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Determination of cytotoxic activity of adamantyl-desmuramyl dipeptides

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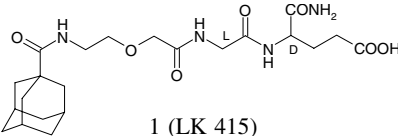
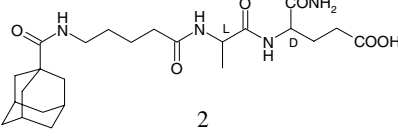
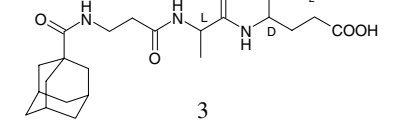
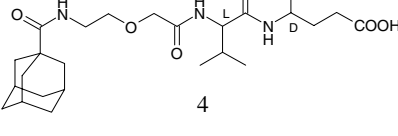
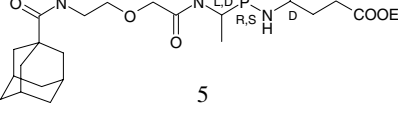
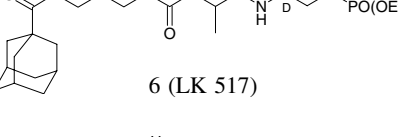
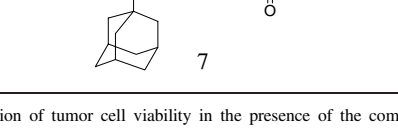
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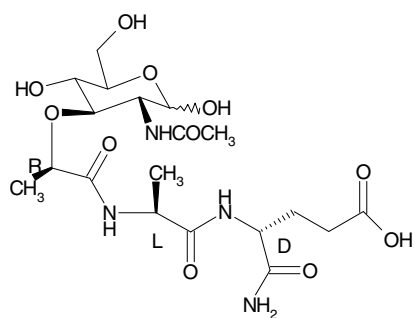
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N-Acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide or MDP) is the smallest peptidoglycan monomer possessing immunoadjuvant activity [1]. In order to improve its pharmaceutical properties, as well as to reduce its side effects, numerous MDP derivatives and analogs have been synthesized and evaluated [2, 3]. Among their many interesting biological activities, MDP analogs can stimulate the tumoricidal activity of macrophages and monocytes and increase the *in vitro* cytotoxicity of NK cells [4–6]. It is well known that the intact *N*-acetyl-D-glucosamine fragment is not essential for the biological activity of MDP analogs. Derivatization of the sugar moiety, as well as its replacement by various acyl groups, is thus an important approach to the design and synthesis of new immunomodulators based on MDP. Recently, we synthesized a series of new adamantyl-desmuramyl dipeptides [3, 7], where the 1-adamantylcarboxamido moi-

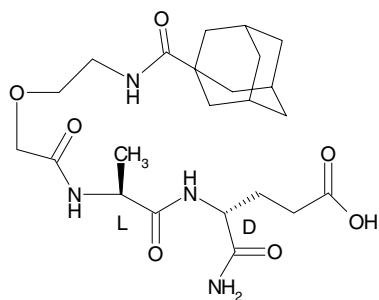
Table 1: Effect of adamantyl-desmuramyl dipeptides on the viability of tumor cell lines

Structure	Reduction %			Synthesis ref.
	(Breast) MDA-MB 231	(Lung) 103H (LCLC)	(Liver) Hep G2	
 1 (LK 415)	—	8	18	[3]
 2	12	—	15	[7]
 3	—	10	—	[7]
 4	22	12	21	[7]
 5	18	—	—	[7]
 6 (LK 517)	16	9	13	[3]
 7	—	23	—	[15]

Values are the reduction of tumor cell viability in the presence of the compound expressed as a percentage of the viability of non-treated cells (—: reduction of the viability not significant)



MDP



LK 415

ety was used as a replacement for MDP's *N*-acetylglucosamine fragment. Two of these were evaluated immunologically (LK 415 and LK 517, Table). LK 415 augmented the capacity to produce interferon- γ and reduced the production of interleukin-10 in cyclophosphamide-treated mice [8]. Both LK 415 and LK 517 modulated the production of cytokines in ionomycin and phorbol-12-myristate-13-acetate activated *in vitro* cultures of human peripheral blood mononuclear cells [3].

Compounds with the adamantane nucleus have recently received considerable attention on account of their antimicrobial, antiviral, and anti-tumor activities [9]. In many cases introduction of the adamantane motif into biologically active substances improved their pharmacodynamic and pharmacokinetic properties. MDP analogs containing the adamantyl residue bound to the essential moiety, L-Ala-D-iGln, exhibited both antiviral and immunomodulatory activity, as demonstrated by the adamantylamide dipeptide [10] and (adamant-2-yl)glycine-L-Ala-D-iGln [11]. The similar approach of combining two biologically active components in a single synthetic compound has recently been used by Dzierzbicka et al. [12, 13]. They synthesized conjugates of MDP with the strong anticancer agents acridine, hydroxyacridine, and acridone which were immunologically active, and exhibited potent *in vitro* cytotoxic activity against a panel of human tumor cell lines [12, 13].

Encouraged by the above results we evaluated the *in vitro* cytotoxicity of a series of adamantyl-desmuramyl dipeptides (compounds 1–6, Table), and one synthetic intermediate (7) against three human tumor cell lines; MDA-MB 231 (breast cancer), 103H (large cell lung cancer), HepG2 (hepatoma) and a normal cell line HUVEC (human endothelial cells). The exponentially growing cells were exposed to the compounds (10^{-4} M) for 24 h and cytotoxic activities were determined by a tetrazolium-based colorimetric assay (MTT assay) [14]. The results are summarized in the Table. All the compounds show weak, but significant cytotoxicity against at least one tumor cell line. Compound 4 is cytotoxic to all three tumor cell lines. None of the compounds exhibited cytotoxic activity against the normal cell line (HUVEC), indicating selective

cytotoxicity against the tumor cell lines studied. Adamantyl-desmuramyl dipeptides are thus highly interesting for further development as potential immunomodulators.

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