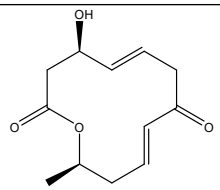
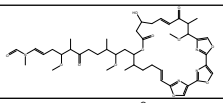
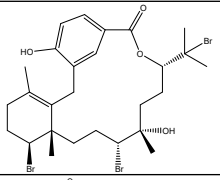
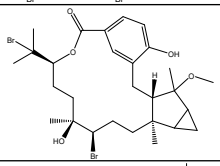
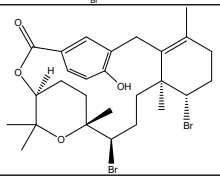
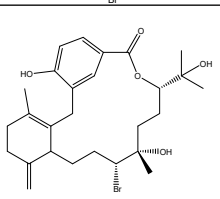
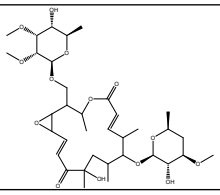
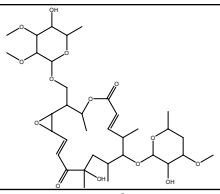
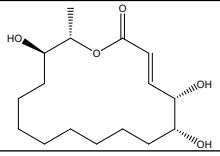
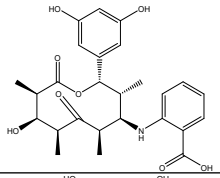
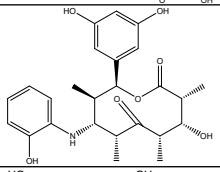
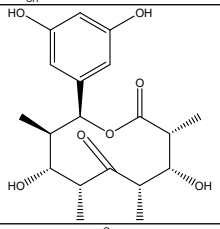
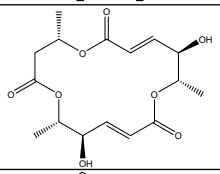
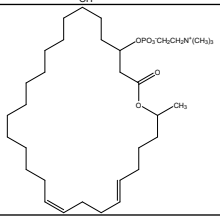
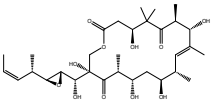
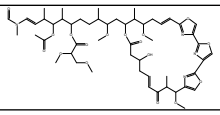
Electronic Supplementary Material (ESI) for RSC Advances.

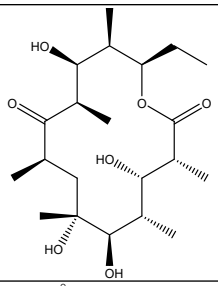
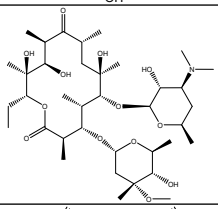
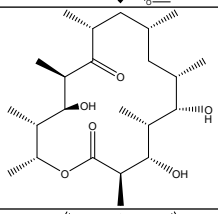
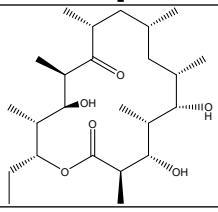
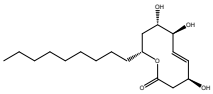
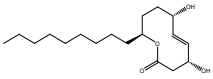
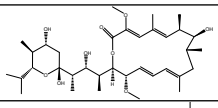
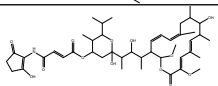
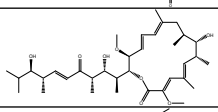
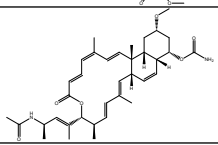
Lipid Polymer Hybrid Nanocarriers as a Combinatory Platform for Different Anti-SARS-CoV-2 Drugs Supported by Computational Studies

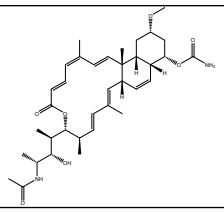
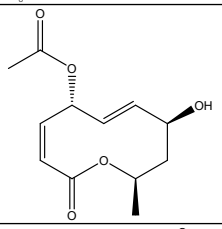
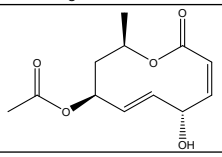
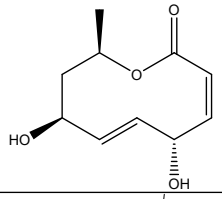
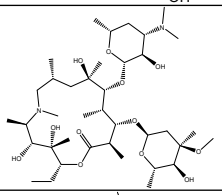
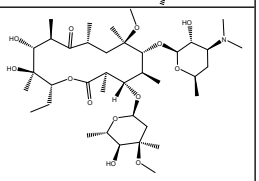
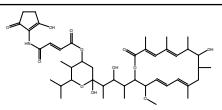
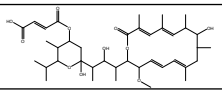
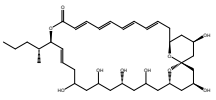
Supplementary Data

Table S1: Macrolides Bioactive Compounds with Amicrobial Activities

Name	Structure	Activity	Natural source	Ref.
Balticolid		Antiviral	*Ascomycetous* Fungus of Marine Origin	1
Halichondramide		Antiviral (anti-malarial)	Marine Sponges	2
Bromophycolide A		HIV strains 96USHIPS7 and UG/92/029 inhibition	red alga *Callophycus serratus*	3
Bromophycolide J		Antimalarial, anti-microbial and anticancer		
Bromophycolide Q				
Bromophycolide K				
Chalcomycin A		macrolide antibiotic	Marine Isolate *Streptomyces* sp. B7064	4

Chalcomycin B				
Aspergillide D			marine-derived Fungus *Aspergillus* sp.	5
Saccharothriolide A		cytotoxicity against human tumor cell lines HeLa and HT1080.	actinomycete *Saccharothrix* sp.	6
Saccharothriolide B				
Saccharothriolide C				
Macrosphelide A		effective against *Staph. aureus*	mycoparasite *Coniothyrium minitans*	7
Eushearilide		antifungal	*Eupenicillium shearii*	8
Tedanolide C		cytotoxicity against HCT-116 cells	Marine Sponge *Ircinia* sp	9
Mycalolide B		Antifungal and cytotoxic effects	Marine Sponge *Sarcotragus* Species	10

Erythronolide B		antibacterial	*Saccharopolyspora erythraea*	11
Erythromycin				
GW479439x				
GW479438x				
Seimatopolide A		activated PPAR-γ with an EC_{50} value of 1.15 μM treatment of diabetes.	*Seimatosporium discosioides*	12
Seimatopolide B		activated PPAR-γ with an EC_{50} value of 11.05 μM treatment of diabetes.		
Bafilomycin A1		macrolide antibiotics	*Streptomyces* sp. strain ZDB	13
Bafilomycin B1				
Bafilomycin D				
Superstolide B		cytotoxic macrolide	deep water sponge *Neosiphonia superste*	14

Superstolide A				
Modiolide D		cytotoxicity	*Microsphaeropsis arundinis*	15
Modiolide E				
Modiolide A				
Azithromycin		antibiotic	available from Nature via fermentation	16
Clarithromycin				
Makinolide		---	*Streptomyces* sp. MK-30	17
JBIR−100		----		
Akaemycin		Activity against Gram-positive bacteria and filamentous fungi	Marine *Streptomyces* sp.	18

Preparation of PLGA polymeric nanoparticles:

Different PLGA nanoparticles co-loaded with azithromycin and piroxicam (PLGA $NPs_{Azi-Pir}$) or niclosamide and piroxicam (PLGA $NPs_{Nic-Pir}$) were prepared by nanoprecipitation method [19]. Briefly, the organic phase was prepared by dissolving PLGA (5 mg/mL), piroxicam (0.75 mg/mL) and azithromycin (0.75 mg/mL) or niclosamide (0.75 mg/mL) into DMF (1 mL). Subsequently, the aqueous phase (9 mL) containing Tween® 80 (10 mg/mL) was titrated dropwise with the organic phase under magnetic stirring (500 rpm) for 2 h at room temperature. The PLGA NPs pellets were collected by ultrafiltration using Amicon® Ultrafilters (MWCO 10K, 12000 rpm for 45 min at 4 °C). The obtained formulations were re-dispersed in PBS (1 mL, pH 7.4) at stored at 4 °C for further analysis. For comparison sake, PLGA NPs loaded with azithromycin, niclosamide or piroxicam were prepared and assigned as PLGA NPs_{Azi}, PLGA NPs_{Nic}, and PLGA NPs_{Pir} respectively [20-26].

Table S2. Particle size, size distribution and zeta potential of the prepared PLGA NPs and LPH formulations.

Formulation	Particle size (nm)[a, c, d]	PDI [a, c, d]	Surface charge (mV) [b, c, d]
PLGA NPs_{Azi} [e]	75.32± 3.11*	0.15±0.02	-30.25± 3.15*
PLGA NPs_{Pir} [e]	69.25± 3.47*	0.19±0.01	-28.65± 4.12*
PLGA $NPs_{Azi-Pir}$ [e]	91.25± 4.52*	0.21±0.03	-26.54± 3.24*
PLGA NPs_{Nic} [e]	67.25± 3.14*	0.23±0.01	-29.35± 2.54*
PLGA $NPs_{Nic-Pir}$ [e]	95.36± 2.14*	0.21± 0.02	-28.33± 3.04*
LPH_{Azi} [f]	110.25± 3.54	0.25±0.02	-15.64± 2.51
LPH_{Pir} [f]	118.59± 4.57	0.18± 0.01	-17.65± 1.87
$LPH_{Azi-Pir}$ [f]	125.32± 7.51	0.17±0.02	-16.54± 2.45
LPH_{Nic} [f]	122.54± 6.41	0.22± 0.02	-18.78± 2.11
$LPH_{Nic-Pir}$ [f]	126.54± 8.45	0.24± 0.03	-16.554± 3.25

[a] Measured by dynamic light scattering.
[b] Surface charge measured by electrophoresis.
[c] Expressed as mean ± SD (n=3).
[d] Statistical analysis was done by student's T-test between each LPH and its PLGA NPs counterpart and * $p< 0.05$ was considered significant.
[e] PLGA NPs composed of PLGA (5 mg/mL), Tween® 80 (10 mg/mL), with the assigned drugs (0.75 mg/mL).
[f] LPH composed of PLGA (5 mg/mL), lecithin (0.5 mg/mL), DSPE-PEG 2000 (0.5 mg/mL), Tween® 80 (10 mg/mL), with the assigned drugs (0.75 mg/mL).

Table S3. Entrapment efficiency of azithromycin, niclosamide and piroxicam in different PLGA NPS and LPH formulations.

Formula	Azithromycin [a, b, c]	Niclosamide [a, b, c]	Piroxicam [a, b, c]
PLGA NPs_{Azi} [d]	65.24± 4.25*	-------	-------
PLGA NPs_{Pir} [d]	-------	-------	47.36± 1.59*
PLGA $NPs_{Azi-Pir}$ [d]	58.12± 3.94*	-------	37.14± 2.58*
PLGA NPs_{Nic} [d]	-------	64.23± 2.01*	-------
PLGA $NPs_{Nic-Pir}$ [d]	-------	59.13± 1.23*	31.59± 2.25*
LPH_{Azi} [e]	83.26± 4.69	-------	-------
LPH_{Pir} [e]	-------	-------	69.11± 6.57
$LPH_{Azi-Pir}$ [e]	74.23± 8.14	-------	51.52± 5.45
LPH_{Nic} [e]	-------	82.11± 4.91	-------
$LPH_{Nic-Pir}$ [e]	-------	85.14± 3.47	48.75± 4.77

[a] Calculated as a percentage of initial drug added, determined by HPLC.
[b] Expressed as mean ± SD (n=3).
[c] Statistical analysis was done by student's T-test between each LPH and its PLGA NPs counterpart and * $p< 0.05$ was considered significant.
[d] PLGA NPs composed of PLGA (5 mg/mL), Tween® 80 (10 mg/mL), with the assigned drugs (0.75 mg/mL).
[e] LPH composed of PLGA (5 mg/mL), lecithin (0.5 mg/mL), DSPE-PEG 2000 (0.5 mg/mL), Tween® 80 (10 mg/mL), with the assigned drugs (0.75 mg/mL).

Table S4: Consensus scores for macrolides compounds with (PDB: ID 6WUU)

Name	Consensus Score
Bafilomycin D	9
Standard ligand of ID 6wuu	33
Bromophycolide	34

Saccharthriolide	37
Umifenovir	40
Bromophyycolide	41
Bafilomycin	45
Chalcomycin A	47
Bafilomycin B1	49
Doxycycline	50
Saccharthriolide B	51
Tedanolide C	54
Remdesivir	56
Seimatopolide B	60
Akaemycin	66
Sofosbuvir	68
N3	68
Bromophycolide	74
Niclosamide	74
Spike ligand	78
Seimatopolide	89
Aspergillide	89
Supertoside A	90
Supertolide	97
Saccharothriolide C	97
JBIR	100
Chalcomycin	102
Erythromycin	102
GW479439	112
Bromophycolide	112
Azithromycin	113
Nitazoxanide	115
Modiolide E	119
Macrophelide	119
Modiolide D	120
Erthronolide B	123
Balticolid	125
Modiolide	146
Halichondramide	160

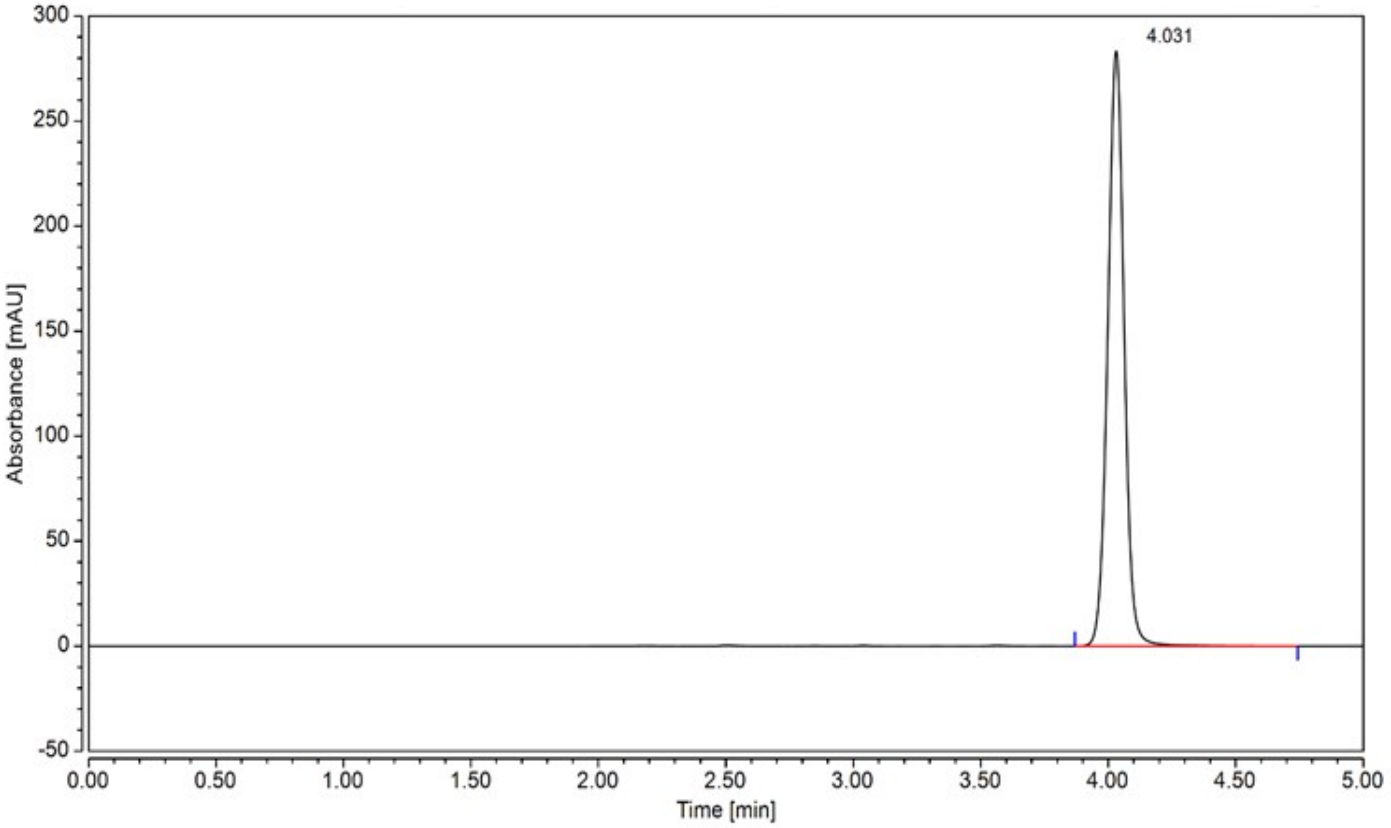


Figure S1: HPLC Chromatogram of 10 µg/mL niclosamide standard solution

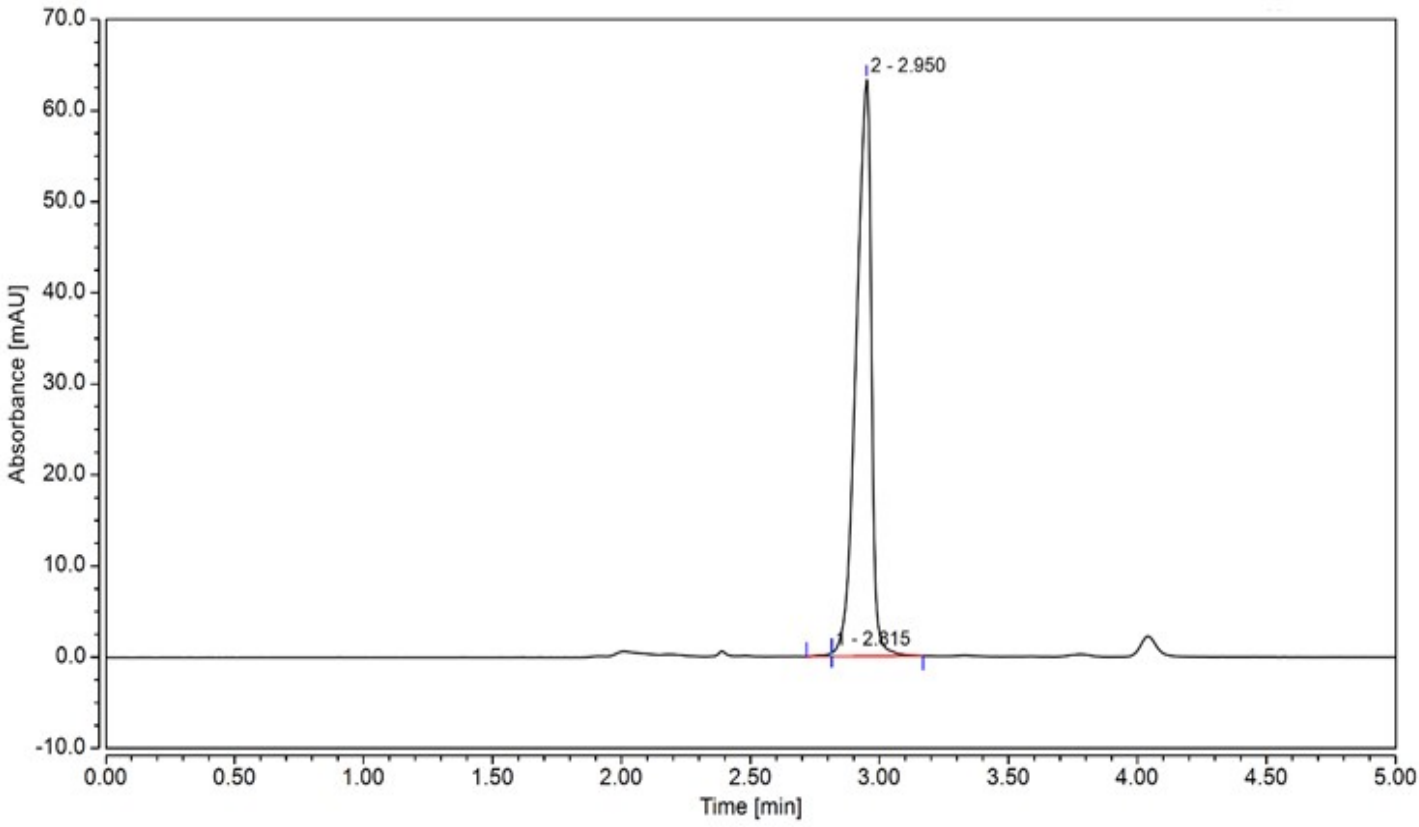


Figure S2: HPLC Chromatogram of 30 µg/mL piroxicam standard solution

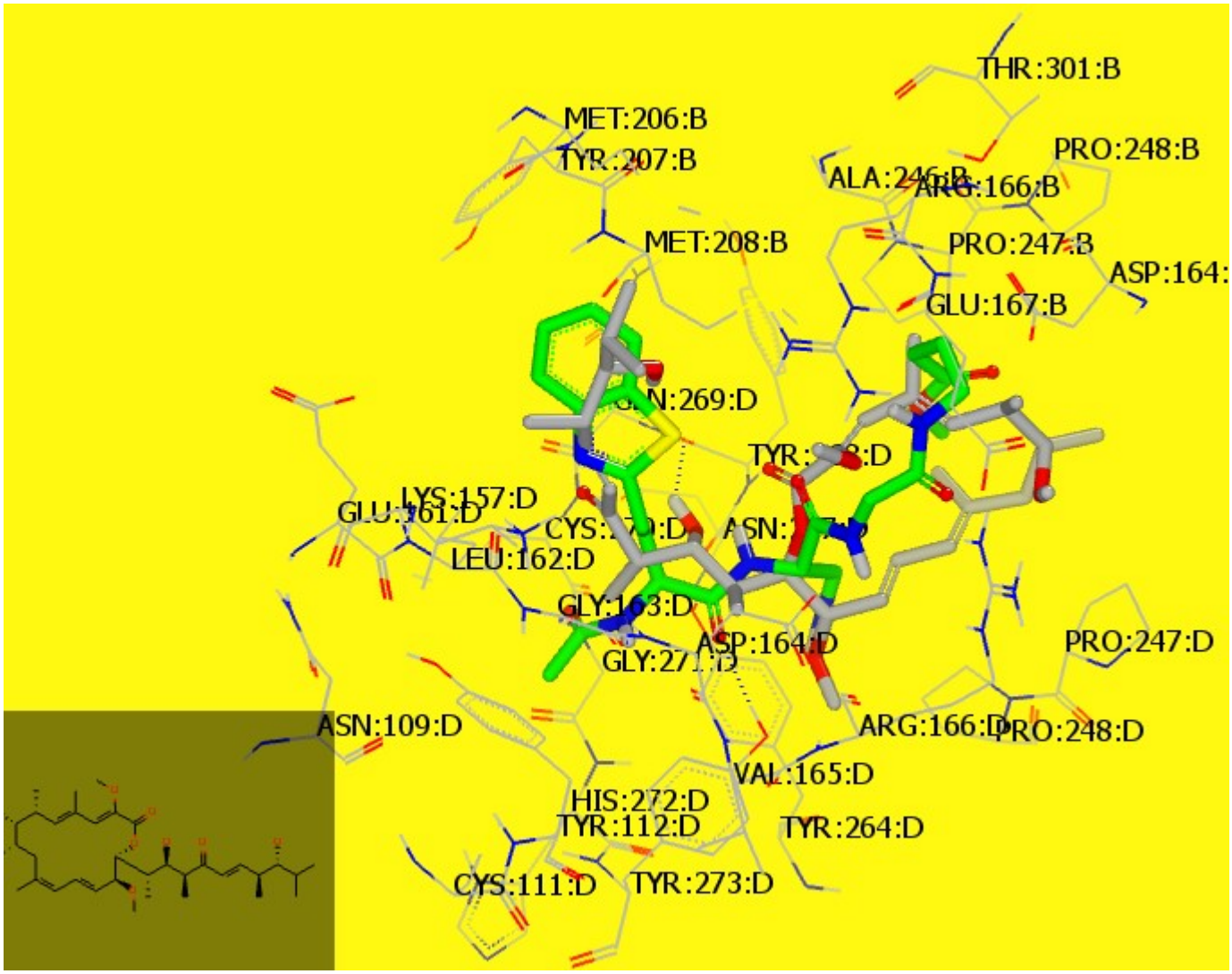


Figure S3: Bafilomycin D showed high overlay to the standard ligand with hydrophobic-hydrophobic interaction.

References

1. M. A. M. Shushni, R. Singh, R. Mentel and U. Lindequist, *Mar Drugs*, 2011, **9**, 844-851.
2. K. Anjum, S. Q. Abbas, S. A. A. Shah, N. Akhter, S. Batool and S. S. u. Hassan, *Biomolecules & Therapeutics*, 2016, **24**, 347-362.
3. H. Zhang, J. Zou, X. Yan, J. Chen, X. Cao, J. Wu, Y. Liu and T. Wang, 2021, **19**, 180.
4. R. N. Asolkar, R. P. Maskey, E. Helmke and H. Laatsch, *The Journal of antibiotics*, 2002, **55**, 893-898.

5. J. Bao, X.-Y. Xu, X.-Y. Zhang and S.-H. Qi, *Natural Product Communications*, 2013, **8**, 1934578X1300800825.
6. S. Lu, S. Nishimura, G. Hirai, M. Ito, T. Kawahara, M. Izumikawa, M. Sodeoka, K. Shin-ya, T. Tsuchida and H. Kakeya, *Chemical Communications*, 2015, **51**, 8074-8077.
7. N. T. M. P. M. R. A. H. J. M. Whipps, *Journal of Applied Microbiology*, 2009, **106**, 2048–2056.
8. T. Hosoe, K. Fukushima, K. Takizawa, T. Itabashi, N. Kawahara, V. Vidotto and K. Kawai, *The Journal of antibiotics*, 2006, **59**, 597-600.
9. C. Chevallier, T. S. Bugni, X. Feng, M. K. Harper, A. M. Orendt and C. M. Ireland, *The Journal of Organic Chemistry*, 2006, **71**, 2510-2513.
10. Y.-H. Liu, Shinde, Pramod B., Hong, Jong-Ki, Lee, Chong-O., Im, Kwang-Sik, Jung, Jee-H., *Natural Product Sciences*, 2005, **11**, 50-53.
11. G. F. Barrie Wilkinson, Brian AM Rudd, Nicholas L Taylor, Andrew P Blackaby, Philip J Sidebottom, David J Cooper, Michael J Dawson, Anthony D Buss, Sabine Gaisser, Ines U Böhm,Christine J Rowe3, Jesús Cortés, Peter F Leadlay and James Staunton, *Chemistry & Biology* 2000, **7**.
12. N. T. Hiep, Y. H. Choi, N. Kim, S. S. Hong, S. B. Hong, B. Y. Hwang, H. J. Lee, S. J. Lee, D. S. Jang and D. Lee, *Journal of natural products*, 2012, **75**, 784-788.
13. Z. T. Dame and P. Ruanpanun, *World journal of microbiology & biotechnology*, 2017, **33**, 139.
14. M. V. D'Auria, L. G. Paloma, L. Minale, A. Zampella and C. Debitus, *Journal of natural products*, 1994, **57**, 1595-1597.
15. M. R. d. A. Weslei Bruno Botero, Iracilda Z. Carlos, Marisa C. Polesib and Lourdes C. dos Santos, *J. Braz. Chem. Soc.*, 2020, **31**, 364-369.
16. S. E. Rossiter, M. H. Fletcher and W. M. Wuest, *Chemical reviews*, 2017, **117**, 12415-12474.
17. S. Kodani, A. Murao, M. Hidaki, K. Sato and N. Ogawa, *The Journal of antibiotics*, 2012, **65**, 331-334.
18. Z. Tao, S. Seizo, K. Hisayuki and I. Yasuhiro, *Current Biotechnology*, 2015, **4**, 249-253.
19. A. Sahin, G. Esendagli, F. Yerlikaya, S. Caban-Toktas, D. Yoyen-Ermis, U. Horzum, Y. Aktas, M. Khan, P. Couvreur and Y. Capan, *Artificial Cells, Nanomedicine, and Biotechnology*, 2017, **45**, 1657-1664.
20. B. Gajra, R. R. Patel and C. Dalwadi, *Drug Development and Industrial Pharmacy*, 2016, **42**, 747-757.
21. J.-S. Baek, C. H. Tan, N. K. J. Ng, Y. P. Yeo, S. A. Rice and S. C. J. Loo, *Nanoscale Horizons*, 2018, **3**, 305-311.
22. S. A. Kulkarni and S.-S. Feng, *Pharmaceutical Research*, 2013, **30**, 2512-2522.
23. K. Yoncheva, E. Lizarraga and J. M. Irache, *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 2005, **24**, 411-419.
24. H. Zhang, K. Wang, X. Xuan, Q. Lv, Y. Nie and H. Guo, *Chemical Communications*, 2016, **52**, 6308-6311.
25. W. S. Cheow and K. Hadinoto, *Colloids and surfaces. B, Biointerfaces*, 2011, **85**, 214-220.
26. J. Lalani, Y. Raichandani, R. Mathur, M. Lalan, K. Chutani, A. K. Mishra and A. Misra, *Journal of biomedical nanotechnology*, 2012, **8**, 918-927.

Figure S3: HPLC Chromatogram of 100 µg/mL azithromycin standard solution

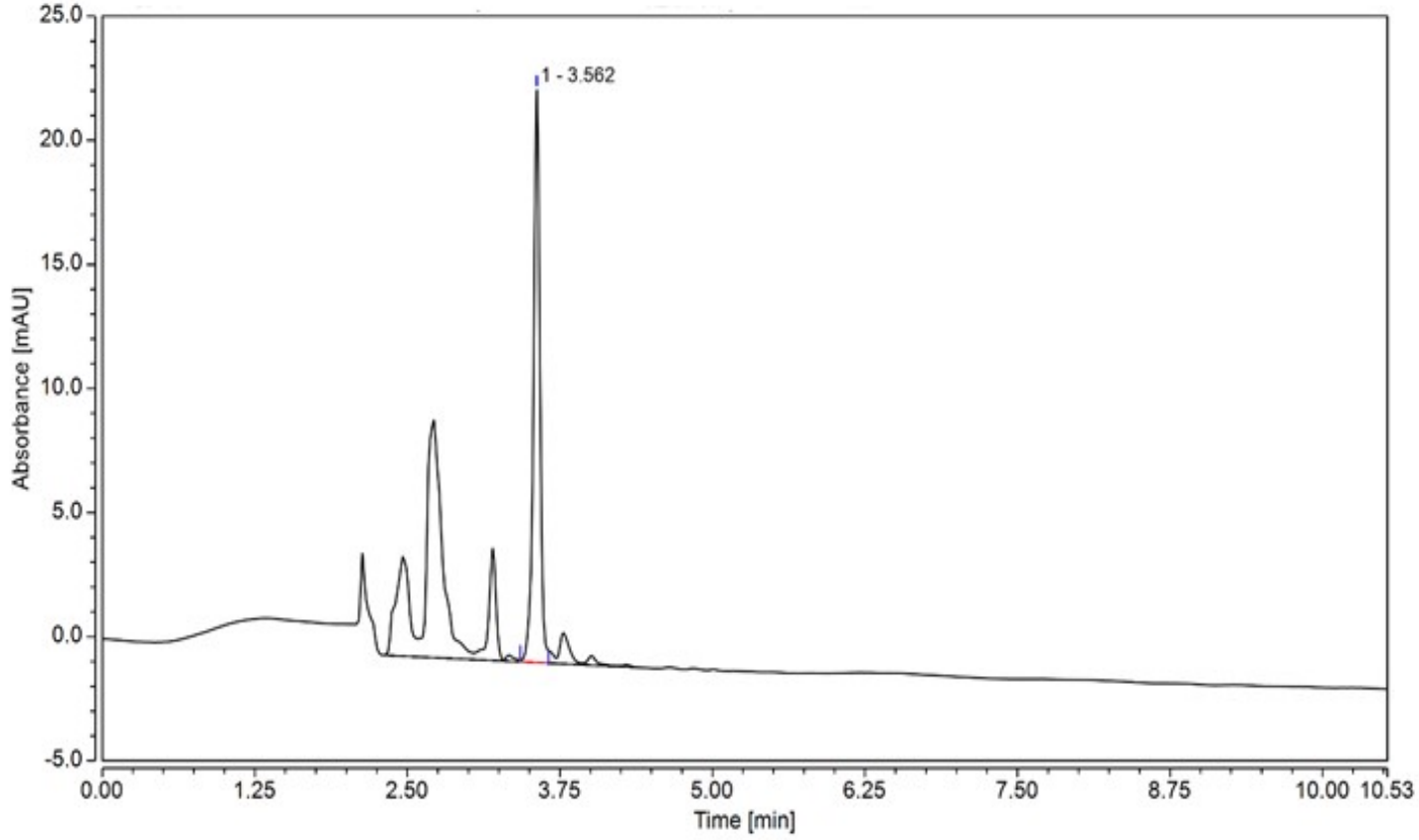

1 - 3.562
Absorbance [mAU]
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
0.00
1.25
2.50
3.75
5.00
6.25
7.50
8.75
10.00
10.53
Time [min]

Figure S 4: Baflimycin D overlay the standard ligand of ID: 6wuu