of amino tautomer also increased (Table 2). Furthermore, the percent amino tautomer was much higher in solvents that can take part in hydrogen bonding compared with in those that can not. This indicated that the amino tautomers were somehow stabilized by hydrogen bonds to the solvent. Hydrogen bonding between the compound in question and the solvent, but also the polarity of the solvent, therefore seemed essential for the tautomeric ratio [9].

Table 2 Dipole moment of six solvents, their hydrogen bond acceptor abilities and the amount of amino tautomer of compound **2e** in each solvent determined by ^{1}H NMR.

Solvent	Dipole moment [20][a]	Hydrogen bond acceptor	% Amino tautomer
Benzene-d_6	0.07[b]	No	13
$CDCl_3$	1.08[c]	No	24
CD_2Cl_2	1.59[c]	No	29
CD_3OD	1.67[c]	Yes	74
Acetone-d_4	2.78[c]	Yes	84
DMSO-d_6	3.94[d]	Yes	92

[a]For nondeuterated species.
[b]Liquid.
[c]In benzene.
[d]Gas.
[e]Determined by ^{1}H NMR spectroscopy at 25 °C.

As a computational counterpart to these experimental results, a molecule of DMSO was added into the model. The hydrogen-bonded complexes were calculated using B3LYP/def2-TZVP. The inclusion of an explicit DMSO molecule appeared to be significant in order to reach approximate quantitative agreement with the experimentally observed tautomeric ratios. Both the BP86 and the B3LYP functionals qualitatively predicted the correct dominating tautomer for all compounds; however, the B3LYP results appeared quantitatively more accurate (Table 1), but with an at least threefold greater computational cost [9].

Molecular geometries

From calculations, the purine ring system was found to be essentially planar, with variation in orientation of the C-6 methoxyamino group, the *N*9 methyl group and in the C-2 substituent for both tautomeric forms of compounds **2**. The methoxyamino group was found to have two distinct minima when rotating around the C–N bond; the methoxy group was either directed toward N-1 in a *syn* orientation (as shown in Scheme 1 and Fig. 2) or toward N-7 in an *anti* orientation [9]. Reported NMR [5,18,21] and UV [18] studies of the imine **2a'** and similar 6-methoxyaminopurines have indicated that these types of imines exist in this *syn* configuration in solution. According to our calculations, the imino tautomers all preferred the *syn* configuration regardless of the identity of the 2-substituent. For the amino tautomers, the *syn* and *anti* conformations were much closer in energy, with energy differences typically 2–5 kJ/mol depending on the computational model and the identity of the 2-substituent, compared with typically 13–16 kJ/mol differences for the amino tautomers. Still, the *syn* conformation was preferred for all amino tautomers in solution [9].

Single-crystal X-ray structures of nitro compound **2i** (CCDC 789455) and methylamino compound **2c** (CCDC 789456) were acquired and indicated that these two compounds had crystallized as their amino and imino tautomeric forms, respectively, the same as the major tautomers for each in DMSO-d_6 solution. The amino tautomer of the nitro compound **2i** showed formation of dimers located on inversion centers, creating layers (Fig. 3, right). The imino tautomer of the methylamino compound **2c'**, on the other hand, was arranged in hydrogen-bonded chains (Fig. 3, left). *Syn* orientation of the methoxyamino group was observed in both these crystals as predicted by calculations for the compounds in solution [9].

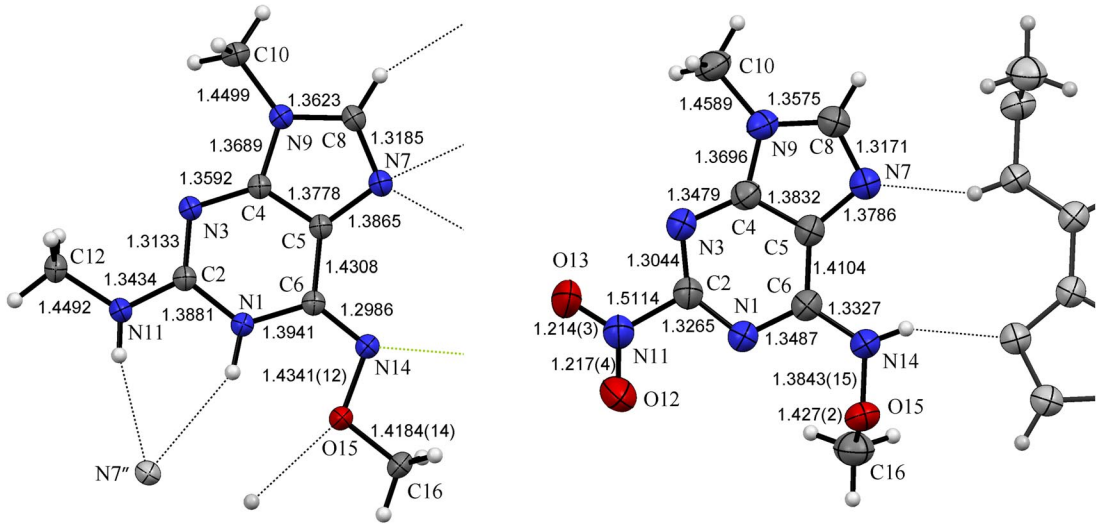


Fig. 3 X-ray crystal structure of methylamino compound **2c** (left) and nitro compound **2i** (right) with bond lengths of covalent bonds. Intermolecular bonds to adjacent molecules are indicated by dotted lines.

BIOLOGICAL ACTIVITY

All agelasine analogs **4** were screened for antimicrobial and antineoplastic activity [8]. All seven compounds showed profound inhibition against *M. tuberculosis* (IC_{90} < 0.2–8.1 μg/mL). The 2-methyl analog **4b** is the most potent antimycobacterial agelasine analog identified to date (IC_{90} < 0.2 μg/mL), and displayed an almost 10-fold improvement compared to the unsubstituted analog **4a**. The remaining 2-substituted analogs were all slightly less active than compound **4a**. These results may indicate that a slightly larger group on C-2 is advantageous for activity against *M. tuberculosis*, but groups that significantly differ from hydrogen in electronic properties show reduction in activity.

Relatively good activities were observed against fungi *C. krusei* (MIC from 2 μg/mL) and *C. albicans* (MIC from 2 μg/mL), protozoa *P. falciparum* (IC_{50} < 0.12–7.5 μg/mL), *L. donovani* (IC_{50} = 0.25–7.5 μg/mL), *T. cruzi* (IC_{50} < 0.12–0.91 μg/mL) and *T. b. rhodesiense* (IC_{50} < 0.12–0.94 μg/mL), and gram-positive bacterium *S. aureus* (MIC = 4–16 μg/mL), for all the 2-substituted analogs. The 2-chloro analog **4h** and the 2-hydroxy analog **4j** generally showed lower activities than the other analogs. All the compounds tested showed low activity against the gram-negative bacterium *E. coli*.

Screening against a series of human cancer cell lines (U-937 GTB, RPMI 8226/s, CEM/s, and ACHN), revealed that the amino analogs **4c,d** showed similar or slightly improved activities compared to the unsubstituted analog **4a** in all cases (IC_{50} = 0.55–4.2 μg/mL). All other changes in the 2-position resulted in lower anticancer activities.

Unfortunately, the cytotoxicity against mammalian cells (VERO cells and/or MRC-5 fibroblasts) was too high for any of the compounds to be of therapeutic use (IC_{50} = 0.45–13 μg/mL).

CONCLUSION

Nine *N*-methoxy-9-methyl-9*H*-purin-6-amines (**2**) with various substituents in the purine 2 position were synthesized either from their corresponding 6-chloro-9-methyl-9*H*-purines (**1**) or 2-chloro-*N*-methoxy-9-methyl-9*H*-purin-6-amine (**2h**), and further treated with benzyl bromide in order to study the reactivity of these compounds. Based on the study, seven of the synthetic intermediates **2** were then treated with geranylgeranyl bromide, and the resulting 7-alkylated agelasine analogs (**4**) were screened for various biological activities, including as antimicrobials and anticancer agents. The results indicated

that the introduction of a methyl group in the 2-position is advantageous for antimycobacterial and antiprotozoal activity, and that an amino function may improve activity against several cancer cell lines. NMR spectroscopy, computational chemistry, and X-ray crystallography were employed to describe the structure of synthetic intermediates **2**. DFT calculations using the B3LYP functional with the def2-TZVP basis set with inclusion of hydrogen bonding to a solvent molecule (DMSO) resulted in the highest quantitative correlation between calculated and experimental results. NBO analysis indicated that the field/inductive withdrawing ability of the C-2 substituents influence the atomic charge on N-7. One or both of these properties affect the reactivity of the compounds in 7-alkylation.

ACKNOWLEDGMENTS

Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases. The antiprotozoal data were determined by the UNDP/WORD BANK/WHO Special Programme for Research and Training in Tropical Diseases. The Norwegian Research Council (NFR) is greatly acknowledged for a scholarship to H.R. (grant 171323/V30). NFR and Faculty of Mathematics and Science, University of Oslo are acknowledged for financing the Bruker instruments used in this study. The authors are grateful to NordForsk (project "Crysteng") for networking and mobility support. The work of T.T. was supported by the Estonian Science Foundation (grant 8255).

REFERENCES

1. M. Gordaliza. *Mar. Drugs* **7**, 833 (2009) and refs. therein.
2. L. Calcul, K. Tenney, J. Ratnam, J. H. McKerrow, P. Crews. *Aust. J. Chem.* **63**, 915 (2010).
3. I. C. Pina, P. Crews, K. Tenney. Abstr. Pap. 232nd ACS Natl. Meet., San Fransisco, Sept. 10–14, 2006, MEDI-544 (2006).
4. A. Vik, E. Hedner, C. Charnock, O. Samuelsen, R. Larsson, L.-L. Gundersen, L. Bohlin. *J. Nat. Prod.* **69**, 381 (2006) and refs. therein.
5. A. K. Bakkestuen, L.-L. Gundersen, D. Petersen, B. T. Utenova, A. Vik. *Org. Biomol. Chem.* **3**, 1025 (2005).
6. A. Proszenyak, C. Charnock, E. Hedner, R. Larsson, L. Bohlin, L.-L. Gundersen. *Arch. Pharm. (Weinheim, Ger.)* **340**, 625 (2007) and refs. therein.
7. H. Roggen, L.-L. Gundersen. *Eur. J. Org. Chem.* 5099 (2008).
8. H. Roggen, C. Charnock, R. Burman, J. Felth, R. Larsson, L. Bohlin, L.-L. Gundersen. *Arch. Pharm.* **344**, 50 (2011).
9. L.-L. Gundersen, C. H. Gørbitz, L. Neier, H. Roggen, T. Tamm. *Theor. Chem. Acc.* In press, doi:10.1007/s00214-010-0850-3.
10. A. Vik, E. Hedner, C. Charnock, L. W. Tangen, O. Samuelsen, R. Larsson, L. Bohlin, L.-L. Gundersen. *Bioorg. Med. Chem.* **15**, 4016 (2007) and refs. therein.
11. T. Krüger, C. Wagner, C. Bruhn, T. Lis, D. Steinborn. *J. Mol. Struct.* **891**, 110 (2008).
12. T. Fujii, K. Ogawa, T. Saito, K. Kobayashi, T. Itaya. *Heterocycles* **38**, 477 (1994).
13. I. S. Marcos, N. Garcia, M. J. Sexmero, P. Basabe, D. Diez, J. G. Urones. *Tetrahedron* **61**, 11672 (2005), and refs. therein.
14. A. Proszenyak, M. Brændvang, C. Charnock, L.-L. Gundersen. *Tetrahedron* **65**, 194 (2009) and refs. therein.
15. M. Brændvang, L.-L. Gundersen. *Bioorg. Med. Chem.* **15**, 7144 (2007) and refs. therein.
16. C. Hansch, A. Leo, R. W. Taft. *Chem. Rev.* **91**, 165 (1991).
17. A. E. Reed, R. B. Weinstock, F. Weinhold. *J. Chem. Phys.* **83**, 735 (1985).
18. T. Fujii, T. Saito, T. Itaya, K. Kizu, Y. Kumazawa, S. Nakajima. *Chem. Pharm. Bull.* **35**, 4482 (1987).

19. M. T. Geballe, A. G. Skillman, A. Nicholls, J. P. Guthrie, P. J. Taylor. *J. Comput.-Aided Mol. Des.* **24**, 259 (2010).
20. A. L. McClellan. *Tables of Experimental Dipole Moment*, Vol. 2, Rahara Enterprises, El Cerrito, CA (1974).
21. F. Hill, D. M. Williams, D. Loakes, D. M. Brown. *Nucleic Acids Res.* **26**, 1144 (1998).

