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Adamantylaminopyrimidines and -pyridines Are Potent Inducers of Tumor Necrosis Factor-α

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Abstract—A series of (1-adamantyl)aminopyrimidine and -pyridine derivatives was prepared by adamantyl cation attack on amino heterocycles. The adamantylated compounds, particularly 2-(1-adamantyl)amino-6-methylpyridine, were found to be potent TNF-α inducers in murine melanoma cells transduced with gene for human TNF-α.

Adamantane derivatives receive considerable attention because of their diverse biological activity. The most known drug of adamantane provenience is amantadine (1-aminoadamantane) that is used for the prophylaxis and treatment of type A influenza.[1] Other aminosubstituted derivatives of amantadine (rimantidine, tromantidine) also show antiviral activity. Amantadine and rimantidine are used for the treatment of Parkinson's disease,[2,3] and memantine (1-amino-3,5-dimethyladamantane) is considered a promising drug for the treatment of certain dementias, particularly Alzheimer's disease.[4] Many adamantane moiety containing molecules show distinct antibacterial activity.[5–7] It has also been reported that a structural analogue of thalidomide, *N*-adamantylphthalimide, showed a potent tumor necrosis factor-α (TNF-α) production-enhancing activity induced by 12-*O*-tetradecamoylphorbol-13-acetate in a human leukemia HL-60 cell line.[8] TNF-α is a well characterized natural protein (cytokine) produced by various cells of the immune system, possessing antitumor and immunomodulatory properties.[9] The hydrophobic, cage-like structure of adamantane has been used to enhance lipophilicity of many biologically active compounds.[10,11]

Recently, a simple method has been developed that enables introduction of the adamantyl group into carboxamides and ureas,[12] as well as into heterocyclic compounds[13,14] by heating with 1-adamantanol in trifluoroacetic acid. However, the synthetic potential of this method may be somewhat limited because of the relative lability of many compounds. The attack of adamantyl cation formed under these harsh conditions can provide a variety of *C*- and *N*-adamantylated derivatives depending on the nature of the starting compounds. In this report, we will present the synthesis of several adamantane derivatives of aminopyridines and aminopyrimidines using this method and provide some data on their biological activity as the inducers of TNF-α in genetically modified mouse melanoma cells.

Chemistry

The synthetic route to (1-adamantyl)aminopyrimidines and -pyridines is based upon the reaction of adamantyl cation formed from 1-adamantanol in refluxing trifluoroactic acid. Aminopyrimidines and -pyridines are usually protonated at their ring heteroatoms, therefore, the acidic medium does not hinder the trapping of the adamantyl cation by the exocyclic amino group. It is of note that aniline or phenylalanine do not react under the above conditions, which indicates that this adamantylation protocol works only with non-protonated amino groups. Using the said method we have previously synthesized a number of methyl- and chlorine-substituted adamantylaminopyrimidines listed in this paper as **1a**–**d**[13,14] (Scheme 1). Here, we extended this list with the newly obtained 2-(1-adamantyl)amino-5-bromopyrimidine **1e**.[15] Similarly to the above mentioned aminopyrimidines, 2- and 4-aminopyridines as well as their methyl- or chlorine-substituted derivatives reacted easily under the conditions described above to form adamantylaminopyridines **2a**–**g** with satisfactory

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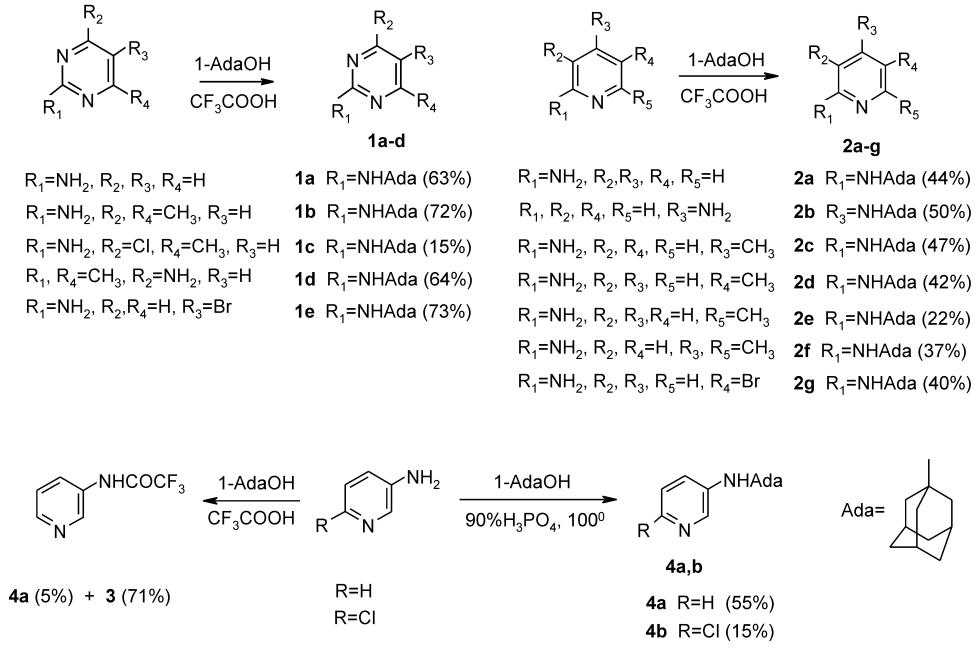
yields. Of the (1-adamantyl)aminopyridines listed in this report, only 2-(1-adamantyl)aminopyridine has been obtained previously by a multistage synthesis.[16]

3-Aminopyridine in trifluoroacetic acid medium yields only small amounts of the expected 3-(1-adamantyl)-aminopyridine **4a**, the main product being 3-trifluoroacetamido derivative **3**.[17] This difficulty has been overcome using 90% orthophosporic acid as the reaction solvent, to providing **4a** and 3-(1-adamantyl)-amino-6-chloropyridine **4b** from respective pyridines. 3-Aminopyridine has aromatic rather than heterocyclic character, and—consequently—is protonated on the exocyclic amino group. The use of orthophosporic acid



Scheme 1.

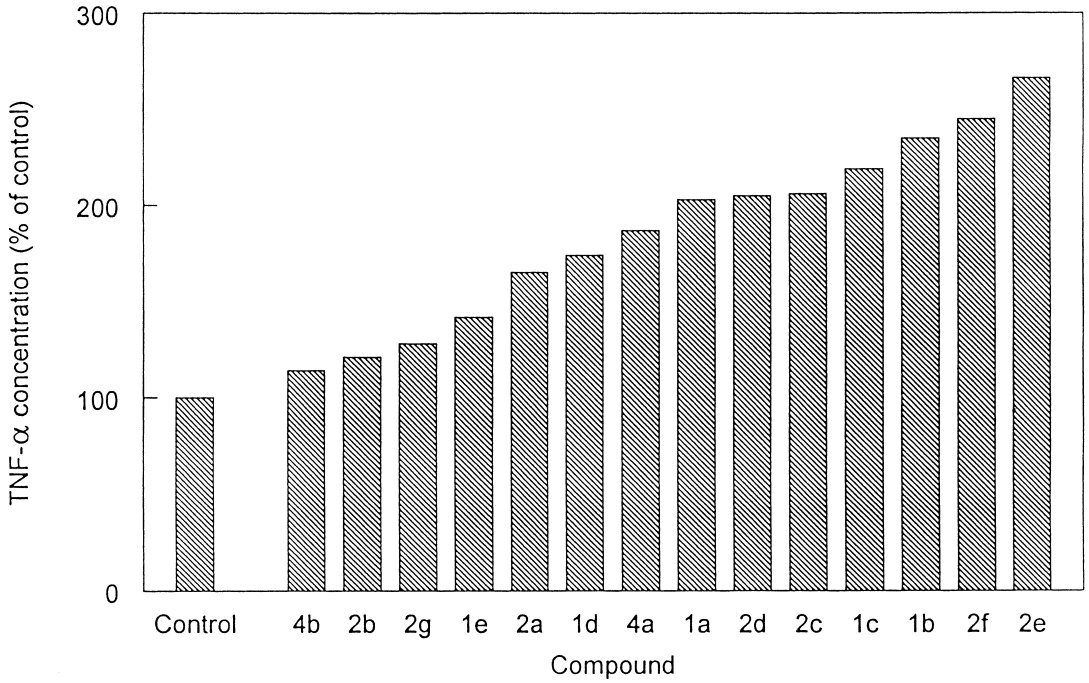


Figure 1. TNF-α stimulatory activity of adamantane derivatives of aminopyrimidines and aminopyridines expressed as percent of TNF-α level measured in control cultures (=100%). Exponentially growing B78/TNF/9 melanoma cells were incubated (2×10^5 cells in 1 mL) in 24-well plates (Nunc) for 24 h in the presence of different adamantane derivatives of aminopyrimidines and aminopyridines (final concentration 10 μM). Concentrations of TNF-α in culture supernatants were assayed by ELISA and were, for example, 916±144 (mean±SD) pg/mL for control cultures (incubated with no compound) and 2534±92 pg/mL for B78/TNF/9 melanoma cultures incubated with the most active compound—**2e** ($p<0.001$, by Student's *t*-test). Compound **2e** was tested for TNF-α stimulatory activity in several independent experiments; similar activity values as presented were obtained.

allows generation of the adamantyl cation without protonation, or with only partial protonation of the exocyclic amino group. The introduction of the bulky adamantyl residue depends strongly on the steric effects. For instance, we were unable to introduce the adamantyl group using the acid reaction conditions (with either orthophosporic acid or trifluoroacetic acid) if there was a methyl group or halogen atom at the *ortho* position relative to the exocyclic amino group. Because of the relatively good solubility of the respective heterocyclic bases in aqueous medium, the use of an excess of heterocycles allowed purification of the products without column chromatography in most cases.[15]

Biological Evaluation

The ability of the (1-adamantyl)aminopyrimidines and (1-adamantyl)aminopyridines described above to stimulate TNF-α production was studied in cultures of B78-H1 murine melanoma cells that had been transduced with the gene for human TNF-α (clone 9, hereafter named B78/TNF/9).[18] This cell line secretes TNF-α at a constant rate, and by this reason appears useful for testing the effect of various chemicals on TNF-α production. TNF-α in 24 h cultures of these cells was assayed using enzyme-linked immunosorbent assay (ELISA).

All the adamantane derivatives tested significantly enhanced TNF-α production in B78/TNF/9 cells at concentrations of 10 μM (Fig. 1). The results obtained reveal certain relationships between chemical structure and biological activity of the adamantane derivative series. First, the adamantylamino group appears practically a 'must' for biological activity in the system employed. The mother compound (2-amino-6-methylpyridine) of the most active derivative **2e** showed only about 10% enhancement of TNF-α production, also amantadine (1-aminoadamantane) was inactive in the tests used. Introduction of the adamantylamino residue at position 4 in the pyrimidine ring, or either at position 3 or 4 of the pyridine ring was much less effective in terms of enhancement of the TNF-α production by the respective derivatives than the corresponding 2-adamantylaminated compounds. The presence of the adamantylamino group at position 2 and of the methyl group at either the 6 position of the pyridine nucleus, or at position 4 in pyrimidines provided the three most potent derivatives **1b**, **2f** and **2e** (Fig. 1). Of note, these latter compounds showed a detectable enhancement of TNF-α production already at 10 nM concentration.[19] Introduction of an extra methyl group or chlorine atom at the heterocyclic ring (e.g., see **2a** and **2f**) resulted in no significant change in TNF-α secretion activity; instead, most such derivatives showed an increased cytotoxicity (data not shown).

Structural similarities among the most active compounds allow speculation that there are two characteristic features that may both be important for their biological activity. One is the presence of two hydrophobic groups, the adamantyl one and the methyl one, on the opposite sides of the pyridine ring. The other is the presence of two nitrogen atoms, which are capable of forming hydrogen bonds in the interconnecting *o*-aminopyridine bridge. Rentgenostructural study on 2-adamantylamino-6-methylpyridine **2e** is in progress (Maurin and Kazimierczuk, in preparation).

Genetically modified cells producing mediators of the immune system (cytokines, interleukines and TNF-α) show promise as 'vaccines' used to stimulate antitumor response in new, experimental forms of cancer therapy.[20] We hope that some of the adamantane derivatives described above, especially **2e**, might be good candidates for adjuvant drugs used to potentiate antitumor efficacy of such therapeutic strategies.

Acknowledgements

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15. Typical procedure for the synthesis of (1-adamantyl)-aminopyrimidines or -pyridines. A solution of aminoheterocyclic base (11 mmol) and 1-adamantanol (10 mmol) in trifluoroacetic acid (10 mL) was stirred under reflux for 5 h. The reaction mixture was poured into ice-cooled water (50 mL) and brought to pH 7 with concd aq NH_3 solution.

The precipitate formed was filtered and crystallized from EtOH/water mixture. Selected data for **1e**: mp 177–180 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.70 (bs, 6H, H-Ada), 2.07 (bs, 9H, H-Ada), 8.21 (s, 1H, H-C(6)). MS: 310 (15), 309 (91), 308 (22), 307 (92), 266 (11), 253 (15), 252 (95), 251 (16), 250 (100). For **2a**: mp 163–166 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.70 (bs, 6H, H-Ada), 2.04 (bs, 6H, H-Ada), 2.12 (bs, 3H, H-Ada), 6.05, 7.35, 8.01 (3m, 4H, H-Pyr). MS: 229 (17), 228 (100), 227 (36), 213 (11), 185 (24), 172 (14), 171 (75), 132 (48). For **2b**: mp 147–148 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.62 (bs, 6H, H-Ada), 1.72 (bs, 6H, H-Ada), 2.03 (bs, 3H, H-Ada), 6.84, 7.95 (2d, 4H, H-Pyr). MS: 229 (8), 228 (41),172 (15), 171 (100), 135 (50), 107 (11). For **2c**: mp 123–125 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.62 (bs, 6H, H-Ada), 1.70 (bs, 6H, H-Ada), 2.14 (bs, 3H, H-Ada), 2.39 (s, 3H, H-Me), 6.45, 6.76, 7.59 (m, s, d, 3H, H-Pyr). MS: 243 (17), 242 (100), 241 (36), 199 (17), 185 (50), 173 (11). For **2d**: mp 209–212 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.60 (bs, 6H, H-Ada), 1.71 (bs, 6H, H-Ada), 2.13 (bs, 3H, H-Ada), 2.40 (s, 3H, H-Me), 6.78, 7.43, 7.74 (m, m, s, 3H, H-Pyr). MS: 243 (17), 242 (100), 241 (32), 227 (10), 199 (19), 185 (58), 173 (12). For **2e**: mp 123–127 °C (sint. >80 °C). ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.61 (bs, 6H, H-Ada), 1.72 (bs, 6H, H-Ada), 2.14 (bs, 3H, H-Ada), 2.49 (s, 3H, H-Me), 6.41, 6.74, 7.52 (3m, 3H, H-Pyr). MS: 243 (17), 242 (100), 241 (23), 227 (10), 199 (16), 186 (11), 185 (59). For **2f**: mp 210–212 °C (sint. >80 °C). ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.60 (bs, 6H, H-Ada), 1.73 (bs, 6H, H-Ada), 2.14 (bs, 3H, H-Ada), 2.36, 2.39 (2s, 6H, H-Me), 6.62, 6.95 (2s, 2H, H-Pyr). MS: 257 (19), 256 (100), 255 (29), 241 (12), 213 (17), 199 (35). For **2g**: mp 115–117 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.71 (bs, 6H, H-Ada), 2.03 (bs, 6H, H-Ada), 2.13 (bs, 3H, H-Ada), 6.48, 7.45, 6.02 (d, d, s, 3H, H-Pyr). MS: 309 (16), 308 (98), 307 (38), 306 (100), 305 (21), 265 (21), 251 (59), 249 (58). For **4a**: mp 227–230 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.60 (bs, 6H, H-Ada), 1.73 (bs, 6H, H-Ada), 2.14 (bs, 3H, H-Ada), 7.10, 8.00, 8.12 (3m, 4H, H-Pyr). MS: 229 (10), 228 (55), 172 (11), 171 (77), 136 (11), 135 (100), 107 (13). For **4b**: mp 124–126 °C. ^{1}H NMR ($CDCl_3$, $SiMe_4$): 1.68 (bs, 6H, H-Ada), 1.85 (bs, 6H, H-Ada), 2.13 (bs, 3H, H-Ada), 7.07, 7.88 (d, s, 3H, H-Pyr). MS: 264 (7), 262 (20), 205 (18), 136 (11), 135 (100).

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