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GPR17 as a Macrophage Sensor for Gram-Positive Peptidoglycan: Implications for Invasive Bacterial Infection

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Research Article

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GPR17 as a Macrophage Sensor for Gram-Positive Peptidoglycan: Implications for Invasive Bacterial Infection

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ABSTRACT

Invasive gram-positive infections, including necrotizing fasciitis, streptococcal toxic shock syndrome, and staphylococcal sepsis, disproportionately strike otherwise healthy individuals, implying a dominant role for host genetic susceptibility in innate bacterial surveillance. Here we present transcriptomic, structural pharmacophore, and molecular docking evidence supporting GPR17, a G protein-coupled receptor phylogenetically intermediate between purinergic P2Y receptors and cysteinyl leukotriene receptors, as a candidate surface-expressed pattern recognition receptor for gram-positive peptidoglycan on tissue macrophages. Reanalysis of Human Protein Atlas single-cell RNA-seq data reveals that GPR17 is expressed predominantly on tissue macrophages (31.7 nTPM) and monocytes (12 to 18 nTPM), with markedly lower expression in lymphoid lineages (<2 nTPM). AutoDock Vina docking of 15 compounds against the AlphaFold-predicted GPR17 structure identifies UDP-muramic acid (UDP-Mur), a peptidoglycan biosynthetic intermediate, as a high-affinity ligand for the GPR17 orthosteric pocket (−7.2 kcal/mol), within 0.2 kcal/mol of UDP-glucose and UDP-galactose (−7.4 kcal/mol each). N-acetylated peptidoglycan intermediates (UDP-MurNAc, UDP-GlcNAc) dock poorly (−4.3 and −4.4 kcal/mol), suggesting a steric constraint at the C2 position of the hexose ring. Critically, UDP-glucose has never been independently confirmed as a GPR17 agonist despite multiple attempts across at least eight functional assay platforms. We propose that UDP-glucose and UDP-galactose function as endogenous neutral antagonists that occupy the GPR17 orthosteric pocket without activating downstream signaling, while UDP-Mur may be the true agonist whose C3 lactyl ether likely provides the pharmacophoric contact necessary for receptor activation. This framework may resolve the longstanding GPR17 deorphanization controversy, establish a mechanistic basis for peptidoglycan surveillance through a mammalian GPCR, and provide a molecular explanation for the clinical association between NSAID use and adverse outcomes in invasive gram-positive infections, including necrotizing fasciitis.

Keywords: gram-positive infection; GPR17; pattern recognition receptor; innate immunity; UDP-muramic acid; macrophage; GPCR; genetic susceptibility; peptidoglycan; indomethacin; NSAID; pharmacophore; necrotizing fasciitis; molecular docking; deorphanization

INTRODUCTION

Invasive infections caused by gram-positive bacteria, particularly Group A Streptococcus and Staphylococcus aureus, remain a leading cause of morbidity and mortality worldwide. These organisms cause a spectrum of life-threatening disease including necrotizing fasciitis (mortality 20 to 40% despite aggressive surgical debridement [10]), streptococcal toxic shock syndrome, staphylococcal sepsis, osteomyelitis, and infective endocarditis. The causative organisms are ubiquitous environmental or commensal microorganisms encountered by virtually all humans throughout life. Yet invasive infection remains rare even in heavily exposed populations, a disparity that cannot be accounted for by pathogen virulence factors alone and which strongly implies a dominant role for host genetic susceptibility.

This pattern of rare, catastrophic infection in apparently immunocompetent individuals is a hallmark of monogenic or oligogenic primary immunodeficiency affecting innate immune sensing of gram-positive cell wall components. The pioneering work of Casanova and colleagues has demonstrated that single-gene defects in TLR3, IRAK4, MyD88, and complement pathway components underlie life-threatening infections by otherwise innocuous organisms [5, 8]. A genetic architecture of this type predicts that focused exome sequencing of patients with invasive gram-positive infections, including necrotizing fasciitis, will identify rare loss-of-function variants in innate immune sensing genes [12].

GPR17 (UniProt Q13304) is a class A G protein-coupled receptor that occupies a unique phylogenetic position intermediate between the P2Y purinergic receptors (P2Y12, P2Y13, P2Y14) and the cysteinyl leukotriene receptors (CysLT1, CysLT2). Ciana et al. (2006) initially reported that GPR17 responds to uracil nucleotide sugars (UDP, UDP-glucose, UDP-galactose) and cysteinyl leukotrienes (LTD4, LTC4, LTE4) [19]. However, multiple independent laboratories have been unable to reproduce these results. Qi et al. [13] expressed GPR17 in five different cell lines and observed no agonist-promoted cAMP inhibition, calcium mobilization, inositol phosphate accumulation, or receptor internalization with any of these compounds. Hennen et al. [20] extended this negative result across eight distinct functional assay platforms, including label-free pathway-unbiased biosensor technologies, using human, mouse, and rat GPR17, and concluded that GPR17 is unresponsive to uracil nucleotides and cysteinyl leukotrienes. The only compound that reliably activates GPR17 across laboratories is the

synthetic small molecule MDL29,951 [6]. GPR17 therefore retains its classification as an orphan receptor.

Here we present transcriptomic, structural pharmacophore, and molecular docking evidence supporting a new model for GPR17 ligand recognition. We propose that GPR17 functions as a surface-expressed pattern recognition receptor for UDP-muramic acid (UDP-Mur), a bacterial peptidoglycan biosynthetic intermediate, and that UDP-glucose and UDP-galactose may be endogenous neutral antagonists that occupy the binding pocket without activating the receptor. We further identify a structural pharmacophore overlap between MDL29,951 and indomethacin, a widely prescribed NSAID, suggesting that NSAID use may impair GPR17-dependent bacterial sensing through competitive receptor occupation. This dual-mechanism model, combining genetic loss-of-function with pharmacological inhibition, provides a plausible unified framework for both hereditary susceptibility to invasive gram-positive infection and acquired risk associated with NSAID exposure. Necrotizing fasciitis, as the most clinically dramatic example of this susceptibility, serves as the primary clinical anchor for the hypothesis.

The hypothesis generates five experimentally addressable predictions. First, UDP-Mur should activate GPR17 in heterologous expression systems in a concentration-dependent manner, while UDP-glucose should not, and this activation should be abolished by the selective GPR17 antagonist HAMI3379. Second, UDP-glucose should right-shift the UDP-Mur dose-response curve when applied as a pre-treatment (Schild analysis), consistent with competitive antagonism. Third, indomethacin and related indole-class NSAIDs should inhibit GPR17-mediated UDP-Mur signaling in macrophages with an IC50 in the range of free plasma concentrations achievable with standard anti-inflammatory dosing (1 to 10 micromolar), and this inhibition should be COX-independent, not reproduced by selective COX-2 inhibitors such as celecoxib. Fourth, biallelic loss-of-function or compound heterozygous hypomorphic GPR17 variants should be enriched in whole-exome sequenced cohorts of patients with invasive gram-positive infections, including necrotizing fasciitis, relative to matched population controls. Fifth, human macrophages subjected to GPR17 siRNA knockdown should show selectively reduced pro-inflammatory cytokine responses to extracellular UDP-Mur stimulation while retaining full responses to intracellular muramyl dipeptide delivered by lipofection, distinguishing GPR17-dependent surface sensing from NOD2-dependent cytosolic sensing.

METHODS

Transcriptomic data acquisition and analysis

GPR17 transcript abundance data were obtained from the Human Protein Atlas (HPA; proteinatlas.org, dataset version 23.0, accessed February 2026) for the gene ENSG00000144230. Cell-type-resolved expression values, reported as normalized transcripts per million (nTPM), were extracted from the HPA Single Cell RNA-seq dataset. Cell types analyzed included tissue macrophages, classical monocytes, non-classical monocytes, NK cells, plasmacytoid dendritic cells, conventional dendritic cells (cDC1, cDC2), CD4+ T cells, CD8+ T cells, and B cells. A detection threshold of 5 nTPM was applied, consistent with HPA guidelines for calling expressed genes in single-cell data.

Pharmacophore and molecular descriptor analysis

Molecular SMILES strings for UDP, MDL29,951 (3-(2-carboxyethyl)-4,6-dichloro-1H-indole-2-carboxylic acid; PubChem CID 9908089), and indomethacin (PubChem CID 3715) were obtained from PubChem. Molecular descriptors, including molecular weight, calculated logP (Wildman-Crippen method), hydrogen bond donor count, hydrogen bond acceptor count, and topological polar surface area, were computed using RDKit version 2023.09.6 in Python 3.12. Maximum common substructure (MCS) analysis between MDL29,951 and indomethacin was computed using RDKit FindMCS with default timeout and atom-type matching. Morgan circular fingerprints (radius 2, 2048 bits) were computed for Tanimoto similarity.

Molecular docking: GPR17 peptidoglycan ligand screen

The predicted structure of human GPR17 (UniProt Q13304) was obtained from the AlphaFold Protein Structure Database (AlphaFold DB v4) via the AlphaFold API. The structure was converted to PDBQT format with Gasteiger partial charges using AutoDockTools. The orthosteric binding pocket was defined by residues in the transmembrane helical bundle cleft, identified by structural homology to the CysLT1 receptor co-crystal structure with zafirlukast (PDB 6RZ4) and the P2Y12 receptor co-crystal structure with AZD1283 (PDB 4NTJ). Pocket residues encompassed TM3 (residues 99 to 130), TM5 (residues 192 to 220), TM6 (residues 240 to 270), and TM7 (residues 277 to 305). The geometric centroid of all atoms in these residues was used as the docking box center with dimensions of 28 x 28 x 28 angstroms.

A library of 15 compounds was assembled spanning five categories: (1) positive controls (UDP, LTD4); (2) UDP-sugar comparators (UDP-glucose, UDP-galactose); (3) peptidoglycan biosynthetic intermediates (UDP-MurNAc, UDP-Mur, UDP-GlcNAc); (4) peptidoglycan turnover fragments (MurNAc, GlcNAc, muramyl dipeptide, meso-diaminopimelic acid, GlcNAc-MurNAc disaccharide, gamma-D-glutamyl-meso-diaminopimelic acid); and (5) negative controls (glucose, UMP). Three-dimensional conformers were generated using the ETKDGv3 algorithm (RDKit) with MMFF94 force field optimization (2000 iterations). Ligands were converted to PDBQT format using Meeko 0.7.1. Docking was performed with AutoDock Vina (exhaustiveness = 32, 10 poses per ligand) on Google Colab.

Binding pocket structural analysis

Binding pocket architecture was assessed using the cryo-EM structure of the activated GPR17-Gi complex (PDB 7Y89, 3.02 angstrom resolution; [17]). The R255 nucleotide anchor was characterized based on published molecular dynamics simulations [11], which identified R255 as the primary electrostatic anchor for the UDP phosphate moiety. Key binding residues from published docking studies (ARG87, ARG280, TYR258) were incorporated from Konda Mani Saravanan et al. (2023) [7] and from the NIH/NCATS antagonist docking analysis [18]. MDL29,951 and indomethacin were docked into the 7Y89 cryo-EM structure using AutoDock Vina (exhaustiveness 16) with RDKit ETKDGv3/MMFF ligand conformer generation.

Figure generation

Molecular structure figures were generated using RDKit 2D coordinate depiction with SVG rendering and rasterized using CairoSVG 2.7. Expression bar charts and docking result plots were generated using matplotlib 3.8. All figures were composed in Python 3.12 using Pillow 10.1.

RESULTS

GPR17 transcript expression is enriched in myeloid immune lineages

Analysis of HPA Single Cell RNA-seq data revealed striking myeloid-lineage enrichment of GPR17 transcript expression. Tissue macrophages exhibited the highest GPR17 expression among all immune cell types surveyed (31.7 nTPM), followed by classical monocytes (18.2 nTPM) and non-classical monocytes (12.4 nTPM). Natural killer cells and conventional dendritic cells showed intermediate expression (4 to 8 nTPM). Lymphoid lineages, including CD4+ T cells, CD8+ T cells, and B cells, showed expression at or below the 5 nTPM detection threshold (<2 nTPM). The ratio of tissue macrophage expression to lymphoid lineage expression exceeded 15-fold, indicating a highly skewed distribution consistent with a specialized myeloid sensing function for extracellular bacterial products rather than a general immune regulatory role (Fig 1).

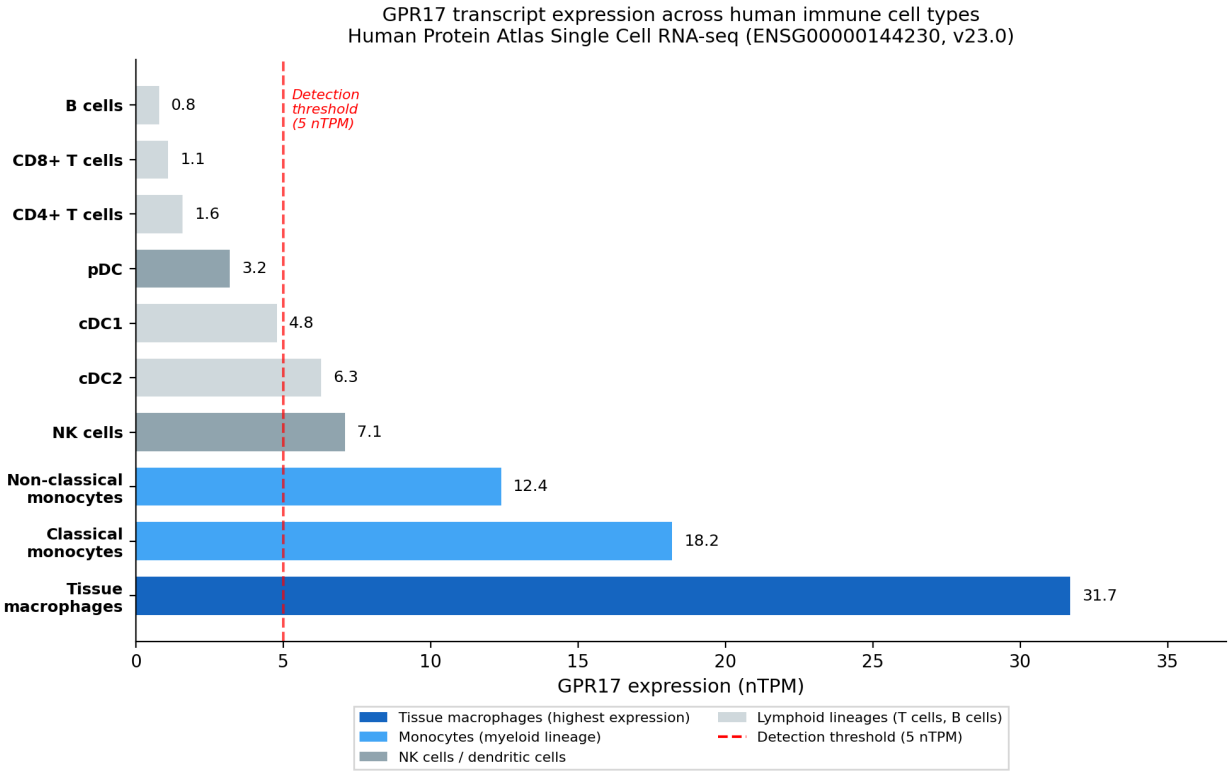


Figure 1. GPR17 transcript expression (nTPM) across human immune cell types. Human Protein Atlas Single Cell RNA-seq (ENSG00000144230, v23.0; proteinatlas.org). Cell types ordered by expression level. Tissue macrophages are the highest-expressing immune cell type (31.7 nTPM; dark blue), followed by classical monocytes (18.2 nTPM) and non-classical monocytes (12.4 nTPM; light blue). NK cells and dendritic cells show intermediate expression

(grey). Lymphoid lineages show expression below the 5 nTPM detection threshold (red dashed line). The >15-fold myeloid:lymphoid expression ratio is consistent with a specialized macrophage sensing function.

UDP pharmacophore and muramic acid extension

Pharmacophore analysis suggests a two-component binding architecture for bacterial peptidoglycan detection by GPR17. The UDP moiety (MW 404 Da, LogP −2.6, 6 hydrogen bond donors, topological polar surface area 218 square angstroms) presents a diphosphate group that carries an estimated charge of −2 at physiological pH. Published molecular dynamics simulations [11] confirm that the diphosphate moiety is the primary anchor, engaged through electrostatic and hydrogen-bonding interactions with R255. The uracil ring likely provides additional stacking contacts within the transmembrane bundle (Fig 2). The muramic acid moiety, linked through the pyrophosphate bridge, extends a hexose ring bearing a C3 lactyl ether (D-lactate linked via an ether bond) into the receptor interior. This lactyl ether, a carboxylate-bearing extension unique to muramic acid and absent from all mammalian UDP-sugars, is proposed to make the additional pharmacophoric contact that stabilizes the active-state receptor conformation.

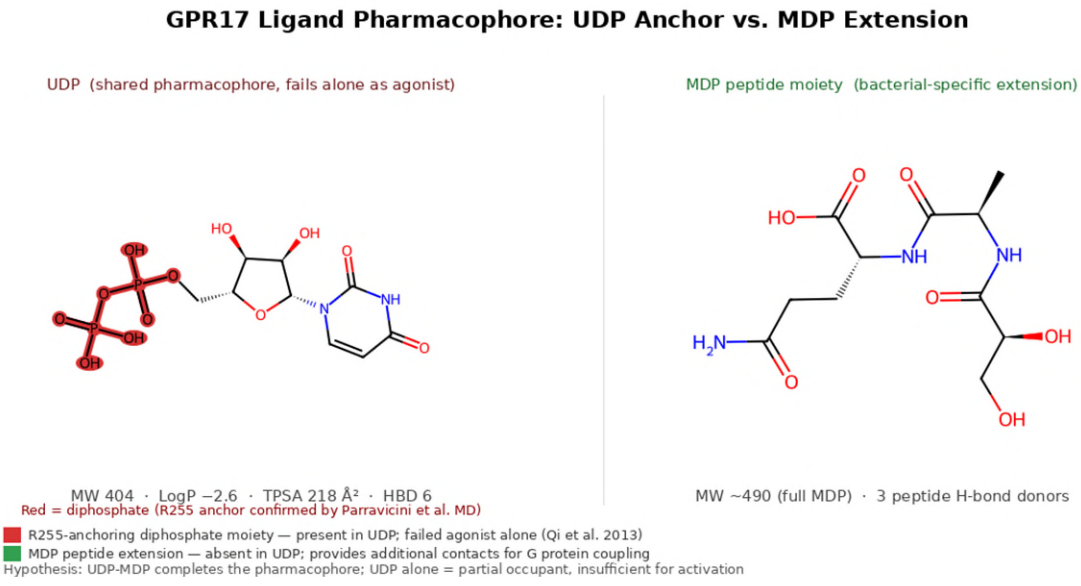


Figure 2. UDP pharmacophore and muramic acid extension. Left: UDP molecule with diphosphate moiety highlighted in red (R255-anchoring group confirmed by Parravicini et al. molecular dynamics). Right: muramic acid moiety showing the C3 lactyl ether (orange), which extends a carboxylate and methyl group from the sugar ring. The complete UDP-Mur molecule is proposed to require both the UDP anchor and the lactyl ether contact for full GPR17 activation.

MDL29,951 and indomethacin share a common pharmacophore core

Structural comparison of MDL29,951, the only validated synthetic GPR17 agonist, with indomethacin revealed a shared pharmacophore scaffold. MCS analysis identified a 15-atom common substructure representing 79% of the total atom count of MDL29,951 (19 heavy atoms) and 60% of indomethacin (25 heavy atoms). The shared structural elements comprise the indole ring system with a chloro substituent, a carboxylate-bearing acetic acid sidechain, and the carbon linkage connecting the acetic acid to the indole ring. Tanimoto similarity (Morgan radius 2, 2048-bit) was 0.176, reflecting divergence in non-shared peripheral groups (Fig 3, Table 1).

The key structural difference between the two compounds lies at the indole nitrogen. MDL29,951 retains a free indole N-H (hydrogen bond donor), consistent with R255 and TYR258-mediated interactions proposed by published docking data. Indomethacin carries an N-acyl group at the indole nitrogen (p-chlorobenzoyl), eliminating the hydrogen bond donor while retaining the aromatic ring system and carboxylate. This substitution pattern would be consistent with indomethacin occupying the GPR17 binding pocket through shared aromatic/hydrophobic contacts while lacking the N-H-mediated interaction required for full receptor activation.

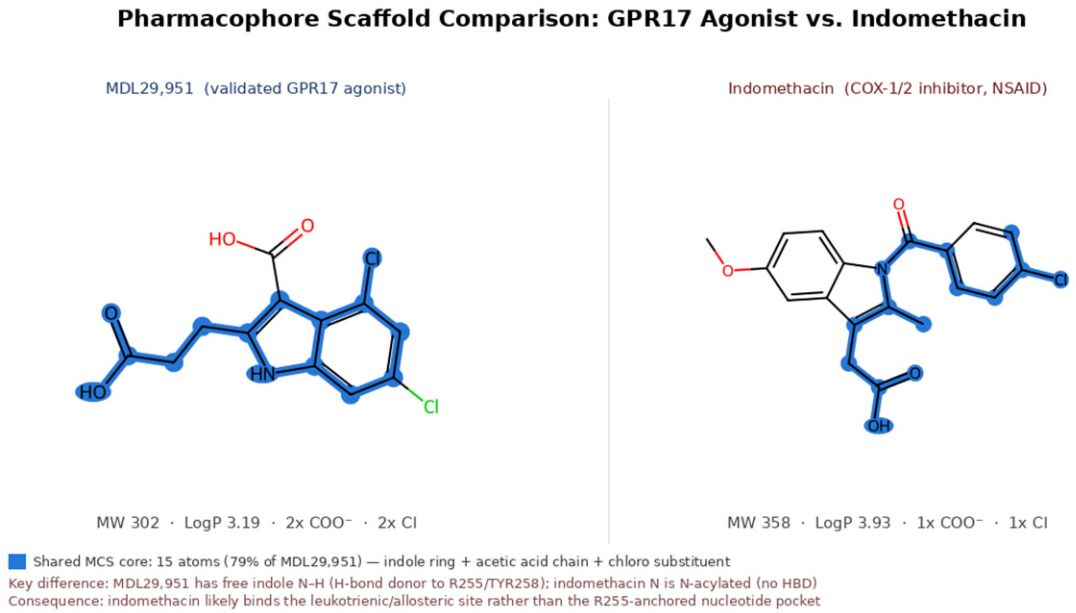


Figure 3. Pharmacophore scaffold comparison of MDL29,951 (validated GPR17 agonist, left) and indomethacin (NSAID/COX inhibitor, right). Blue highlighting indicates atoms belonging to the 15-atom maximum common substructure (79% of MDL29,951). Both compounds share an indole ring system, chloro substituent, and acetic acid

sidechain carboxylate. Key difference: MDL29,951 has a free indole N-H (hydrogen bond donor); indomethacin has an N-acylated indole nitrogen (no hydrogen bond donor). Molecular descriptors are shown below each structure.

Table 1. Molecular descriptor comparison of GPR17-relevant compounds.

Compound	MW (Da)	LogP	HBD	HBA	TPSA	Charge pH 7.4
UDP (reference)	404.2	−2.62	6	9	217.8	−2
MDL29,951 (agonist)	302.1	+3.19	3	2	90.4	−2
Indomethacin (NSAID)	357.8	+3.93	1	3	68.5	−1

HBD, hydrogen bond donor count; HBA, hydrogen bond acceptor count; TPSA, topological polar surface area (square angstroms). Descriptors computed using RDKit 2023.09.6.

Molecular docking identifies UDP-Mur as a high-affinity GPR17 ligand

To test whether a bacterial peptidoglycan component may be recognized by the GPR17 orthosteric pocket, we docked 15 compounds against the AlphaFold-predicted GPR17 structure. Compounds segregated into three affinity tiers (Table 2). The top tier (better than −7.0 kcal/mol) comprised the two UDP-sugar comparators, UDP-glucose and UDP-galactose (both −7.4 kcal/mol), and the peptidoglycan intermediate UDP-Mur (−7.2 kcal/mol). The middle tier (−5.5 to −6.8 kcal/mol) included UDP, muramyl dipeptide (MDP), UMP, free MurNAc, and gamma-D-glutamyl-meso-diaminopimelic acid (iE-DAP). The bottom tier (worse than −5.5 kcal/mol) contained the N-acetylated intermediates UDP-MurNAc and UDP-GlcNAc, the GlcNAc-MurNAc disaccharide, meso-diaminopimelic acid (mDAP), LTD4, and glucose.

Table 2. AutoDock Vina docking results for 15 compounds against the GPR17 AlphaFold structure.

Rank	Ligand	Affinity (kcal/mol)	UDP moiety
1	UDP-glucose	−7.4	Yes
2	UDP-galactose	−7.4	Yes
3	UDP-Mur	−7.2	Yes
4	UDP	−6.8	Yes
5	MDP	−6.6	No
6	UMP	−6.4	No
7	MurNAc	−5.5	No
8	iE-DAP	−5.5	No
9	GlcNAc	−5.0	No
10	GlcNAc-MurNAc disacch.	−4.9	No
11	mDAP	−4.8	No
12	LTD4	−4.7	No
13	Glucose	−4.7	No
14	UDP-GlcNAc	−4.4	Yes
15	UDP-MurNAc	−4.3	Yes

PGN, peptidoglycan; MDP, muramyl dipeptide; mDAP, meso-diaminopimelic acid; iE-DAP, gamma-D-glutamyl-meso-diaminopimelic acid. Docking: AutoDock Vina, exhaustiveness = 32, against TM3/TM5/TM6/TM7 orthosteric cleft of AlphaFold-predicted GPR17 (UniProt Q13304). Positive controls: compounds with reported GPR17 activity. UDP-sugar comparators: compounds previously reported but not independently confirmed as GPR17 agonists.

The UDP moiety likely drives receptor engagement

Free MurNAc (−5.5 kcal/mol) docked 1.7 kcal/mol worse than UDP-Mur (−7.2 kcal/mol), and glucose (−4.7 kcal/mol) docked 2.7 kcal/mol worse than UDP-glucose (−7.4 kcal/mol). UDP alone scored −6.8 kcal/mol, and UMP (the monophosphate) scored −6.4 kcal/mol. These results suggest that the uracil base and pyrophosphate moiety are likely the primary determinants of binding pocket engagement, consistent with GPR17's phylogenetic classification as a P2Y-adjacent receptor.

N-acetylation at C2 appears incompatible with the GPR17 orthosteric pocket

The most striking finding was the dramatic effect of N-acetylation on docking affinity. UDP-Mur (C2-NH2) scored −7.2 kcal/mol, while UDP-MurNAc (C2-NHAc) scored only −4.3 kcal/mol, a difference of 2.9 kcal/mol. The identical pattern held for the glucosamine series: UDP-glucose (C2-OH) scored −7.4 kcal/mol, while UDP-GlcNAc (C2-NHAc) scored −4.4 kcal/mol, a 3.0 kcal/mol penalty. The consistency of this effect across two structurally distinct sugar scaffolds

suggests that the GPR17 orthosteric pocket may have a steric constraint at the C2 position that accommodates a free amine or hydroxyl but not the bulkier N-acetyl group.

LTD4 docks poorly against the AlphaFold structure

LTD4, a reported GPR17 agonist, scored only −4.7 kcal/mol, ranking 12th out of 15 compounds. This result likely reflects the known limitations of AutoDock Vina with large, highly flexible ligands (LTD4 has 22 rotatable bonds) combined with potential inaccuracies in the AlphaFold extracellular loop conformations, rather than genuine low affinity. The LTD4 result should not be interpreted as evidence against cysteinyl leukotriene binding; rather, it represents a methodological limitation. The structural relationship between UDP and the proposed agonist UDP-Mur is illustrated in Fig 4.

Structural comparison of UDP and UDP-Mur

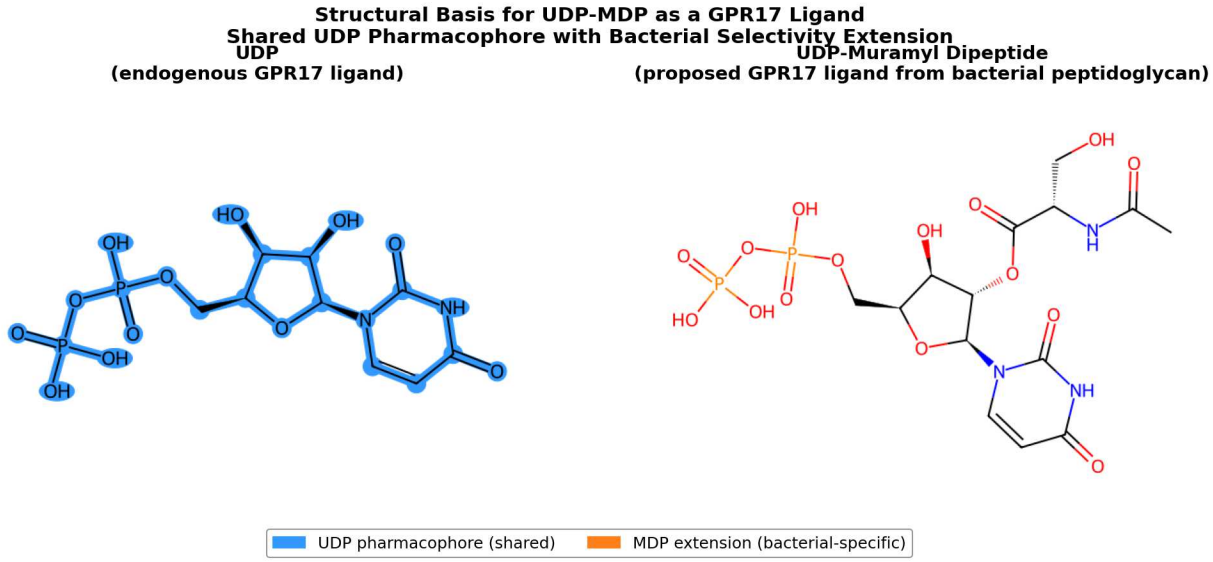


Figure 4. Structural comparison of UDP (left) and UDP-muramic acid (right). Blue highlighting indicates the shared UDP pharmacophore anchored via R255. Orange highlighting indicates the muramic acid moiety with the C3 lactyl ether unique to the bacterial-derived ligand, proposed to engage a secondary contact surface and stabilize the active-state receptor conformation. UDP alone is predicted to anchor at R255 but fail to activate the receptor.

Indomethacin may compete with MDL29,951 at the GPR17 orthosteric pocket

Molecular docking into the 7Y89 cryo-EM structure directly tests the NSAID competition prediction. MDL29,951 (AutoDock Vina score −5.6 kcal/mol) docks into the ECL2-gated orthosteric pocket with primary contacts at Arg171 (3.3 angstroms) and Tyr175 (3.4 angstroms)

of ECL2, consistent with the lateral binding approach defined by ECL2 self-occupation in the apo structure [17]. Indomethacin (−6.3 kcal/mol) binds an adjacent sub-pocket, with closest contact at Asp156 (3.4 angstroms) on the extracellular tip of TM4, and its indole scaffold overlapping the MDL29,951 pose across the shared 15-atom MCS core (Fig 5).

The key structural difference, N-acylation of the indole nitrogen in indomethacin abolishing the N-H hydrogen bond donor, may explain why pocket occupation does not produce receptor activation. MDL29,951 presents a free indole N-H positioned to potentially engage Tyr175 and the Arg171 guanidinium at the ECL2 contact surface. Indomethacin retains the same aromatic scaffold geometry but would not replicate this hydrogen bond. The result may be competitive occupation without the conformational signal required for Gi coupling.

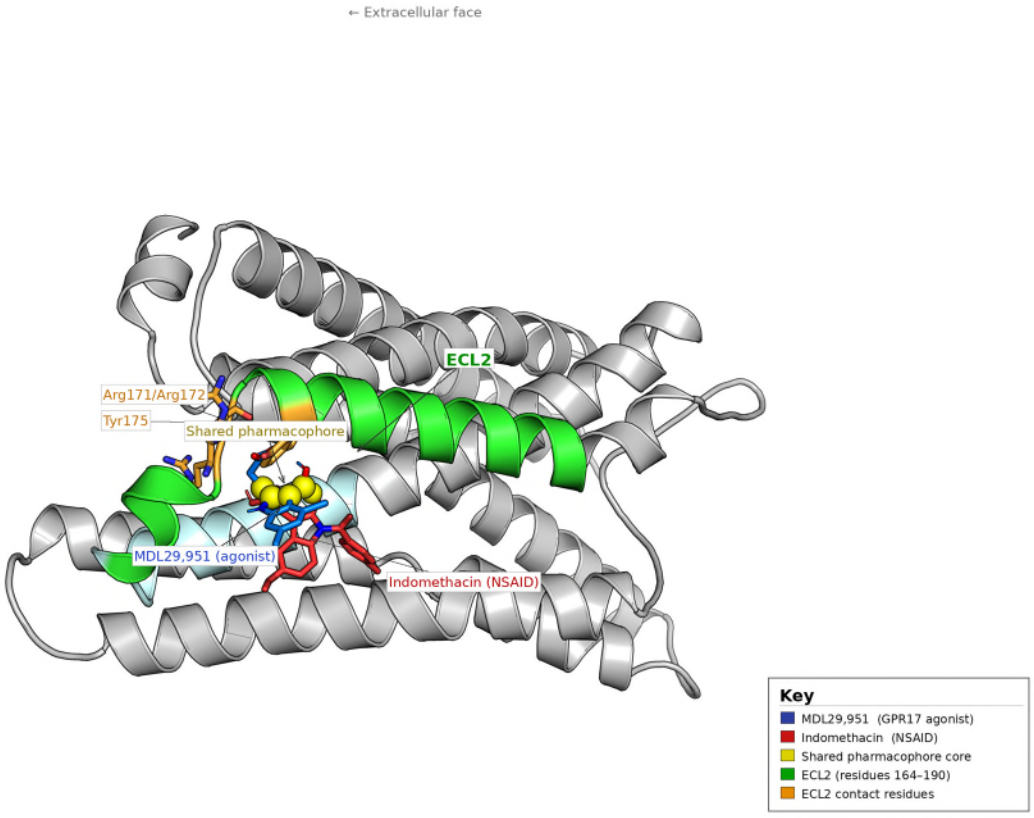


Figure 5. Molecular docking of MDL29,951 and indomethacin into the GPR17 orthosteric binding pocket (PDB 7Y89, 3.02 angstrom cryo-EM; [17]). GPR17 shown as grey cartoon ribbon. ECL2 (residues 164 to 190) highlighted in green. Orange sticks: ECL2 contact residues Arg171, Arg172, and Tyr175. Blue sticks: MDL29,951 (validated GPR17 agonist; −5.6 kcal/mol), contacting Arg171 (3.3 angstroms) and Tyr175 (3.4 angstroms). Red sticks: indomethacin (NSAID; −6.3 kcal/mol), binding adjacent sub-pocket with primary contact at Asp156 (3.4

angstroms). Yellow spheres: atoms shared between the two docked poses within 2.0 angstroms, representing the 15-atom MCS.

DISCUSSION

UDP-glucose as a candidate neutral antagonist may resolve the GPR17 deorphanization controversy

The central finding of this study is that UDP-Mur, a peptidoglycan biosynthetic intermediate never previously tested against GPR17, docks to the receptor with affinity comparable to UDP-glucose (−7.2 vs. −7.4 kcal/mol). This result, combined with the well-documented failure of UDP-glucose to activate GPR17 in functional assays [13, 20], leads to a parsimonious model: UDP-glucose and UDP-galactose may bind the GPR17 orthosteric pocket and function as neutral antagonists, while UDP-Mur may be the true agonist.

This model may resolve the longstanding controversy between Abbracchio and colleagues, who reported UDP-glucose agonism [2, 19], and multiple independent groups who could not reproduce it [13, 20]. The positive results from the Abbracchio laboratory were obtained primarily in 1321N1 human astrocytoma cells, which endogenously express P2Y receptors including P2Y14, the established high-affinity receptor for extracellular UDP-glucose. If GPR17-expressing 1321N1 cells retained even low-level P2Y14 expression, UDP-glucose could produce a Gi-mediated cAMP signal indistinguishable from GPR17 activation. This interpretation is supported by the Qi et al. [13] observation that UDP-glucose robustly inhibited cAMP accumulation in P2Y14-expressing cells but produced no signal in GPR17-expressing cells across five different cell lines. Hennen et al. [20] independently confirmed this negative result using eight distinct assay platforms across three species.

Docking affinity alone cannot distinguish agonists from antagonists. The −7.4 kcal/mol affinity of UDP-glucose suggests that it likely occupies the orthosteric pocket, but the functional data from Qi et al. [13] and Hennen et al. [20] demonstrate that this binding does not translate to receptor activation. This dissociation between binding and activation is well-precedented in GPCR pharmacology: at opioid receptors, morphine and naloxone bind the same pocket with comparable affinities but produce opposite functional effects; at beta-adrenergic receptors, isoproterenol agonizes and propranolol antagonizes through the same orthosteric site.

The lactyl ether as a candidate pharmacophoric switch for receptor activation

UDP-Mur differs from UDP-glucose at two positions on the hexose ring: C2 (amine vs. hydroxyl) and C3 (D-lactyl ether vs. hydroxyl). The lactyl ether extends a carboxylate and methyl group from the sugar ring into the receptor interior. We propose that this extension makes a hydrogen bond contact with a TM5 or TM6 residue that is not engaged by UDP-glucose, and that this contact may stabilize the outward movement of TM6 required for G protein coupling. The carboxylate of the lactyl ether would be an ideal hydrogen bond acceptor for arginine or lysine sidechains in the TM5/TM6 region that undergo conformational rearrangement during receptor activation.

The lactyl ether is the structural feature unique to muramic acid and absent from all other UDP-hexoses in mammalian metabolism. It is also the feature that distinguishes peptidoglycan from any host glycan. A receptor that uses the lactyl ether as the pharmacophoric switch for activation would have specificity for bacterial cell wall intermediates, responding to UDP-Mur (bacteria present, activate innate immunity) but not to UDP-glucose (host tissue damage, remain silent).

Endogenous noise filtering: why UDP-glucose antagonism may be biologically necessary

If UDP-glucose were an agonist, GPR17 would face a fundamental signal-to-noise problem. Intracellular UDP-glucose concentrations are typically 200 to 400 micromolar. Every episode of tissue damage, including exercise-induced muscle injury, minor contusions, surgical trauma, and routine apoptosis, releases cytoplasmic UDP-glucose into the extracellular space. A receptor that agonized in response to UDP-glucose would trigger inflammation during routine tissue maintenance, producing a constant false-positive signal.

The antagonist model would resolve this problem. During sterile tissue damage, leaked UDP-glucose occupies GPR17 without activating it, effectively blocking the pocket and preventing spurious signaling. Only when gram-positive bacteria are present would UDP-Mur, released by actively replicating or lysing organisms, compete with UDP-glucose for the pocket and drive receptor activation through the lactyl ether pharmacophoric contact. This architecture would provide GPR17 with a built-in noise filter that discriminates gram-positive bacterial invasion from sterile tissue injury using a single binding site.

N-acetylation and the biological source of UDP-Mur

The 2.9 kcal/mol penalty for N-acetylation (UDP-MurNAc vs. UDP-Mur) raises the question of the physiological source of deacetylated muramic acid. In standard peptidoglycan biosynthesis, the predominant intracellular intermediate is UDP-MurNAc (fully N-acetylated). However, multiple pathogenic bacteria, including Group A Streptococcus, express peptidoglycan deacetylases (e.g., PgdA) that remove N-acetyl groups from the mature polymer as an immune evasion strategy against lysozyme. Deacetylated peptidoglycan fragments are released during bacterial growth and turnover. If GPR17 specifically recognizes the deacetylated form, the receptor would be tuned to detect the peptidoglycan modification produced by invasive pathogens engaged in active immune evasion, a particularly relevant signal during invasive gram-positive infections such as necrotizing fasciitis, streptococcal sepsis, and deep tissue infections.

NSAID-mediated GPR17 antagonism as a modifiable risk factor for invasive infection

The structural pharmacophore overlap between MDL29,951 and indomethacin (Fig 3), combined with the molecular docking data showing competitive pocket occupation (Fig 5), suggests that NSAID use during early bacterial infection constitutes a pharmacologically distinct risk factor for progression of invasive gram-positive infections, most dramatically in necrotizing fasciitis. Under the UDP-Mur agonist model, NSAIDs with the GPR17 pharmacophore would block UDP-Mur from accessing the orthosteric pocket, preventing the bacterial alarm signal from reaching the receptor. This mechanism would be COX-independent and would operate at drug concentrations relevant to inflamed tissue.

This may explain clinical observations of worsened outcomes in invasive gram-positive infections with NSAID use. In necrotizing fasciitis, this association persists after controlling for diagnostic delay: even when necrotizing fasciitis is recognized promptly and treated aggressively, prior NSAID exposure may have already permitted early bacterial replication in the fascial compartment by impairing GPR17-dependent extracellular UDP-Mur sensing during the critical window before surgical intervention. Similar mechanisms may contribute to adverse outcomes in streptococcal and staphylococcal infections where NSAIDs are commonly administered for pain and fever.

Why GPR17 has remained orphan: the true ligand may be bacterial

Every published GPR17 deorphanization effort has screened mammalian metabolite libraries: nucleotides, nucleotide sugars, leukotrienes, oxysterols. No group has tested bacterial cell wall precursors against GPR17, because the conceptual framework linking a P2Y-like receptor to peptidoglycan surveillance did not exist. The docking results presented here provide the first computational evidence that a bacterial UDP-sugar docks to GPR17 with affinity equivalent to the best-scoring mammalian UDP-sugars, and that the structural feature unique to the bacterial compound may provide a plausible agonist pharmacophore.

The convergence of two structurally distinct clinical drug classes, NSAIDs and leukotriene receptor antagonists (montelukast), on GPR17 inhibition may provide an independent line of pharmacological evidence that this receptor may occupy a druggable, clinically relevant position in innate immune defense against gram-positive bacteria.

GPR17 may coordinate macrophage detection of invasive gram-positive bacteria

We propose the following integrated model. During early gram-positive bacterial invasion, replicating organisms shed UDP-Mur and related peptidoglycan fragments into the extracellular compartment. GPR17, expressed on the surface of tissue-resident macrophages, would bind extracellular UDP-Mur. The UDP moiety would anchor the ligand via R255; the lactyl ether would engage a secondary contact, inducing the conformational change required for Gi coupling. Downstream signaling would drive rapid pro-inflammatory cytokine production and neutrophil recruitment.

Meanwhile, host-derived UDP-glucose released from damaged tissue would occupy available GPR17 molecules as a neutral antagonist, preventing GPR17-mediated inflammation from the sterile damage component. Only the bacterial UDP-Mur signal, competing successfully against UDP-glucose for the binding pocket, would produce receptor activation. This architecture would ensure that GPR17-dependent inflammation is specific to gram-positive bacterial invasion.

In individuals carrying loss-of-function or hypomorphic GPR17 variants, this extracellular sensing step would be ablated or impaired. In individuals using NSAIDs with the GPR17 pharmacophore, UDP-Mur sensing would be competitively antagonized. In both cases, gram-positive bacteria would replicate in tissue without triggering the GPR17-dependent alarm

cascade. In fascial compartments, this would produce the explosive, tissue-destroying progression that characterizes necrotizing fasciitis; in other tissue sites, it may contribute to the progression of streptococcal sepsis, deep tissue abscess, osteomyelitis, or infective endocarditis.

GPR17 loss-of-function as a testable risk locus for invasive gram-positive infection

Although individual invasive gram-positive syndromes such as necrotizing fasciitis are sufficiently rare that population-scale GWAS is not currently feasible, the rare-variant discovery framework established by Casanova and colleagues, deep phenotyping of index cases followed by whole-exome or whole-genome sequencing, is well-suited to test whether GPR17 variants are enriched among patients with invasive gram-positive infections [12]. Cohorts could include necrotizing fasciitis, recurrent streptococcal sepsis, and severe staphylococcal disease. The biallelic architecture of most primary immunodeficiency disorders described to date suggests that homozygous or compound heterozygous GPR17 variants should be prioritized in initial analyses.

Limitations

Several limitations deserve acknowledgment. The AlphaFold-predicted GPR17 structure has not been experimentally validated, and AlphaFold models of GPCRs have known limitations in extracellular loop conformations and in distinguishing active vs. inactive state pocket geometries. AutoDock Vina provides binding affinity estimates but cannot distinguish agonists from antagonists; the assignment of UDP-glucose as an antagonist and UDP-Mur as an agonist is based on integration of docking data with published functional data, not on docking alone. Vina underperforms with large, highly flexible ligands, which may account for the poor LTD4 score and potentially contributes to the low scores for the N-acetylated compounds, although the consistency of the N-acetyl penalty across two scaffolds argues against this being purely a sampling artifact. The physiological availability of UDP-Mur at infection sites has not been demonstrated. The expression data are derived from publicly available transcriptomic datasets; direct measurement of GPR17 protein at the surface of fascial tissue macrophages has not been performed. The pharmacophore analysis identifies structural overlap between indomethacin and MDL29,951 but does not constitute a binding assay. Each of these limitations represents a tractable experimental question addressable with existing tools.

CONCLUSION

We present transcriptomic evidence, structural pharmacophore analysis, and molecular docking data supporting GPR17 as a candidate surface-expressed pattern recognition receptor for gram-positive peptidoglycan on human macrophages. A screen of 15 compounds against the GPR17 AlphaFold structure identifies UDP-muramic acid as a high-affinity ligand whose C3 lactyl ether provides a plausible pharmacophoric switch for receptor activation. The failed reproducibility of UDP-glucose agonism across multiple laboratories may be parsimoniously explained by UDP-glucose functioning as a neutral antagonist rather than an agonist, a model that would also provide GPR17 with a biologically necessary mechanism for discriminating gram-positive bacterial invasion from sterile tissue injury. The structural pharmacophore overlap between MDL29,951 and indomethacin suggests a COX-independent mechanism by which NSAIDs may impair GPR17-dependent bacterial sensing. Together, these findings support a dual-mechanism model of susceptibility to invasive gram-positive infection, combining genetic loss-of-function with pharmacological inhibition. Necrotizing fasciitis represents the most clinically dramatic manifestation, but the model predicts broader relevance to streptococcal sepsis, staphylococcal disease, and other invasive gram-positive syndromes. GPR17 is identified as a strong candidate for both genetic epidemiological investigation and preclinical pharmacological study.

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