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N′-Substituted-2′-O,3′-N-carbonimidoyl Bridged Macrolides: Novel Anti-inflammatory Macrolides without Antimicrobial Activity

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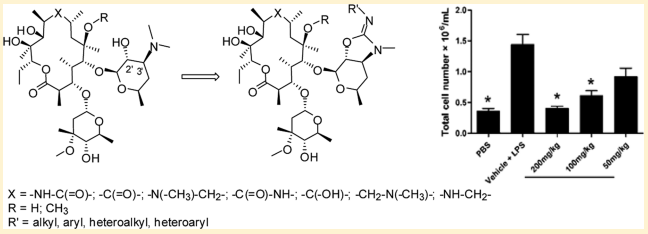
Supporting Information

ABSTRACT: Macrolide antibiotics, like erythromycin, clarithromycin, and azithromycin, possess anti-inflammatory properties. These properties are considered fundamental to the efficacy of these three macrolides in the treatment of chronic inflammatory diseases like diffuse panbronchiolitis and cystic fibrosis. However, long-term treatment with macrolide antibiotics presents a considerable risk for promotion of bacterial resistance. We have examined antibacterial and anti-inflammatory effects of a novel macrolide class: *N*′-substituted 2′-*O*,3′-*N*-carbonimidoyl bridged erythromycin-derived 14- and 15-membered macrolides. A small focused library was prepared, and compounds without antimicrobial activity, which inhibited IL-6 production, were selected. Data analysis led to a statistical model that could be used for the design of novel anti-inflammatory macrolides. The most promising compound from this library retained the anti-inflammatory activity observed with azithromycin in lipopolysaccharide-induced pulmonary neutrophilia in vivo. Importantly, this study strongly suggests that antimicrobial and anti-inflammatory activities of macrolides are independent and can be separated, which raises development plausibility of novel anti-inflammatory therapeutics.



INTRODUCTION

Macrolide antibiotics ("macrolides") are well-established class of antimicrobial agents characterized by the presence of a highly substituted macrocyclic lactone ring. Erythromycin, a secondary metabolite isolated from *Saccharopolyspora erythraea,* was the first macrolide to be introduced to clinical use over 50 years ago. Afterward, several semisynthetic derivatives of erythromycin, such as clarithromycin (6-*O*-methylerythromycin A)[1] and azithromycin (9-deoxy-9a-aza-9a-methyl-9a-homoerythromycin A),[2] were designed to broaden the antimicrobial spectrum, reduce gastrointestinal side effects, and increase acid-stability and bioavailability in this class of antibiotics. Telithromycin, a representative of a new macrolide subclass called ketolides was approved for clinical use some 10 years ago.[3] This new subclass of 14-membered macrolides is characterized by a keto group at the C-3 position instead of the cladinose sugar.[4] This change further increased acid stability and prevented induction of macrolide−lincosamide−streptogramin B resistance.[5]

Macrolides are broad-spectrum antibiotics that exhibit antibacterial activity against aerobic Gram-positive bacteria, certain Gram-negative bacteria, anaerobic bacteria, and intracellular pathogens such as *Mycoplasma*, *Chlamydia*, and *Legionella*.[6] Macrolides reversibly bind to the 50S subunit of the bacterial ribosome adjacent to the peptidyl transferase center, thereby blocking the egress of nascent polypeptides and consequently destabilizing the ribosome−peptidyl−tRNA interactions.[7] Nowadays, macrolides are widely used in the treatment of respiratory and urogenital tract, skin, and soft tissue infections.[8]

In addition to efficacy in treatment of bacterial infections, many studies over the last 20 years have demonstrated that certain macrolides are effective in the treatment of various chronic inflammatory disorders of the respiratory tract.[9] Introduction of erythromycin to the treatment of diffuse panbronchiolitis (DPB) in the 1980s drastically increased 10-year survival rate, decreased frequency of exacerbations, and restored lung function.[10] Afterward, azithromycin was successfully used in the treatment of cystic fibrosis (CF), which shares a number of similarities in clinical and pathological characteristics with DPB. In CF patients, azithromycin treatment was shown to significantly improve lung function and reduce the frequency of exacerbations. Consequently, certain macrolides, such as erythromycin, clarithromycin, and azithromycin, are now the first-line therapy for DPB and recommended for patients with CF. Clinical efficacy of these macrolides in diseases such as DPB and CF cannot be directly linked to their antimicrobial activity.[11] They are also being evaluated in the therapy of chronic obstructive pulmonary disease (COPD), chronic sinusitis, asthma, bronchiectasis, and bronchiolitis

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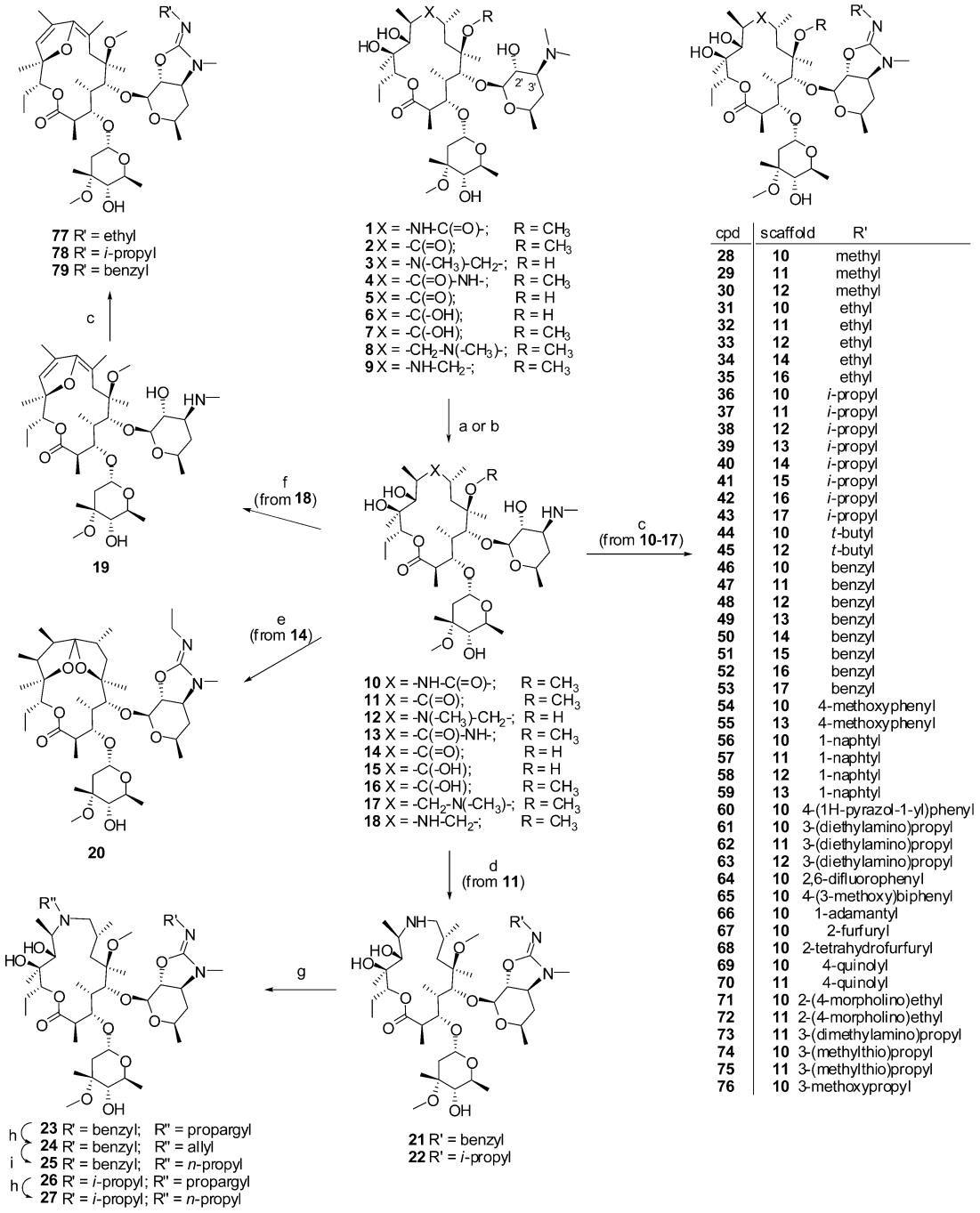
Scheme 1. Synthesis of *N*′-Substituted-2′-*O*,3′-*N*-carbonimidoyl Bridged Macrolides[a]



cpd	scaffold	R'
28	10	methyl
29	11	methyl
30	12	methyl
31	10	ethyl
32	11	ethyl
33	12	ethyl
34	14	ethyl
35	16	ethyl
36	10	*i*-propyl
37	11	*i*-propyl
38	12	*i*-propyl
39	13	*i*-propyl
40	14	*i*-propyl
41	15	*i*-propyl
42	16	*i*-propyl
43	17	*i*-propyl
44	10	*t*-butyl
45	12	*t*-butyl
46	10	benzyl
47	11	benzyl
48	12	benzyl
49	13	benzyl
50	14	benzyl
51	15	benzyl
52	16	benzyl
53	17	benzyl
54	10	4-methoxyphenyl
55	13	4-methoxyphenyl
56	10	1-naphtyl
57	11	1-naphtyl
58	12	1-naphtyl
59	13	1-naphtyl
60	10	4-(1H-pyrazol-1-yl)phenyl
61	10	3-(diethylamino)propyl
62	11	3-(diethylamino)propyl
63	12	3-(diethylamino)propyl
64	10	2,6-difluorophenyl
65	10	4-(3-methoxy)biphenyl
66	10	1-adamantyl
67	10	2-furfuryl
68	10	2-tetrahydrofurfuryl
69	10	4-quinolyl
70	11	4-quinolyl
71	10	2-(4-morpholino)ethyl
72	11	2-(4-morpholino)ethyl
73	11	3-(dimethylamino)propyl
74	10	3-(methylthio)propyl
75	11	3-(methylthio)propyl
76	10	3-methoxypropyl

[a]Reagents and conditions: (a) Sodium acetate trihydrate, iodine, methanol, 500 W halogen lamp irradiation, 4−5 h, or (b) *N*-iodosuccinimide, acetonitrile, 1.5 h. (c) Step 1, R′-NCS, triethylamine, acetonitrile, 60 °C, 1−2 h; step 2, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 60 °C, 24−72 h. (d) **11**, glacial acetic acid.[18] (e) Byproduct isolated from the reaction: **14**, ethyl isothiocyanate, triethylamine, acetonitrile, 60 °C, 1−2 h; step 2, EDC, 60 °C, 44 h. (f) **18**, benzyl or isopropyl isothiocyanate, triethylamine, acetonitrile, 60 °C, 1−2 h; step 2, EDC, 60 °C, 24−72 h. (g) Propargyl bromide, *N*,*N*-diisopropylethylamine, DMF, 80 °C, MW, 20 min. (h) H_2, Pd/C, ethanol, RT, 40 min. (i) H_2, Pd/C, ethanol, 2 bar, 2 h.

obliterans.[12] However, the long-term low-dose treatment of chronic inflammatory disorders of the respiratory tract with macrolide antibiotics promotes bacterial resistance.[13] This risk limits current use of macrolide antibiotics only to a small number of inflammatory conditions associated with high mortality rate despite of existing solid evidence confirming their efficacy in other numerous inflammatory conditions. The majority of these diseases exhibit neutrophil-dominated inflammation and thus do not have adequate therapy coverage. Therefore, macrolide drugs which would retain, e.g., azithromycin type of anti-inflammatory activity, without antimicrobial effects, could provide therapy for a number of chronic inflammatory diseases which are currently not adequately treated. There are examples of 12-, 14-, and 15-membered macrolides where immunomodulatory and antibacterial activity can be separated by appropriate chemical derivatization.[14]

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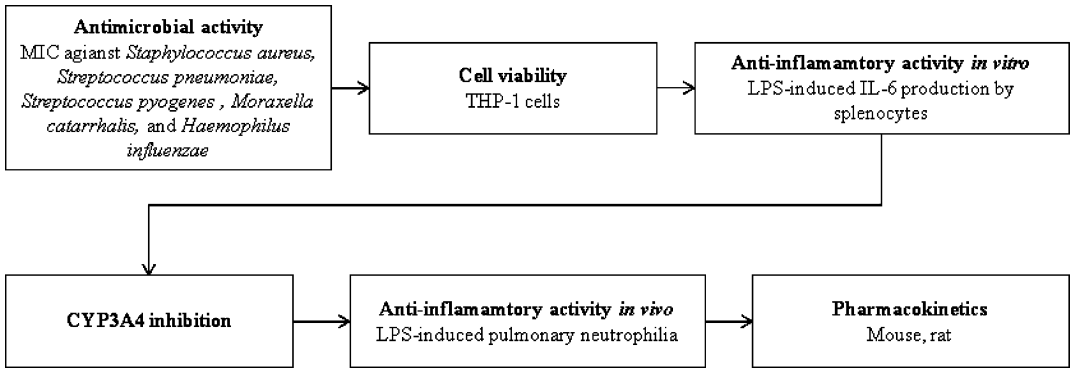

Figure 1. Screening cascade. Criteria for progression were: no antimicrobial activity (MIC >64 μg/mL), no influence on cell viability (less than 10% inhibition at 50 μM concentration), anti-inflammatory activity in vitro (at least 60% inhibition of LPS-stimulated IL-6 production at 50 μM), no significant potential to induce drug−drug interaction (CYP3A4 IC_{50} >5 μM), anti-inflammatory activity in vivo (significant inhibition of neutrophil number in bronchoalveolar lavage fluid following intranasal LPS challenge), pharmacokinetic profile (PK) that would allow once daily oral dosing ($t_{1/2}$ >3 h, F > 20%).

Consequently, we have synthesized a focused library of erythromycin-derived 14- and 15-membered macrolides characterized by *N*′-substituted 2′-*O*,3′-*N*-carbonimidoyl bridged desosamine sugar and have examined their antibacterial and anti-inflammatory activities. Because desosamine is directly involved in the binding of macrolides to ribosomes through a network of hydrogen bonds and ionic interactions,[15] 2′-*O*,3′-*N*-carbonimidoyl bridging should diminish the antibacterial activity of macrolide antibiotics.[16] Moreover, various substituents at the nitrogen of the 2′-*O*,3′-*N*-carbonimidoyl bridge could further affect antibacterial activity but also fine-tune the macrolide physicochemical properties.

RESULTS AND DISCUSSION

In the course of our research focused on novel macrolide transformations, we described the synthesis of a new macrolide class marked by *N*′-substituted-2′-*O*,3′-*N*-carbonimidoyl bridged desosamine sugar.[17] Such macrolides can be synthesized in an easy and straightforward manner in only two steps, 3′-*N*-demethylation and subsequent one-pot cyclization via intermediary formed thiourea moiety, from commercially available standard antibacterial macrolides such as azithromycin and clarithromycin. Accordingly, we expanded the synthesis of 2′-*O*,3′-*N*-carbonimidoyl bridged macrolides to cover a wider chemical space and fine-tune physicochemical properties, mostly by introducing a broader range of substituents at *N*′-position but also by modifying aglycone rings of standard macrolide scaffolds (Scheme 1). A small focused library containing 60 different molecules was prepared. These compounds were screened for antimicrobial activity against a panel of common respiratory pathogens (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, and *Haemophilus influenzae*), cytotoxicity against a human acute monocytic leukemia cell line (THP-1), and inhibition of lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) production by murine splenocytes (Figure 1). Antimicrobially inactive (minimal inhibitory concentration (MIC) > 64 μg/mL) compounds with anti-inflammatory activity (at least 60% inhibition of LPS-induced IL-6 production at 50 μM concentration), which did not influence cell viability (less than 10% reduction of viability at 50 μM concentration), were further progressed. Compounds with MIC values higher than 64 μg/mL were considered antimicrobially inactive based on microbiological susceptibility breakpoints[19] for macrolide antibiotics as well as pharmacokinetic data found in literature which suggests that this concentration is above the maximal concentration achieved after standard and chronic azithromycin treatment. Namely, C_{max} of azithromycin in plasma and blood of CF patients following long-term treatment with azithromycin (500 mg daily) was 0.67 ± 0.31 and 2.01 ± 0.74 mg/L, respectively.[20] In addition, the 2 g azithromycin extended release formulation achieved C_{max} of 0.94 and 3.2 mg/L in serum and epithelial lining fluid, respectively.[21] As effective concentrations are substantially lower, compounds are not tested above this breakpoint level as it is considered biologically irrelevant. Physicochemical properties were determined for a diverse subset from this library in order to establish a model for the prediction of in vitro activities.

Results from antibacterial activity screening showed that around 30% of all prepared macrolides still exhibit rather weak antibacterial activity while the rest had MIC values greater than 64 μg/mL (Table S1, Supporting Information). Complete suppression of antibacterial activity was best achieved with the clarithromycin 9a-lactam **1** scaffold because all 2′-*O*,3′-*N*-carbonimidoyl bridged clarithromycin-9a-lactams (compounds derived from **10**) have MIC values >64 μg/mL. The antibacterial activity of clarithromycin (**2**) and erythromycin (**5**) derivatives can also be successfully suppressed by 2′-*O*,3′-*N*-carbonimidoyl bridging in most (∼90%) cases. On the other hand, suppression of antibacterial activity on 9a- or 8a-azalide scaffolds (compounds derived from **12** and **17**) was less successful because ∼45% of such 2′-*O*,3′-*N*-carbonimidoyl bridged compounds had some MIC values below 64 μg/mL, including three compounds showing an MIC = 1 μg/mL against *S. pneumoniae*. Similar results, ∼60% success in suppression of antibacterial activity, were obtained for compounds derived from 9-hydroxy scaffolds **15** and **16**. Interestingly, 2′-*O*,3′-*N*-carbonimidoyl bridging of rearranged compounds **77**−**79** and **20** could not completely suppress their antibacterial activity (some MICs = 8 μg/mL). The above results suggest that 2-imino-1,3-oxazolidine condensation to desosamine is an easy and efficient way of suppressing or at least diminishing antibacterial activities of standard antibacterial macrolides.

Less than a half of the synthesized macrolides (23 compounds) had no significant effect on viability of THP-1 cells. Of the 18 macrolides that passed both antibacterial and

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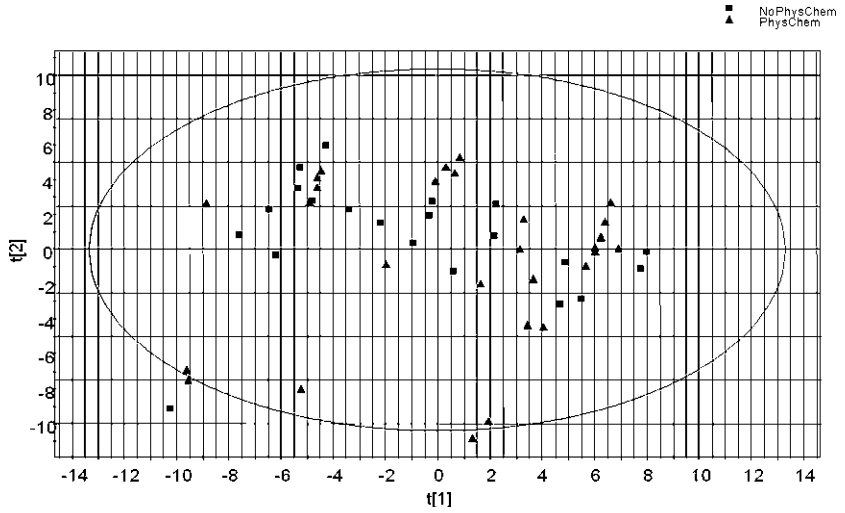

Figure 2. PCA analysis of synthesized compounds. A group of 28 diverse compounds were selected for physicochemical profiling (shown in triangles).

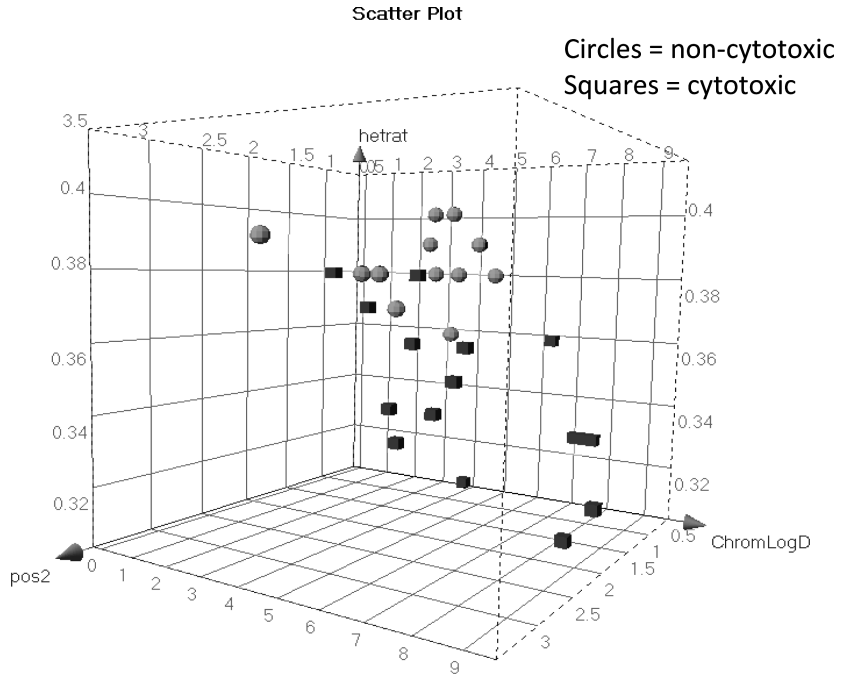

Figure 3. Analysis of THP-1 viability in terms of ChromlogD, pos, and hetrat descriptors.

THP-1 screening, 10 compounds also showed a desired level of IL-6 inhibition (Table S1, Supporting Information).

In an attempt to establish a correlation between physicochemical properties of the novel macrolide derivatives, their cytotoxic effect and IL-6 inhibition, a group of 28 diverse compounds, out of all compounds synthesized, were selected for physicochemical profiling (Figure 2). Compounds were further described applying simple properties and 2D descriptors as described in the Experimental Section (Table S2, Supporting Informations).

Experimentally obtained values for the physicochemical properties, together with calculated descriptors, were used to analyze and explain the effect of these compounds on THP-1 cell viability and inhibition of IL-6 production. Reliable separation between compounds which do and do not influence cell viability of THP-1 cells was achieved by using ChromlogD, pos (number of positively ionizable atoms), and hetrat (ratio of polar N, O, S vs C atoms in molecule) descriptors (Figure 3).

More polar compounds with a higher hetrat ratio, lower ChromlogD, and lower pos tended to have weaker effect on cell viability. As ChromlogD and pos variables are mutually dependent, compounds with lower lipophilicity can have a larger number of positively ionizable atoms. To develop quantitative models that can predict influence of compounds on THP-1 cell viability, partial least-squares (PLS) analysis was done using all generated descriptors. A two component model was developed. The number of descriptors was reduced by eliminating nonsignificant ones, improving Q^2 and stability of the generated PLS models. Two chromatographic lipophilicity measures turned out to have the largest impact on the THP-1 cell viability: ChromlogD and HSA %binding (binding to human serum albumin) as shown in Figure 4. An increase in

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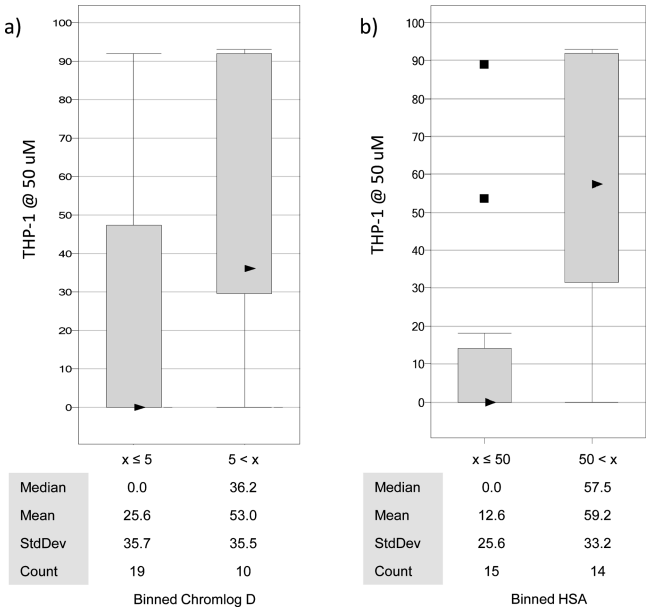

Figure 4. Box plot analysis of influence of (a) ChromlogD (ChromlogD <5, and ChromlogD >5) and (b) HSA %binding data (HSA %binding <50 and HSA %binding >50) on THP-1 cell viability for the set of oxazolidine compounds.

lipophilicity clearly decreases THP-1 cell viability. Better statistics were obtained with HSA %binding as a lipophilicity measure. It was shown previously that biomimetic chromatographic partition systems may prove to be better models then standard log *P*/log *D* variants[22] as it was found in this case as well.

A quantitative PLS model for THP-1 cell viability was developed with four descriptors (Figure 5): HSA %binding as a

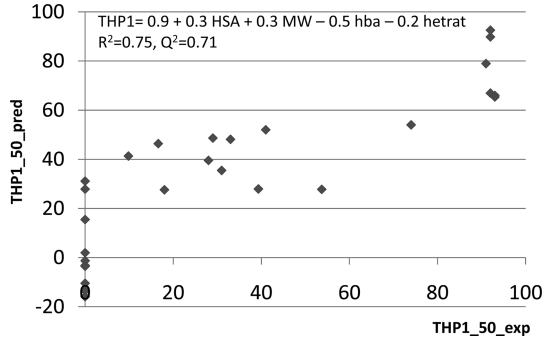

Figure 5. PLS model for THP-1 cell viability based on calculated and physicochemical descriptors.

measure of lipophilicity, MV as a measure of molecular size, number of hydrogen bond acceptors (hba), and hetrat ratio. The first two descriptors contribute positively to THP-1 cytotoxicity, while the last two describe the lipophilic character of molecules that decrease effect on THP-1 cell viability of studied compounds. Whereas this model exhibited significant accuracy in prediction (R^2 = 0.75 Q^2 = 0.77), an analogous model was developed using ChromlogD as a main lipophilicity measure, with somewhat lower statistical significance (R^2 = 0.52 Q^2 = 0.45).

Available THP-1 data were further compared with IL-6 inhibition (Figure S1, Supporting Informations) showing that THP-1 cell viability overlaps with IL-6 inhibition, i.e., compounds that decrease cell viability also produce artificial IL-6 inhibition. We were interested in compounds that show significant inhibition of IL-6 without effect on cell viability. There are 12 such compounds in our compound set, with measured physicochemical properties, which were used to build up the model for IL-6 inhibition.

A PLS model for IL-6 inhibition based on measured and calculated descriptors was developed. Experimental vs calculated IL-6 values and coefficient contribution of most important descriptors are shown in Figure 6. As in the case of THP-1 cell

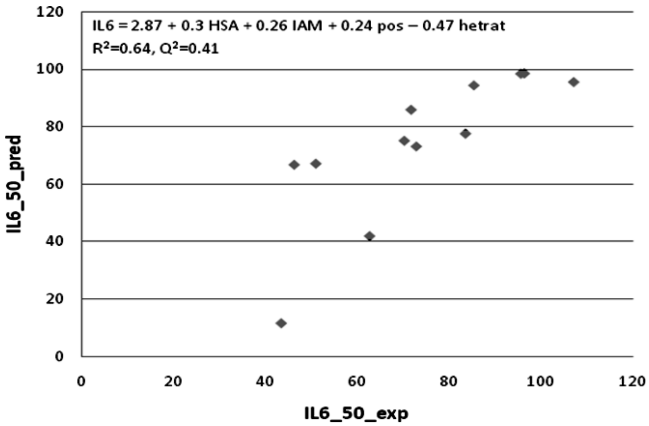

Figure 6. PLS model for IL-6 inhibition based on calculated and measured descriptors.

viability, the main parameter governing IL-6 inhibition is lipophilicity expressed as ChromlogD or HSA %binding, with HSA giving better correlations. Additional descriptors are CHI IAM (chromatographic hydrophobicity indices obtained on immobilized artificial membrane) lipophilicity, number of hetero atoms in the molecule, and number of positive charges. CHI IAM lipophilicity and number of positive charges contributes positively, while number of hetero atoms contributes negatively to the IL-6 model.

The developed models were externally validated on a set of 10 standard and subsequently synthesized compounds structurally different from the structures in the training set. Very good predictions were obtained for IL-6, confirming stability and prediction power of the developed model (Figure 7). Somewhat less accurate predictions were obtained for THP-1, with four compounds out of 10 incorrectly classified as toxic while being nontoxic. However, only one compound had

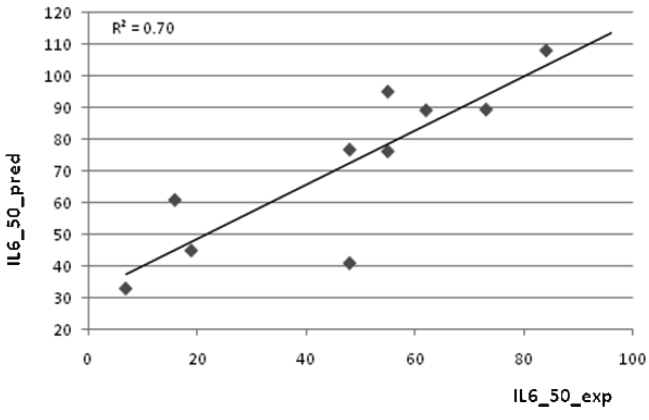

Figure 7. External validation of the IL-6 model.

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Table 1. Combined in Vitro Screening Data for 10 Best Compounds (AZI = Azithromycin)

	antibacterial activity (MIC, μg/mL)					THP-1 reduction of viability (%)	IL-6 inhibition (%)			CYP3A4 (IC_{50}, μM)	
compd	*S. aureus*	*S. penumoniae*	*S. pyogenes*	*M. catarrhalis*	*H. influenzae*	50 μM	50 μM	25 μM	12 μM	DEF	7BQ
AZI	0.5	<0.125	<0.125	<0.125	2	0	84	74	61	>10	>10
32			>64			0	73	64	55		2.9
33			>64			0	99	96	86	7.9	>10
34			>64			0	86	80	70	0.5	5.7
38			>64			9	95	90	79	7.8	>10
39			>64			0	67	55	45	1.0	>10
40			>64			0	94	81	61	1.1	9.8
44			>64			0	86	62	52	1.4	>10
54			>64			6	71	44	39	0.7	4.7
73			>64			0	74	62	55	0.7	8
74			>64			0	67	48	36	1.33	>10

significant error in prediction while three others are within the error of the model (<30%). It is also important to say that validation set was biased toward nontoxic compounds that were overestimated in the initial model.

Screening results for this focused library and corresponding developed PLS models for THP-1 cell viability and IL-6 inhibition will be used to design the next generation of anti-inflammatory macrolides.

At this point, 10 molecules fulfilled the required criteria for further progression toward anti-inflammatory candidates (Table 1). Compounds were screened in for their potential to inhibit the CYP3A4 recombinant human enzyme in a fluorimetry based high-throughput inhibition assay, in order to filter out molecules with high risk for drug−drug interactions (DDI). On the basis of their inhibition potential, within our organization, we typically classify compounds as follows: very high risk IC_{50} < 1 μM, high risk IC_{50} 1−5 μM, moderate risk IC_{50} 5−10 μM, and low risk IC_{50} > 10 μM for DDI. Results from the recombinant CYP inhibition screen are used to filter out potent inhibitors and are crosschecked later on in human liver microsomes using multiple probes. Within this program, 5 μM was selected as a cutoff value to filter compounds with high risk for DDI.[23] Screening of these 10 molecules against CYP3A4 inhibition revealed that only two molecules (**33** and **38**) fulfilled this additional criterion (Table 1). Furthermore, activity of the selected macrolides **33** and **38** was examined in an in vivo model of LPS-induced pulmonary neutrophilia and compared to standard macrolide antibiotic azithromycin (Figure 8).

Compound **38** dose dependently reduced total cell and neutrophil numbers in bronchoalveolar lavage fluid (Figure 8c,d). Compound **33** had lower anti-inflammatory activity in this animal model (Figure 8a,b). Accordingly, compound **38** was selected as the most promising anti-inflammatory compound from this novel macrolide class.

Compound **38** was further extensively studied in an in vivo model of LPS-induced pulmonary neutrophilia. In line with previously published results for azithromycin,[24] inhibition of LPS-induced pulmonary neutrophilia by compound **38** seems to be mediated through inhibition of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 β (IL-1β), chemokine (C-X-C motif) ligand 2 (CXCL2), tumor necrosis factor α (TNFα), IL-6, and chemokine (C−C motif) ligand 2 (CCL2) production (Figure 9). All of these cytokines are reported to be involved in activation, recruitment, and survival of neutrophils (Figure 9). Therefore, inhibition of these cytokines would be considered beneficial in the suppression of neutrophil-dominated inflammatory disorders.

Molecular mechanism underlying macrolide anti-inflammatory activity has not been unambiguously elucidated yet. However, many studies performed in the last two decades suggest that the activity of a number of molecules belonging to the 14- and 15-membered ring macrolide class is mediated through inhibition of nuclear factor kappa B (NFκB), activator protein 1 (AP-1), and extracellular signal-regulated kinase 1/2 (ERK1/2).[25] Moreover, we have suggested that the inhibition of LPS-induced neutrophil accumulation in lungs by azithromycin is primarily caused by inhibition of IL-1β and AP-1 in alveolar macrophages.[26] Because azithromycin[24] and compound **38** have similar patterns of cytokine inhibition in LPS-induced pulmonary neutrophilia, one can assume that the anti-inflammatory effects of both compounds are mediated through the same molecular mechanism, shared with other 14- and 15-membered ring macrolides.

To further estimate suitability of compound **38** for possible development as a drug candidate, in vivo disposition kinetics and bioavailability for compound **38** were evaluated in Balb/c mice and Sprague−Dawley rats. As shown in Table 2, compound **38** showed an encouraging profile in both species. It is characterized by a low systemic clearance (ca. 22% and 14% of liver blood flow in mice and rats, respectively), a large volume of distribution, indicating extensive distribution into tissues, and a prolonged half-life of ca. 17 h in mice and ca. 20 h in rats. Following oral administration, compound **38** showed a good bioavailability in mice (62%) and in rats (33%). Allometric scaling to humans with these data indicated the potential to achieve once daily oral dosing. The pharmacokinetic profile for compound **38** in rodents closely resembles profiles observed with the macrolide antibiotic azithromycin in rodents (internal data) and justifies its progression.

CONCLUSION

In this study, we have examined antibacterial and anti-inflammatory effects of a novel macrolide class: *N*′-substituted 2′-*O*,3′-*N*-carbonimidoyl bridged erythromycin-derived 14- and 15-membered macrolides. A small focused library of 60 compounds was prepared and tested in a screening cascade designed to select compounds that inhibit IL-6 production yet be without antibacterial activity and effects on THP-1 cell viability. Analysis of experimental results resulted in PLS

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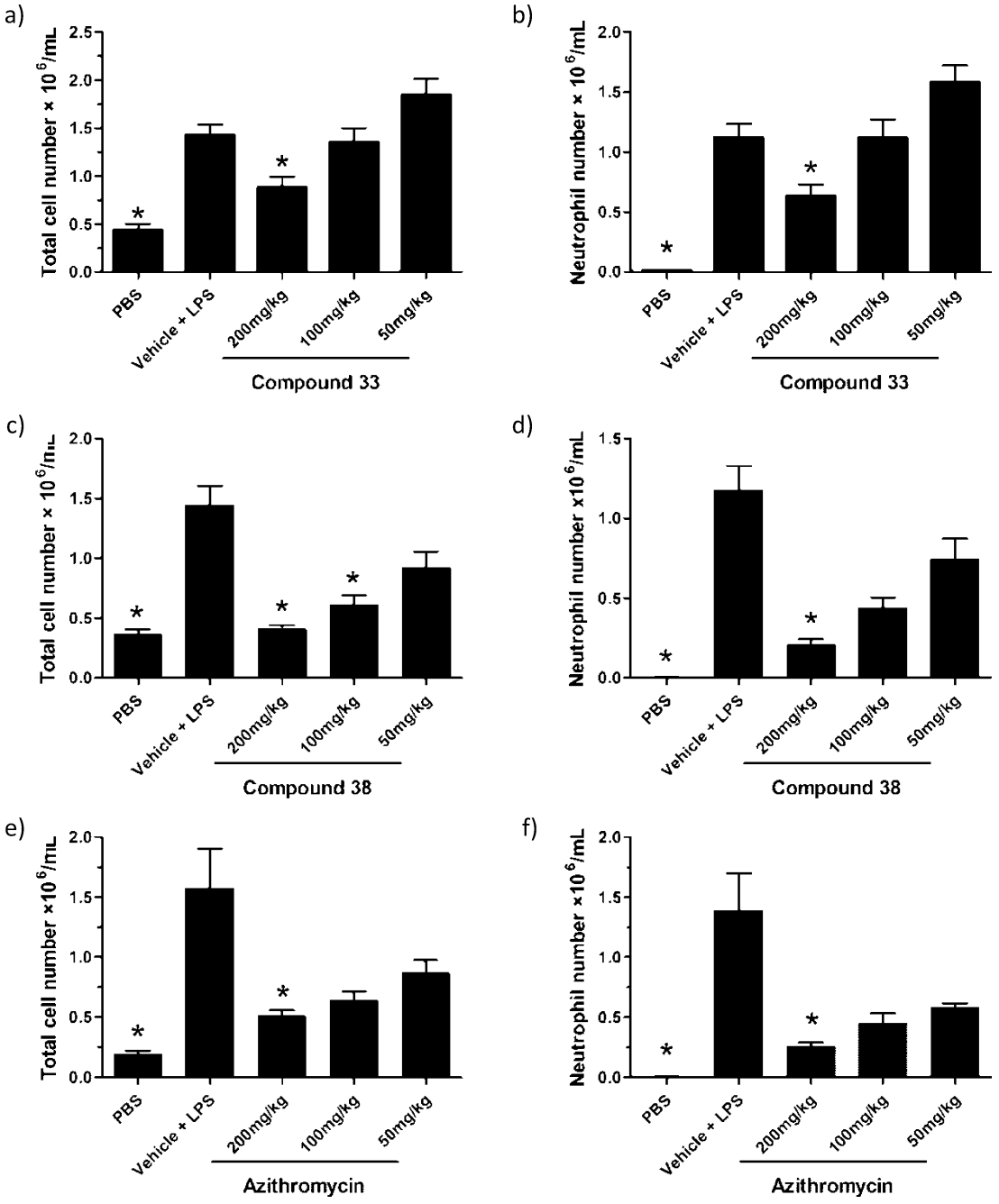

Figure 8. Total cell (a, c, and e) and neutrophil (b, d, and f) number in bronchoalveolar lavage fluid (BALF) 24 h after LPS challenge. Compounds were administered intraperitoneally 2 h prior LPS challenge at a dose of 200 mg/kg. Data are presented as means ± SEM of eight animals per group Asterisk represents $p < 0.05$, Kruskal–Wallis test followed by Dunn's multiple comparison test.

models that can be used for the design of next generation anti-inflammatory macrolides. Compound **38** (2′-*O*,3′-*N*-(*N*′-isopropylcarbonimidoyl)-3′-*N*-demethyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A) was selected as the most promising representative and has been extensively studied in an in vivo model of LPS-induced pulmonary neutrophilia. In accordance with the results for azithromycin, inhibition of LPS-induced pulmonary neutrophilia by compound **38** seems to be mediated through inhibition of GM-CSF, IL-1β, CXCL2, TNFα, IL-6, and CCL2. Pharmacokinetic studies in rodents revealed that compound **38** has good oral bioavailability and a half-life that supports once-daily dosing in humans. In summary, the described novel antimicrobially inactive macrolide derivative **38** retained the anti-inflammatory activity of azithromycin, thereby demonstrating that these two types of activities are independent and can be separated. Such finding provides basis for the development of novel anti-inflammatory agents, which will complement existing anti-inflammatory therapeutics and significantly broaden treatment possibilities.

EXPERIMENTAL SECTION

Chemistry. All solvents and reagents were used as supplied, unless noted otherwise. Mass spectra were recorded on Varian MAT 311 instrument (FAB) and Platform LCZ or LCQ Deca instruments (ESI). HRMS (ESI) were recorded on Micromass Qtof2. NMR spectra were recorded at 25 °C in $CDCl_3$ and DMSO-d_6 with TMS as the internal standard on Bruker Avance DRX500 and Bruker Avance III 600

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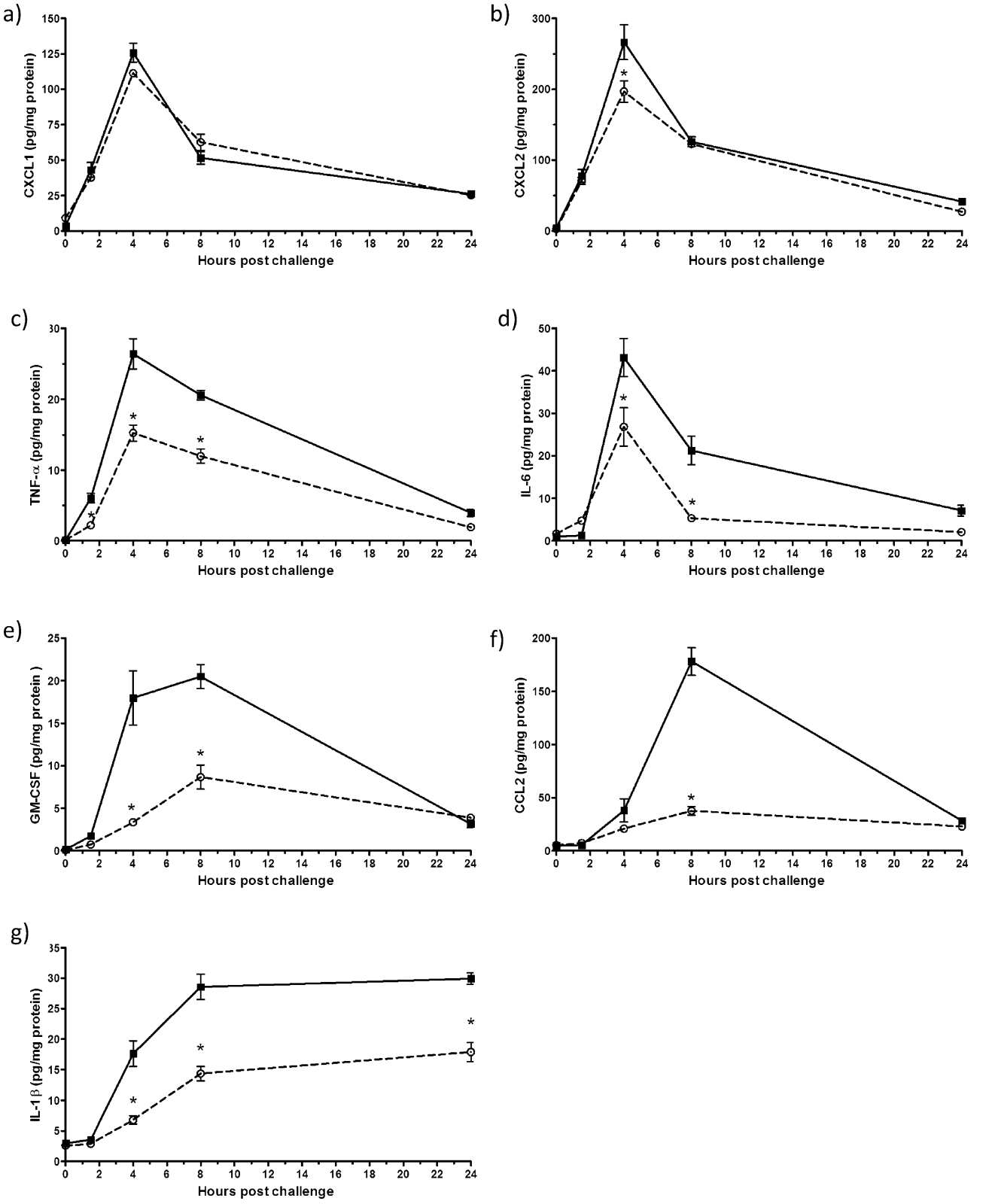

Figure 9. Concentration of CXCL1 (a), CXCL2 (b), TNF-α (c), IL-6 (d), GM-CSF (e), CCL2 (f), and IL-1β (g) in lung homogenates at different time points after LPS challenge (solid squares). Compound **38** (open circles) was administered intraperitoneally 2 h prior LPS challenge at a dose of 200 mg/kg. Data are presented as means ± SEM of five animals per group Asterisk represents $p < 0.05$, Two-way ANOVA; Bonferroni post test.

spectrometers equipped with 5 mm diameter inverse detection probe with *z*-gradient accessory, as well as Bruker Avance DPX300 spectrometer using dual $^{1}H/^{13}C$ probe. For characterization of complex organic structures, one-dimensional (1D) (^{1}H and APT) and two-dimensional (2D) (COSY, HMQC and HMBC) NMR techniques were used. The purity of all prepared compounds was determined as ≥95% using HPLC-MS method.

Detailed descriptions of compounds **36**−**38**, **46**−**48**, **55**−**58**, **60**−**62**, and **64**−**67** have been given in ref 17. Detailed characterization of the other 43 compounds can be found in Supporting Information. 3′-

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Table 2. Pharmacokinetic Properties (CL, Clearance; Vss, Volume of Distribution; $t_{1/2}$, Half-Life; *F*, Bioavailability) of Compound 38. Data are presented as means ± S.D. of 3 mice per timepont and 3 rats per group

	CL (mL/min/kg)	Vss (L/kg)	$t_{1/2}$ (h)	oral *F* (%)
mouse	19.8 ± 1.1	23 ± 1.8	17.3 ± 1.5	62 ± 10.5
rat	12.3 ± 1.9	8.6 ± 1.3	20.3 ± 6.2	33 ± 5

N-Demethylated compounds **10**−**19** were prepared using described procedures.[17,27] A general procedure for 2′-*O*,3′-*N*-carboinimidoyl formation is provided. The procedures for the synthesis of **33** and **38** as well as key intermediate **12** are described in details bellow.

*3′-N-Demethyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**12**).* A solution of **3** (10 g, 13.3 mmol) and *N*-iodosuccinimide (7.5 g, 33.3 mmol) in acetonitrile (250 mL) was stirred at room temperature for 1.5 h. Acetonitrile was evaporated and solid residue dissolved in CH_2Cl_2 (300 mL), washed with saturated aqueous Na_2SO_3 (5 × 70 mL) and saturated aqueous $NaHCO_3$ (5 × 70 mL), dried over Na_2SO_4, and evaporated to afford 10.8 g of yellowish solid. The solid residue was dissolved in the mixture of water (100 mL) and CH_2Cl_2 (50 mL), pH was adjusted to 4.1, and layers separated. Extraction at pH 4.1 was repeated two times with CH_2Cl_2 (2 × 50 mL). To the aqueous layer, fresh CH_2Cl_2 (200 mL) was added, pH was adjusted to 9.3, and then extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts at pH 9.3 were dried over Na_2SO_4 and evaporated to afford title product (8.8 g, purity = 89% according to LC-MS) as a white solid, which was used in subsequent reactions without further purification. MS (ES+) *m*/*z*: 735.2 $[MH]^+$.

General Procedure for 2′-O,3′-N-Carbonimidoyl bridging:[17] To a solution of 3′-*N*-demethyl derivatives **10**−**19** in acetonitrile (*c* = 0.02 g/mL), corresponding isothiocyanate (3 equiv) and triethylamine (6 equiv) were added. The reaction was stirred at 60 °C for 2 h, and then EDC (4 equiv) was added. The reaction mixture was further stirred at 60 °C for 24−48 h. Completion of the reaction was checked by LC-MS. Solvent was evaporated and the residue purified on the Flashmaster personal solid-phase extraction techniques (eluents 1.5% $MeOH/CH_2Cl_2$−$MeOH/CH_2Cl_2/NH_4OH$ (4.5:90:0.25)) to afford chromatographically homogeneous fractions of title products.

*2′-O,3′-N-(N′-Ethylcarbonimidoyl)-3′-N-demethyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**33**).* According to the general procedure for sequential one-pot synthesis, reaction of **12** (0.9 g, 1.23 mmol) and ethyl isothiocyanate afforded **33** (0.31 g, 32%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.07 (1″-H), 4.91 (1′-H), 4.68 (13-H), 4.22 (3-H), 4.05 (5″-H), 3.70 (5′-H), 3.66 (11-H), 3.66 (5-H), 3.55 (2′-H), 3.36 (C═N-C$\underline{H}_2$-CH_3), 3.27 (3″-OCH_3), 3.07 (4″-H), 2.77 (3′-H), 2.80 (2-H), 2.72 (10-H), 2.70 (3′-NCH_3), 2.53 (9-H_a), 2.35 (2″-H_a), 2.35 (9a-NCH_3), 2.09 (9-H_b), 1.97 (8-H), 1.97 (4-H), 1.92 (4′-H_a), 1.88 (14-H_a), 1.74 (7-H_a), 1.61 (2″-H_b), 1.51 (14-H_b), 1.42 (4′-H_b), 1.33 (6-CH_3), 1.31 (5″-CH_3), 1.30 (5′-CH_3), 1.26 (7-H_b), 1.26 (3″-CH_3), 1.22 (2-CH_3), 1.13 (C═N-CH_2-$C\underline{H}_3$)1.13 (10-CH_3), 1.10 (12-CH_3), 1.00 (4-CH_3), 0.93 (8-CH_3), 0.89 (15-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.5 (1-C), 155.9 (C═N), 99.9 (1′-C), 95.6 (1″-C), 84.8 (5-C), 79.9 (2′-C), 79.0 (3-C), 77.9 (4″-C), 77.5 (13-C), 74.5 (11-C), 74.2 (12-C), 73.3 (3″-C), 73.2 (6-C), 70.1 (5′-C), 70.1 (9-C), 65.7 (5″-C), 62.8 (3′-C), 62.1 (10-C), 49.3 (3″-OCH_3), 44.9 (2-C), 41.9 (7-C), 40.7 (4-C), 40.6 (C═N-$\underline{C}H_2$-CH_3), 36.4 (9a-NCH_3), 36.4 (4′-C), 35.3 (2″-C), 32.1 (3′-NCH_3), 27.1 (6-CH_3), 26.6 (8-C), 21.9 (8-CH_3), 21.5 (3″-CH_3), 20.9 (14-C), 20.7 (5′-CH_3), 18.0 (5″-CH_3), 16.6 (C═N-CH_2-$\underline{C}H_3$)16.1 (12-CH_3), 15.3 (2-CH_3), 10.1 (15-C), 8.5 (4-CH_3), 7.3 (10-CH_3) ppm. HRMS (ES) calcd for C_{40} $H_{73}N_3O_{12}$ (M + H^+) 788.5277, found 788.5301.

*2′-O,3′-N-(N′-Isopropylcarbonimidoyl)-3′-N-demethyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**38**).* According to the general procedure for sequential one-pot synthesis, reaction of **12** (0.47 g, 0.64 mmol) and isopropyl isothiocyanate afforded **38** (0.29 g, 57%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.09 (1″-H), 4.92 (1′-H), 4.68 (13-H), 4.19 (3-H), 4.05 (5″-H), 3.81 (C═N-C$\underline{H}$-$(CH_3)_2$), 3.69 (5′-H), 3.64 (11-H), 3.63 (5-H), 3.61 (2′-H), 3.27 (3″-OCH_3), 3.07 (4″-H), 2.82 (3′-H), 2.80 (2-H), 2.78 (3′-NCH_3), 2.70 (10-H), 2.54 (9-H_a), 2.39 (2″-H_a), 2.33 (9a-NCH_3), 2.13 (9-H_b), 2.00 (8-H), 1.98 (4′-H_a), 1.96 (4-H), 1.89 (14-H_a), 1.72 (7-H_a), 1.60 (2″-H_b), 1.47 (14-H_b), 1.42 (4′-H_b), 1.32 (6-CH_3), 1.30 (5″-CH_3), 1.28 (5′-CH_3), 1.28 (7-H_b), 1.23 (3″-CH_3), 1.20 (2-CH_3), 1.16 (C═N-CH-($C\underline{H}_3$)$_2$), 1.11 (12-CH_3), 1.08 (10-CH_3), 1.06 (C═N-CH-($C\underline{H}_3$)$_2$), 0.97 (4-CH_3), 0.94 (8-CH_3), 0.89 (15-CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.7 (1-C), 155.9 (C═N), 99.9 (1′-C), 95.3 (1″-C), 84.9 (5-C), 80.4 (2′-C), 78.8 (3-C), 77.6 (4″-C), 77.5 (13-C), 74.3 (11-C), 73.3 (6-C), 73.2 (3″-C), 74.2 (12-C), 70.2 (5′-C), 70.1 (9-C), 65.8 (5″-C), 62.9 (3′-C), 62.4 (10-C), 49.4 (3″-OCH_3), 46.8 (C═N-$\underline{C}$H-$(CH_3)_2$), 45.1 (2-C), 41.9 (7-C), 41.0 (4-C), 36.5 (9a-NCH_3), 36.4 (4′-C), 34.8 (2″-C), 32.3 (3′-NCH_3), 27.3 (6-CH_3), 26.7 (8-C), 24.3 (C═N-CH-($\underline{C}H_3$)$_2$), 24.2 (C═N−CH-($\underline{C}H_3$)$_2$), 21.9 (8-CH_3), 21.6 (3″-CH_3), 21.1 (14-C), 20.8 (5′-CH_3), 18.1 (5″-CH_3), 16.2 (12-CH_3), 15.2 (2-CH_3), 11.2 (15-CH_3), 8.5 (4-CH_3), 7.3 (10-CH_3) ppm. HRMS (ES) calcd for C_{41} $H_{75}N_3O_{12}$ (M + H^+) 802.5429, found 802.5444.

Physicochemical Measurements. Chromatographic hydrophobicity index (CHI) log *D* and CHI immobilized artificial membrane (IAM) values were determined by using fast-gradient high-performance liquid chromatographic (HPLC) method and MS as detector on the Waters Early Candidate Profiling (ECP) 4-way MUX LC/MS system (Waters Corp, UK). The CHI[28] values were determined using Luna C18(2) (Phenomenex, UK) HPLC columns. The CHI IAM[29] values were measured using an immobilized artificial membrane column (IAM PC DD column from Regis Analytical, West Lafayette, IL, USA) with fast acetonitrile gradient at starting mobile phase of pH = 7.4. CHI values were derived directly from a gradient reversed phase chromatographic retention time by using a calibration line obtained for standard compounds, separately for each considered property. The CHI value approximates to the volume % organic concentration when the compound elutes. CHI was linearly transformed into ChromlogD by least-squares fitting experimental CHI values to calculated ClogP values for over 20K research compounds using the following formula: ChromlogD = 0.0857CHI-2.00. Chemically bonded human serum albumin (HSA) and α-1-acidglycoprotein stationary phases were used in the remaining two channels of the 4-way HPLC/MS system. Samples were injected onto the four HPLC columns from the same sample solution at the same time. Isopropyl alcohol gradient was used on the protein column up to 30% using linear gradient. The run time was 6 min including the re-equilibration of the stationary phases with the 50 mM pH 7.4 ammonium acetate buffer. As most of the macrolides do not contain UV chromophore, it was essential to use the mass spectrometric detection of the peak retention times. The obtained gradient retention times were standardized using a calibration set of mixtures as described in the references (CHI,[28] IAM,[29] HSA/AGP[30]).

Statistical Analysis. Descriptive statistics and graphical analysis were done by SpotFire DecisionSite software, 8.2.1 (2006, Spotfire, Inc., US, http://spotfire.tibco.com). Data were analyzed by principal component analysis (PCA) and partial least-squares (PLS) techniques implemented within SIMCA-P software 11.0 (Umetrics, Umeå, Sweden). All descriptors were scaled to unit variance. PLS analysis was done using all generated descriptors. Two component models were developed. The number of descriptors was reduced by sequentially eliminating nonsignificant ones, improving Q^2 and stability of the generated PLS models. Elimination of descriptors was based on the descriptor coefficients and their importance in the projection (VIP). VIP is defined as a sum over all model dimensions of the variable contributions (VIN). For a given PLS dimension, *a*, $(VIN)_{ak}^2$ is equal to the squared PLS weight $(w_{ak})^2$ of that term, multiplied by the explained sum-of-squares of that PLS dimension. Descriptors with large VIP, larger than 1, are the most relevant for explaining *Y*. Models were validated by random permutation of dependent variables and by leave-four-out cross-validation used to calculate uncertainty measures (standard errors and confidence intervals) of scores, loadings, PLS-regression coefficients, predicted *Y*-values, and important variables.

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All methods were employed with default settings within corresponding software. Biological properties of macrolides were analyzed and modeled in terms of various experimentally determined physicochemical and calculated descriptors. A number of two-dimensional (2D) structural descriptors was calculated, like counts of H-bond donor (hbd) and acceptor (hba) atoms, count of positively charged atoms (pos), ratio of number of polar atoms (O,N,S), and number of carbon atoms (hetrat), atom-based E-state descriptors[31] as well as Abraham descriptors (solute excess molar refractivity ($R2$), dipolarity/polarizability (π), summation hydrogen bond acidity (α) and basicity (β), and the McGowan characteristic volume (Vx).[32] Because of macrolide complex structure, calculation of 3D descriptors is time-consuming and not applicable for a larger number of compounds. Available force fields are not able to reliably reproduce conformations of macrocyclic ring and flexible substituents. All employed descriptors were either measured or calculated by the in-house developed approaches.

Biology. *Materials: Chemicals, Antibodies and Drugs.* LPS from *Escherichia coli* serotype 0111:B4 was obtained from Sigma Chemical Co. (St Louis, MO, US). Luminex kits were purchased from R&D Systems (Minneapolis, MN, US). Azithromycin dihydrate was from PLIVA Inc. (Zagreb, Croatia). All other reagents, if not indicated otherwise, were from Sigma Chemical Co. (St Louis, MO, US).

Antimicrobial Activity. Whole-cell antimicrobial activity of compounds against *Staphylococcus aureus* (ATCC13709), *Streptococcus pneumoniae* (ATCC49619), *Streptococcus pyogenes* (ATCC700294), *Moraxella catarrhalis* (ATCC23246), and *Haemophilus influenzae* (ATCC49247) was determined by broth microdilution test using the Clinical and Laboratory Standards (CLSI) recommended procedure (Document M7-A6A7, Methods for Dilution Susceptibility Tests for Bacteria that Grow Aerobically). The minimum inhibitory concentration (MIC) is determined as the lowest concentration of compound that inhibited visible growth.

Cell Viability. THP-1 cells (TIB-202, ATCC) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in the presence of test compounds at concentration of 50 μM for 24 h. Afterward, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] (Promega, US), a detection reagent, was added and cells were incubated for an additional 0.5−2 h. The amount of MTS-formazan produced was determined using a spectrophotometer at 490 nm.[33]

Percentage of inhibition of cell viability was calculated using the following formula:

$$\%\text{inhibition of cell viability} = (OD_{490}\text{treated cells}/OD_{490}\text{untreated cells}) \times 100$$

LPS-Induced IL-6 Production by Murine Splenocytes. After cervical dislocation, mouse spleens were removed by using sterile dissection tools. Spleens were transferred to a prewetted cell strainer in a 50 mL sterile conical tube, and cell suspension was made by gentle puddle. Cells were centrifuged (20 min, 300*g*) and resuspended in 2 mL of sterile phosphate buffered saline (PBS) (Sigma Chemical Co., USA). Red blood cells were lysed by addition of 3 mL of sterile water and occasionally gentle shaking for 1 min. Afterward, the tube was filled to 40 mL with DMEM medium and centrifuged (20 min, 300*g*). Cells were resuspended in DMEM supplemented with 1% FBS and seeded in a 24-well plate, 10^6 cells per mL medium. Cells were preincubated with the test compounds for 2 h at 37 °C in an atmosphere of 5% CO_2 and 90% humidity. Afterward, cells were stimulated with 1 μg/mL lipopolysaccharide (LPS, *E. coli* 0111:B4, Sigma Chemical Co., USA) and incubated overnight. Concentration of IL-6 was determined in cell supernatants by sandwich ELISA using capture and detection antibodies (R&D Systems, USA) according to the manufacturer's recommendations.

Inhibition (as percentage) was calculated using the following formula:

$$\%\text{inhibition} = [1 - (\text{concentration of IL-6 in sample} - \text{concentration of IL-6 in negative control}) / (\text{concentration of IL-6 in positive control} - \text{concentration of IL-6 in negative control})] \times 100$$

The positive control refers to LPS-stimulated samples that were not preincubated with the compounds. The negative control refers to unstimulated and untreated samples.

Animals. Studies were performed on 10-week-old male BALB/cJ mice and 7-week-old CD Sprague−Dawley rats (both from Charles River, Lyon, France). Mice and rats were kept on wire mesh floors with irradiated maize granulate bedding (Scobis Due, Mucedola, Settimo Milanese, Italy) and maintained under standard laboratory conditions (temperature 23−24 °C, relative humidity 60 ± 5%, 15 air changes per h, artificial lighting with circadian cycle of 12 h). Pelleted food (VRF-1, Altromin, Charles River, Isaszag, Hungary) and tap water were provided ad libitum.

All procedures on animals were approved by the ethics committee of GlaxoSmithKline Research Centre Zagreb Limited and performed in accordance with the European Economic Community Council Directive 86/609.

LPS-Induced Pulmonary Neutrophilia. Experimental pulmonary neutrophilia was induced as described earlier.[34] Briefly, mice (eight animals per treatment group in the experiments aimed at determination of cell counts in BALF and five animals per treatment group in the experiment where cytokine concentration in lung homogenates was determined), under light anesthesia, were instilled intranasally with 2 μg of LPS from *E. coli*/60 μL PBS. Vehicle, azithromycin, and compounds **33** and **38** were administered intraperitoneally 2 h before intranasal challenge with LPS at a dose of 200 mg/kg (body weight). We have previously demonstrated that 600 mg/kg po is an effective dose for inhibition of inflammation in this animal model.[24] On the basis of the oral bioavailability of 35−55% (internal data), the estimated effective dose of azithromycin after ip administration would be 200−300 mg/kg. The dosing regimen was chosen as the lowest effective dose according to the results of preliminary studies. For administration, macrolides were first dissolved in dimethlysulfoxide (DMSO) and then diluted with 0.5% (w/v) methylcellulose (final concentration of DMSO was 5% (v/v)). Solutions obtained were applied in a volume of 20 mL/kg (body weight).

Bronchoalveolar Lavage and Determination of Total and Relative Cell Number in Bronchoalveolar Lavage Fluid (BALF). The animals were euthanized by an intraperitoneal overdose of thiopental (Inresa Arzneimittel GmbH, Freiburg, Germany) 24 h after LPS application. After preparation and cannulation of tracheas, bronchoalveolar lavage was performed with phosphate buffered saline (PBS) in a total volume of 1 mL (0.4, 0.3, and 0.3 mL). The bronchoalveolar lavage samples were centrifuged (4 °C, 100*g*, 5 min) and the pellet of cells resuspended in an equal volume of fresh PBS and used for total and differential cell counts. Total number of cells in BALF was counted with a hematological analyzer (Sysmex SF 3000, Sysmex Co., Kobe, Japan). Percentages of neutrophils were determined by morphological examination of at least 200 cells on smears prepared by cytocentrifugation (Cytospin-3, Thermo Fisher Scientific Inc., Pittsburgh, PA, US) and stained with Kwik-Diff staining set (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). Number of neutrophils (and macrophages) in BALF was calculated for each sample according to the formula:

$$\text{number of neutrophils} = \text{total number of cells} \times (\text{neutrophil percentage}/100\%)$$

Preparation of Lung Homogenates for Determination of Cytokines. In separate groups of mice, nonlavaged lungs were collected immediately prior (0 h) or at various time points after LPS application for determination of the concentrations of cytokines.

Lungs were homogenized on ice in PBS with protease inhibitors (1 μg/mL leupeptin, 2 μg/mL aprotinin, 1 μg/mL pepstatin, and 17 μg/mL phenylmethylsulfonyl fluoride); 4 mL of PBS with protease inhibitors were added per gram of lung tissue. Homogenates were centrifuged (4 °C, 2500*g*, 15 min) and stored at −80 °C until analysis.

Determination of Protein Concentration. Protein concentration in lung homogenates was determined by BCA Protein Assay (Thermo Fisher Scientific Inc., Waltham, MA, US) according to the manufacturer's recommendation.

Measurement of Inflammatory Mediators in Lungs. Samples were analyzed using xMAP technology, which enables simultaneous measurement of multiple biomarkers. Concentrations of GM-CSF, IL-1β, IL-6, CCL2 (JE), CXCL1 (KC), CXCL2 (MIP-2), and TNF-α were determined using Fluorokine MAP multiplex kit (R&D Systems, Minneapolis, MN, US) according to the manufacturer's protocol. Briefly, 50 μL of samples were incubated with antibody-coated microparticles for 3 h at room temperature. Afterward, washed beads were incubated with biotinylated detection antibody cocktail for 1 h at room temperature, washed, and incubated for 30 min with streptavidin−phycoerythrin conjugate. After the final wash, the microparticles were resuspended in buffer and analyzed with the Luminex 200TM (Luminex, Austin, TX, US) and STarStation software v2.3 (Applied Cytometry Systems, Sacramento, CA, US) using a five-parameter-logistic-curve fitting.

Concentration of cytokines in lung homogenates was further normalized to protein concentration in the samples and expressed as pg of analyte per mg of protein.

Statistical Analysis. All values are presented as means ± SEM. To define statistically significant differences in cell numbers among vehicle-treated and macrolide-treated mice 24 h after LPS challenge, the data were subjected to Kruskal−Wallis test followed by Dunn's multiple comparison test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, US). To define statistically significant differences in cytokine concentrations among vehicle-treated and macrolide-treated mice at different time points after LPS challenge, the data were subjected to two-way ANOVA followed by Bonferroni post test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, US). The level of significance was set at $p < 0.05$ in all cases.

In Vitro CYP3A4 Inhibition. Test compounds were incubated using a concentration range from 0.1 to 10 μM with recombinant human CYP3A4 (Cypex Bactosomes, CYPEX, Dundee, UK). The compounds were preincubated ($n = 2$) at 37 °C for 5 or 10 min with the CYP 3A4 isoform in the presence of 50 mM phosphate buffer (pH 7.4) and two fluorescent probes (diethoxyfluorescein (DEF) and 7-benzyloxyquinoline (7-BQ)) in a 96-well plate. The incubations were started by addition of a cofactor solution (7.65 mg glucose-6-phosphate, 1.7 mg NADP, 6 units glucose-6-phosphate dehydrogenase per mL of 2% sodium bicarbonate) prepared freshly the day of the experiment. The plates (black polypropelene 96-well) were incubated at 37 °C for 10 min, and fluorescent readings were taken on a Tecan Infinite F500 plate reader (excitation wavelength: DEF 485 nm, 7BQ 405 nm; emission wavelength DEF 535 nm, 7BQ 535 nm; gain DEF 47 nm, 7BQ 50 nm) over a 10 min time-course. The fluorescence data were then used to calculate the IC_{50} values for each compound.

Pharmacokinetics. Pharmacokinetic profiles were determined in male Balb/c mice ($n = 3$/time point) and CD Sprague−Dawley rats ($n = 3$) following single oral and single intravenous administration. In the mouse, doses of 5 and 25 mg/kg were used of iv and po, respectively. In the rat, doses of 2 and 10 mg/kg were used for iv and po, respectively. For oral dosing, the compound was formulated as a solution in DMSO−PEG 200−PBS (10:20:70) and for intravenous dosing as a solution in DMSO/acetate buffer (0.1 M)/PBS (4:48:48). Profiles in the mouse were obtained by taking terminal bleeding over a range of time points up to 30 h postdose (three animals/time point). Profiles in the rat (3 animals/group) were obtained by serial blood sampling over a range of time points up to 30 h postdose. Blood samples were diluted with water (1:1), prepared by protein precipitation, and subjected to quantitative analysis by LCMS/MS using compound-specific mass transitions. Drug concentration−time profiles were generated and noncompartmental PK analysis (WinNonlin 4.2, Pharsight) used to generate estimates of half-life, clearance, volume of distribution, and oral bioavailability.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

In vitro data (antibacterial activity, THP-1 viability, IL-6 inhibition) for all *N*′-substituted-2′-*O*,3′-*N*-carbonimidoyl bridged macrolides prepared as well as detailed characterization of 2′-*O*-3′-*N*-carbonimidoyl bridged macrolides not reported previously. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

IL-6, interleukin-6; THP-1, human acute monocytic leukemia cell line; pos, number of positively ionizable atoms; hetrat, ratio of polar N, O, S vs C atoms in molecule; HSA %binding, binding to human serum albumin; CHI IAM, chromatographic hydrophobicity indices obtained on immobilized artificial membrane; MV, measure of molecular size; hba, number of hydrogen bond acceptors; drug−drug interactions; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1β, interleukin-1β; CXCL2, chemokine (C-X-C motif) ligand 2; TNFα, tumor necrosis factor α; CCL2, chemokine (C−C motif) ligand 2; BALF, bronchoalveolar lavage fluid

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