Structure-Activity Relationship in NOD2 Agonistic Muramyl Dipeptides

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Abstract

Nucleotide-binding oligomerization domain 2 (NOD2) is a receptor of the innate immune system that is capable of perceiving bacterial and viral infections. Muramyl dipeptide (MDP, *N*-acetyl muramyl L-alanyl-D-isoglutamine), identified as the minimal immunologically active component of bacterial cell wall peptidoglycan (PGN), is recognized by NOD2. In terms of biological activities, MDP demonstrated vaccine adjuvant activity and stimulated non-specific protection against bacterial, viral, and parasitic infections and tumors. However, MDP has certain drawbacks including pyrogenicity, rapid elimination, andlack of oral bioavailability. Several detailed structure-activity relationship (SAR) studies around MDP scaffolds are being carried out to identify better NOD2 ligands. The present review elaborates a comprehensive SAR summarizing structural aspects of MDP derivatives in relation to NOD2 agonistic activity.

Keywords: NOD2, muramyl dipeptide,MDP, vaccine adjuvant, PAMPs, innate immunity.

1. Introduction

The innate immune system is the bedrock of the body's ability to fight early infection and comprises various families of pattern recognition receptors (PRRs): promising targets for immunomodulation and vaccine adjuvant discovery.[1–5]One such family is the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) which included NOD2, the target of MDP, with other major families including the toll-like receptors (TLRs), and retinoic acid-inducible gene (RIG)-like receptors, and the C type lectin receptors.[6] PRRs detect invading microorganisms *via* recognition of pathogen-associated molecular patterns (PAMPs). PAMPs can comprise specific features in bacterial or viral DNA or RNA or cell wall components like peptidoglycan (PGN) and lipopolysaccharides (LPS).[7] NLRs, such as NOD2, play a major role in the formation of caspase-1 activation complexes, known as inflammasomes.

NLR family

In humans, there are 22 known NOD-like receptors (NLRs)[8,9] which are divided into four subfamilies based on the type of *N*-terminal domain: the acidic transactivation domain (NLRA), the baculoviral inhibitory repeat-like domain (NLRB), the caspase activation and recruitment domain-CARD (NLRC), and the pyrin domain (NLRP). The NLRA subfamily includes the MHC-II trans activator (CIITA, Fig. 1) as the only member. Similarly, the human NLRB subfamily has only one member, NAIP. The NLRC subfamily consists of six members: NLRC1 (NOD1), NLRC2 (NOD2), NLRC3 (NOD3), NLRC4 (NOD4), NLRC5 (NOD5), and NLRX1; while the NLR-P subfamily consists of 14 members, NLRP1–14.[10,11]

The sole member of the NLRA family, CIITA, is unique in the fact that it acts as a transcription factor.[12] Mutations in this gene are responsible for the bare lymphocyte syndrome, in which the immune system is severely compromised and cannot effectively

fight infection. The NLRB subfamily consists of NAIP (NLR family, apoptosis inhibitory protein), which is responsible for triggering interleukin 1β (IL-1β) secretion in response to intracellular flagellin.[13] The most studied NLRs belong to the NLRC and NLRP families. The Nucleotide-binding oligomerization domain, Leucine-Rich repeat, and Pyrin domain (NLRP) containing family, also referred to as the NALP family, play a role in apoptosis and inflammation with members of the NLRP subfamily expressing *N*-terminal pyrin domain and are crucial for the organization of IL-1β inflammasomes. The NLRC subfamily members display either *N*-terminal caspase recruitment domain (CARD) or an undefined domain that has no apparent homology with other proteins.[14,15]

The present review focuses on NOD2, one of the receptors of the NLRC family. Among the NLRC receptors, NOD2 has two CARD domains, NOD1 and NOD4 have one, whereas NOD3, NOD5, and NLRX1 do not have an identified *N*-terminus domain.[16] The NOD1 and NOD2 receptors are known to recognize different bacterial and viral PAMPs.[17]NOD1 recognizes gamma-glutamyl diaminopimelic acid (iE-DAP), a peptidoglycan product of Gram-negative bacteria. Whereas, NOD2 recognizes muramyl dipeptide (MDP, **1**) present in the bacterial peptidoglycan which consist of *N*-acetyl muramic acid linked by its lactic acid moiety to a dipeptide comprising L-alanine (first amino acid, AA1) and D-isoglutamine (second amino acid, AA2).[18,19] Recent studies have shown that NOD2 can react with viral single-stranded RNA (ssRNA), leading to type I interferon production[20,21] and activation of some inflammasomes. In humans, a frameshift mutation resulting in truncated NOD2 protein is found to be associated with susceptibility to Crohn's disease.[22]Conversely, mutations that lead to overexpression of NOD2 and excess nuclear factor-kappa B (NF-κB) activation results inBlau syndrome, an autosomal dominant disorder.[23]

2. Mechanistic details of NOD2 recognition and activation

NOD2 is a bona fide receptor for MDP, a cell wall component of both Gram-positive and Gram-negative bacteria. NOD2 is known to reside in an inactivated state with the leucine-rich repeats (LRRs) folded back onto the NACHT domain (or NOD domain). On exposure to MDP, NOD2 undergoes conformational changes allowing it to self-oligomerize through the kinase receptor-interacting protein 2 (RIP2) *via* homophilic CARD–CARD interaction. The RIP2 then mediates the recruitment and activation of the TAK1, which is a prerequisite for activating the IKK kinase complex and mitogen-activated protein kinases (MAPK) pathway. This allows NF-κB to translocate to the nucleus and start transcription of proinflammatory genes, including cytokines, growth factors, and factors responsible for stimulating immune cells.[24–30]

While the structure of human NOD2 is yet to be determined, Maekawa *et al.* reported the crystal structure of rabbit NOD2 in the ADP-bound state.[31] The crystal structure showed that ADP-bound NOD2 folded into a hook-shaped structure with dimensions of`~70 x 80 x 40 Å, consisting of a NOD domain (residues 195–744) and a LRR domain (residues 745–1,020). The NOD domain can be further sub-divided into the nucleotide-binding domain (NBD, residues 195-425), helical domain 1 (HD1, residues 426–485), the winged helix domain (WHD, residues 486–602), and helical domain 2 (HD2, residues 603–744).[31]Lauro*et al*. and others have proposed that the recognition of MDP occurs within the LRR domain.[32–34] However, this remains unproven as the crystal structure of NOD2 bound to MDP has not yet been reported, so the molecular details of this interaction remain poorly understood.

The mutations of NOD2 are associated with inflammatory diseases including,Blau syndrome, early-onset sarcoidosis and Crohn's disease.[35] Apart from the role of NOD receptors in pathogen recognition and inflammation, they also play an important role in cancer progression.[10] For example, Mifamurtide (**2**, Fig. 4), a NOD2 agonist, has been approved for immunotherapy in combination with chemotherapy for patients with osteosarcoma,[36–38]

subsequent to surgical resection of the primary tumor.[10] A synthetic derivative of MDP, murabutide (**3**, Fig 4) was shown to suppress HIV-1 replication in monocyte-derived macrophages. Romurtide, a synthetic stearoyl-MDP derivative (also known under the names MDP-Lys and muroctasin) (**4**, Fig 4) is being used in the treatment of leukopenia that occurs during radiotherapy.[39] Recently, another role of NOD2 in stem cell protection *via* recognition of commensal microbiota was highlighted by Nigro and co-workers.[40]

3. Structure Activity Relationship Investigations

The NOD2 activity of MDP (**1)** is higher than that of murabutide (MB) (**3**)but has low clinical utility due to drawbacks, including pyrogenicity, rapid elimination, and lack of oral availability.[41]Identification of new NOD2 ligands with greater clinical utility will be possible with better understanding of the SAR in MDP derivatives.

3.1. Modifications at the C1 and C2 position of the *N*-acetyl muramyl moiety

Chen and co-workers generated a library of compounds having modifications at the C1 and C2 position of MDP scaffold (**5-24**, Fig. 5) and evaluatedat 1μM concentration in the NOD2-dependent NF-κB activation assay using HEK293T cells.[42] The first set of compounds was prepared by *N*-acylation at C2 position and by keeping hydroxyl functionality at the C1 position (**5-12**,Fig.5A). All the newly synthesized analogs were found to be potent stimulators than MDP except compound **10**confirming the non-tolerance to long acyl chain at C2 position. Further, analogs bearing the methoxy group at the C1 equatorial (β) position (**13-19**) were synthesized, and it was interesting to observe that all these analogs were more potent than their respective anomeric (axial) hydroxy analogs (**5-12**). Compounds bearing *O*-alkyl chains (C5-C11, **20-22**) at the equatorial C1 position showed similar potency to that of **5**, whereas the replacement by polar methylaminoxy amines (**23** and **24**) at the C1 position abrogated the activity. Surprisingly, compound **25**bearing a C1 methoxy group with no

substituent attached to the C2 amine group was found to be the most potent stimulator of NOD2. In contrast, thecompound **26**, which is the salt form of compound **6**, showed diminished NOD2 agonistic activity.[43,44]

The compounds having acylation at the C2 position (**27**-**31**, Fig. 6)still retained the NOD2 agonist activity two-fold greater than the untreated control. The fluorescent derivatives, bearing dansyl at the C2 position (**32**) or linked on the isoglutamine residuewith small ethylenediamine linker (**34**, **35**)did not activate NOD2. In contrast, compound **33**,with biotin at the C2 position was still active, although to a lesser extent than other acylated derivatives.As the fused heterocycle component in biotin is linked to carboxylic acid with four methylene spacers, installing a fluorophore on NOD2 ligands at C2 without losing their NOD2 activity might be possible with increased linker length. Recently, Reddy *et al*. synthesized the azido derivatives with *O*-benzyl moiety at C1 position (**36**-**39**, Fig. 5C). Among the synthesized derivatives, compound **37**showed better NOD2 agonistic activity, whereas the compound **36** exhibited comparable activity tostandard MDP. The compounds bearing C8 and C14 chains (**38** and **39**, respectively) showed minimal NOD2 activity.

3.2. Modifications at the C4 position of the *N*-acetyl muramyl moiety

Cheng and co-workers next modified the C4 position of the carbohydrate moiety.[41]A dramatic loss of NOD2 activity was observed for **40**,**41**,and**46** (Fig. 7) due to the masking of the hydroxy group by the benzyl group at the C1 axial position as compared to MDP, suggesting that the orientation and substituents at the C1 position might play an important role in NOD2 activity. Also, the compounds with a substituted triazole moiety (**42**-**46**) at the C4 position dramatically weaken the interactions with the NOD2 receptor and thus showed diminished activity.This shows that a substituted triazole moiety causes a significant weakening of NOD2 activity at the C4 position.

3.3. Modifications at the C6 position of the *N*-acetyl muramyl moiety

Sansonetti*et al.* showed that MDP activates NOD2 in a stereospecific manner. Substitution ofD-isoglutamate by L-isoglutamate in MDP failed to stimulate NOD2, suggesting that the specific peptide portion of MDP is important for the ligand recognition.[18,45] To keep the recognition region of the MDP molecule intact and available for interaction, Grimes and co-workers reasoned that a modification would best be made at the C6 position of the carbohydrate, far off from the potential binding site C3. Accordingly, C6-amino versions of both MDP-L-D (**47**, Fig. 8) and MDP-L-L (**48**) were synthesized, but only L-D derivatives could activate NOD2. In addition, 6-amino MDP derivatives containing biotin with disulfide bond as a cleavable linker (**49**, **50**) as well as with polyethyleneoxylinkers of variable lengths (**51**-**54**) were also synthesized. The synthesized analogs were tested for their ability to activate NOD2 in an established NF-κB luciferase assay.Among these compounds, **49** and **51** with a shorter linker (length 20 and 32 Å, respectively) as well as with L-D configuration only were found to be the most active. Compound **53**, with a longer linker (length 56 Å) was the least active in the series.

Azuma *et al*. also synthesized MDP analogs having modifications at the C6 position (6-*O*-acyl derivatives) in which the muramyl moiety was conjugated with various lipophilic molecules. These compounds were evaluated for their antitumor activities. It was observed that administration of 100 µg each of 6-*O*-mycoloyl-MDP (**55**, Fig. 9), 6-*O*-nocardomycololyl (**56**), 6-*O*-corynomycoloyl-MDP (**57**), or 6-*O*-mycoloyl-*N*-acetylmuramyl-Gly-D-isoGln (**58**) in an oil-based vehicle suppressed the growth of fibrosarcoma (tumor) in mice while 6-*O*-mycoloyl-*N*-acetylmuramyl-L-Ser-D-isoGln (**59**) was active in terms of regression of an established line 10 hepatoma in guinea pigs.[46]In addition to these analogs, B30-MDP (**60**) was also synthesized in which natural mycolic acid was substituted by a synthetic fatty acid

chain of higher molecular weight resulting in stronger antitumor immunity.[47,48] Further modification led to the synthesis of quinonyl-MDP-66 (**61**), which suppressed the growth of Meth A fibrosarcoma in mice.[49–52]

3.4. Modifications at the first amino acid

To further assess the effect of modifications at the first amino acid, L-alanine of MDP was replaced by L-valine (**62**,Fig. 10) or L-serine (**63**), which showed comparable NOD2 activity. However, *N*-methyl L-alanine (**64**) or L-threonine (**65**), L-proline (**66**), and glycine (**67**)had reduced NOD2 activity. MDP analogs**69-71** having D-configuration of the first amino acid were utterly inactive. While these analogs demonstrated less NOD2 activity than MDP, they were still reported to have significant activity when injected *in vivo*.[53,54,55]

3.5.Modifications at second amino acid

When MDP derivatives having modifications at the second amino acid were investigated, it was found that compounds having *mono*-ester moiety at the α-position (**72-78**, and **86-88**, Fig. 11) were more active than MDP. It was interesting to observe that NOD2 activity increases with increase in the chain length, with the C12 alkyl chain (**76**) having the highest NOD2 activity. Interestingly, **72** induced approximately five-fold more NF-kB activation than MDP when stimulated at the lowest dose of 1 ng/mL. On the other hand, compounds with *mono*-ester moiety at γ-position (**79**and **80**) exhibited less NOD2 activity than MDP. The compounds possessing *di*-ester moiety (**81** and **82**) were slightly more active than MDP, but less active than those with a mono-ester moiety (**72** and **73**). The compound possessing carboxylic acid moiety on both sides (**83**) showed similar activity to MDP but the compound having a terminal amide group (**84**)on both sides was inactive. Surprisingly, compound **85** with methyl ester at γ-position and amino group at the α-position showed lower NOD2 agonistic activity than MDP. Theanalogs which contained a *N*-monosubstituted amide moiety

having alkyl chains of varied lengths (**89** and **90**), a cyclohexyl moiety (**91**), and a fluorine-containing alkane (**92**)were inactive.The compound with D-alanyl residue on the γ-carboxyl (**93**) resulted in diminished NOD2 activity, while the compound with L-lysyl residue (**94**)retainedNOD2 activity but was lower than MDP. Further, when biosteric replacement of α amide of D-isoglutamine of MDP is carried out using with 2,5-substituted tetrazoles, it was observed that among the varied chain lengths 2, 5-disubstituted tetrazole derivatives (**95-100**), the tetrazole analogues**96** bearing the -Butyl (C4) and**97** having octyl (C8) chain showed the best NOD2 stimulation potency equivalent with reference compoundMDP.The biological results also showed that the NOD2 activity was lost for the MDP analog (**101**), which possessed a shorter alkane spacer.[56–60]

3.6.MDP derivatives having modifications at both the dipeptide and carbohydrate moiety

Cai *et al.* synthesized MDP analogues having modifications at both the dipeptide and carbohydrate moiety.[61]In the small set of compounds (**102-105**, Fig. 12), the dipeptide synthesized using D-isoglutamine benzyl ester and L-alanine was linked to C1 benzyl *N*-acetylglucosamine (GlcNAc) or its acetonide *via*lactic or glycolic acid. Among the four derivatives, only compound **104** was similar in activity to that of MDP. Interestingly, **104** gradually reduced TNF-α as a result of an increased concentration and induced AP1/A20 gene expression. At high doses, **104** reduced the transcription of RIP2, NOD2, and NF-κB in macrophages to a greater extent than MDP treatment. This indicates that, based on its efficacy, analog**104** is indeed an improved compound in comparison to MDP.

3.7. Carbocyclic analogs of MDP

In order to understand the importance of carbohydratemoiety in MDP, the first carbocyclic analog of MDP, *N*-[D-2-(cyclohexyloxy) propionyl]-L-alanyl-D-isoglutamine (**106**, Fig. 13) was synthesized by Kikelj, D.*et al.*[62]Compound **106**in which the carbohydrate moiety of

MDP was replaced with cyclohexanol derivatives, was inactive as an adjuvant for the induction of delayed-type hypersensitivity to azobenzenearsonate*N*-acetyl-*L*-tyrosine in guinea pigs.Kikelj*et al.* then synthesized a variety of carbocyclic MDP analogs(**107-117**) and these compounds were tested for their ability to enhance non-specific resistance in mice immunosuppressed with cyclophosphamide and infected with *Candida albicans*. The results were evaluated in reference to azimexon and MDP. The analogs with D-glutamic acid moiety (**107-109**) displayed significant immunorestorant activity, while the analogs with D-isoglutamine (**108-110**) were less active.Also, all the four diastereomers of*N*-{trans-2-[[2'-(acetylamino)cyclohexyl]oxy]propionyl}-L-alanyl-D-isoglutamine*i.e.*, **111-114** were inactive, thus confirming the importance of D-glutamic acid. Analogs with increased lipophilicity **115** and **116** displayed activity at higher doses, being the most for **109** with a longer alkyl chain. The rigidified analog**117** showed reduced activity suggesting that rigidification is not beneficial for immunorestorative activity.

3.8.Desmuramylpeptides

Zhao *et al.* synthesized a new series of analogs known as desmuramylpeptides wherein the *N*-acetylmuramyl moiety is replaced by a variety of aryl moieties with different spacers or linking functionalities (**118-137**, Fig. 14). The library was synthesized using solid phase strategy and their adjuvant activities were evaluated *ex-vivo* to determine the synergism with $S_{28–39}$ peptide,a MHC class I binding epitope of recombinant hepatitis B surface antigen (HBsAg) for both human and mice. In the first series, where L-alanine was used as the first amino acid, it was observed that compounds having a free amino group (**118** and **119**, Fig.14A) at the C2 position did not improve the activity. Further, compound **120** having a phenethyl group at the R3 position and chlorine at R1 position was potentin regard to the enhancement of the immunological activity than the other analogs of this series.It was also observed that substitution with an electron-donating group, such as a methoxy group, at R3

position was unable to contribute significantly to the activity of compound **121**. Also, the compound with no substitution on benzene ring (**122**) were not effective. Further, introduction of astrong electron-withdrawing group, such as a nitro group, onto a phenethyl moiety at R4 position (**123** and**124**) did not improve the activity. Similar observations were obtained when L-phenylalanine and L-isoleucine were used as the first amino acids. The compound with phenethyl group at R3 position (**127**,Fig 14Band **131**, Fig.14D),showed enhancement of immunological activity. On the other hand, the analog with L-leucine as the first amino acid and phenethyl group at R3 position (**128** Fig 14C) showed moderate activity. Next, when L-valine was used as the first amino acid, only the compounds **134** (Fig. 14E) and **135** showed moderate activity. Surprisingly, the analog**137** with phenethyl group at R3 position showed diminished activity. All the other analogs did not show any improvement in the immunological activity.[63] However, the direct NOD2 agonistic activity of the synthesized library of compounds was not reported by the authors.

With an aim to find the appropriate surrogate moiety for *N*-acetyl muramyl moiety, Khan and co-workers also synthesized a new class of amphiphilic desmuramylpeptides wherein carbohydrate moiety of the parent molecule was replaced by hydrophilic arenes.[64] A lipophilic chain was also introduced at the *C*-terminus of dipeptide moiety while conserving L-D configuration. These desmuramylpeptides were highly active at 15 µM concentration. When 2-hydroxyethyl containing desmuramylpeptides (**138-145**,Fig. 15) were used at 15 µM concentration, they showed slightly higher activity than Murabutide. Similarly, 3-hydroxypropyl containing desmuramylpeptides (**142-145**)showed higher activity than all 2-hydroxyethyl containing desmuramylpeptides except **140**.[64]

Further,Jakopin*et al*. synthesized a series of desmuramylpeptides, in which*N*-acetylmuramyl moiety was replaced by saccharin and indole heterocycles and their derivatives (**146-181**, Fig.16).[65] These compounds were screened for their ability to induce TNF-α release from

naive THP-1 cells and to modulate LPS-induced TNF-α release. All these analogs, including MDP and MB, were not able to induce significant TNF-α release on their own, but a synergistic effect on lipopolysaccharide induced TNF-α secretion was observed. None of these analogs were as effective as MDP and MB. The MDP and MB enhanced LPS-induced TNF-α release by 87.4 and 82.6 %, respectively. All these analogs showed only weak activity,with compound **162** having an indole-scaffold being the most active. Further, to address the influence of lipophilicity, desmuramyldipeptide**166** and its corresponding benzyl (**168**) and ethyl esters (**169**)were investigated.However, the highly lipophilic benzyl ester (**168**) did not increase activity compared to the value of the parent compound **166**.

To address the lipophilicity of the saccharin and indole bearing desmuramylpeptides, the carboxylic acid functionalities of dipeptide moiety were replaced by ethyl ester functionalities (**182-188**, Fig. 17). These derivatives were screened for the NOD2 agonistic activity at 20 μM. The positive control MB **3** and the synthetic compounds 1**83** and **184** significantly increased NF-κB transcriptional activity with respect to untreated cells. However, other desmuramyldipeptides showed nearly the same activity as of untreated cells.[7]

Further structural modifications to compound **184** were made involving the replacement of the indole-2-yl moiety by its closelyrelated mimetics (**188-206**, Fig. 18), along with the modifications in the peptide Gly-L-Ala-D-Glu moiety. The compounds derived by the methylation of secondary nitrogen of indole moiety (**188**) and substitution with 4-fluorobenzyl (**190**) demonstrated NOD2 activation, although to a lesser extent. Surprisingly, in regioisomeric derivative **191**, formed by linking the peptide fragment at the C3 position of indole moiety instead of the C2 position, the NOD2 agonistic activity was completely abolished. Similarly, the analog lacking the benzene ring (**192**)and pyrrole ring (**193**) of the indole heterocycle resulted in the complete loss of activity. The partial saturation of the indole heterocycle resulted in indoline derivatives **194** and **195** with decreased NOD2

agonistic activities. It was interesting to observe that most of the 5-substituted indole derivatives were active. Replacement withelectron-withdrawing groups, such as fluorine (**196**) and bromine (**197**), did notcontribute significantly to the activity of the parent compound. Interestingly, the 5-phenyl substituted derivative **198** proved to be a potent NOD2 agonist with 10.4-fold activation. Thus, itsregioisomeric derivatives **199**-**201** were also explored for their NOD2 agonistic activity. Among thesecompounds, the 6-phenylsubstituted regioisomer**200** showed slightly improved activity than **198**.Elongating the glycine spacer by one methylenegavecompound **202** with significantly diminished NOD2 activity. The hydrolysis of diethylester groups afforded compound **203**, which exhibited decreased NOD2activity and the bioisosteric replacement of dicarboxylic acids with 1,2,4-oxadiazoles (**205**) also resulted in diminished NOD2 activity. As expected, the dibenzyl ester derivative **204** showed a remarkable increase in the NOD2-agonistic activity. Interestingly, the introduction of cinnamoyl moiety afforded the analog**206**,which showed enhanced NOD2 stimulatory capacitysuggesting that the cinnamoyl structural fragment is an important feature forNOD2 recognition.[66]

The compounds **200** and **206** were selected as the lead compounds and a detailed SAR study was then performed by Jakopin and co-workers. The synthesized compounds were evaluated for their ability to activate NOD2 in a reporter cell assay employing HEK-Blue NOD2-dependent NF-κB inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene assay at 2 µM. As the compound **200** (Fig. 19) showed 11.2- fold activation, so its analogs with L-valine (**207**, Fig. 19) and L-phenylalanine (**208**) were also explored, but none of these derivatives showed better NOD2 activation. So, this class of desmuramylpeptide was not explored further.When desmuramylpeptides having 3,4-methylenedioxy **209**, benzofuran **210**, 6-bromobenzofuran **211**, and 4,5-dimethyl-2-phenyloxazole **214** were explored for their

NOD2 activity, the compounds showed diminished activity. However, substituting the benzofuran by bromo (**212**) and nitro (**213**) at 5-position moderately improved the activity.

Next, the SAR study was performed on the desmuramylpeptide**206**. This SAR study analyzed the derivatization of the R_1 and R_2 position of cinnamoyl moiety as well as the replacement of the first (R_3) and second amino acid (R_4 and R_5) of the peptide part. Initially, the variation on the first amino acid was carried out while keeping the cinnamoyl moiety intact. It was observed that the derivative with L-valine (**216**, Fig. 20A), instead of L-alanine (**215**) at the first amino acid showed maximum NOD2 activity (**216**) followed by the L-phenylalanine derived analogue **216**.[33] Further, the cinnamoyl moiety was replaced with trans ferulic acid, and a variation of the first amino acid was also made. Among these compounds, compound **219** (Fig. 20B) with L-valine amino acid was identified as a potent NOD2 agonist. It was observed that analogs having L-serine (**221**),*O*-benzyl L-serine (**222**), L-threonine (**223**),L-cyclohexylalanine (**234**), L-pyridylalanine (**225**), (*S*)-adamantylglycine (**226**)and L-homophenylalanine (**227**) amino acids showed relatively poor activities while analogs bearing L-alanine (**218**) and L-phenylalanine (**220**) showed moderate NOD2 activity. Next, chemical space around the C3 and C4 positions of cinnamoyl moiety was also explored.[67]

The compound **219** with R_1 methoxy and R_2 hydroxy group on cinnamoyl moiety was found to be a potent NOD2 agonist. On substitution with dihydroxy (**228**, Fig. 21)and dimethoxy (**229**) at R_1 and R_2 positions did not improve the activity, while the introduction of two fluorine atoms (**230**) on the phenyl ring of cinnamoyl moiety showed comparable activity as that of lead compound (**219**). Furthermore, the authors tested the effect of substituents at R_2 by installing amino, nitro, methyl, trifluoromethyl, isopropyl, isopropoxy and phenyl groups. The 4-nitro and 4-isopropyl group bearingcompounds(**231** and **232**), showed maximum NOD2 activity. The derivatives with smaller functional groups *i.e.*, 4-chloro (**233**), 4-bromo (**234**), 4-amino (**235**) and 4-methyl (**236**), exhibited the strongest activity, followed by

derivatives incorporating trifluoromethyl (**237**) or an isopropoxy (**238**) groups. A further increase in the size of the substituent at the R_2 position to phenyl group (**239**) and 4-flouro phenoxy group (**240**)resulted in inactive analogs. In contrast, the functionalization at R_1 potion was better tolerated with phenyl (**241**) and phenoxy (**242**) derivatives exhibiting the same potency as lead compound **219** followed by nitro (**243**) and bromo (**244**) derivatives. Acylation of the 4-hydroxy group of the lead compound with acetyl (**245**) and lauroyl tail (**246**) on the aromatic ring retained the activity. However, increasing the C12 chain of **246** to C18 (**247**) resulted in markedly diminished activity. Furthermore, acylation with lipophilic adamantane-moiety produced compound **248**, which was identified as the firstdesmuramylpeptide with NOD2 stimulating activity in thesingle-digit nanomolar range.

Jakopin and co-workers also synthesized cyclopentyl derivatives which served a dual role. In addition to increasing the lipophilicity, analogs with cyclopentyl esters have been reported to be selectively cleaved by human carboxylesterase-1 enzyme. The cyclopentyl congener of **206** *i.e*., compound **249** (Fig. 22A) showed a two-fold improvement over **206**. Among the *O*-acyl derivatives of the cinnamoyl moiety, the acetyl derivative **250** showed the maximum NOD2 activity, the lauroyl derivative (**251**)retained the activity and the activity was diminished by extending the lipophilic tail to C18 (**252**). Hence NOD2 activation by these compounds is most likely linked directly to their lipophilicity.The analog with lipophilic adamantane (**253**) showed maximum NOD2 activity in this series, whereas the compound **255** with phenyl ring substituted at the R_1 position of cinnamoyl moietyshowed moderate activity. Furthermore, the authors tested the effect of cyclopropanation of the double bond of cinnamoyl moiety (**256-260**,Fig. 22B). However, this approach provided only marginal improvement in the NOD2 activity. It was also observed that the reduction of the double bond (**259**)decreased NOD2 agonistic activity by a factorof 70. Similarly, the NOD2 agonistic activity was reduced bya factor of 156 with the spacer prolonged to a propylene

group (**260**) which indicated that the earlier identified lead **206** provides the optimal positioning of the aromatic ring.

at the R_1 and R_2 position along with modifications at the second amino acid.

Hydrolysis of the diethyl ester groups of **206**to free carboxyl groups resulted in compound **261**, with significantly diminished NOD2 activity. Similarly, when the diethyl ester groups were replaced by bulkier *t*-butyl ester (**262**,Fig. 23A), and lauroyl ester (**263**) resulted in markedly reduced NOD2activation, may be because these bulky ester functionalities are considerably less hydrolyzable by human carboxylesterase-1.In addition to this, when functionalized carboxamates and amides (**264-273**) were evaluated as potential bioisosteric replacements of the carboxylic acid functionality, the compounds showed pronounced loss in NOD2 agonistic activity. It has been speculated that both the carboxylate groups of D-Glu formimportant interactions with the receptor. Thus, replacement with the rigidified moieties (**274-279**,Fig. 23B) completely abolished the NOD2 activity.

3.9. MannosylatedDesmuramylpeptides

Ribic*et al.* synthesized two series of mannosylateddesmuramylpeptides wherein mannose was coupled to dipeptides containing lipophilic adamantane on N- orC-terminus through a glycolyl or hydroxyisobutyryl linker. The adjuvant activities of synthesized compounds were investigated in themouse model using ovalbumin as an antigen. Their activities were compared to peptidoglycan monomer (PGM, **280**,Fig. 24). In the *in vivo* experiments, analogswith a glycolyl linker showed higher adjuvantactivity than the analogswith a hydroxyisobutyryl linker indicatingthat the introduction of the glycolyl moiety plays a significantrole in the stimulation of the immune response. In particular,compound **286** was identified, as the most potent adjuvantin this class of mannosylateddesmuramylpeptides. A significant boost in activity was observed in all groups containing glycolyl linker (**284-286**)

than with the analogs having a hydroxyisobutyryl linker (**281**-**283**), implying the importance of glycolyl linker. Compound **286** led to the highest increase in activity with mannosylateddesmuramylpeptides containing a glycolyl linker. In both series, the introduction of the bulky and lipophilic adamantane did not affect activity. Also, it was interesting to observe that the adjuvant activity changes with respect to the position of adamantane moiety. The stimulation was higher in the groups containing the adamant-1-yl moiety at the *N*-terminus (**283** and **286**) compared to the analogs containing adamant-1-yl moiety at C- terminus (**282** and **285**).[68,69]

3.10. Peptidoglycan fragment library containing two types of glycan sequence

Wang *et al*. synthesized a peptidoglycan fragment library containing two types of glycan sequences: Mur-NAc-Glc-NAc (MG) and Glc-NAc-Mur-NAc (GM). Among both the sequences, the disaccharides withdipeptides, **287** and **294**, showed potent activities comparableto the activation of the monosaccharide dipeptide, MDP. In both the glycan sequences, the tetrasaccharide compounds with dipeptide (L-Ala-D-isoGln)**290** (Fig. 25) and **295** exhibited stronger activities than the same glycan sequence groups with longer peptide chains. Among the dipeptide-containing fragments, MGMG2 **290** showed stronger activity than GMGM2 **295**. MGMG2 **290** had an approximately ten-fold higher activity than GMGM2 **295**; MGMG3 **291** had an approximately 65-fold higher activity than GMGM3 **296**; and MGMG4 **292** had an approximately 26-fold higher activity than GMGM4 **297**.[70–72]

3.11. MDP-antigen conjugates

Based on the immunostimulatory properties of lipophilic MDP analogs, Williams *et al.* synthesized MDP-antigen conjugates. The NOD2 activating capacity of these conjugates was then determined by NOD2 transfected HEK293 cells. The antigen $DEVA_5K$ and the Pam_3Cys-antigen conjugate were used as controls. The compound **298** (Fig. 26)showed a substantialamount of IL-8 production, confirming that the β-azidopropanolmodification on

the anomeric position is allowed. Among the conjugates **299**–**302**,the conjugates **299** and **300** showed diminished NOD2 activity, whereas conjugates **301** and **302** induced IL-8 production at a level comparable to **299**. The difference in the activity of conjugates **299** and**300** compared to **301** and **302** indicates that the attachment point of theMDP to the antigenic peptide is important.[73,72]

3.12. MDP-conjugates with biomolecules and small molecule drugs

Various conjugates of MDP analogs with biomolecules and other small-molecule drugs have been synthesized. The conjugation of MDP-L-Ala with cholesterol resulted in a lipophilic derivative (**303**,Fig. 27).[75,76] It was observed that when **303** was incorporated into DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine):PS (Polystyrene) (7:3 molar ratio) liposomes, it induced cytotoxic activity of mouseperitoneal macrophages against P815 mastocytoma cells whereas treatment with free MDP at the concentration of50 μg/mL had no effect. Compound **303** was 7400-fold more active than free MDP. Dzierzbicka*et al*.conjugated MDP analogs with small molecule drugs like acridine and acridone derivatives (**304**-**310**).[77]Among these, compound **304** stimulated the cytotoxic activity of NK cells, with compounds **305**-**308** induced cytotoxicactivity against several human cell lines. Moreover, compounds **305**, **307**, and **308** were also active *in vivo* in ahollow fiber assay, and compound **293** showed *in vivo* activity against melanoma in mice. Also, several nor-MDPs were conjugated to a heterocyclic aryl amine batracylin (**309** and **310**). These compounds inhibited theproliferation of melanoma cells.[78] Furthermore, DMPs were also coupled to3′-amino, 2′-hydroxyl, or 7-hydroxyl group of PTX forming 3′-N-MTC-01,2′-*O*-MTC-01, and 7-*O*-MTC-01. Among them, compound 2′-*O*-MTC-01 **311** showed the mostpotent antitumor activity *in vitro*. Further experiments demonstrated the ability of compound **311** to induce expression and production of TNF-α and IL-12.[79] To obtain analogs of MDP with antitumor and antimetastatic activities, compound **311** was further modified by replacing the muramic acid

moiety by various aromatic groups, leading to MTC-220 (**312**).[80] Compound **312** inhibited growth of various tumor cell lines but it was speculated that **312** might also act *via* TLR4 as it contains the PTX motif, which has been shown to bind to TLR4 receptors.

4. Conclusion

To date, a large number of MDP derivatives have been synthesized, among which several compounds showed promising activity when compared to MDP. It was observed that L-configuration of the first amino acid *i.e.*,L-alanine and D-configuration of the glutamic acid residue of second amino acid (D-*iso*glutamine) are essential for NOD2 activity. The modification at the C2 position of carbohydrate moiety also play an important role in NOD2 activity. Compounds devoid of *N*-acetylation were inactive or drastically lost the NOD2 activity. Compounds with NH_2group at the C2 position carbohydrate moiety showed potent activity, although its salt form showed no activity. Also, the replacement of *N*-acetyl group with *N*-glycolyl improved activity. Analogs in which the OH group at the C1 position of carbohydrate moiety was substituted by the OMe group had higher activity than *N*-acetyl MDP. Compounds with the *O*-benzyl moiety at the C1 axial position lost activity suggesting that the orientation and substituent at the C1 position play an important role in NOD2 activity. Compounds bearing successively longer alkyl chains (C5–C11) at the equatorial C1 position, showed similar activity to *N*-glycolyl MDP. Dramatic loss in the activity was observed for the compounds with substituted triazole moiety at the C4 position. Substituting a methyl ester for the amide of the D-isoGln residue generated Glu-MDP(D-Glu)-OCH_3, which had greater activity than MDP. When the first amino acid was replaced by glycine, L-threonine, L-serine, L-proline, L-cyclohexylalanine, (*S*)-adamantyl glycine, L-homophenylalanine, and L-pyridylalanine, activity was reduced. However, substitution with valine and phenylalanine retained activity. Substituted cinnamic acid moiety with 4-OH and 3-OMe groups could replace *N*-acetyl muramic acid in desmuramylpeptides without loss of

activity. Introduction of two ethyl esters, a bulkier cyclopentyl ester on carboxylic acid of D-glutamic acid of desmuramylpeptide improved activity. However, two bulkier stearyl (C18) groups led to diminished activity. Similar effects on activity were obtained when phenolic hydroxyl of ferulic acid amide moiety was functionalized with the same groups through esterification. However, attachment of the lipophilic adamantane group to the cinnamoyl moiety greatly improved the activity.The literature clearly highlights many critical features of different substituents or derivatives of MDP which contribute to their ability to activate NOD2, which may hopefully assist in the design of further modifications to improve NOD2 agonist activity. However, acomplete understanding of how these modifications affect NOD2 activity awaits the determination of their actual binding mode to the NOD2 structure, either through the determination of a crystal structure of a NOD2-MDP complex or through more detailed *in silico* molecular modelling.

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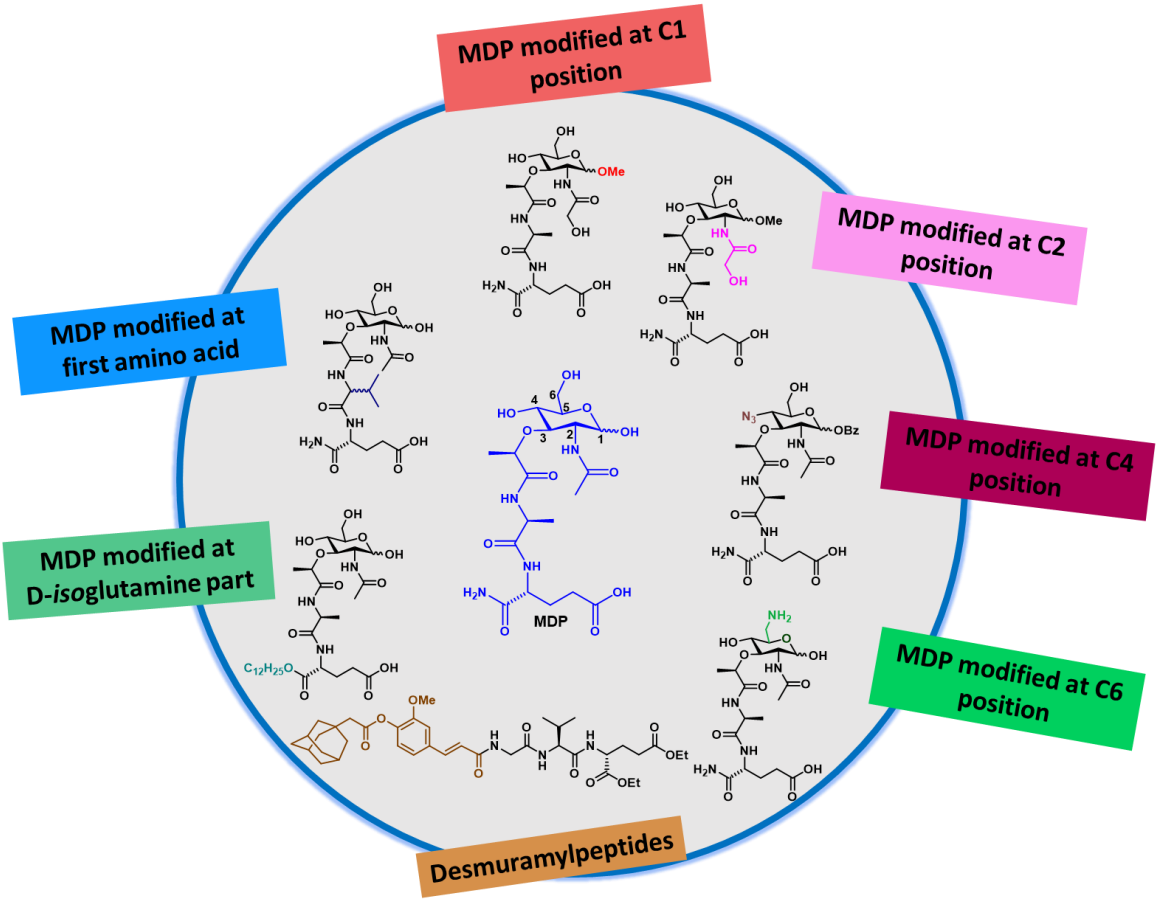


TOC graphic

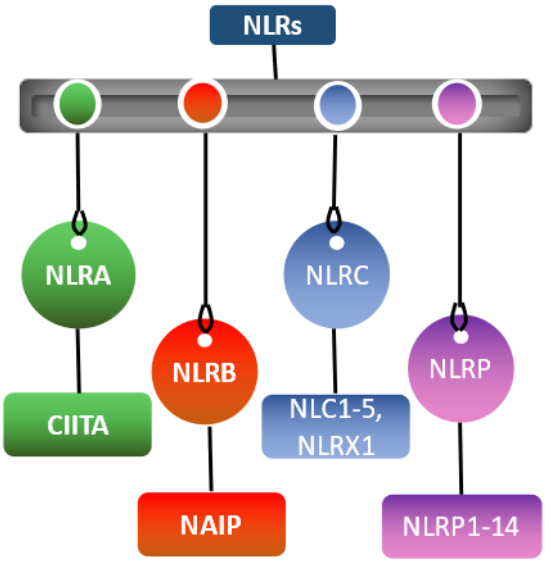


Fig. 1. Types of NLRs.

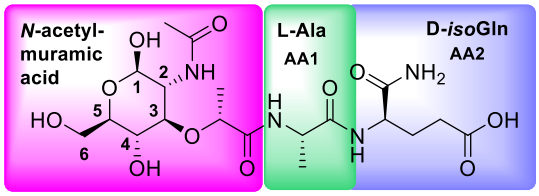


Fig. 2. Muramyl dipeptide (MDP) structure

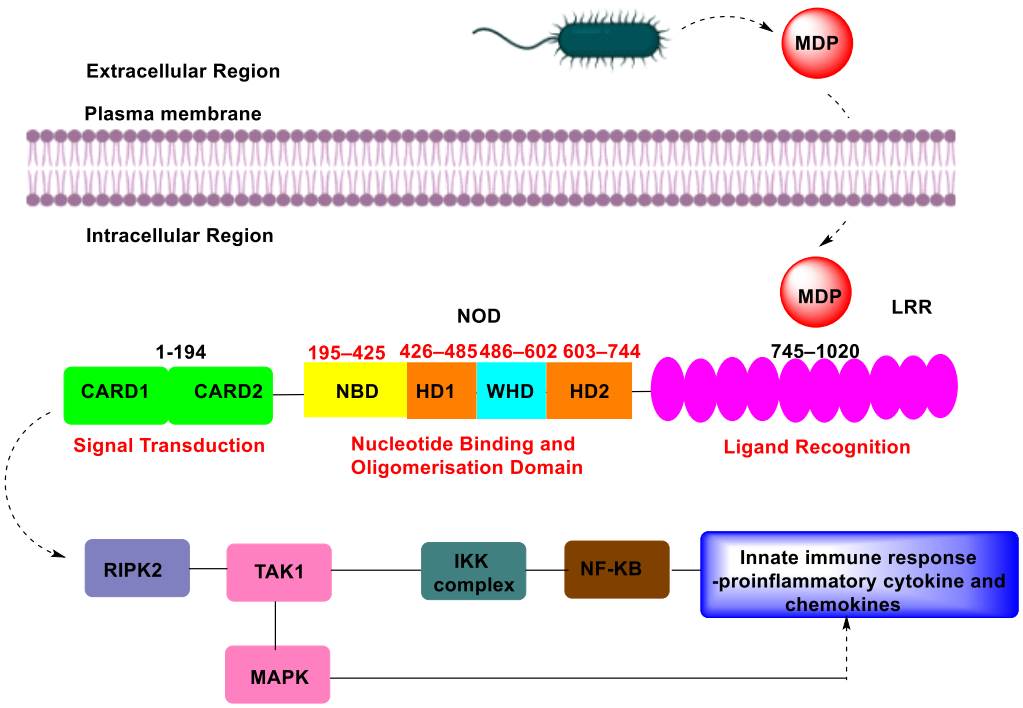


Fig. 3. Structure and signalling pathway of NOD2 activation.

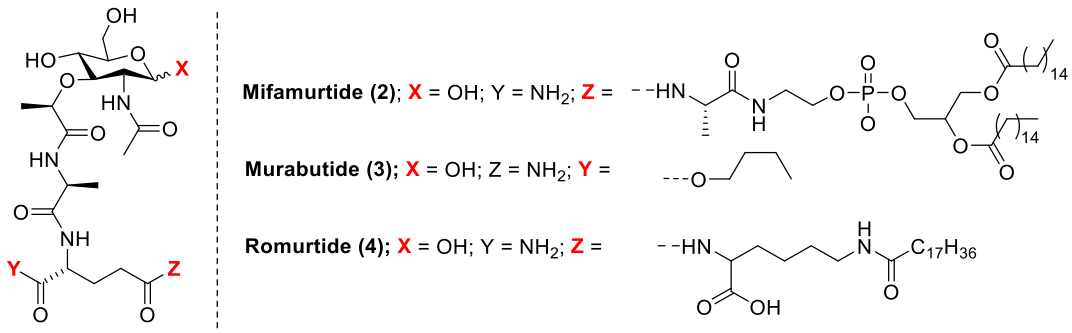
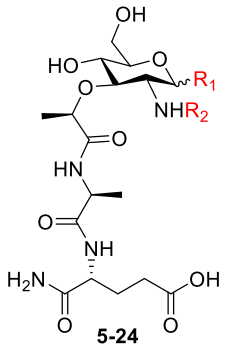
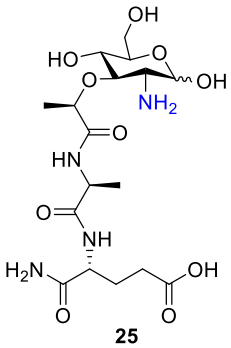


Fig. 4. Synthetic derivatives of MDP in clinical use.



5-24



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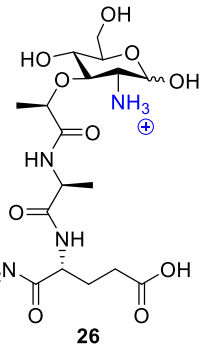


26

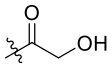
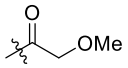
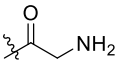
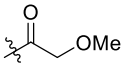
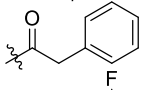
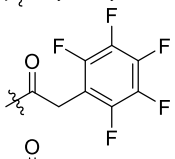
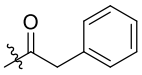
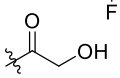
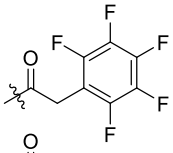
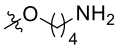
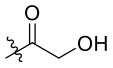
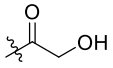
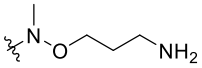
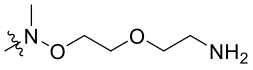
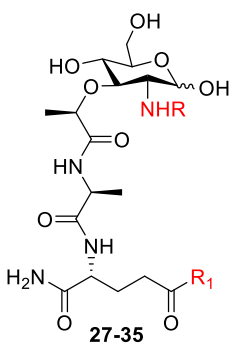
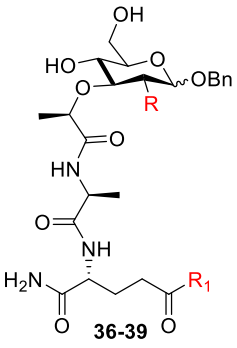
Compound	R_1	R_2	Compound	R_1	R_2
5	OH		**15**	OMe	
6	OH		**16**	OMe	
7	OH		**17**	OMe	
8	OH		**18**	OMe	
9	OH		**19**	OMe	
10	OH				
11	OH		**20**		
12	OH		**21**		
			22		
13	OMe		**23**		
14	OMe	NH_2	**24**		

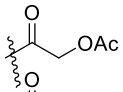
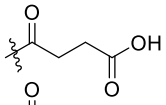
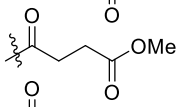
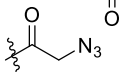
Fig. 5. Analogs of MDP having modifications at the C2 and C1 positions.

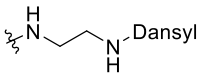
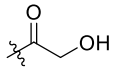
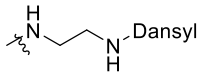


27-35



36-39

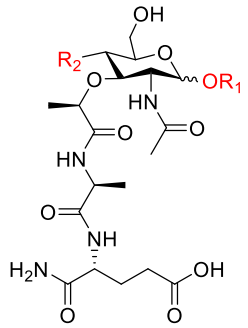
Compound	R	R_1
27		OH
28		OH
29		OH
30		OH
31		OH
32	Dansyl	OH
33	Biotin	OH

Compound	R	R_1
34		
35		
36	N_3	OH
37	N_3	OBn
38	N_3	OC_8H_{17}
39	N_3	$OC_{14}H_{29}$

Compounds (**27-35**) were evaluated at 20 μM concentration

HEK NOD-2 cells were incubated with 20 μg of test compound (**36-40**).

Fig. 6. C2 modified MDP analogs by Melnyk and Reddy et al.



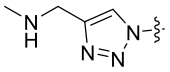
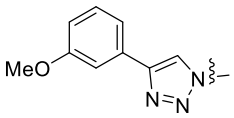
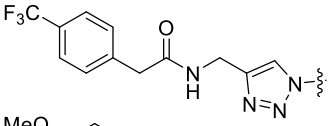
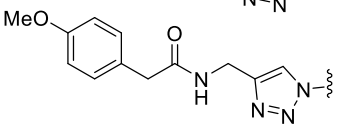
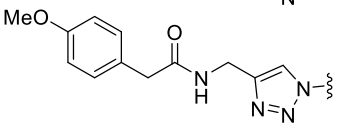
Compound	R_1	R_2
40	Bn	N_3
41	Bn	NH_2
42	H	
43	H	
44	H	
45	H	
46	Bn	

Fig. 7. Modifications at the C4 position of the carbohydrate moiety.

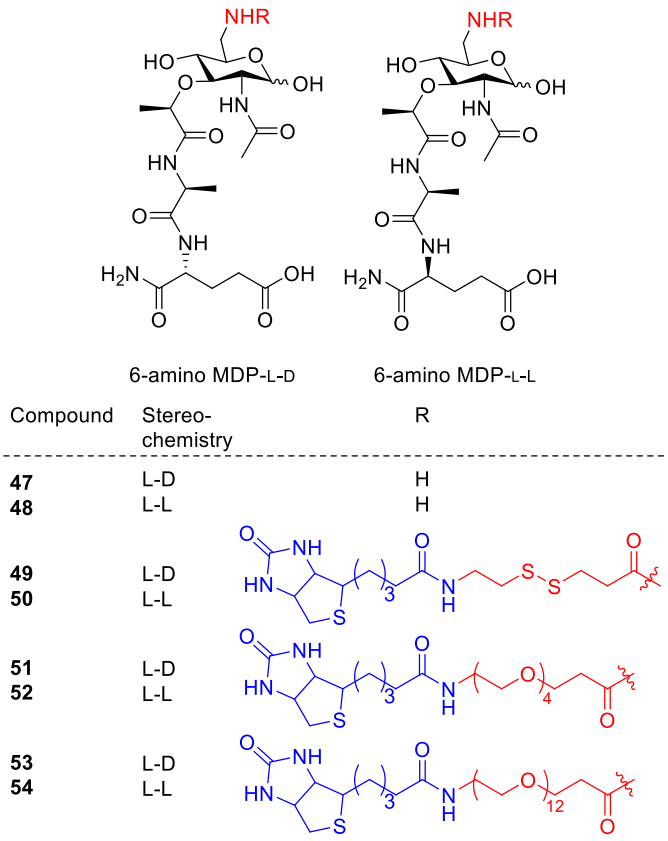
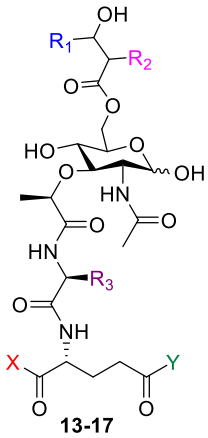
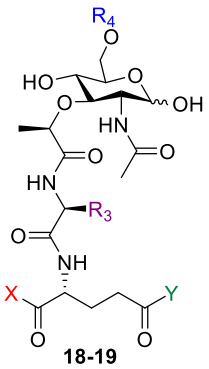


Fig. 8. NOD2 agonists having modifications at the C6 position of MDP.



13-17



18-19

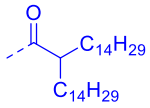
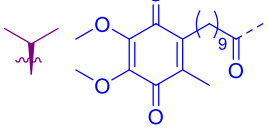
No.	Compound	R_1	R_2	R_3	R_4	X	Y
55	6-*O*-mycoloyl-MDP	C_{43-57}	C_{24}	CH_3	-	NH_2	OH
56	6-*O*-nocardomycololyl-MDP	C_{31-43}	C_{10-14}	CH_3	-	NH_2	OH
57	6-*O*-corynomycoloyl-MDP	C_{11-15}	C_{10-14}	CH_3	-	NH_2	OH
58	6-*O*-mycoloyl-*N*-acetyl muramyl-Gly-D-*iso*Gln	C_{43-57}	C_{24}	H	-	NH_2	OH
59	6-*O*-mycoloyl-*N*-acetyl muramyl-L-Ser-D-isoGln	C_{43-57}	C_{24}		-	NH_2	OH
60	B-30 MDP	-	-	CH_3		NH_2	OH
61	Quinonyl MDP-66	-	-			OCH_3	NH_2

Fig. 9. MDP analogs having modifications at the C6 position.

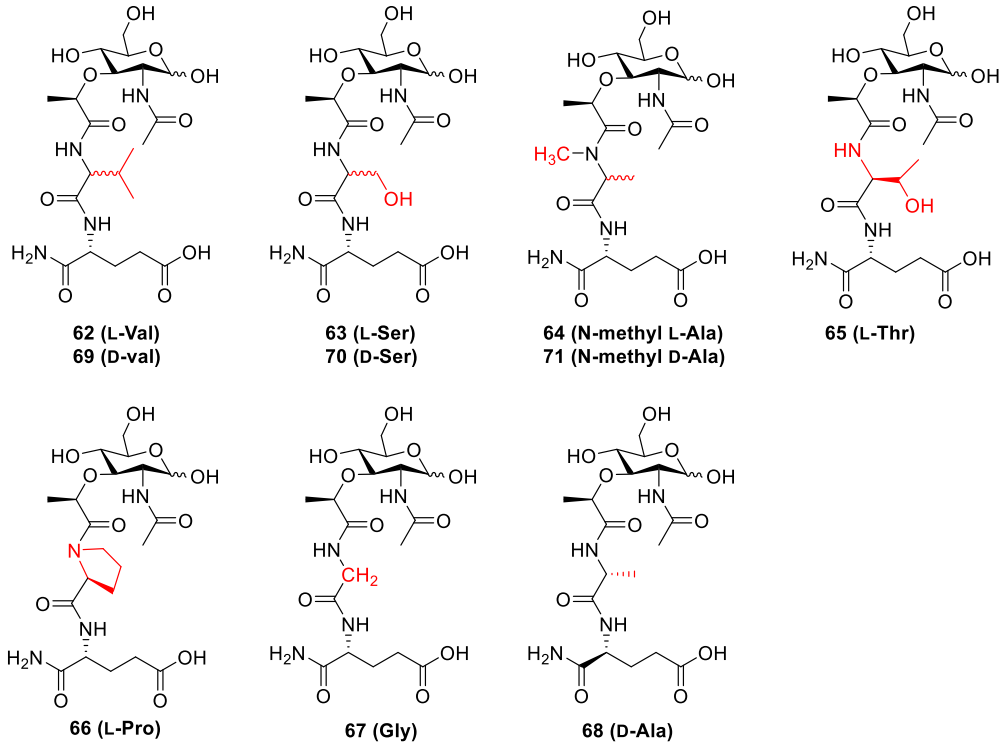
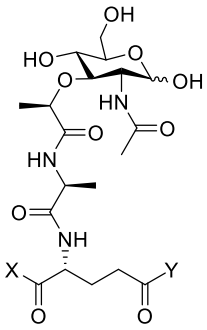
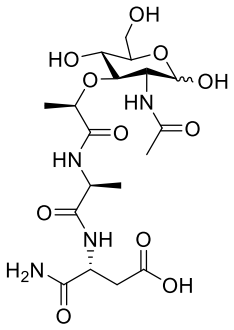


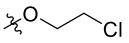
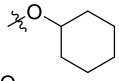
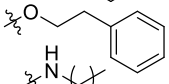
Fig. 10. Modifications at the first amino acid.



73-95



101

Compound	X	Y
72	OCH_3	OH
73	OEt	OH
74	OC_4H_9	OH
75	OC_8H_{17}	OH
76	$OC_{12}H_{25}$	OH
77	$OC_{16}H_{33}$	OH
78	$OC_{20}H_{41}$	OH
79	NH_2	OCH_3
80	NH_2	OEt
81	OCH_3	OCH_3
82	OEt	OEt
83	OH	OH
84	NH_2	NH_2
85	OCH_3	NH_2
86		OH
87		OH
88		OH
89		OH
90		OH

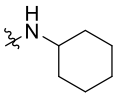
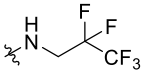
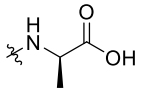
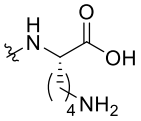
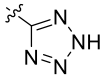
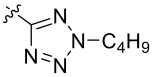
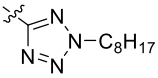
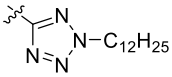
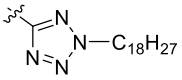
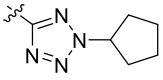
Compound	X	Y
91		OH
92		OH
93	NH_2	
94	NH_2	
95		OH
96		OH
97		OH
98		OH
99		OH
100		OH

Fig. 11. MDP analogs having modification at the D-iso-glutamine residue (second amino acid) of the peptide part.

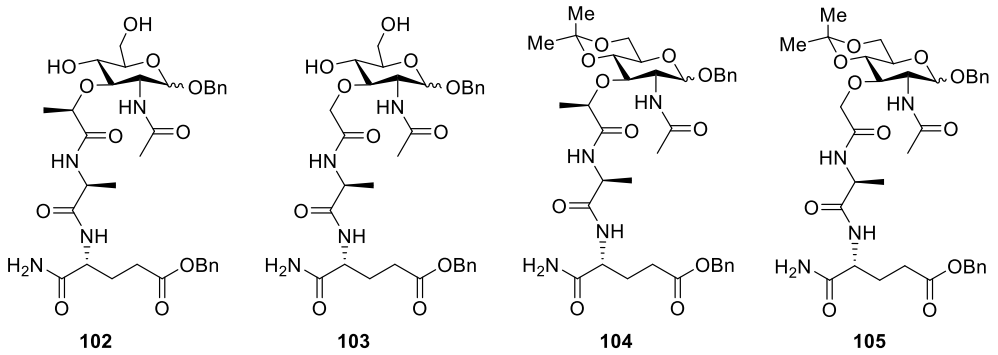


Fig. 12. Modification at dipeptide and carbohydrate moiety.

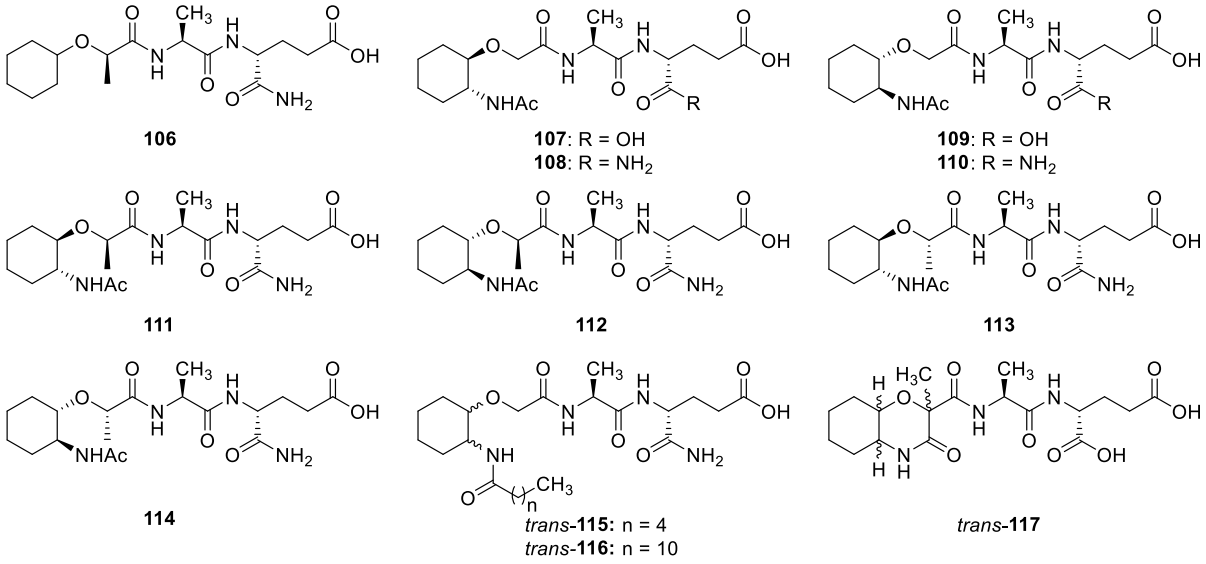


Fig. 13. Carbocyclic analogs of MDP.

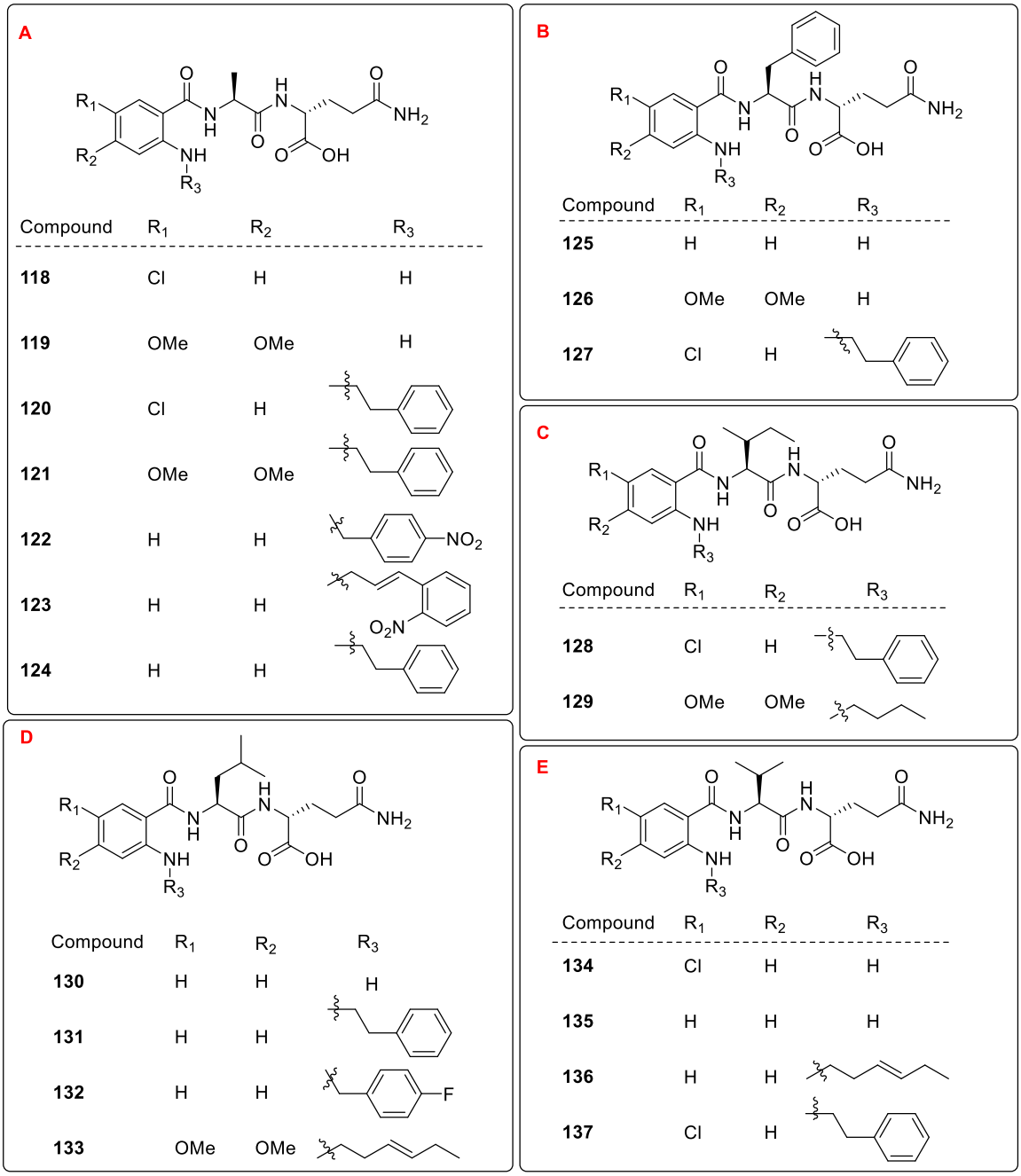
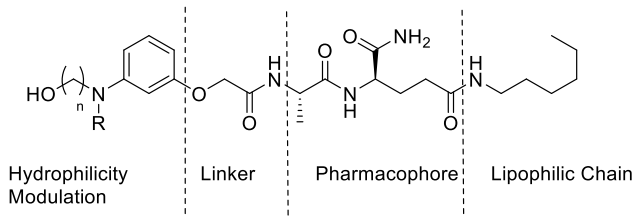
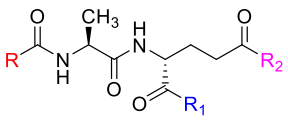


Fig. 14. Aromatic ring containing desmuramylpeptides.



R	H	$(CH_2)_nOH$	CH_3CHO	$CH_3(CH_2)_2CO$
n=2	**138**	**139**	**140**	**141**
n=3	**142**	**143**	**144**	**145**

Fig. 15. Desmuramylpeptides having hydrophilic arene as a substitute for carbohydrate moiety.



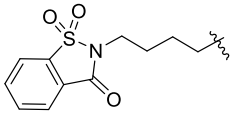
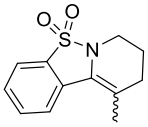
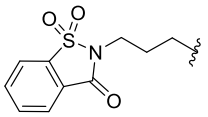
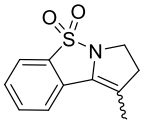
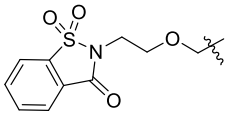
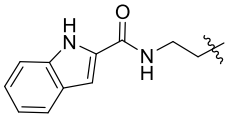
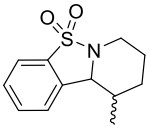
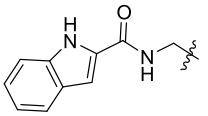
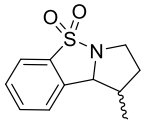
Compound	R	R_1	R_2	Compound	R	R_1	R_2
146		NH_2	OH	**166**		NH_2	OH
147		OH	OH	**167**		OH	OH
148		NH_2	OCH_2Ph	**168**		NH_2	OCH_2Ph
149		OCH_2Ph	OCH_2Ph	**169**		NH_2	OEt
				170		OCH_2Ph	OEt
150		NH_2	OH	**171**		OEt	OCH_2Ph
151		OH	OH				
152		NH_2	OCH_2Ph				
153		OCH_2Ph	OCH_2Ph	**172**		NH_2	OH
				173		OH	OH
154		NH_2	OH	**174**		NH_2	OCH_2Ph
155		OH	OH	**175**		OCH_2Ph	OCH_2Ph
156		NH_2	OCH_2Ph	**176**		OEt	OEt
157		OCH_2Ph	OCH_2Ph	**177**		NH_2	OEt
158		NH_2	OH				
159		OH	OH	**178**		NH_2	OH
160		NH_2	OCH_2Ph	**179**		OH	OH
161		OCH_2Ph	OCH_2Ph				
162		NH_2	OH				
163		OH	OH	**180**		NH_2	OH
164		NH_2	OCH_2Ph	**181**		OH	OH
165		OCH_2Ph	OCH_2Ph				

Fig. 16. Saccharin and indole heterocycles containing desmuramylpeptides.

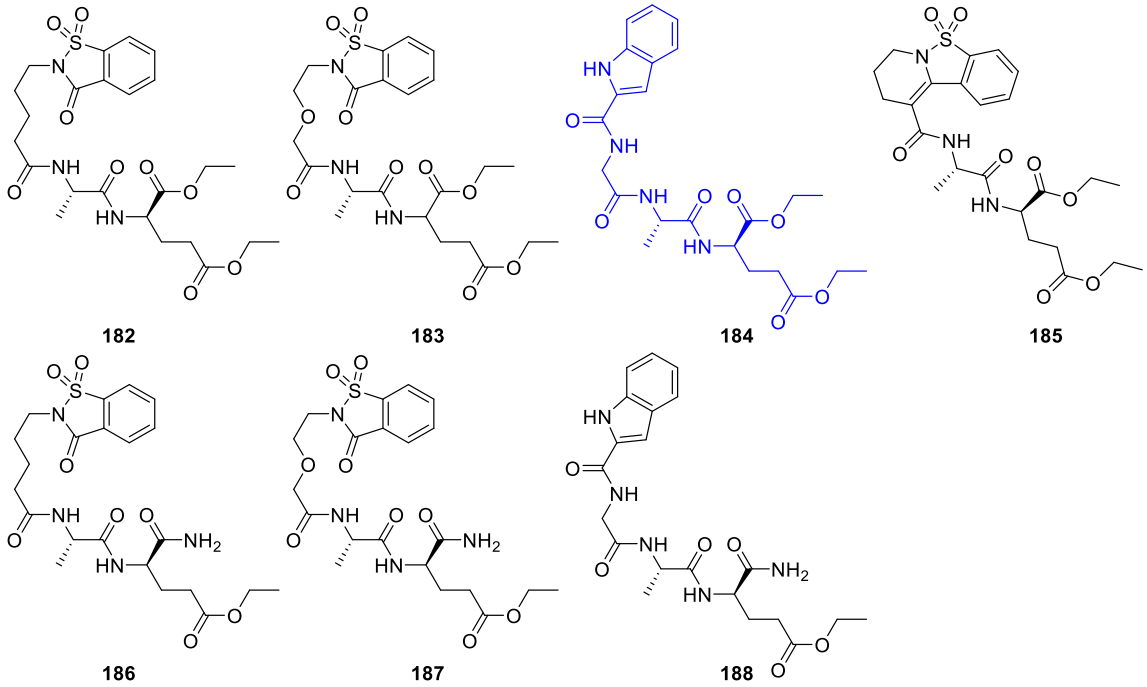
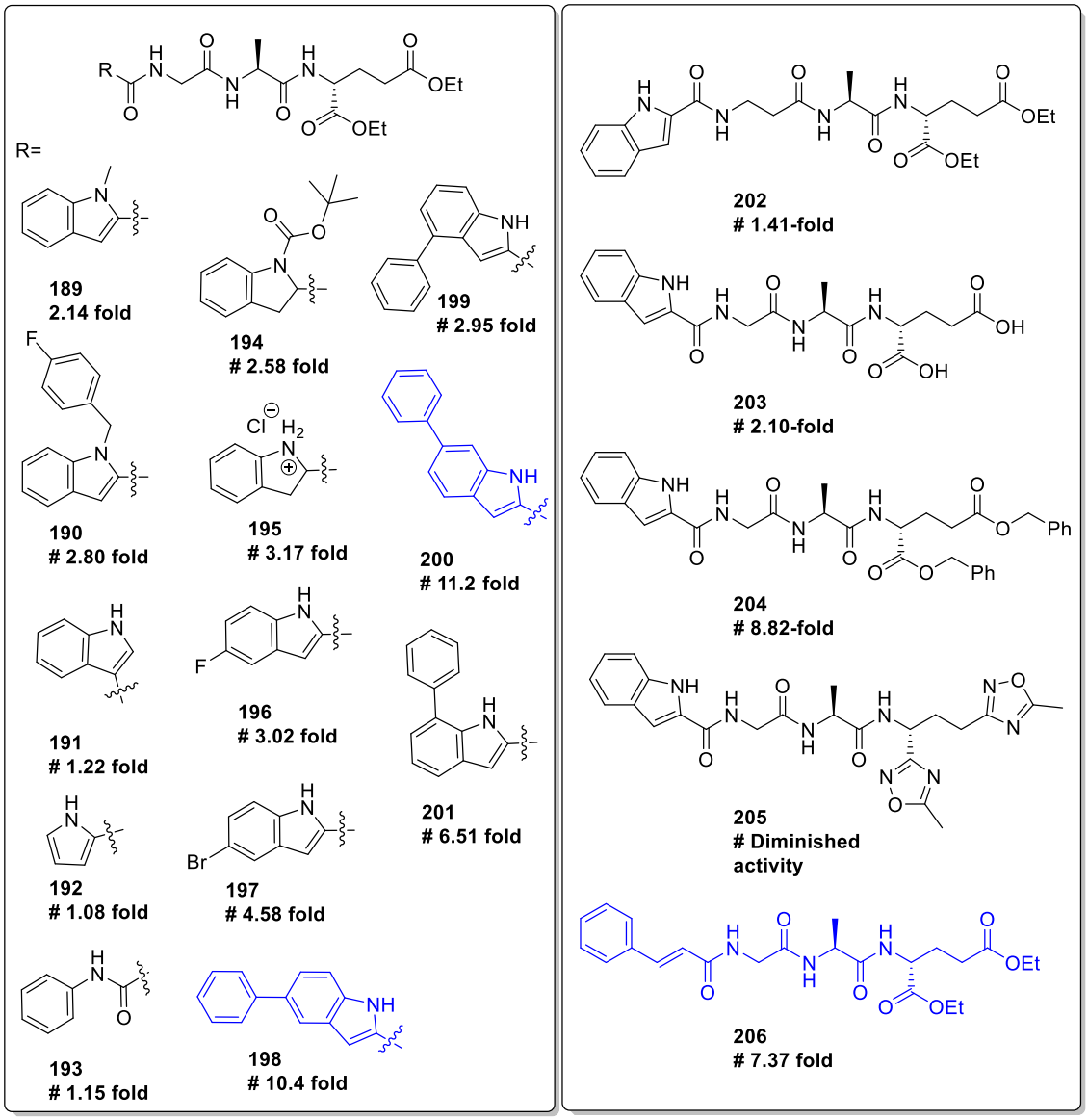


Fig. 17. Novel desmuramylpeptides with ester functionalities.



refers to fold-increase in NOD2 activity at 20μM (fold increase) with respect to untreated cells and reference compound MDP (**1**) and **177 MDP #** 11 fold, **177 #** 5 fold

Fig. 18. Desmuramylpeptides and their NOD2 agonistic activity.

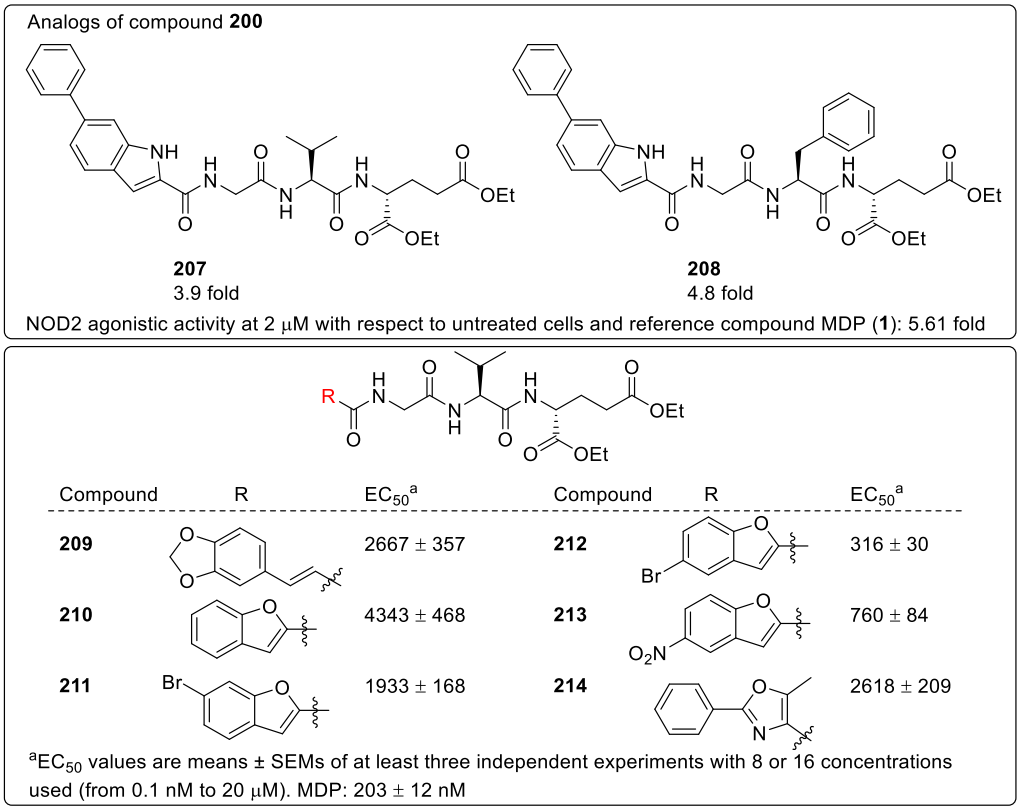


Fig. 19. Desmuramylpeptides having modifications at the C3 and C4 position along with modifications at first amino acid.

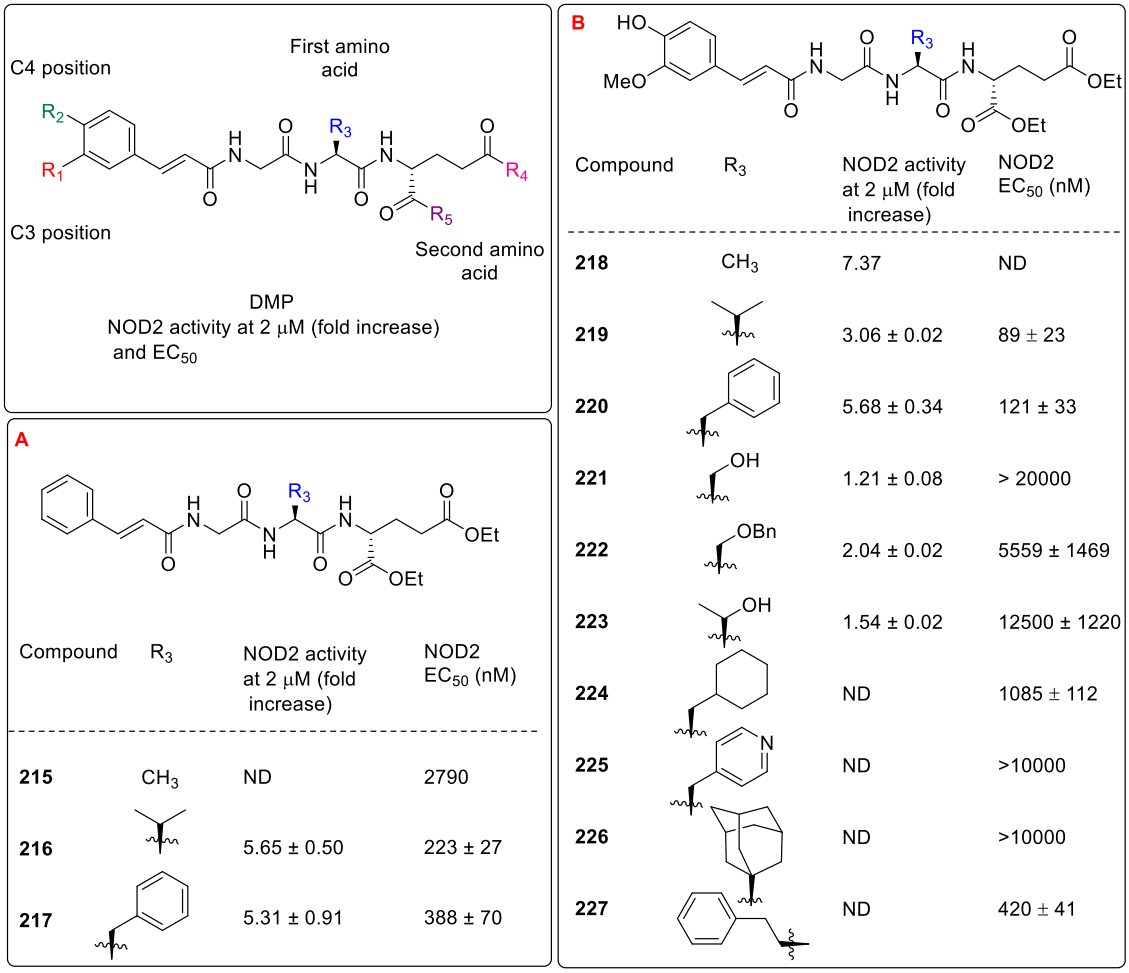
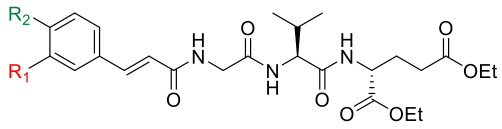
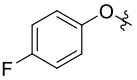
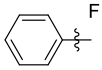
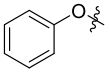
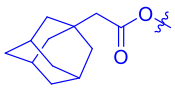


Fig. 20. Desmuramylpeptides having cinnamoyl moiety and variation of first amino acid.



Compound	R_1	R_2	NOD2 activity at 2 μM (fold increase)	NOD2 EC_{50} (nM)
228	OH	OH	2.67 ± 0.19	ND
229	OMe	OMe	2.64 0.01	763 ± 88
230	F	F	5.60 ± 0.35	ND
231	H	NO_2	3.02 ± 0.07	49 ± 4
232	H		2.83 ± 0.06	71 ± 4
233	H	Cl	ND	412 ± 65
234	H	Br	ND	534 ± 39
235	H	NH_2	2.45 ± 0.18	542 ± 48
236	H	CH_3	ND	402 ± 89
237	H	CF_3	ND	1192 ± 261
238	H		ND	795 ± 126

Compound	R_1	R_2	NOD2 activity at 2 μM (fold increase)	NOD2 EC_{50} (nM)
239	H		ND	>10000
240	H		ND	1929 ± 230
241		H	ND	47 ± 10
242		H	ND	61 ± 8
243	NO_2	H	ND	82 ± 14
244	Br	H	ND	135 ± 20
245	OMe	CH_3COO	3.05 ± 0.03	62 ± 27
246	OMe	$C_{11}H_{23}CO$	3.17 ± 0.03	30 ± 5
247	OMe	$C_{17}H_{35}CO$	2.37 ± 0.01	2828 ± 206
248	OMe		ND	4.5 ± 1.1

MDP (**1**): # 2.83 ± 0.16; *148 ± 26

refers to NOD2 activity at 2 μM (fold increase) and * refers to EC_{50} (nM)

Fig. 21. Desmuramylpeptides having C3 and C4 substituted cinnamoyl moiety.

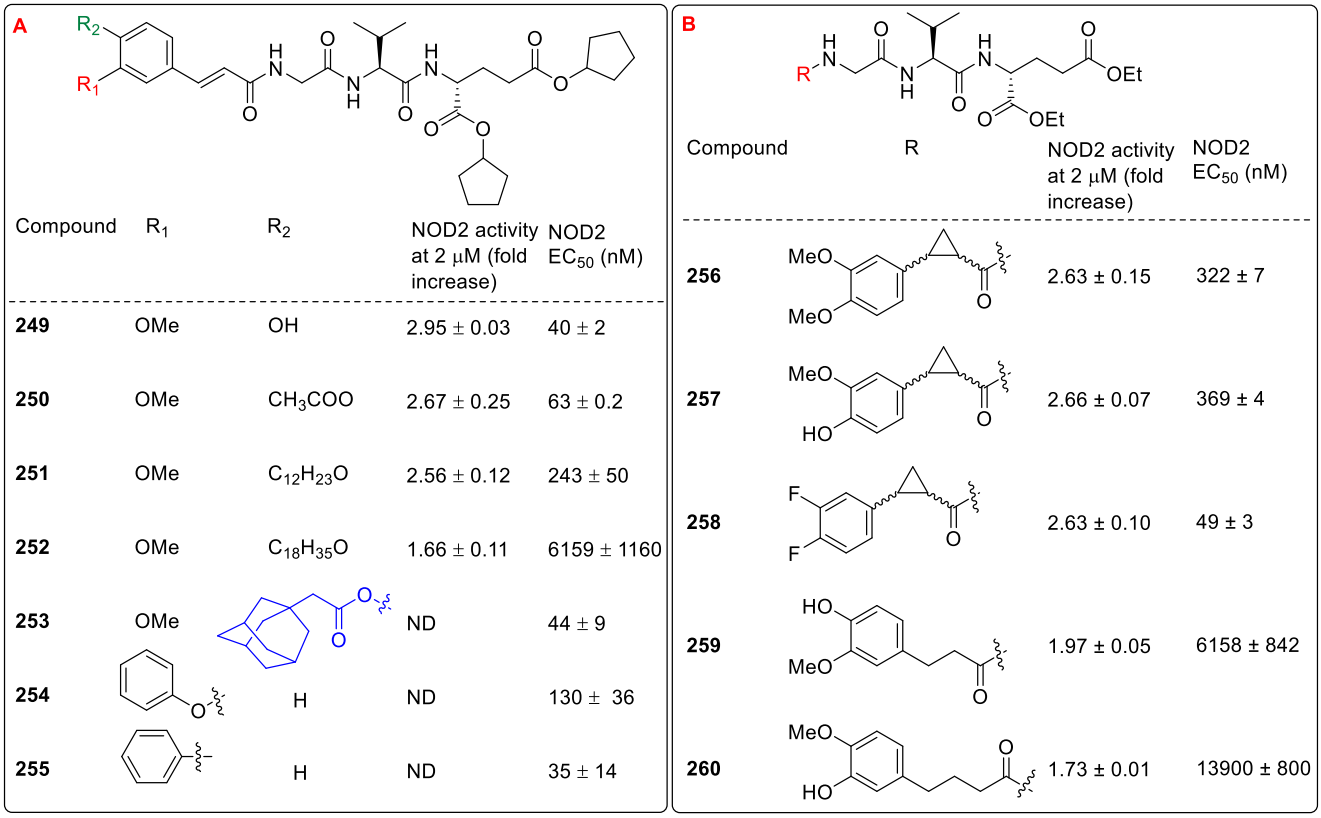


Fig. 22. Desmuramylpeptides having modifications

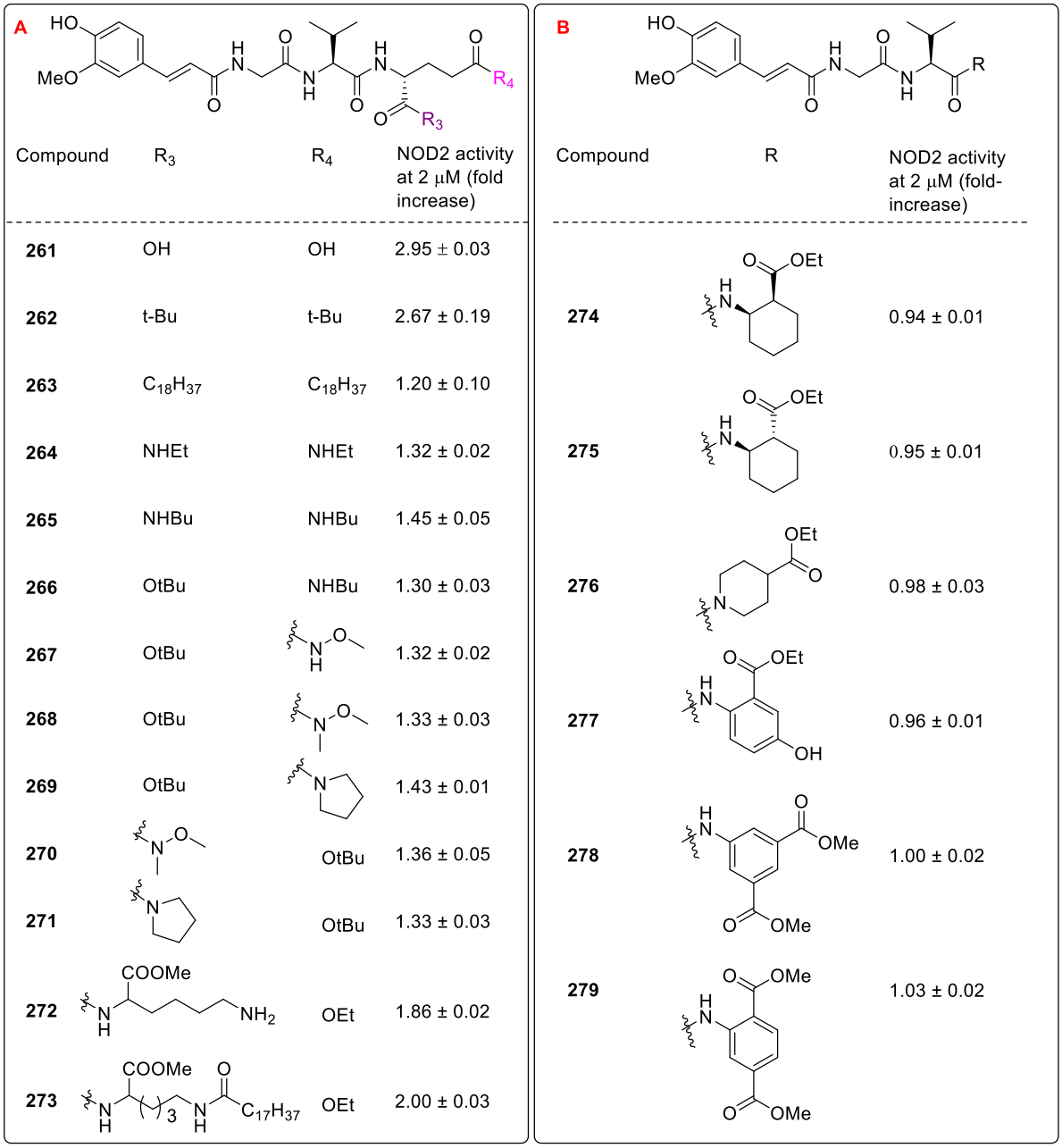
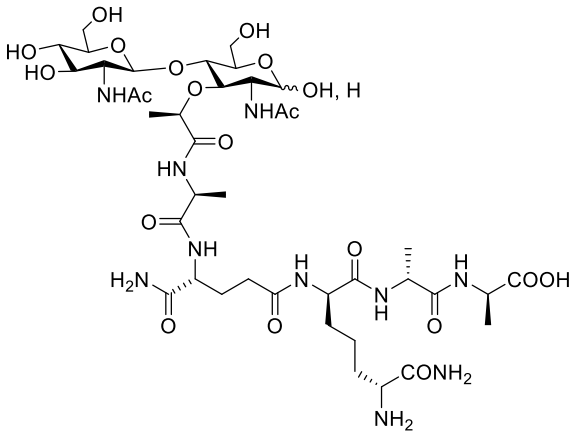
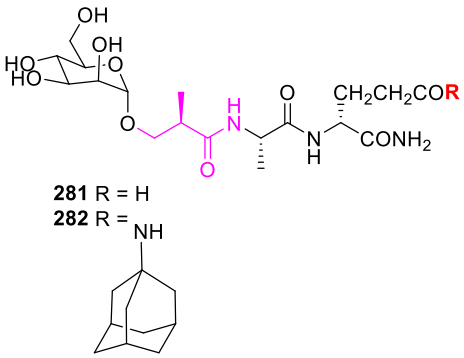


Fig. 23. Desmuramylpeptides having at the second amino acid.

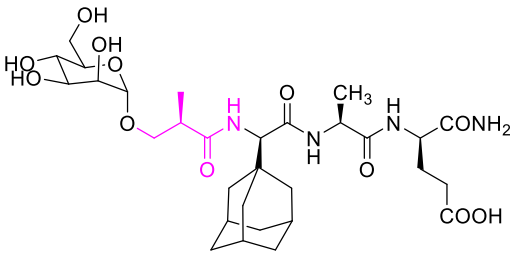


Peptidoglycan monomer (PGM; **280**)

A. Mannosylated peptides with hydoxyisobutyryl linker

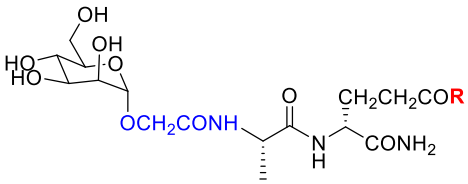


281 R = H
282 R =

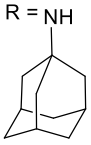


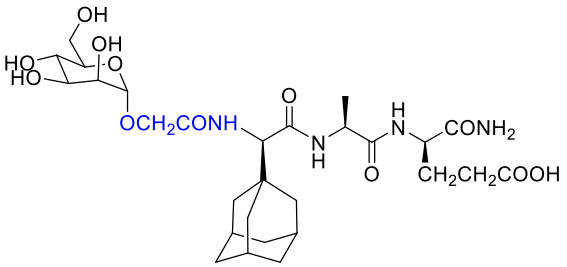
283 ManAdTP

B. Mannosylated peptides with glycolyl linker



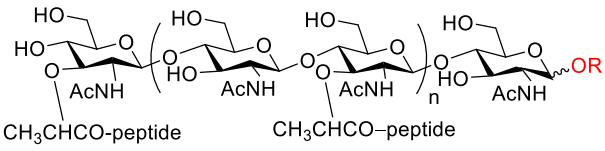
284 R = H
285 R =





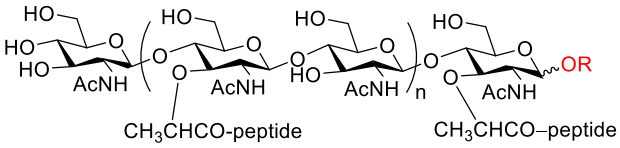
286

Fig. 24. Mannosylated desmuramylpeptides.



287-293

Compound	R	n	peptide
287	H	0	L-Ala-D-isoGln
288	H	0	L-Ala-D-isoGln-L-Lys
289	H	0	L-Ala-D-isoGln-L-Lys-D-Ala
290		1	L-Ala-D-isoGln
291		1	L-Ala-D-isoGln-L-Lys
292		1	L-Ala-D-isoGln-L-Lys-D-Ala
293		1	L-Ala-D-isoGln-L-Lys-D-Ala-D-Ala



294-297

Compound	R	n	peptide
294	H	0	L-Ala-D-isoGln
295		1	L-Ala-D-isoGln
296		1	L-Ala-D-isoGln-L-Lys
297		1	L-Ala-D-isoGln-L-Lys-D-Ala

Fig. 25. Peptidoglycan fragment library containing two types of glycan sequence.

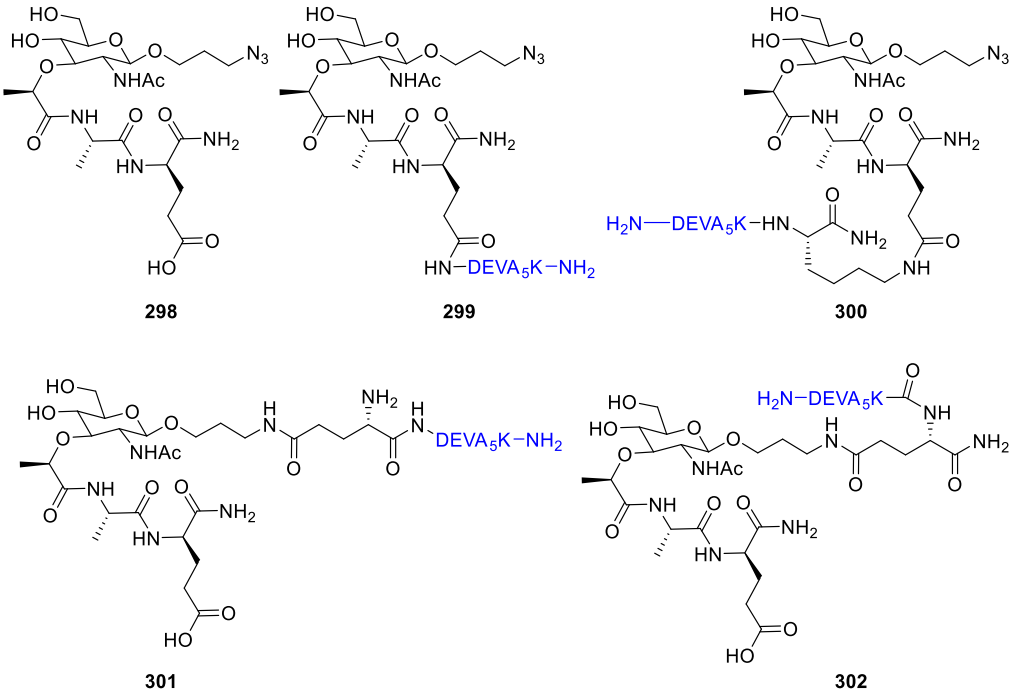


Fig. 26. Muramyl dipeptide antigen conjugates.

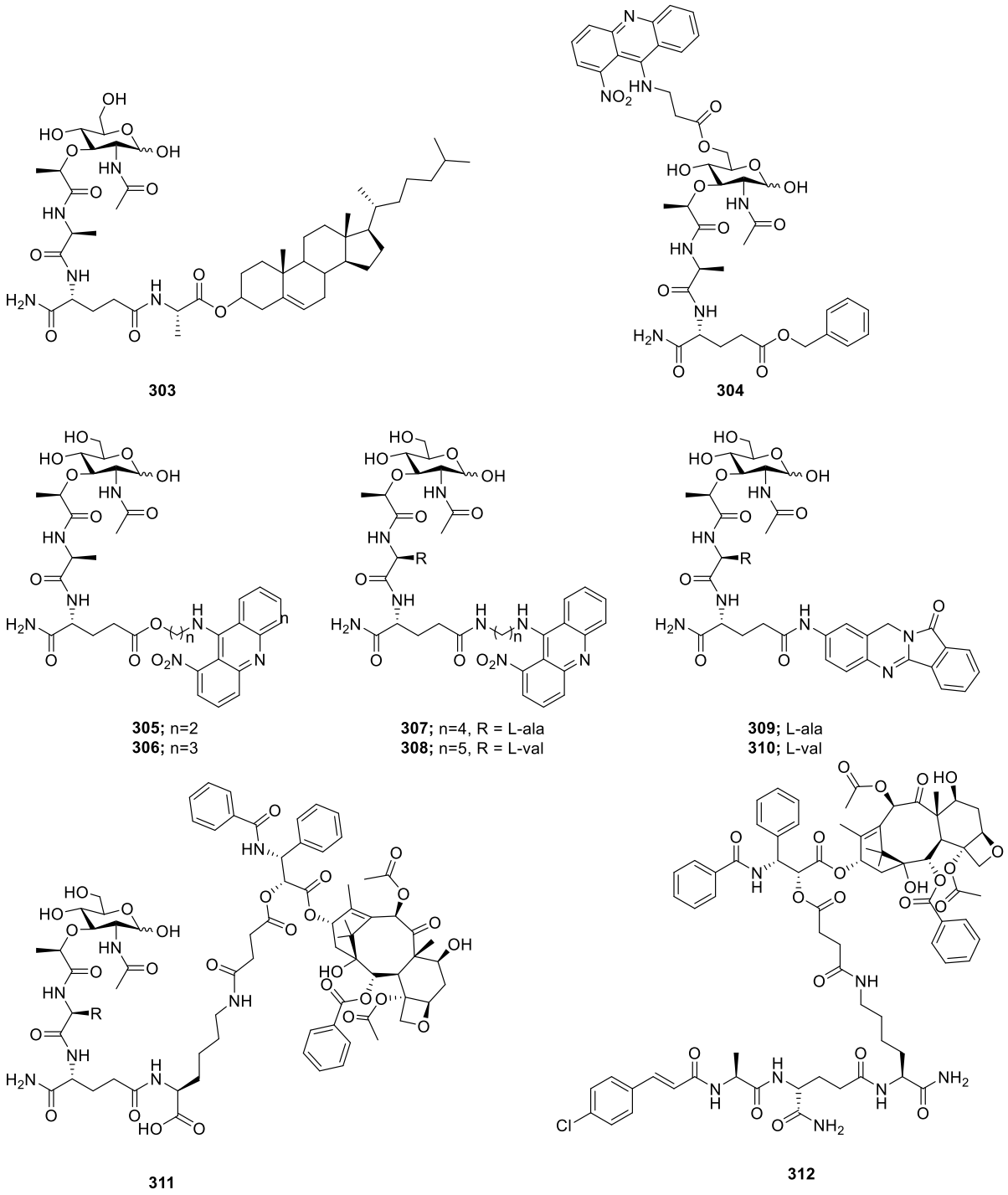


Fig. 27. Muramyl dipeptide conjugates with biomolecules and small molecule drugs.

Figure Legends

Fig. 1. Types of NLRs.

Fig. 2. Muramyl dipeptide (MDP) structure

Fig. 3. Structure and signalling pathway of NOD2 activation.

Fig. 4. Synthetic derivatives of MDP in clinical use.

Fig. 5. Analogs of MDP having modifications at the C2 and C1 positions.

Fig. 6. C2 modified MDP analogs by Melnyk and Reddy et al.

Fig. 7. Modifications at the C4 position of the carbohydrate moiety.

Fig. 8. NOD2 agonists having modifications at the C6 position of MDP.

Fig. 9. MDP analogs having modifications at the C6 position.

Fig. 10. Modifications at the first amino acid.

Fig. 11. MDP analogs having modification at the D-iso-glutamine residue (second amino acid) of the peptide part.

Fig. 12. Modification at dipeptide and carbohydrate moiety.

Fig. 13. Carbocyclic analogs of MDP.

Fig. 14. Aromatic ring containing desmuramylpeptides.

Fig. 15. Desmuramylpeptides having hydrophilic arene as a substitute for carbohydrate moiety.

Fig. 16. Saccharin and indole heterocycles containing desmuramylpeptides.

Fig. 17. Novel desmuramylpeptides with ester functionalities.

Fig. 18. Desmuramylpeptides and their NOD2 agonistic activity.

Fig. 19. Desmuramylpeptides having modifications at the C3 and C4 position along with modifications at first amino acid.

Fig. 20. Desmuramylpeptides having cinnamoyl moiety and variation of first amino acid.

Fig. 21. Desmuramylpeptides having C3 and C4 substituted cinnamoyl moiety.

Fig. 22. Desmuramylpeptides having modifications

Fig. 23. Desmuramylpeptides having at the second amino acid.

Fig. 24. Mannosylateddesmuramylpeptides.

Fig. 25. Peptidoglycan fragment library containing two types of glycan sequence.

Fig. 26. Muramyl dipeptide antigen conjugates.

Fig. 27. Muramyl dipeptide conjugates with biomolecules and small molecule drugs.

Author Biosketches

Aarzoo Kamboj obtained her Master of Science (2019) degree from Multani Mal Modi College, Patiala. She is currently pursuing research for her doctoral degree in Chemistry under the supervision of Dr. Deepak B. Salunke at the Department of Chemistry, Panjab University, Chandigarh. Her research interest involves the design and synthesis of new NOD2 agonists and their conjugates for exploration as vaccine adjuvants.

Dr. Madhuri T. Patil completed her Ph.D. from the CSIR National Chemical Laboratory, Pune, India and also worked as a post-doctoral researcher at the Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, USA. Before taking her current position at the Post Graduate Department of Chemistry, Mehr Chand Mahajan DAV College for Women, Chandigarh as Assistant Professor, Dr. Patil worked at the Dr. D. Y. Patil Institute of Engineering and Technology, Pune. Dr. Patil is also a recipient of the DST Women Scientist Fellowship of Govt. of India. Her research interest is in the field of inositol-based surfactants and heterocyclic chemistry.

Dr. Deepak B. Salunke completed Ph.D. in Chemistry from CSIR-National Chemical Laboratory, Pune and was awarded Indo-French Sandwich Thesis Scholarship during his PhD to work at the ICSN, CNRS, Gif-sur-Yvette, France. He completed his postdoctoral studies at the NCTU Taiwan and University of Kansas, Lawrence USA. Dr. Salunke is also a recipient of Assistant Research Professorship at the Higuchi Biosciences Centre, University of Kansas and a Ramalingaswami Fellowship of DBT India. Dr. Salunke also worked at Chembiotech Ltd., Advinus Therapeutics Pvt. Ltd. and SAI Life Sciences before taking his current position as faculty at the Department of Chemistry, Panjab University, Chandigarh. Dr. Salunke has recently established a National Interdisciplinary Centre of Vaccine, Immunotherapeutics and Antimicrobials at Panjab University and working towards the development of new vaccine adjuvants and immunotherapeutics targeting Pattern Recognition Receptors.

Professor Nikolai Petrovsky is a physician and vaccine researcher. He is the founder of Vaxine, an Australian biotechnology company applying novel approaches including artificial intelligence to vaccine and adjuvant development. His research has been awarded multi-million dollar contracts from the US National Institutes of Health and he has authored over 200 peer-reviewed research papers. He developed SpikoGen®, a vaccine against COVID-19 that received emergency use authorization in the Middle East in October 2021, making it the first recombinant protein vaccine against COVID-19 to achieve regulatory approval.